B.Sc. Microbiology **2018-2019**

Semester – III

18MBU303 **ADVANCED BIOCHEMISTRY**

COURSE OBJECTIVES

- To provide the informative understanding on Advances in Biochemistry and its applications.
- It serves as good research knowledge on various metabolic pathways that prevails inside the human body.

COURSEOUTCOME (CO'S)

1. A candidate can able to understand metabolic pathways of carbohydrates, proteins, Lipids and Nucleic acid.
2. This skill based course will provide clear understanding about the Biological oxidation.

Unit I- Introduction to metabolism

Bioenergitics - Thermodynamics principles, Concepts of free energy, Standard free energy, Biological oxidation- reduction reactions, redox potential, High Energy phosphate compounds.

Unit II- Carbohydrate metabolism

Glycolysis, TCA cycle, Glycogenesis, Glycogenolysis, HMP shunt, Gluconeogenesis, Glucuronic acid pathway.

Unit III- Lipid metabolism

Fatty acid oxidation – α, β, ω oxidation. Biosynthesis of saturated and unsaturated fatty acids. Metabolism ofcholesterol, triglycerides and ketone bodies.

Unit IV- Protein metabolism

Ketogenic and Glucogenic amino acids. Degradation of proteins: Deamination, Transamination and Decarboxylation, Urea cycle.

Unit V- Nucleic acid metabolism and Biological oxidation

Biosynthesis and degradation of purine and pyrimidine nucleotides. Mitochondrial Electron Transport Chain: electron carriers, sites of ATP production, inhibitors of ETC, Oxidative phosphorylation:- structure of ATPase complex, chemiosmotic theory, inhibitors of oxidative phosphorylation and uncouplers, Mitochondrial shuttle system.

SUGGESTED READINGS

1. Fundamentals of Biochemistry, J.L. Jain, S.Chand publications, 2004.
2. Lehninger's Principles of Biochemistry (2000) by Nelson, David l. and Cox, M.M. Macmillan /Worth, NY.
3. Harper's Biochemistry Robert K. Murray, Daryl K. Granner, Peter A. Mayes, Victor W. Rodwell, 24th edition, Prentice Hall International. Inc.
4. Principles of Biochemistry, Geoffrey L. Zubay, 3rd edition William W. Parson, Dennis E. Vance, W.C. Brown Publishers, 1995.
5. Principles of Biochemistry, David L. Nelson, Michael M.Cox, Lehninger, 4th edition, W.H. Freeman and company.
6. Biochemistry, LubertStryer, 4th edition, W.H. Freeman & Co, 1995.
7. Fundamentals of Biochemistry (1999) by Donald Voet, Judith G.Voet and Charlotte W Pratt, John Wiley & Sons, NY.

KARPAGAM ACADEMY OF HIGHER EDUCATION

(Deemed to be University)
(Established Under Section 3 of UGC Act 1956)
Coimbatore - 641021.
(For the candidates admitted from 2018 onwards)
DEPARTMENT OF BIOCHEMISTRY

SUBJECT: ADVANCED BIOCHEMISTRY **SEMESTER: III**

SUBJECT CODE: 18MBU303 **CLASS: II B.Sc., MB**

LECTURE PLAN
DEPARTMENT OF BIOCHEMISTRY

S. No	Duration of period	Topics covered	Books referred	Page No	Web page referred
UNIT-I (Introduction to metabolism)					
1	1	Bioenergitics			-
2		Principles of Thermodynamics			
3	1	Concepts of free energy			-
4		Standard free energy			
5	1	Biological oxidation			-
6	1	Reduction reactions			-
7	1	Redox potential			-
8	1	High Energy phosphate compounds			
9	1	Revision and QP discussion			-
		Total No. of Hours Planned for Unit I: 09			
UNIT-II (Carbohydrate metabolism)					
1	1	Glycolysis			-
2	1	TCA cycle,			-
3	1	Glycogenesis			-
4	1	Glycogenolysis			-
5	1	HMP shunt			-
6	1	Gluconeogenesis			-
7	1	Glucuronic acid pathway			-
8	1	Revision and QP discussion			-

		Total No. of Hours Planned for Unit II: 08			
UNIT-III (Lipid metabolism)					
1	1	Fatty acid oxidation- β Oxidation			-
2	1	α and ω oxidation of fatty acids			-
3	1	Biosynthesis of saturated fatty acids			-
4	1	Biosynthesis of unsaturated fatty acids			-
5	1	Metabolism of cholesterol			-
6	1	Metabolism of triglycerides			-
7	1	Metabolism of ketone bodies			-
8	1	Revision and QP discussion			-
		Total No of Hours Planned for Unit III: 08			-
UNIT-IV (Protein metabolism)					
1	1	Ketogenic amino acids			-
2		Glucogenic amino acids			
3	1	Degradation of proteins: Deamination			-
4	1	Transamination of proteins			-
5	1	Decarboxylation of proteins			-
6	1	Urea cycle			-
7	1	Revision and discussion of possible question			
		Total No of Hours Planned for Unit IV: 07			
	UNIT-V (Nucleic acid metabolism and Biological oxidation)				
1	1	Biosynthesis of purine nucleotides			
2	1	Biosynthesis of pyrimidine nucleotides			
3	1	Degradation of purine nucleotides			
4	1	Degradation of pyrimidine nucleotides			
5	1	Mitochondrial Electron Transport Chain electron carriers			
6	1	sites of ATP production			
7	1	Inhibitors of ETC			
8	1	Oxidative phosphorylation			
9	1	structure of ATPase complex			

9	1	Chemiosmotic theory			
10	1	Inhibitors of oxidative phosphorylation and uncouplers			
11	1	Mitochondrial shuttle system			
12	1	Revision and discussion of possible question			
		Total No of Hours Planned for Unit V: 12			
1	1	Previous year End Semester Exam- QP discussion	-	-	-
2	1	Previous year End Semester Exam- QP discussion	-	-	-
3	1	Previous year End Semester Exam- QP discussion	-	-	-
Total	**03**	**Hours planned for QP discussion: 03**			
	Total No of Hours Planned For this syllabi: 48				

TEXT BOOK

Lehninger Principles of Biochemistry, David L. Nelson, Michael M. Cox. Fourth edition.

REFERENCES

Harper's Illustrated Biochemistry, Robert K. Murray, Daryl K. Granner, Peter A. Mayes, Victor W. Rodwell. Twenty sixth edition.

Biochemistry, Jeremey M. Berg, John L. Tymoczko, Lubert Stryer. Fifth Edition.

UNIT-I

SYLLABUS

Introduction to metabolism: Bioenergetics- Thermodynamics principles, Concepts of free energy, Standard free energy, Biological oxidation-reduction reactions, redox potential, High energy phosphate compounds.

Bioenergetics and Thermodynamics

Bioenergetics is the quantitative study of the energy transductions that occur in living cells and of the nature and function of the chemical processes underlying these transductions. Although many of the principles of thermodynamics have been introduced in earlier chapters and may be familiar to you, a review of the quantitative aspects of these principles is useful here.

Laws of Thermodynamics

The first law is the principle of the conservation of energy: *for any physical or chemical change, the total amount of energy in the universe remains constant; energy may change form or it may be transported from one region to another, but it cannot be created or destroyed.*

The second law of thermodynamics, which can be stated in several forms, says that the universe always tends toward increasing disorder: *in all natural processes, the entropy of the universe increases.*

Three thermodynamic quantities that describe the energy changes occurring in a chemical reaction:

Gibbs free energy, *G,* expresses the amount of energy capable of doing work during a reaction at constant temperature and pressure. When a reaction proceeds with the release of free energy (that is, when the system changes so as to possess less free energy), the free-energy change, ΔG, has a negative value and the reaction is said to be exergonic. In endergonic reactions, the system gains free energy and ΔG is positive.

Enthalpy, *H,* is the heat content of the reacting system. It reflects the number and kinds of chemical bonds in the reactants and products. When a chemical reaction releases heat, it is said to

be exothermic; the heat content of the products is less than that of the reactants and ΔH has, by convention, a negative value. Reacting systems that take up heat from their surroundings are endothermic and have positive values of ΔH.

Entropy, *S,* is a quantitative expression for the randomness or disorder in a system. When the products of a reaction are less complex and more disordered than the reactants, the reaction is said to proceed with a gain in entropy.

The units of ΔG and ΔH are joules/mole or calories/mole (recall that 1 cal _ 4.184 J); units of entropy are joules/mole _ Kelvin (J/mol _ K). Under the conditions existing in biological systems (including constant temperature and pressure), changes in free energy, enthalpy, and entropy are related to each other quantitatively by the equation $\Delta G = \Delta H - T\Delta S$ in which ΔG is the change in Gibbs free energy of the reacting system, ΔH is the change in enthalpy of the system, T is the absolute temperature, and ΔS is the change in entropy of the system. By convention, ΔS has a positive sign when entropy increases and ΔH, as noted above, has a negative sign when heat is released by the system to its surroundings. Either of these conditions, which are typical of favorable processes, tend to make ΔG negative. In fact, ΔG of a spontaneously reacting system is always negative. The second law of thermodynamics states that the entropy of the universe increases during all chemical and physical processes, but it does not require that the entropy increase take place in the reacting system itself. The order produced within cells as they grow and divide is more than compensated for by the disorder they create in their surroundings in the course of growth and division. In short, living organisms preserve their internal order by taking from the surroundings free energy in the form of nutrients or sunlight, and returning to their surroundings an equal amount of energy as heat and entropy.

Free Energy

Cells are isothermal systems—they function at essentially constant temperature (they also function at constant pressure). Heat flow is not a source of energy for cells, because heat can do work only as it passes to a zone or object at a lower temperature. The energy that cells can and must use is free energy, described by the Gibbs free-energy function *G*, which allows prediction of the direction of chemical reactions, their exact equilibrium position, and the amount of work they can in theory perform at constant temperature and pressure. Heterotrophic cells acquire free energy

from nutrient molecules, and photosynthetic cells acquire it from absorbed solar radiation. Both kinds of cells transform this free energy into ATP and other energy-rich compounds capable of providing energy for biological work at constant temperature.

The Standard Free-Energy Change Is Directly Related to the Equilibrium Constant

The composition of a reacting system (a mixture of chemical reactants and products) tends to continue changing until equilibrium is reached. At the equilibrium concentration of reactants and products, the rates of the forward and reverse reactions are exactly equal and no further net change occurs in the system. The concentrations of reactants and products *at equilibrium* define the equilibrium constant, *K*eq (p. 26). In the general reaction $aA + bB = cC + dD$, where *a*, *b*, *c*, and *d* are the number of molecules of A, B, C, and D participating, the equilibrium constant is given by

$$K_{eq} = \frac{[C]^c\,[D]^d}{[A]^a\,[B]^b}$$

where [A], [B], [C], and [D] are the molar concentrations of the reaction components at the point of equilibrium.

Physical constants based on this biochemical standard state are called **standard transformed constants** and are written with a prime (such as $\Delta G'$ and K'_{eq}) to distinguish them from the untransformed constants used by chemists and physicists. (Notice that most other textbooks use the symbol ΔG rather than $\Delta G'$. Our use of $\Delta G'$, recommended by an international committee of chemists and biochemists, is intended to emphasize that the transformed free energy G' is the criterion for equilibrium.) By convention, when H2O, H+, and/or Mg2+ are reactants or products, their concentrations are not included in equations such as Equation 13–2 but are instead incorporated into the constants K'_{eq} and $\Delta G'$.

Biological Oxidation-Reduction Reactions

Chemically, **oxidation** is defined as the removal of electrons and **reduction** as the gain of electrons. Thus, oxidation is always accompanied by reduction of an electron acceptor. This principle of oxidation-reduction applies equally to biochemical systems and is an important concept underlying understanding of the nature of biologic oxidation. Note that many biologic oxidations can take place without the participation of molecular oxygen, eg, dehydrogenations.

The life of higher animals is absolutely dependent upon a supply of oxygen for **respiration,** the process by which cells derive energy in the form of ATP from the controlled reaction of hydrogen with oxygen to form water. In addition, molecular oxygen is incorporated into a variety of substrates by enzymes designated as **oxygenases;** many drugs, pollutants, and chemical carcinogens (xenobiotics) are metabolized by enzymes of this class, known as the **cytochrome P450 system.** Administration of oxygen can be lifesaving in the treatment of patients with respiratory or circulatory failure.

FREE ENERGY CHANGES CAN BE EXPRESSED IN TERMS OF REDOX POTENTIAL

In reactions involving oxidation and reduction, the free energy change is proportionate to the tendency of reactants to donate or accept electrons. Thus, in addition to expressing free energy change in terms of ΔG0′, it is possible, in an analogous manner, to express it numerically as an **oxidation-reduction** or **redox potential** (E′0). The redox potential of a system (E0) is usually compared with the potential of the hydrogen electrode (0.0 volts at pH 0.0). However, for biologic systems, the redox potential (E′0) is normally expressed at pH 7.0, at which pH the electrode potential of the hydrogen electrode is −0.42 volts. The relative positions of redox systems in the table allows prediction of the direction of flow of electrons from one redox couple to another. Enzymes involved in oxidation and reduction are called **oxidoreductases** and are classified into four groups: **oxidases, dehydrogenases, hydroperoxidases,** and **oxygenases.**

OXIDASES USE OXYGEN AS A HYDROGEN ACCEPTOR

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

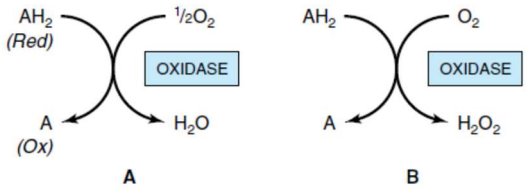


Figure 11–1. Oxidation of a metabolite catalyzed by an oxidase **(A)** forming H_2O, **(B)** forming H_2O_2.

SOME OXIDASES CONTAIN COPPER

Cytochrome oxidase is a hemoprotein widely distributed in many tissues, having the typical heme prosthetic group present in myoglobin, hemoglobin, and other cytochromes (Chapter 6). It is the terminal component of the chain of respiratory carriers found in mitochondria and transfers electrons resulting from the oxidation of substrate molecules by dehydrogenases to their final acceptor, oxygen. The enzyme is poisoned by carbon monoxide, cyanide, and hydrogen sulfide. It has also been termed cytochrome *a*3. It is now known that cytochromes *a* and *a*3 are combined in a single protein, and the complex is known as **cytochrome *aa*3.** It contains two molecules of heme, each having one Fe atom that oscillates between Fe3+ and Fe2+ during oxidation and reduction. Furthermore, two atoms of Cu are present, each associated with a heme unit.

Other Oxidases Are Flavoproteins

Flavoprotein enzymes contain flavin mononucleotide (FMN) or flavin adenine dinucleotide (FAD) as prosthetic groups. FMN and FAD are formed in the body from the vitamin riboflavin (Chapter 45). FMN and FAD are usually tightly—but not covalently—bound to their respective apoenzyme proteins. Metalloflavoproteins contain one or more metals as essential cofactors. Examples of flavoprotein enzymes include L-amino acid oxidase, an FMN-linked enzyme found in kidney with general specificity for the oxidative deamination of the naturally occurring L-amino acids; **xanthine oxidase,** which contains molybdenum and plays an important role in the conversion of purine bases to uric acid**,** and is of particular significance in uricotelic animals and **aldehyde dehydrogenase,** an FAD-linked enzyme present in mammalian livers, which contains molybdenum and nonheme iron and acts upon aldehydes and N-heterocyclic substrates. The mechanisms of oxidation and reduction of these enzymes are complex. Evidence suggests a two step reaction shown below.

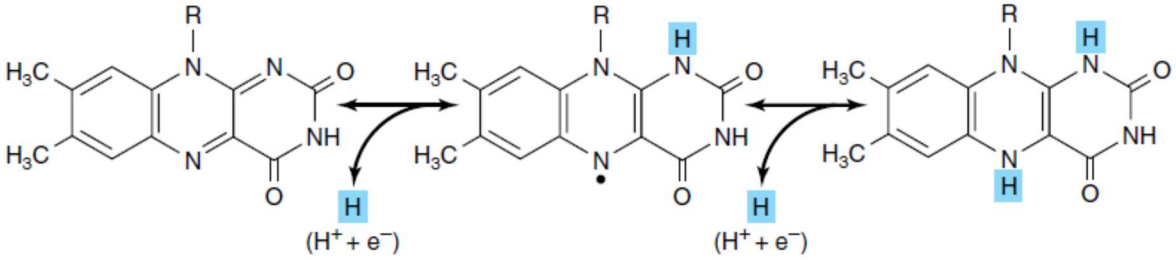


Figure 11–2. Oxidoreduction of isoalloxazine ring in flavin nucleotides via a semi-quinone (free radical) intermediate (center).

DEHYDROGENASES CANNOT USE OXYGEN AS A HYDROGEN ACCEPTOR

There are a large number of enzymes in this class. They perform two main functions:

1. Transfer of hydrogen from one substrate to another in a coupled oxidation-reduction reaction (Figure 11–3). These dehydrogenases are specific for their substrates but often utilize common coenzymes or hydrogen carriers, eg, NAD+. Since the reactions are reversible these properties enable reducing equivalents to be freely transferred within the cell. This type of reaction, which enables one substrate to be oxidized at the expense of another, is particularly useful in enabling oxidative processes to occur in the absence of oxygen, such as during the anaerobic phase of glycolysis.

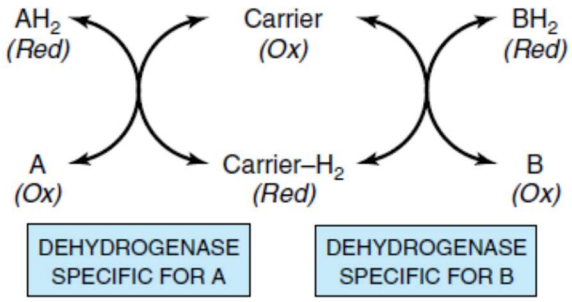


Figure 11–3. Oxidation of a metabolite catalyzed by coupled dehydrogenases.

2. As components in the **respiratory chain** of electron transport from substrate to oxygen (Figure 12–3).

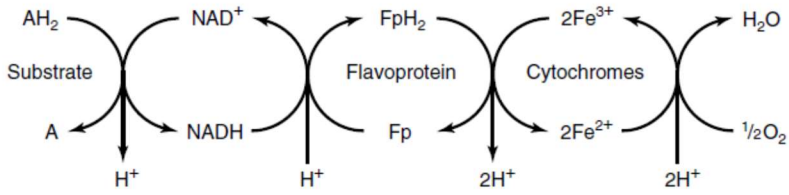
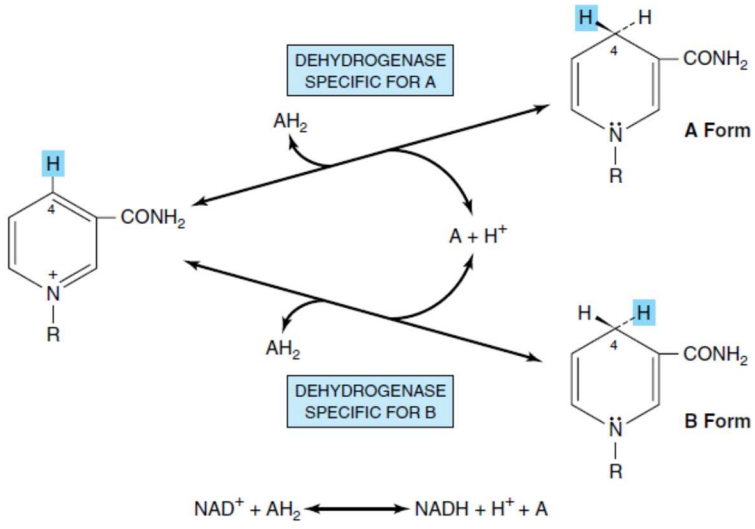


Figure 12–3. Transport of reducing equivalents through the respiratory chain.

Many Dehydrogenases Depend on Nicotinamide Coenzymes

These dehydrogenases use **nicotinamide adenine dinucleotide (NAD+)** or **nicotinamide adenine dinucleotidephosphate (NADP+)**—or both—and are formed in the body from the vitamin **niacin** (Chapter 45). The coenzymes are reduced by the specific substrate of the dehydrogenase and reoxidized by a suitable electron acceptor (Figure 11–4).They may freely and reversibly dissociate from their respective apoenzymes. Generally, NAD-linked dehydrogenases catalyze oxidoreduction reactions in the oxidative pathways of metabolism, particularly in glycolysis, in the citric acid cycle, and in the respiratory chain of mitochondria. NADP-linked dehydrogenases are found characteristically in reductive syntheses, as in the extramitochondrial pathway of fatty acid synthesis and steroid synthesis and also in the pentose phosphate pathway.

Figure 11–4. Mechanism of oxidation and reduction of nicotinamide coenzymes. There is stereospecificity about position 4 of nicotinamide when it is reduced by a substrate AH_2. One of the hydrogen atoms is removed from the substrate as a hydrogen nucleus with two electrons (hydride ion, H^-) and is transferred to the 4 position, where it may be attached in either the A or the B position according to the specificity determined by the particular dehydrogenase catalyzing the reaction. The remaining hydrogen of the hydrogen pair removed from the substrate remains free as a hydrogen ion.



Other Dehydrogenases Depend on Riboflavin

The flavin groups associated with these dehydrogenases are similar to FMN and FAD occurring in oxidases. They are generally more tightly bound to their apoenzymes than are the nicotinamide coenzymes. Most of the riboflavin-linked dehydrogenases are concerned with electron transport in (or to) the respiratory chain (Chapter 12). NADH dehydrogenase acts as a carrier of electrons between NADH and the components of higher redox potential (Figure 12–3). Other dehydrogenases such as succinate dehydrogenase, acyl-CoA dehydrogenase, and mitochondrial glycerol-3-phosphate dehydrogenase transfer reducing equivalents directly from the substrate to the respiratory chain (Figure 12–4). Another role of the flavin-dependent dehydrogenases is in the dehydrogenation (by dihydrolipoyl dehydrogenase) of reduced lipoate, an intermediate in the oxidative decarboxylation of pyruvate and α-ketoglutarate (Figures 12–4 and 17–5). The electron-transferring flavoprotein is an intermediary carrier of electrons between acyl-CoA dehydrogenase and the respiratory chain.

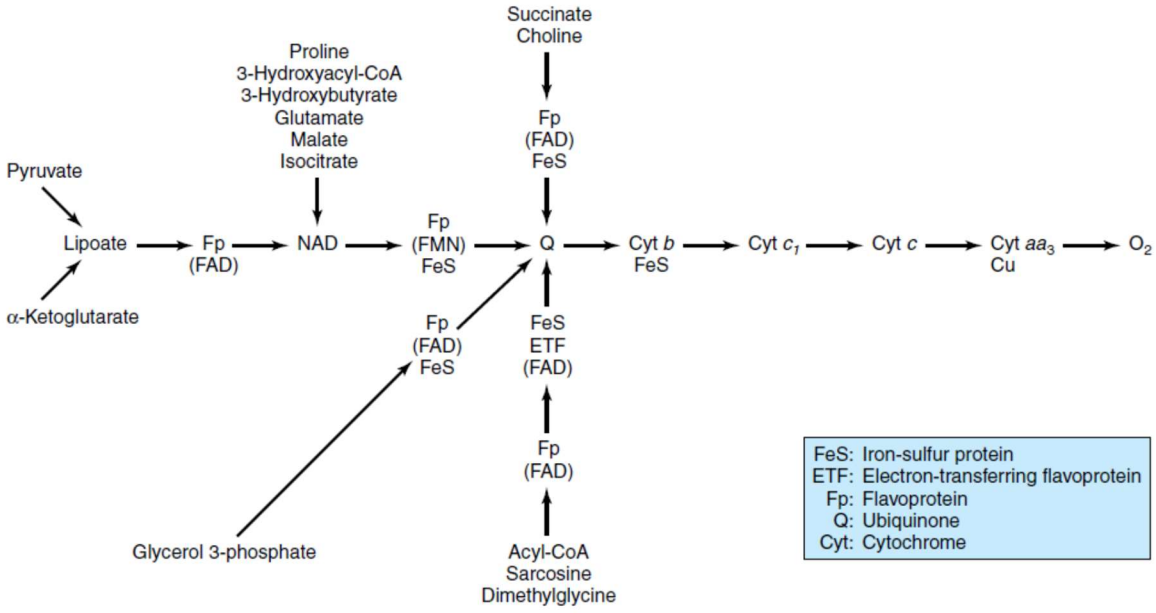


Figure 12–4. Components of the respiratory chain in mitochondria, showing the collecting points for reducing equivalents from important substrates. FeS occurs in the sequences on the O_2 side of Fp or Cyt *b*.

HIGH-ENERGY PHOSPHATES PLAY A CENTRAL ROLE IN ENERGY CAPTURE AND TRANSFER

In order to maintain living processes, all organisms must obtain supplies of free energy from their environment. **Autotrophic** organisms utilize simple exergonic processes; eg, the energy of sunlight (green plants), the reaction $Fe^{2+} \rightarrow Fe^{3+}$ (some bacteria). On the other hand, **heterotrophic** organisms obtain free energy by coupling their metabolism to the breakdown of complex organic molecules in their environment. In all these organisms, ATP plays a central role in the transference of free energy from the exergonic to the endergonic processes (Figure 10–3). ATP is a nucleoside triphosphate containing adenine, ribose, and three phosphate groups. In its reactions in the cell, it functions as the Mg^{2+} complex.

The importance of phosphates in intermediary metabolism became evident with the discovery of the role of ATP, adenosine diphosphate (ADP), and inorganic phosphate (Pi) in glycolysis.

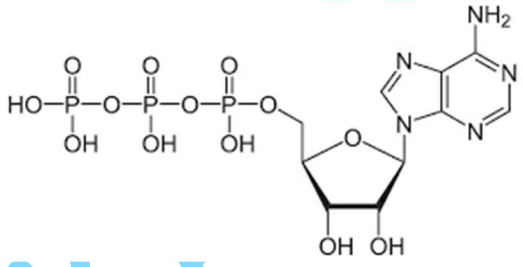


Fig. Molecular structure of ATP

The standard free energy of hydrolysis of a number of biochemically important phosphates. An estimate of the comparative tendency of each of the phosphate groups to transfer to a suitable acceptor may be obtained from the $\Delta G0'$ of hydrolysis at 37 °C. The value for the hydrolysis of the terminal phosphate of ATP divides the list into two groups. **Low-energy phosphates,** exemplified by the ester phosphates found in the intermediates of glycolysis, have $\Delta G0'$ values smaller than that of ATP, while in **high-energy phosphates** the value is higher than that of ATP. The components of this latter group, including ATP, are usually anhydrides (eg, the 1-phosphate of 1,3-bisphosphoglycerate), enolphosphates (eg, phosphoenolpyruvate), and phosphoguanidines (eg, creatine phosphate, arginine phosphate). The intermediate position of ATP allows it to play an important role in energy transfer. The high free energy change on hydrolysis of ATP is due to relief of charge repulsion of adjacent negatively charged oxygen atoms and to stabilization of the

reaction products, especially phosphate, as resonance hybrids. Other "high-energy compounds" are thiol esters involving coenzyme A (eg, acetyl-CoA), acyl carrier protein, amino acid esters involved in protein synthesis, *S*-adenosylmethionine (active methionine), UDPGlc (uridine diphosphate glucose), and PRPP (5-phosphoribosyl-1-pyrophosphate).

High-Energy Phosphates Are Designated by ~ _P

The symbol ~_P indicates that the group attached to the bond, on transfer to an appropriate acceptor, results in transfer of the larger quantity of free energy. For this reason, the term **group transfer potential** is preferred by some to "high-energy bond." Thus, ATP contains two high-energy phosphate groups and ADP contains one, whereas the phosphate in AMP (adenosine monophosphate) is of the low-energy type, since it is a normal ester link.

HIGH-ENERGY PHOSPHATES ACT AS THE "ENERGY CURRENCY" OF THE CELL

ATP is able to act as a donor of high-energy phosphate to form those compounds below it in Table 10–1. Likewise, with the necessary enzymes, ADP can accept high-energy phosphate to form ATP from those compounds above ATP in the table. In effect, an **ATP/ ADP cycle** connects those processes that generate ~_P to those processes that utilize ~_P (Figure 10–6), continuously consuming and regenerating ATP. This occurs at a very rapid rate, since the total ATP/ADP pool is extremely small and sufficient to maintain an active tissue for only a few seconds.

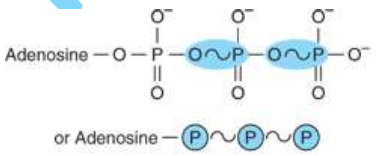


Fig. Structure of ATP, ADP, and AMP showing the position and the number of high-energy phosphates (~_P).

There are three major sources of ~_P taking part in **energy conservation** or **energy capture:**

(1) Oxidative phosphorylation: The greatest quantitative source of ~_P in aerobic organisms. Free energy comes from respiratory chain oxidation using molecular O2 within mitochondria.

(2) Glycolysis: A net formation of two ~_P results from the formation of lactate from one molecule of glucose, generated in two reactions catalyzed by phosphoglycerate kinase and pyruvate kinase, respectively.

(3) The citric acid cycle: One ~_P is generated directly in the cycle at the succinyl thiokinase step.

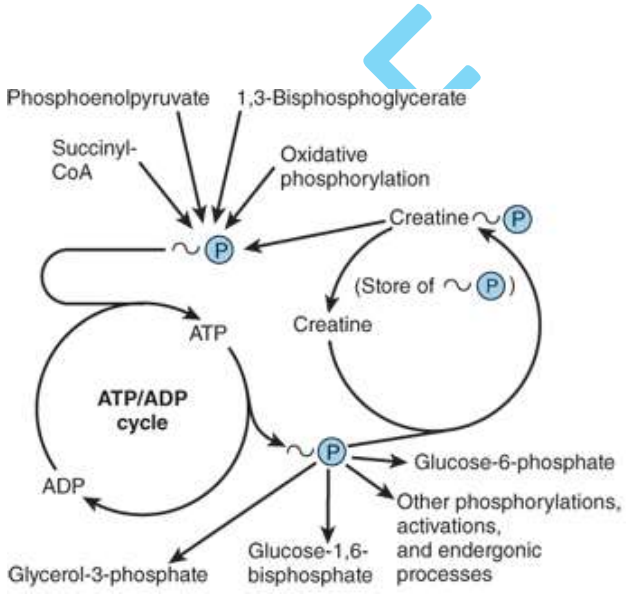


Fig. Role of ATP/ADP cycle in transfer of high-energy phosphate

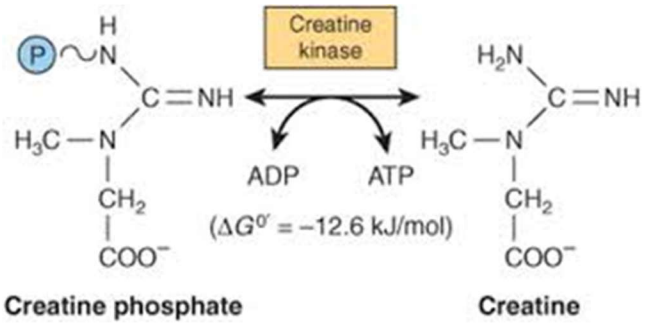


Fig. Transfer of high-energy phosphate between ATP and creatine

Phosphagens act as storage forms of high-energy phosphate and include creatine phosphate, occurring in vertebrate skeletal muscle, heart, spermatozoa, and brain; and arginine phosphate, occurring in invertebrate muscle. When ATP is rapidly being utilized as a source of energy for muscular contraction, phosphagens permit its concentrations to be maintained, but when the ATP/ADP ratio is high, their concentration can increase to act as a store of high-energy phosphate.

ATP Allows the Coupling of Thermodynamically Unfavorable Reactions to Favorable Ones

The phosphorylation of glucose to glucose 6-phosphate, the first reaction of glycolysis, is highly endergonic and cannot proceed under physiologic conditions.

(1) Glucose+Pi → Glucose 6- phosphate+ H2O (ΔG0′ = +13.8 kJ/ mol)

To take place, the reaction must be coupled with another—more exergonic—reaction such as the hydrolysis of the terminal phosphate of ATP.

(2) ATP→ADP+Pi (ΔG0′ = −30.5 kJ /mol)

When (1) and (2) are coupled in a reaction catalyzed by hexokinase, phosphorylation of glucose readily proceeds in a highly exergonic reaction that under physiologic conditions is irreversible. Many “activation” reactions follow this pattern.

Adenylyl Kinase (Myokinase) Interconverts Adenine Nucleotides

This enzyme is present in most cells. It catalyzes the following reaction:

$$ATP + AMP \xrightarrow{\text{Adenylyl Kinase}} 2ADP$$

This allows:

(1) High-energy phosphate in ADP to be used in the synthesis of ATP.

(2) AMP, formed as a consequence of several activating reactions involving ATP, to be recovered by rephosphorylation to ADP.

(3) AMP to increase in concentration when ATP becomes depleted and act as a metabolic (allosteric) signal to increase the rate of catabolic reactions, which in turn lead to the generation of more ATP.

When ATP Forms AMP, Inorganic Pyrophosphate (PPi) Is Produced

This occurs, for example, in the activation of long chain fatty acids:

$$ATP + CoA.SH + R.COOH \xrightarrow{\text{Acyl CoA synthetase}} AMP + PPi + R.CO\text{--}SCoA$$

This reaction is accompanied by loss of free energy as heat, which ensures that the activation reaction will go to the right; and is further aided by the hydrolytic splitting of PPi, catalyzed by **inorganic pyrophosphatase,** a reaction that itself has a large $\Delta G0'$ of -27.6 kJ/mol.

Note that activations via the pyrophosphate pathway result in the loss of two ~_P rather than one ~_P as occurs when ADP and Pi are formed.

$$PPi + H_2O \xrightarrow{\text{Inorganic pyrophosphatase}} 2pi$$

A combination of the above reactions makes it possible for phosphate to be recycled and the adenine nucleotides to interchange.

Other Nucleoside Triphosphates Participate in the Transfer of High-Energy Phosphate

By means of the enzyme **nucleoside diphosphate kinase,** UTP, GTP, and CTP can be synthesized from their diphosphates, eg,

$$ATP + UDP \xrightarrow{\text{Nucleoside diphosphate Kinase}} ADP + UTP$$

All of these triphosphates take part in phosphorylations in the cell. Similarly, specific nucleoside monophosphate kinases catalyze the formation of nucleoside diphosphates from the corresponding monophosphates.

POSSIBLE QUESTIONS
UNIT I

PART A (1 mark)
Question number 1-20 (From given 300 MCQs)

PART B (2 Marks)

1. What is Gibbs free energy?
2. Define Enthalpy
3. Define Entropy
4. What is free energy?
5. Brief about the first law of thermodynamics
6. Brief about the second law of thermodynamics
7. Write a short note on equilibrium constant
8. Write a brief note on biological oxidation reaction
9. Write a brief note on reduction reaction
10. What is redox potential?
11. Explain about high energy phosphate compounds

PART C (6 Marks)

12. Discuss in detail about the basic concepts of Bioenergetics
13. Give a detail account on biological oxidation- reduction reaction
14. Elaborate about high energy phosphate compunds

UNIT I	QUESTION	Option 1	Option 2	Option 3	Option 4	Option 5	Option 6	Answer
1	If enthalpy change for a reaction is zero, then ΔG° equals to	$-T\Delta S^{\circ}$	$T\Delta S^{\circ}$	$-\Delta H^{\circ}$	lnkeq			**$-T\Delta S^{\circ}$**
2	ΔG° is defined as the	Residual energy present in the reactants at equilibrium	Residual energy present in the products at equilibrium	Difference in the residual energy of reactants and products at equilibrium	Energy required in converting one mole of reactants to one mole of products			**Energy required in converting one mole of reactants to one mole of products**
3	The study of energy relationships and conversions in biological systems is called as	Biophysics	Biotechnology	Bioenergetics	Microbiology			**Bioenergetics**
4	For a reaction if ΔG° is positive, then	The products will be favored	The reactants will be favored	The concentration of the reactants and products will be equal	All of the reactant will be converted to products			**The reactants will be favored**
5	Unfolding of regular secondary protein structure causes	Large decrease in the entropy of the protein	Little increase in the entropy of protein	No change in the entropy of the protein	Large increase in the entropy of the protein			**Large increase in the entropy of the protein**
6	What does the first law of thermodynamics state?	Energy can neither be destroyed nor created	Energy cannot be 100 percent efficiently transformed from one type to another	All living organisms are composed of cells	Input of heat energy increases the rate of movement of atoms and molecules			**Energy can neither be destroyed nor created**
7	The relationship between K'eq and $\Delta G'^{\circ}$ is	$\Delta G'^{\circ} = RTlnK'eq$	$\Delta G'^{\circ} = -RTK'eq$	$\Delta G'^{\circ} = RK'eq$	$\Delta G'^{\circ} = -RK'eq$			**$\Delta G'^{\circ} = -RTK'eq$**
8	What is the relationship between ΔG and $\Delta G'^{\circ}$?	$\Delta G = \Delta G'^{\circ} + RTln([products]/[reactants])$	$\Delta G = \Delta G'^{\circ} - RTln([products]/[reactants])$	$\Delta G = \Delta G'^{\circ} + RTln([reactants]/[products])$	$\Delta G = \Delta G'^{\circ} - RTln([reactants]/[products])$			**$\Delta G = \Delta G'^{\circ} + RTln([products]/[reactants])$**
9	If $\Delta G'^{\circ}$ of the reaction A → B is -40kJ/mol under standard conditions then the reaction	Will never reach equilibrium	Will not occur spontaneously	Will proceed at a rapid rate	Will proceed from left to right spontaneously			**Will proceed from left to right spontaneously**
10	Which of the following statements is false?	The reaction tends to go in the forward direction if ΔG is large and positive	The reaction tends to move in the backward direction if ΔG is large and negative	The system is at equilibrium if $\Delta G = 0$	The reaction tends to move in the backward direction if ΔG is large and positive			**The reaction tends to move in the backward direction if ΔG is large and positive**
11	The biochemical reaction A B has a large and positive change in free-energy ($\Delta G >> 1$) under physiological conditions; which of the following is the best estimate for the equilibrium constant Keq\text{K}_{eq}*Keq* K, start subscript, e, q, end subscript?	Keq> 1	Keq< 1	Keq=0	Keq>> 1			**Keq< 1**
12	Chemical reactions can be classified according to free energy changes. A chemical reaction has a ΔG of -686/kcal/mol. Is this an endergonic or exergonic reaction? How would the addition of catalyst change the ΔG this reaction?	Exergonic, the catalyst would reduce the ΔG	Endergonic, the catalyst would increase the ΔG	Exergonic, the catalyst would not reduce ΔG	Endergonic, the catalyst would reduce the ΔG			**Exergonic, the catalyst would not reduce ΔG**
13	What can be said about the levels of energy of products and reactants, and ΔG in cellular respiration.	Reactants are glucose and carbon dioxide have low levels of energy, and the products oxygen and water have high levels of energy, ΔG is positive	Reactants are water and oxygen have high levels of energy, and products carbon dioxide and glucose have low levels of energy, ΔG is negative.	Reactants are glucose and oxygen have low levels of energy, and the products carbon dioxide and water levels of energy, ΔG is positive	Reactants are glucose and oxygen have high levels of energy, and the products carbon dioxide and water have low levels of energy, ΔG is negative.			**Reactants are glucose and oxygen have high levels of energy, and the products carbon dioxide and water have low levels of energy, ΔG is negative.**
14	Photuris lucicrescens is a bioluminescent coleopterIn simple words, this just means the lightning bug is a beetle that can glow in the dark. Is bioluminescence an endergonic or exergonic reaction and what is the ΔG of bioluminescence?	Exergonic reaction with positive ΔG.	Exergonic reaction with negative ΔG	Endergonic reaction with negative ΔG	Endergonic reaction with positive ΔG			**Endergonic reaction with positive ΔG**
15	In human physiology endergonic and exergonic reaction are often coupleHow does the ATP/ADP cycle couple these reactions?	Endergonic hydrolysis of ADP	Exergonic phosphorylation of ADP	Endergonic phosphorylation of molecule by ATP	Exergonic hydrolysis of ATP			**Exergonic hydrolysis of ATP**
16	ATP cycle refers to a set of reactions that promotes reversible changes of ATP into ADP via endergonic and exergonic reactions. According to Le Chatellier's principle, what would you expect to occur if ADP levels increased?	Decreased levels of ATP	Decreased levels of inorganic phosphate	No change in delta G	Increased rate of the hydrolysis reaction			**Decreased levels of inorganic phosphate**
17	What would it mean if an antibiotic fails to distort the binding site of bacterial enzymes?	Increased bacterial resistance to the antibiotic	Decreased enzymatic activities on bacteria	Increased levels of substrate on bacteria	Increased activation energy of bacterial chemical reactions			**Increased bacterial resistance to the antibiotic**
18	The symptoms of a certain disease are a result of increased competitive enzymatic inhibition. What kind of chemical manipulations with enzyme, substrate, or products would minimize the symptoms?	Addition of non-competitive inhibitor	Decreased levels of product	Removal of an allosteric activator	Increased levels of substrate			**Increased levels of substrate**
19	The electron transport chain is an important chemical reaction of cellular respiration. What would occur if oxygen was not present in the electron transport chain (anaerobic conditions)?	The anaerobic reaction would be endergonic with magnitude of ΔG smaller than in the presence of oxygen	The anaerobic reaction would be exergonic with magnitude of ΔG smaller than in the presence of oxygen	The anaerobic reaction would be endergonic with magnitude of ΔG larger than in the presence of oxygen	The anaerobic reaction would be exergonic with magnitude of ΔG larger than in the presence of oxygen			**The anaerobic reaction would be exergonic with magnitude of ΔG smaller than in the presence of oxygen**
20	Why does muscle contraction result in higher body temperature?	Because ATP phosphorylation has negative ΔG	Because ATP phosphorylation has positive ΔG	Because ATP hydrolysis has negative ΔG	Because ATP hydrolysis has positive ΔG			**Because ATP hydrolysis has negative ΔG**
21	Organization of photosynthetic pigments into clusters is	photosynthesis	photosynthetic clusters	cluster arrangement	photo system			**photo system**
22	Chlorophylls are soluble in	organic solvents	inorganic solvents	organic solutes	inorganic solutes			**organic solvents**
23	A compound which is found in all living cells and play a key role in energy transformations is	ADP	ATP	chlorophyll	granum			**ATP**
24	Chlorophyll converts light energy into	heat energy	chemical energy	potential energy	electrical energy			**chemical energy**
25	Photosynthesis is process in which inorganic compounds are reduced to organic compounds using	heat energy	light energy	chemical energy	electrical energy			**light energy**
26	Phases of Calvin cycle involves	carbon fixation	reduction	regeneration	All of the Above			**All of the Above**
27	In photosynthesis carbon dioxide, light and water are	reactants	products	by-products	catalysts			**reactants**
28	Energy is measured in which of the following units?	Kelvin	Joule	Pascal	mol			**Joule**
29	Which one of the following statements regarding energy is false?	The total energy in a chemical universe (a system and its surroundings) is constant	Energy can be converted from one form to another	The energy stored in chemical bonds is referred to as kinetic energy	An object suspended in the air has more energy than an object lying on the ground			**The energy stored in chemical bonds is referred to as kinetic energy**
30	In which direction does the transfer of energy as heat happen spontaneously?	From cold to hot	From hot to cold	From cold to cold	From hot to hot			**From hot to cold**
31	Which one of the following statements best describes the enthalpy change of a reaction?	The energy released when chemical bonds are formed during a chemical reaction	The energy consumed when chemical bonds are broken during a chemical reaction	The difference between the energy released by bond formation and the energy consumed by bond cleavage during a chemical reaction	The increase in disorder of the system as a reaction proceeds			**The difference between the energy released by bond formation and the energy consumed by bond cleavage during a chemical reaction**
32	Enthalpy is represented by which of the following symbols?	H	K	S	U			**H**
33	The enthalpy of combustion of glucose is -2808 kJ mol-1. Which one of the following statements regarding this process is false?	The products of the combustion of glucose are less stable than glucose itself	This process is exothermic	Overall, energy is liberated when this process happens	None of the above			**The products of the combustion of glucose are less stable than glucose itself**
34	Which of the following statements regarding the Gibbs free energy change for a reaction is false?	The Gibbs free energy change is the proportion of the enthalpy change of a reaction that is used to increase the entropy	If the Gibbs free energy change for a reaction is negative, the reaction happens spontaneously	The Gibbs free energy is represented by the symbol G	A reaction with a negative Gibbs free energy change of reaction is called an exergonic reaction			**The Gibbs free energy change is the proportion of the enthalpy change of a reaction that is used to increase the entropy**
35	The Standard Gibb's free energy, ΔG°, is	the residual energy present in the reactants at equilibrium	the residual energy present in the products at equilibrium	the difference in the residual energy of reactants and products at equilibrium	the energy required to convert one mole of reactants to one mole of products			**the energy required to convert one mole of reactants to one mole of products**
36	If the Standard Gibb's free energy, ΔG°, for a reaction is positive then	the products will be favored	the reactants will be favored	the concentration of the reactants and products will be equal	all of the reactant will be converted to product			**the reactants will be favored**
37	Carbon dioxide (CO_2) and water (H_2O) are produced as output when	oxidation of glucose occurs	when oxidation does not occur at all	when oxidation is accompanied with cell division	when oxidation is accompanied with water			**oxidation of glucose occurs**
38	Hydrogen peroxide (H_2O_2)	helps line up cell proteins	is poisonous to tissues	is often needed as a catalyst	aids in oxidation of glucose			**is poisonous to tissues**
39	More hydrogen peroxide (H_2O_2) in urine, the	lighter the color of the strip	acidic the urine be	darker the color of the strip	more healthier a person be			**darker the color of the strip**
40	Out of the following respiratory chain components indicate which one is a mobile carriers of electrons?	Cytochrome oxidase	NADH-Q reductase	Ubiquinone	Succinate dehydrogenase			**Ubiquinone**
41	Choose the incorrect statement about redox potential	The redox potential of a system (E0) is usually compared with the potential of the hydrogen electrode	The components of electron transport chain are organized in terms of their redox potential.	NADH/NAD+ redox pair has the least redox potential of -0.42 volts	Oxygen/H2O redox pair has the highest redox potential of +.82 volts			**NADH/NAD+ redox pair has the least redox potential of -0.42 volts**
42	All are flavoproteins except one, choose the odd one out	Xanthine oxidase	NADH dehydrogenase –Q reductase	Succinate dehydrogenase	Cytochrome c			**Cytochrome c**
43	All except one are incorrect about oxidases	Oxidases catalyze reactions involving hydrogen peroxide	Oxidases catalyze reaction using oxygen as a hydrogen acceptor	Oxidases catalyze reactions using Niacin as coenzyme	Oxidases catalyze reactions of direct incorporation of oxygen in to the substrate			**Oxidases catalyze reaction using oxygen as a hydrogen acceptor**
44	All of the following are NAD+ requiring enzymes except one –	Acyl co A dehydrogenase	Glyceraldehyde-3-P dehydrogenase	Glyceraldehyde-3-P dehydrogenase	Malate dehydrogenase			**Acyl co A dehydrogenase**
45	Which of the components of electron transport chain does not contain Iron sulfur center?	NADH dehydrogenase complex	Cytochrome a-a3 oxidase	Succinate dehydrogenase	Cytochrome bc1-c reductase			**Cytochrome a-a3 oxidase**
46	The enzymes of mitochondrial matrix include all except	Enzymes of fatty acid oxidation	Creatine kinase	Enzymes of TCA cycle	Pyruvate dehydrogenase complex			**Creatine kinase**
47	Patients with inherited defects of mitochondria involving components of the respiratory chain and oxidative phosphorylation present with all except	Myopathy	Lactic acidosis	Encephalopathy	Hepatomegaly			**Hepatomegaly**
48	The inner mitochondrial membrane is rich in which of the following phospholipids?	Cardiolipin	Lecithin	Cephalin	Acyl co A			**Cardiolipin**
49	All are true about ATP synthase complex, except	F1projects into the inter membranous space	F0 spans the membrane and forms a proton channel	F0 is inhibited by Oligomycin	F1contains the phosphorylation mechanism			**F1projects into the inter membranous space**
50	The energy yield during the conversion of succinate to Fumarate isa	1 ATP	2 ATP	3 ATP	4 ATP			**2 ATP**
51	The electron flow from complex I to complex III is through	Cytochrome c	Ubiquinone	Complex II	Complex IV			**Ubiquinone**
52	Which one of the following enzymes catalyzes substrate level phosphorylation in TCA cycle	Malate dehydrogenase	Succinate Thiokinase	Succinate dehydrogenase	Alpha keto glutarate dehydrogenase complex			**Succinate Thiokinase**
53	One out of the following is an inhibitor of complex I	Rotenone	H2S	BAL	CN			**Rotenone**
54	Which out of the following statements concerning the components of electron transport chain is true?	Oxygen directly oxidizes Cytochrome c	Succinate dehydrogenase directly reduces Cytochrome c	All of the components are embedded in the inner mitochondrial membrane	Cyanide does not inhibit proton pumping but inhibits ETC			**Cyanide does not inhibit proton pumping but inhibits ETC**
55	The free energy released during the transport of a pair of electrons in electron transport chain is	7.3 Kcal/mol	52.6 Kcal/mol	21.9 Kcal/mol	12.6 Kcal/mol			**52.6 Kcal/mol**
56	Which one of the following is a high energy phosphate	ATP	ADP	NADH	both ATP and ADP			**ATP and ADP**
57	NADH stands for	Niacin dehydrogenase	Nucleic acid analogue	Nicotinamide adenine dinucleotide	Nucleoside acid analogue			**Nicotinamide adenine dinucleotide**
58	Which one of the following thermodynamic quantities is **not** a state function?	Gibbs free energy	enthalpy	entropy	work			work
59	Which statement is **incorrect**?	The thermodynamic symbol for entropy is S	Gibbs free energy is a state function	For an endothermic process, ΔH is negative	If the work done by the system is greater than the heat absorbed by the system, ΔE is negative			For an endothermic process, ΔH is negative
60	Which statement is **false**?	The thermodynamic quantity most easily measured in a "coffee cup" calorimeter is ΔH.	ΔH is sometimes exactly equal to ΔE	ΔH is neraly equal to ΔE	ΔH is equal to ΔE for the reaction			ΔH is equal to ΔE for the reaction

UNIT-II

SYLLABUS

Carbohydrate metabolism: Glycolysis, TCA cycle, Glycogenesis, Glycogenolysis, HMP shunt, Gluconeogenesis, Glucuronic acid pathway

GLYCOLYSIS

Glycolysis is an almost universal pathway for extraction of the energy available from carbohydrates, shared among prokaryotes and eukaryotes, aerobes and anaerobes alike. In anaerobes, glycolysis is the only significant source of energy from carbohydrates. In aerobic organisms, considerably more energy can be harvested downstream from glycolysis in the citric acid cycle. Glycolysis produces energy in the form of ATP and NADH. The glycolytic pathway consists of 10 enzyme-catalyzed steps. During glycolysis, glucose, a six-carbon carbohydrate, is oxidized to form two molecules of pyruvate, a three-carbon molecule. For each glucose molecule metabolized, the pathway produces two molecules of ATP and two molecules of NADH.

Glycolysis-A universal pathway

Glycolysis is not isolated from other metabolic pathways. Other molecules besides glucose can enter at a few points along the glycolytic pathway. For example, the product of glycogen breakdown, glucose-6-phosphate, can enter the glycolytic pathway at the second step. Glyceraldehyde-3-phosphate, which is produced by photosynthesis, is also a glycolytic intermediate, so it can be directed from this anabolic pathway into glycolysis when energy is needed. Additionally, intermediates can be drawn out of the glycolytic pathway when energy levels are high, for use in biosynthetic pathways. For instance, during active energy production pyruvate, the product of glycolysis, enters the citric acid cycle, but when energy is not needed pyruvate serves as a substrate in amino acid synthesis.

Reactions of glycolysis

It consist of two phases namely preparatory phase and pay off phase

a)Preparatory phase

The first five steps are regarded as the preparatory (or investment) phase, since they consume energy to convert the glucose into two three-carbon sugar phosphates. The first step in glycolysis is phosphorylation of glucose by a family of enzymes called hexokinases to form glucose 6-phosphate (G6P). This reaction consumes ATP, but it acts to keep the glucose concentration low, promoting continuous transport of glucose into the cell through the plasma membrane transporters. In addition, it blocks the glucose from leaking out – the cell lacks transporters for G6P, and free diffusion out of the cell is prevented due to the charged nature of G6P. Glucose may alternatively be formed from the phosphorolysis or hydrolysis of intracellular starch or glycogen. In animals, an isozyme of hexokinase called glucokinase is also used in the liver, which has a much lower affinity for glucose (Km in the vicinity of normal glycemia), and differs in regulatory properties. The different substrate affinity and alternate regulation of this enzyme are a reflection of the role of the liver in maintaining blood sugar levels.cofactors: $Mg2^{+}$.

G6P is then rearranged into fructose 6-phosphate (F6P) by glucose phosphate isomerase. Fructose can also enter the glycolytic pathway by phosphorylation at this point.The change in structure is an isomerization, in which the G6P has been converted to F6P. The reaction requires an enzyme, phosphohexose isomerase, to proceed. This reaction is freely reversible under normal cell conditions. However, it is often driven forward because of a low concentration of F6P, which is constantly consumed during the next step of glycolysis. Under conditions of high F6P concentration, this reaction readily runs in reverse.

The energy expenditure of another ATP in this step is justified in 2 ways: The glycolytic process (up to this step) is now irreversible, and the energy supplied destabilizes the molecule. Because the reaction catalyzed by Phosphofructokinase 1 (PFK-1) is coupled to the hydrolysis of ATP, an energetically favorable step, it is, in essence, irreversible, and a different pathway must be used to do the reverse conversion during gluconeogenesis. This makes the reaction a key regulatory point (see below). This is also the rate-limiting step. Furthermore, the second phosphorylation event is necessary to allow the formation of two charged groups (rather than only one) in the subsequent step of glycolysis, ensuring the prevention of free diffusion of

substrates out of the cell.

The same reaction can also be catalyzed by pyrophosphate-dependent phosphofructokinase (PFP or PPi-PFK), which is found in most plants, some bacteria, archea, and protists, but not in animals. This enzyme uses pyrophosphate (PPi) as a phosphate donor instead of ATP. It is a reversible reaction, increasing the flexibility of glycolytic metabolism. A rarer ADP-dependent PFK enzyme variant has been identified in archaean species.

Cofactors: $Mg2^{+}$.

Destabilizing the molecule in the previous reaction allows the hexose ring to be split by aldolase into two triose sugars, dihydroxyacetone phosphate, a ketone, and glyceraldehyde 3-phosphate, an aldehyde. There are two classes of aldolases: class I aldolases, present in animals and plants, and class II aldolases, present in fungi and bacteria; the two classes use different mechanisms in cleaving the ketose ring.

Electrons delocalized in the carbon-carbon bond cleavage associate with the alcohol group. The resulting carbanion is stabilized by the structure of the carbanion itself via resonance charge distribution and by the presence of a charged ion prosthetic group. Triosephosphate isomerase rapidly interconverts dihydroxyacetone phosphate with glyceraldehyde 3-phosphate (GADP) that proceeds further into glycolysis. This is advantageous, as it directs dihydroxyacetone phosphate down the same pathway as glyceraldehyde 3-phosphate, simplifying regulation.

b) Pay-off phase

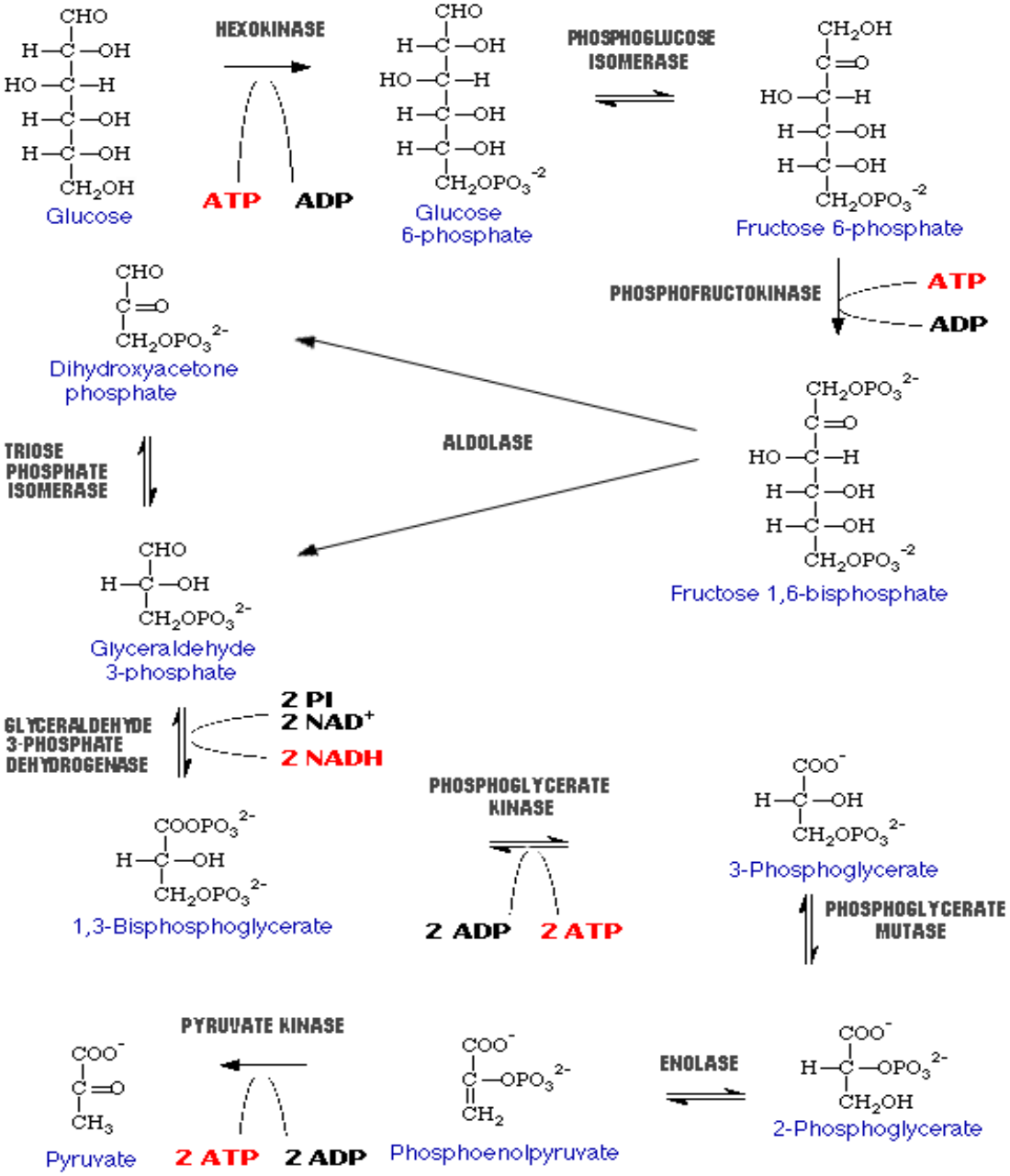
The second half of glycolysis is known as the pay-off phase, characterised by a net gain of the energy-rich molecules ATP and NADH. Since glucose leads to two triose sugars in the preparatory phase, each reaction in the pay-off phase occurs twice per glucose molecule. This yields 2 NADH molecules and 4 ATP molecules, leading to a net gain of 2 NADH molecules and 2 ATP molecules from the glycolytic pathway per glucose. The triose sugars are dehydrogenated and inorganic phosphate is added to them, forming 1, 3-bisphosphoglycerate. The hydrogen is used to reduce two molecules of NAD+, a hydrogen carrier, to give NADH + H^{+} for each triose. Hydrogen atom balance and charge balance are both maintained because the phosphate (Pi) group actually exists in the form of a hydrogen phosphate anion (HPO42-), which dissociates to contribute the extra H+ ion and gives a net charge of -3 on both sides.

This step is the enzymatic transfer of a phosphate group from 1,3-bisphosphoglycerate to

ADP by phosphoglycerate kinase, forming ATP and 3-phosphoglycerate. At this step, glycolysis has reached the break-even point: 2 molecules of ATP were consumed, and 2 new molecules have now been synthesized. This step, one of the two substrate-level phosphorylation steps, requires ADP; thus, when the cell has plenty of ATP (and little ADP), this reaction does not occur. Because ATP decays relatively quickly when it is not metabolized, this is an important regulatory point in the glycolytic pathway. ADP actually exists as ADPMg-, and ATP as $ATPMg^{2}$-, balancing the charges at -5 both sides. A final substrate-level phosphorylation now forms a molecule of pyruvate and a molecule of ATP by means of the enzyme pyruvate kinase. This serves as an additional regulatory step, similar to the phosphoglycerate kinase step.

Regulation

Glycolysis is regulated by slowing down or speeding up certain steps in the glycolysis pathway. This is accomplished by inhibiting or activating the enzymes that are involved. The steps that are regulated may be determined by calculating the change in free energy, G, for each step. If a step's products and reactants are in equilibrium, then the step is assumed not to be regulated. Since the change in free energy is zero for a system at equilibrium, any step with a free energy change near zero is not being regulated. If a step is being regulated, then that step's enzyme is not converting reactants into products as fast as it could, resulting in a build-up of reactants, which would be converted to products if the enzyme were operating faster. Since the reaction is thermodynamically favorable, the change in free energy for the step will be negative. A step with a large negative change in free energy is assumed to be regulated.


Glucose
HEXOKINASE
ATP
ADP
Glucose 6-phosphate
PHOSPHOGLUCOSE ISOMERASE
Fructose 6-phosphate
PHOSPHOFRUCTOKINASE
ATP
ADP
Dihydroxyacetone phosphate
ALDOLASE
Fructose 1,6-bisphosphate
TRIOSE PHOSPHATE ISOMERASE
Glyceraldehyde 3-phosphate
GLYCERALDEHYDE 3-PHOSPHATE DEHYDROGENASE
2 PI
2 NAD+
2 NADH
1,3-Bisphosphoglycerate
PHOSPHOGLYCERATE KINASE
2 ADP
2 ATP
3-Phosphoglycerate
PHOSPHOGLYCERATE MUTASE
PYRUVATE KINASE
Pyruvate
2 ATP
2 ADP
Phosphoenolpyruvate
ENOLASE
2-Phosphoglycerate

Post-glycolysis processes

The overall process of glycolysis is:

Glucose + 2 NAD^+ + 2 ADP + 2 Pi 2 Pyruvate + 2 NADH + 2 H^+ + 2 ATP + 2 H_2O

If glycolysis were to continue indefinitely, all of the NAD+ would be used up, and glycolysis would stop. To allow glycolysis to continue, organisms must be able to oxidize NADH back to NAD+. How this is performed depends on which external electron acceptor is available.

Fermentation

In this pyruvate is converted to lactate (the conjugate base of lactic acid) in a process called lactic acid fermentation:

Pyruvate + NADH + H^+ Lactate + NAD^+

This process occurs in the bacteria involved in making yogurt (the lactic acid causes the milk to curdle). This process also occurs in animals under hypoxic (or partially anaerobic) conditions, found, for example, in overworked muscles that are starved of oxygen, or in infarcted heart muscle cells. In many tissues, this is a cellular last resort for energy; most animal tissue cannot tolerate anaerobic conditions for an extended period of time. Some organisms, such as yeast, convert NADH back to NAD+ in a process called ethanol fermentation. In this process, the pyruvate is converted first to acetaldehyde and carbon dioxide, then to ethanol.

Lactic acid fermentation and ethanol fermentation can occur in the absence of oxygen. This anaerobic fermentation allows many single-cell organisms to use glycolysis as their only energy source. Anoxic regeneration of NADH is only an effective means of energy production during short, intense exercise, providing energy for a period ranging from 10 seconds to 2 minutes and is dominant from about 10–30 seconds during a maximal effort. It replenishes very quickly over this period and produces 2 ATP molecules per glucose molecule, or about 5% of glucose's energy potential (38 ATP molecules in bacteria). The speed at which ATP is produced is about 100 times that of oxidative phosphorylation. The pH in the cytoplasm quickly drops when hydrogen ions accumulate in the muscle, eventually inhibiting enzymes involved in glycolysis.

The burning sensation in muscles during hard exercise can be attributed to the production of hydrogen ions during a shift to lactic acid fermentation as oxygen is converted to carbon dioxide by aerobic respiration faster than the body can replenish it. These hydrogen ions form a part of lactic acid along with lactate. The body falls back on this less efficient but faster method of

producing ATP under low oxygen conditions. This is thought to have been the primary means of energy production in earlier organisms before oxygen was at high concentration in the atmosphere and thus would represent a more ancient form of energy production in cells. The liver later gets rid of this excess lactate by transforming it back into an important glycolytic intermediate called pyruvate; see Cori cycle. Fermenation of pyruvate to lactate is sometimes also called "anaerobic glycolysis", however, glycolysis ends with the production of pyruvate regardless in the presence or absence of oxygen.

Anaerobic respiration

In the above two examples of fermentation, NADH is oxidized by transferring two electrons to pyruvate. However, anaerobic bacteria use a wide variety of compounds as the terminal electron acceptors in cellular respiration: nitrogenous compounds, such as nitrates and nitrites; sulfur compounds, such as sulfates, sulfites, sulfur dioxide, and elemental sulfur; carbon dioxide; iron compounds; manganese compounds; cobalt compounds; and uranium compounds.

Aerobic respiration

In aerobic organisms, a complex mechanism has been developed to use the oxygen in air as the final electron acceptor.

- First, pyruvate is converted to acetyl-CoA and CO2 within the mitochondria in a process called pyruvate decarboxylation.
- Second, the acetyl-CoA enters the citric acid cycle, also known as Krebs Cycle, where it is fully oxidized to carbon dioxide and water, producing yet more NADH.
- Third, the NADH is oxidized to NAD+ by the electron transport chain, using oxygen as the final electron acceptor. This process creates a hydrogen ion gradient across the inner membrane of the mitochondria.
- Fourth, the proton gradient is used to produce about 2.5 ATP for every NADH oxidized in a process called oxidative phosphorylation.

Fates of pyruvate

The catabolic role of glycolysis with regard to converting potential chemical energy to usable chemical energy during the oxidation of glucose to pyruvate is evidenced. Many of the metabolites in the glycolytic pathway are also used by anabolic pathways, and, as a consequence, flux through the pathway is critical to maintain a supply of carbon skeletons for biosynthesis. In

addition, not all carbon entering the pathway leaves as pyruvate and may be extracted at earlier stages to provide carbon compounds for other pathways. These metabolic pathways are all strongly reliant on glycolysis as a source of metabolites: and many more.

- Gluconeogenesis
- Lipid metabolism
- Pentose phosphate pathway
- Citric acid cycle, which in turn leads to:
- Amino acid synthesis
- Nucleotide synthesis
- Tetrapyrrole synthesis

From an anabolic metabolism perspective, the NADH has a role to drive synthetic reactions, doing so by directly or indirectly reducing the pool of NADP+ in the cell to NADPH, which is another important reducing agent for biosynthetic pathways in a cell.

THE CITRIC ACID CYCLE

The cycle starts with reaction between the acetyl moiety of acetyl-CoA and the four-carbon dicarboxylic acid oxaloacetate, forming a six-carbon tricarboxylic acid, citrate. In the subsequent reactions, two molecules of CO_2 are released and oxaloacetate is regenerated (Figure 16–1). Only a small quantity of oxaloacetate is needed for the oxidation of a large quantity of acetyl-CoA; oxaloacetate may be considered to play a **catalytic role.** The citric acid cycle is an integral part of the process by which much of the free energy liberated during the oxidation of fuels is made available. During oxidation of acetyl-CoA, coenzymes are reduced and subsequently reoxidized in the respiratory chain, linked to the formation of ATP (oxidative phosphorylation; see Figure 16–2 and also Chapter 12). This process is **aerobic,** requiring oxygen as the final oxidant of the reduced coenzymes. The enzymes of the citric acid cycle are located in the **mitochondrial matrix,** either free or attached to the inner mitochondrial membrane, where the enzymes of the respiratory chain are also found.

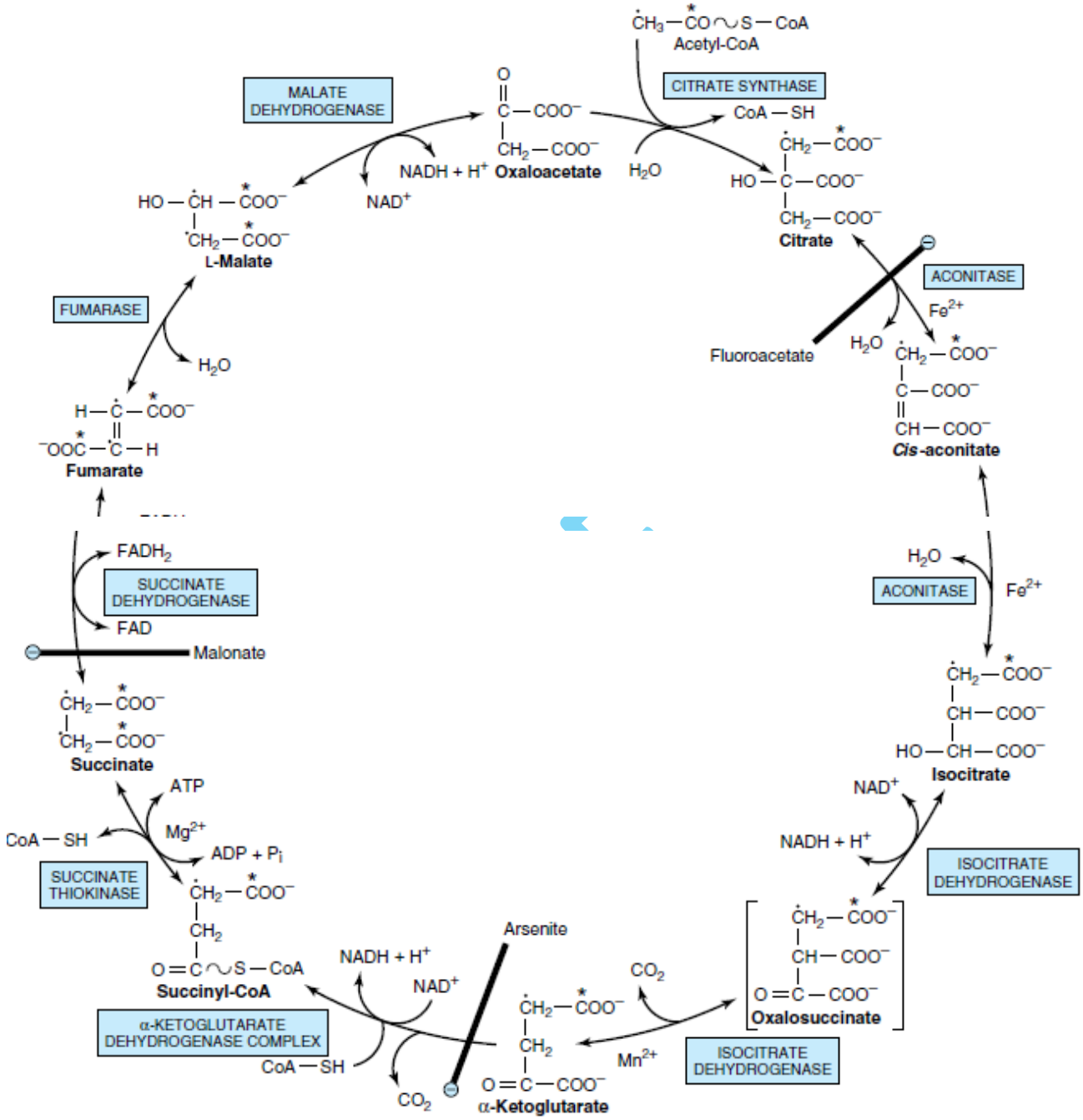
REACTIONS OF THE CITRIC ACID CYCLE LIBERATE REDUCING EQUIVALENTS & CO_2

The initial reaction between acetyl-CoA and oxaloacetate to form citrate is catalyzed by **citrate synthase** which forms a carbon-carbon bond between the methyl carbon of acetyl-CoA and the carbonyl carbon of oxaloacetate. The thioester bond of the resultant citryl- CoA is hydrolyzed, releasing citrate and CoASH—an exergonic reaction. Citrate is isomerized to isocitrate by the enzyme **aconitase** (aconitate hydratase); the reaction occurs in two steps: dehydration to *cis*-aconitate, some of which remains bound to the enzyme; and rehydration to isocitrate. Although citrate is a symmetric molecule, aconitase reacts with citrate asymmetrically, so that the two carbon atoms that are lost in subsequent reactions of the cycle are not those that were added from acetyl- CoA. This asymmetric behavior is due to **channeling**— transfer of the product of citrate synthase directly onto the active site of aconitase without entering free solution. This provides integration of citric acid cycle activity and the provision of citrate in the cytosol as a source of acetyl-CoA for fatty acid synthesis. The poison **fluoroacetate** is toxic because fluoroacetyl-CoA condenses with oxaloacetate to form fluorocitrate, which inhibits aconitase, causing citrate to accumulate.

Isocitrate undergoes dehydrogenation catalyzed by **isocitrate dehydrogenase** to form, initially, oxalosuccinate, which remains enzyme-bound and undergoes decarboxylation to α-ketoglutarate. The decarboxylation requires Mg2+ or Mn2+ ions. There are three isoenzymes of isocitrate dehydrogenase. One, which uses NAD+, is found only in mitochondria. The other two use NADP+ and are found in mitochondria and the cytosol. Respiratory chain-linked oxidation of isocitrate proceeds almost completely through the NAD+-dependent enzyme. α-Ketoglutarate undergoes **oxidative decarboxylation** in a reaction catalyzed by a multi-enzyme complex similar to that involved in the oxidative decarboxylation of pyruvate (Figure 17–5). The _-**ketoglutarate dehydrogenase complex** requires the same cofactors as the pyruvate dehydrogenase complex—thiamin diphosphate, lipoate, NAD+, FAD, and CoA—and results in the formation of succinyl-CoA. The equilibrium of this reaction is so much in favor of succinyl-CoA formation that it must be considered physiologically unidirectional. As in the case of pyruvate oxidation (Chapter 17), arsenite inhibits the reaction, causing the substrate, _-**ketoglutarate,** to accumulate. Succinyl-CoA is converted to succinate by the enzyme **succinate thiokinase (succinyl-CoA synthetase).** This is the only example in the citric acid cycle of substrate-level phosphorylation. Tissues in which gluconeogenesis occurs (the liver and kidney)

contain two isoenzymes of succinate thiokinase, one specific for GDP and the other for ADP. The GTP formed is used for the decarboxylation of oxaloacetate to phosphoenolpyruvate in gluconeogenesis and provides a regulatory link between citric acid cycle activity and the withdrawal of oxaloacetate for gluconeogenesis. Nongluconeogenic tissues have only the isoenzyme that uses ADP.

When ketone bodies are being metabolized in extrahepatic tissues there is an alternative reaction catalysed by **succinyl-CoA–acetoacetate-CoA transferase (thiophorase)**— involving transfer of CoA from succinyl- CoA to acetoacetate, forming acetoacetyl-CoA (Chapter 22). The onward metabolism of succinate, leading to the regeneration of oxaloacetate, is the same sequence of chemical reactions as occurs in the β-oxidation of fatty acids: dehydrogenation to form a carbon-carbon double bond, addition of water to form a hydroxyl group, and a further dehydrogenation to yield the oxo- group of oxaloacetate. The first dehydrogenation reaction, forming fumarate, is catalyzed by **succinate dehydrogenase,** which is bound to the inner surface of the inner mitochondrial membrane. The enzyme contains FAD and iron-sulfur (Fe:S) protein and directly reduces ubiquinone in the respiratory chain. **Fumarase (fumarate hydratase)** catalyzes the addition of water across the double bond of fumarate, yielding malate. Malate is converted to oxaloacetate by **malate dehydrogenase,** a reaction requiring NAD+. Although the equilibrium of this reaction strongly favors malate, the net flux is toward the direction of oxaloacetate because of the continual removal of oxaloacetate (either to form citrate, as a substrate for gluconeogenesis, or to undergo transamination to aspartate) and also because of the continual reoxidation of NADH.


Acetyl-CoA
CITRATE SYNTHASE
Oxaloacetate
Citrate
MALATE DEHYDROGENASE
L-Malate
FUMARASE
Fumarate
ACONITASE
Fluoroacetate
Cis-aconitate
Isocitrate
ISOCITRATE DEHYDROGENASE
Oxalosuccinate
α-Ketoglutarate
α-KETOGLUTARATE DEHYDROGENASE COMPLEX
Succinyl-CoA
SUCCINATE THIOKINASE
Succinate
SUCCINATE DEHYDROGENASE
Malonate
Arsenite

TWELVE ATP ARE FORMED PER TURN OF THE CITRIC ACID CYCLE

As a result of oxidations catalyzed by the dehydrogenases of the citric acid cycle, three molecules of NADH and one of FADH2 are produced for each molecule of acetyl-CoA catabolized in one turn of the cycle. These reducing equivalents are transferred to the respiratory chain (Figure 16–2), where reoxidation of each NADH results in formation of 3 ATP and reoxidation of FADH2 in formation of 2 ATP. In addition, 1 ATP (or GTP) is formed by substrate-level phosphorylation catalyzed by succinate thiokinase.

VITAMINS PLAY KEY ROLES IN THE CITRIC ACID CYCLE

Four of the B vitamins are essential in the citric acid cycle and therefore in energy-yielding metabolism: (1) **riboflavin,** in the form of flavin adenine dinucleotide (FAD), a cofactor in the α-ketoglutarate dehydrogenase complex and in succinate dehydrogenase; (2) **niacin,** in the form of nicotinamide adenine dinucleotide (NAD), the coenzyme for three dehydrogenases in the cycle— isocitrate dehydrogenase, α-ketoglutarate dehydrogenase, and malate dehydrogenase; (3) **thiamin (vitamin B1),** as thiamin diphosphate, the coenzyme for decarboxylation in the α-ketoglutarate dehydrogenase reaction; and (4) **pantothenic acid,** as part of coenzyme A, the cofactor attached to "active" carboxylic acid residues such as acetyl-CoA and succinyl-CoA.

THE CITRIC ACID CYCLE PLAYS A PIVOTAL ROLE IN METABOLISM

The citric acid cycle is not only a pathway for oxidation of two-carbon units—it is also a major pathway for interconversion of metabolites arising from **transamination** and **deamination** of amino acids. It also provides the substrates for **amino acid synthesis** by transamination, as well as for **gluconeogenesis** and **fatty acid synthesis**. Because it functions in both oxidative and synthetic processes, it is **amphibolic.**

The Citric Acid Cycle Takes Part in Gluconeogenesis, Transamination, & Deamination

All the intermediates of the cycle are potentially glucogenic, since they can give rise to oxaloacetate and thus net production of glucose (in the liver and kidney, the organs that carry out gluconeogenesis; see Chapter 19). The key enzyme that catalyzes net transfer out of the cycle into gluconeogenesis is **phosphoenolpyruvate carboxykinase,** which decarboxylates oxaloacetate to phosphoenolpyruvate, with GTP acting as the donor phosphate (Figure 16–4).

Net transfer into the cycle occurs as a result of several different reactions. Among the most important of such **anaplerotic reactions** is the formation of oxaloacetate by the carboxylation of pyruvate, catalyzed by **pyruvate carboxylase.** This reaction is important in maintaining an adequate concentration of oxaloacetate for the condensation reaction with acetyl-CoA. If acetyl-CoA accumulates, it acts both as an allosteric activator of pyruvate carboxylase and as an inhibitor of pyruvate dehydrogenase, thereby ensuring a supply of oxaloacetate. Lactate, an important substrate for gluconeogenesis, enters the cycle via oxidation to pyruvate and then carboxylation to oxaloacetate. **Aminotransferase** (transaminase) reactions form pyruvate from alanine, oxaloacetate from aspartate, and α-ketoglutarate from glutamate. Because these reactions are reversible, the cycle also serves as a source of carbon skeletons for the synthesis of these amino acids. Other amino acids contribute to gluconeogenesis because their carbon skeletons give rise to citric acid cycle intermediates. Alanine, cysteine, glycine, hydroxyproline, serine, threonine, and tryptophan yield pyruvate; arginine, histidine, glutamine, and proline yield α-ketoglutarate; isoleucine, methionine, and valine yield succinyl-CoA; and tyrosine and phenylalanine yield fumarate. In ruminants, whose main metabolic fuel is short chain fatty acids formed by bacterial fermentation, the conversion of propionate, the major glucogenic product of rumen fermentation, to succinyl-CoA via the methylmalonyl-CoA pathway (Figure 19–2) is especially important.

The Citric Acid Cycle Takes Part in Fatty Acid Synthesis

Acetyl-CoA, formed from pyruvate by the action of pyruvate dehydrogenase, is the major building block for long-chain fatty acid synthesis in nonruminants. (In ruminants, acetyl-CoA is derived directly from acetate.) Pyruvate dehydrogenase is a mitochondrial enzyme, and fatty acid synthesis is a cytosolic pathway, but the mitochondrial membrane is impermeable to acetyl-CoA. Acetyl-CoA is made available in the cytosol from citrate synthesized in the mitochondrion, transported into the cytosol and cleaved in a reaction catalyzed by **ATP-citrate lyase.**

Regulation of the Citric Acid Cycle Depends Primarily on a Supply of Oxidized Cofactors

In most tissues, where the primary role of the citric acid cycle is in energy-yielding metabolism, **respiratory control** via the respiratory chain and oxidative phosphorylation regulates citric acid

cycle activity (Chapter 14). Thus, activity is immediately dependent on the supply of NAD+, which in turn, because of the tight coupling between oxidation and phosphorylation, is dependent on the availability of ADP and hence, ultimately, on the rate of utilization of ATP in chemical and physical work. In addition, individual enzymes of the cycle are regulated. The most likely sites for regulation are the nonequilibrium reactions catalyzed by pyruvate dehydrogenase, citrate synthase, isocitrate dehydrogenase, and α-ketoglutarate dehydrogenase. The dehydrogenases are activated by Ca2+, which increases in concentration during muscular contraction and secretion, when there is increased energy demand. In a tissue such as brain, which is largely dependent on carbohydrate to supply acetyl-CoA, control of the citric acid cycle may occur at pyruvate dehydrogenase. Several enzymes are responsive to the energy status, as shown by the [ATP]/[ADP] and [NADH]/[NAD+] ratios. Thus, there is allosteric inhibition of citrate synthase by ATP and long-chain fatty acyl-CoA. Allosteric activation of mitochondrial NAD-dependent isocitrate dehydrogenase by ADP is counteracted by ATP and NADH. The α-ketoglutarate dehydrogenase complex is regulated in the same way as is pyruvate dehydrogenase (Figure 17 6). Succinate dehydrogenase is inhibited by oxaloacetate, and the availability of oxaloacetate, as controlled by malate dehydrogenase, depends on the [NADH]/[NAD+] ratio. Since the *K*m for oxaloacetate of citrate synthase is of the same order of magnitude as the intramitochondrial concentration, it is likely that the concentration of oxaloacetate controls the rate of citrate formation. Which of these mechanisms are important in vivo has still to be resolved.

GLYCOGENESIS

The Pathway of Glycogen Biosynthesis Involves a Special Nucleotide of Glucose

As in glycolysis, glucose is phosphorylated to glucose 6-phosphate, catalyzed by **hexokinase** in muscle and **glucokinase** in liver. Glucose 6-phosphate is isomerized to glucose 1-phosphate by **phosphoglucomutase.**The enzyme itself is phosphorylated, and the phosphogroup takes part in a reversible reaction in which glucose 1,6-bisphosphate is an intermediate. Next, glucose 1-phosphate reacts with uridine triphosphate (UTP) to form the active nucleotide **uridine diphosphate glucose (UDPGlc)*** and pyrophosphate (Figure 18–2), catalyzed by **UDPGlc pyrophosphorylase. Pyrophos-phatase** catalyzes hydrolysis of pyrophosphate to 2 mol of inorganic phosphate, shifting the equilibrium of the main reaction by removing one of its

products. **Glycogen synthase** catalyzes the formation of a glycoside bond between C1 of the activated glucose of UDPGlc and C4 of a terminal glucose residue of glycogen, liberating uridine diphosphate (UDP). A pre-existing glycogen molecule, or "glycogen primer," must be present to initiate this reaction. The glycogen primer may in turn be formed on a primer known as **glycogenin,** which is a 37-kDa protein that is glycosylated on a specific tyrosine residue by UDPGlc. Further glucose residues are attached in the 1→4 position to make

a short chain that is a substrate for glycogen synthase. In skeletal muscle, glycogenin remains attached in the center of the glycogen molecule (Figure 13–15), whereas in liver the number of glycogen molecules is greater than the number of glycogenin molecules.

Branching Involves Detachment of Existing Glycogen Chains

The addition of a glucose residue to a preexisting glycogen chain, or "primer," occurs at the nonreducing, outer end of the molecule so that the "branches" of the glycogen "tree" become elongated as successive 1→4 linkages are formed (Figure 18–3). When the chain has been lengthened to at least 11 glucose residues, **branching enzyme** transfers a part of the 1→4 chain (at least six glucose residues) to a neighboring chain to form a 1→6 linkage, establishing a **branch point.** The branches grow by further additions of 1→4-glucosyl units and further branching.

GLYCOGENOLYSIS

Glycogen phosphorylase catalyzes the rate-limiting step in glycogenolysis by promoting the phosphorylytic cleavage by inorganic phosphate (phosphorylysis; cf hydrolysis) of the 1→4 linkages of glycogen to yield glucose 1-phosphate. The terminal glucosyl residues from the outermost chains of the glycogen molecule are removed sequentially until approximately four glucose residues remain on either side of a 1→6 branch (Figure 18–4). Another enzyme **(_-[1v4]v_-[1v4] glucan transferase)** transfers a trisaccharide unit from one branch to the other, exposing the 1→6 branch point. **Hydrolysis** of the 1→6 linkages requires the **debranching enzyme.** Further phosphorylase action can then proceed. The combined action of phosphorylase and these other enzymes leads to the complete breakdown of glycogen. The reaction catalyzed by phosphoglucomutase is reversible, so that glucose 6-phosphate can be formed from glucose 1-phosphate. In **liver** (and **kidney**), but not in muscle, there is a specific enzyme, **glucose-6-**

phosphatase, that hydrolyzes glucose 6-phosphate, yielding glucose that is exported, leading to an increase in the blood glucose concentration.

THE REGULATION OF GLYCOGENOLYSIS & GLYCOGENESIS

The principal enzymes controlling glycogen metabolism— glycogen phosphorylase and glycogen synthase— are regulated by allosteric mechanisms and covalent modifications due to reversible phosphorylation and dephosphorylation of enzyme protein in response to hormone action (Chapter 9). Cyclic AMP (cAMP) (Figure 18–5) is formed from ATP by **adenylyl cyclase** at the inner surface of cell membranes and acts as an intracellular **second messenger** in response to hormones such as **epinephrine, norepinephrine,** and **glucagon.** cAMP is hydrolyzed by **phosphodiesterase,** so terminating hormone action. In liver, insulin increases the activity of phosphodiesterase.

Phosphorylase Differs Between Liver & Muscle

In liver, one of the serine hydroxyl groups of active **phosphorylase a** is phosphorylated. It is inactivated by hydrolytic removal of the phosphate by **protein phosphatase- 1** to form **phosphorylase b.** Reactivation requires rephosphorylation catalyzed by **phosphorylase kinase.** Muscle phosphorylase is distinct from that of liver. It is a dimer, each monomer containing 1 mol of pyridoxal phosphate (vitamin B6). It is present in two forms: **phosphorylase a,** which is phosphorylated and active in either the presence or absence of 5′-AMP (its allosteric modifier); and **phosphorylase b,** which is dephosphorylated and active only in the presence of 5′-AMP. This occurs during exercise when the level of 5′-AMP rises, providing, by this mechanism, fuel for the muscle. Phosphorylase a is the normal physiologically active form of the enzyme.

cAMP Activates Muscle Phosphorylase

Phosphorylase in muscle is activated in response to epinephrine (Figure 18–6) acting via cAMP. Increasing the concentration of cAMP activates **cAMP-dependent protein kinase,** which catalyzes the phosphorylation by ATP of inactive **phosphorylase kinase b** to active **phosphorylase kinase a,** which in turn, by means of a further phosphorylation, activates phosphorylase b to phosphorylase a.

Ca2+ Synchronizes the Activation of Phosphorylase With Muscle Contraction

Glycogenolysis increases in muscle several hundred-fold immediately after the onset of contraction. This involves the rapid activation of phosphorylase by activation of phosphorylase kinase by Ca2+, the same signal as that which initiates contraction in response to nerve stimulation. Muscle phosphorylase kinase has four types of subunits—α, β, γ, and δ—in a structure represented as (αβγδ)4. The α and β subunits contain serine residues that are phosphorylated by cAMP-dependent protein kinase. The δ subunit binds four Ca2+ and is identical to the Ca2+-binding protein **calmodulin** (Chapter 43). The binding of Ca2+ activates the catalytic site of the γ subunit while the molecule remains in the dephosphorylated b configuration. However, the phosphorylated a form is only fully activated in the presence of Ca2+. A second molecule of calmodulin, or TpC (the structurally similar Ca2+-binding protein in muscle), can interact with phosphorylase kinase, causing further activation. Thus, activation of muscle contraction and glycogenolysis are carried out by the same Ca2+-binding protein, ensuring their synchronization.

Glycogenolysis in Liver Can Be cAMP-Independent

In addition to the action of **glucagon** in causing formation of cAMP and activation of phosphorylase in liver, **_1-adrenergic** receptors mediate stimulation of glycogenolysis by epinephrine and norepinephrine. This involves a **cAMP-independent** mobilization of Ca2+ from mitochondria into the cytosol, followed by the stimulation of a **Ca2+**/calmodulin-sensitive phosphorylase kinase. cAMP-independent glycogenolysis is also caused by vasopressin, oxytocin, and angiotensin II acting through calcium or the phosphatidylinositol bisphosphate pathway (Figure 43–7).

Protein Phosphatase-1 Inactivates Phosphorylase

Both phosphorylase a and phosphorylase kinase a are dephosphorylated and inactivated by **protein phosphatase- 1.** Protein phosphatase-1 is inhibited by a protein, **inhibitor-1,** which is active only after it has been phosphorylated by cAMP-dependent protein kinase. Thus, cAMP controls both the activation and inactivation of phosphorylase (Figure 18–6). **Insulin** reinforces this effect by inhibiting the activation of phosphorylase b. It does this indirectly by increasing uptake of glucose, leading to increased formation of glucose 6-phosphate, which is an inhibitor of phosphorylase kinase.

Glycogen Synthase & Phosphorylase Activity Are Reciprocally Regulated (Figure 18–7)

Like phosphorylase, glycogen synthase exists in either a phosphorylated or nonphosphorylated state. However, unlike phosphorylase, the active form is dephosphorylated **(glycogen synthase a)** and may be inactivated to **glycogen synthase b** by phosphorylation on serine residues by no fewer than six different protein kinases. Two of the protein kinases are Ca2+/calmodulindependent (one of these is phosphorylase kinase). Another kinase is cAMP-dependent protein kinase, which allows cAMP-mediated hormonal action to inhibit glycogen synthesis synchronously with the activation of glycogenolysis. Insulin also promotes glycogenesis in muscle at the same time as inhibiting glycogenolysis by raising glucose 6-phosphate concentrations, which stimulates the dephosphorylation and activation of glycogen synthase. Dephosphorylation of glycogen synthase b is carried out by protein phosphatase-1, which is under the control of cAMP-dependent protein kinase.

REGULATION OF GLYCOGEN METABOLISM IS EFFECTED BY A BALANCE IN ACTIVITIES BETWEEN GLYCOGEN SYNTHASE & PHOSPHORYLASE

Not only is phosphorylase activated by a rise in concentration of cAMP (via phosphorylase kinase), but glycogen synthase is at the same time converted to the inactive form; both effects are mediated via **cAMP dependent protein kinase.** Thus, inhibition of glycogenolysis enhances net glycogenesis, and inhibition of glycogenesis enhances net glycogenolysis. Furthermore, the dephosphorylation of phosphorylase a, phosphorylase kinase a, and glycogen synthase b is catalyzed by a single enzyme of wide specificity—**protein phosphatase-1.** In turn, protein phosphatase-1 is inhibited by cAMP-dependent protein kinase via inhibitor-1. Thus, glycogenolysis can be terminated and glycogenesis can be stimulated synchronously, or vice versa, because both processes are keyed to the activity of cAMP-dependent protein kinase. Both phosphorylase kinase and glycogen synthase may be reversibly phosphorylated in more than one site by separate kinases and phosphatases. These secondary phosphorylations modify the sensitivity of the primary sites to phosphorylation and dephosphorylation **(multisite phosphorylation).** What is more, they allow insulin, via glucose 6-phosphate elevation, to have effects that act reciprocally to those of cAMP (Figures 18–6 and 18–7).

CLINICAL ASPECTS Glycogen Storage Diseases Are Inherited

"Glycogen storage disease" is a generic term to describe a group of inherited disorders characterized by deposition of an abnormal type or quantity of glycogen in the tissues. The principal glycogenoses are summarized in Table 18–2. Deficiencies of **adenylyl kinase** and **cAMP-dependent protein kinase** have also been reported. Some of the conditions described have benefitedfrom liver transplantation.

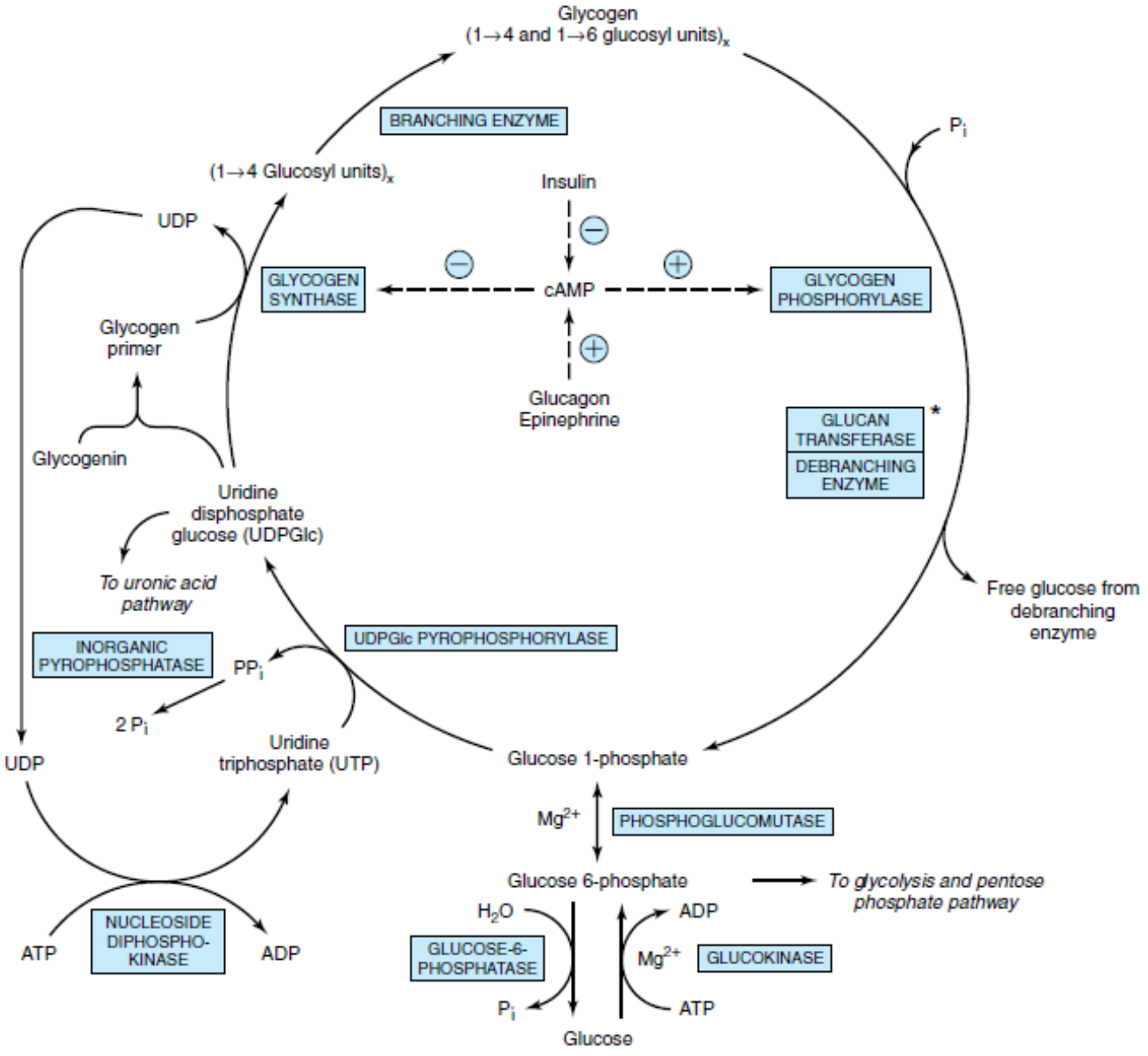


Figure 18–1. Pathway of glycogenesis and of glycogenolysis in the liver.

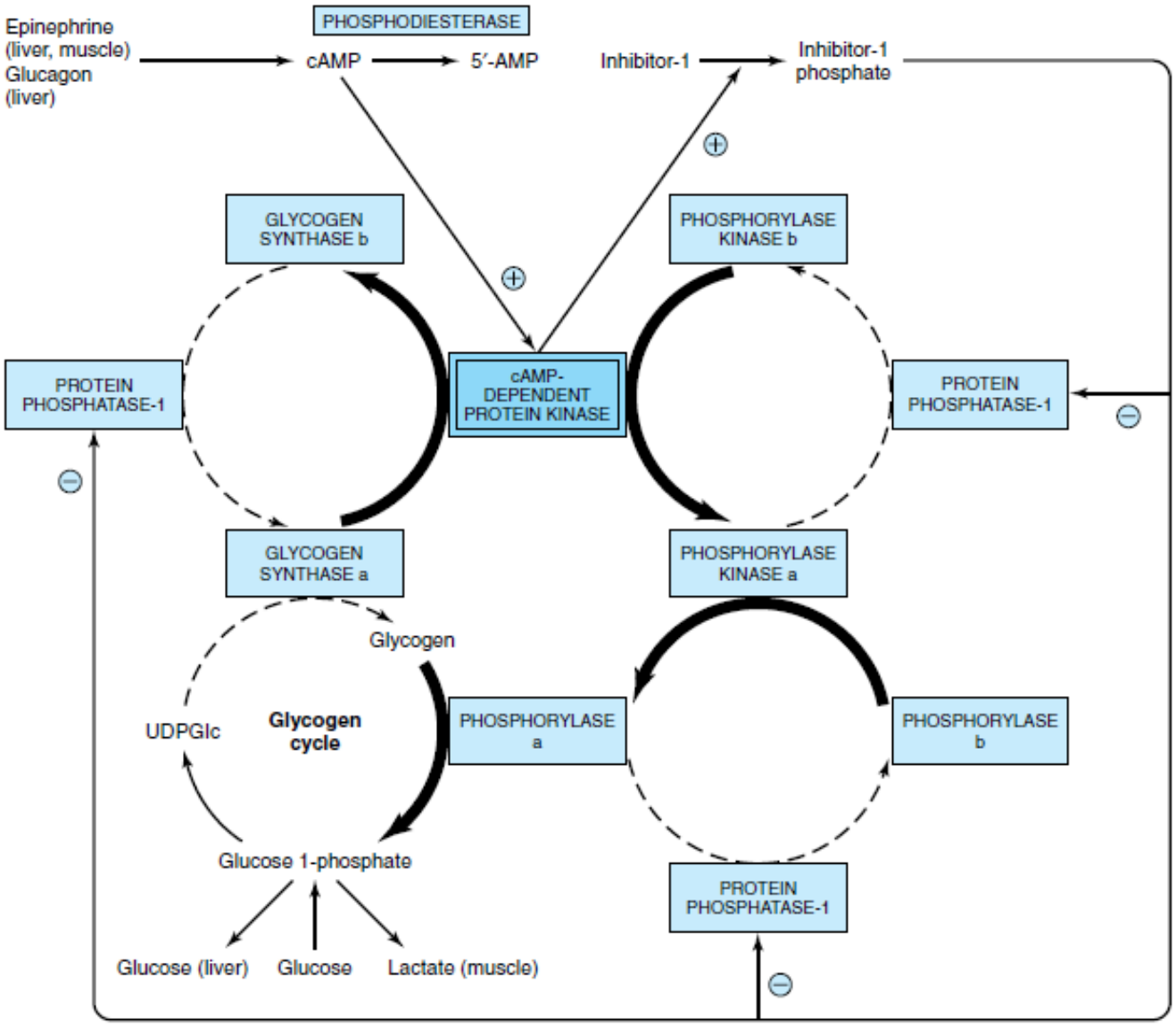


Figure 18–8. Coordinated control of glycogenolysis and glycogenesis by cAMP-dependent protein kinase. The reactions that lead to glycogenolysis as a result of an increase in cAMP concentrations are shown with bold arrows, and those that are inhibited by activation of protein phosphatase-1 are shown as broken arrows. The reverse occurs when cAMP concentrations decrease as a result of phosphodiesterase activity, leading to glycogenesis.

GLUCONEOGENESIS

Gluconeogenesis is the biosynthesis of new glucose, (i.e. not glucose from glycogen). This process is frequently referred to as endogenous glucose production (EGP). The production of glucose from other carbon skeletons is necessary since the testes, erythrocytes and kidney medulla exclusively utilize glucose for ATP production. The brain also utilizes large amounts of the daily glucose consumed or produced via gluconeogenesis. However, in addition to glucose, the brain can derive energy from ketone bodies which are converted to acetyl-CoA and shunted into the TCA cycle. The primary carbon skeletons used for gluconeogenesis are derived from pyruvate, lactate, glycerol, and the amino acids alanine and glutamine. The liver is the major site of gluconeogenesis, however, as discussed below, the kidney and the small intestine also have important roles to play in this pathway. Synthesis of glucose from three and four carbon precursors is essentially a reversal of glycolysis

Precursor

In humans the main gluconeogenic precursors are lactate, glycerol (which is a part of the triacylglycerol molecule), alanine and glutamine. Altogether, they account for over 90% of the overall gluconeogenesis. Other glucogenic amino acid as well as all citric acid cycle intermediates, the latter through conversion to oxaloacetate, can also function as substrates for gluconeogenesis. In ruminants, propionate is the principal gluconeogenic substrate.

Lactate is transported back to the liver where it is converted into pyruvate by the Cori cycle using the enzyme lactate dehydrogenase. Pyruvate, the first designated substrate of the gluconeogenic pathway, can then be used to generate glucose. Transamination or deamination of amino acids facilitates entering of their carbon skeleton into the cycle directly (as pyruvate or oxaloacetate), or indirectly via the citric acid cycle.

Whether even-chain fatty acids can be converted into glucose in animals has been a longstanding question in biochemistry. It is known that odd-chain fatty acids can be oxidized to yield propionyl CoA, a precursor for succinyl CoA, which can be converted to pyruvate and enter into gluconeogenesis. In plants, specifically seedlings, the glyoxylate cycle can be used to convert fatty acids (acetate) into the primary carbon source of the organism. The glyoxylate cycle produces four-carbon dicarboxylic acids that can enter gluconeogenesis.

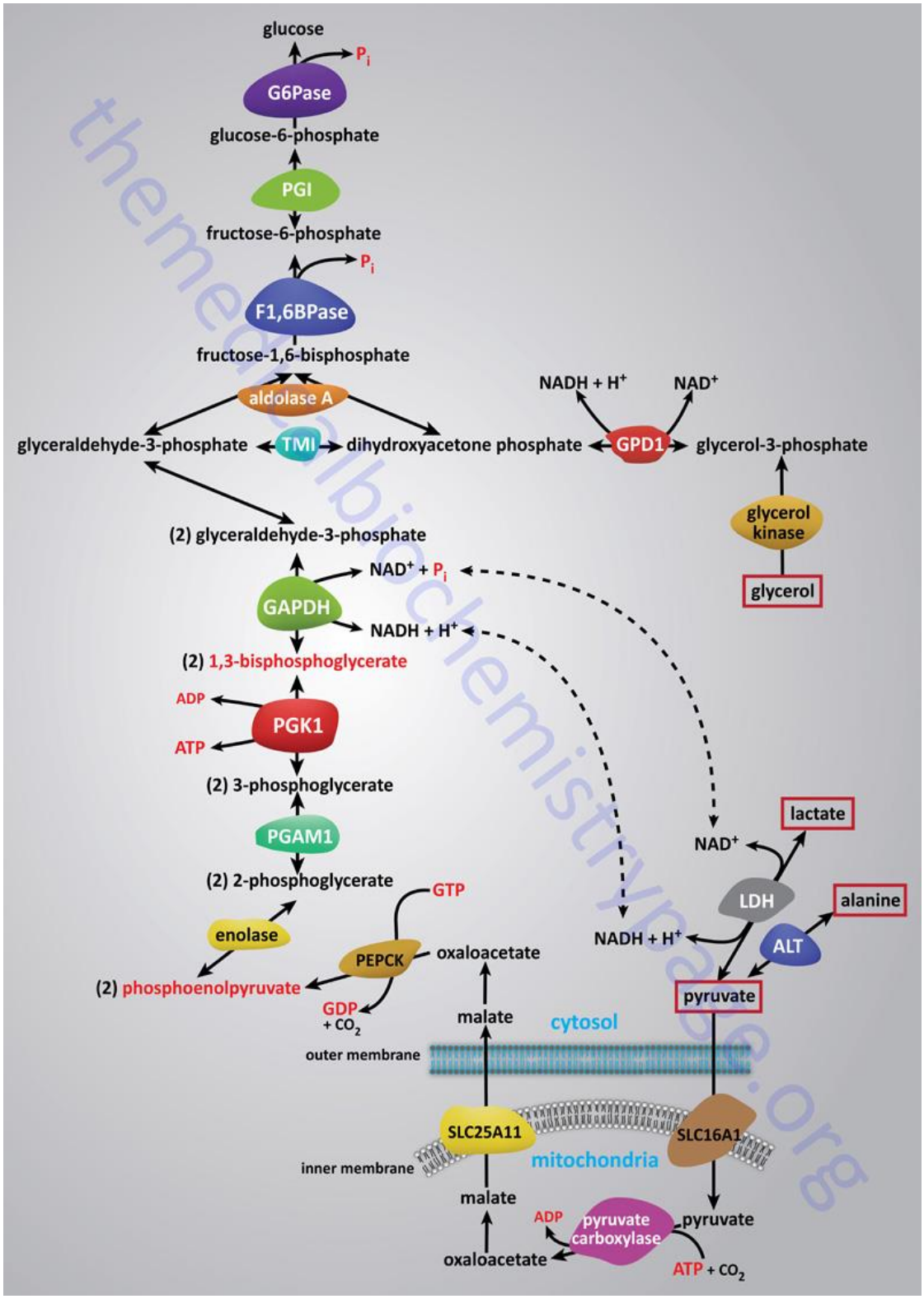
The existence of glyoxylate cycles in humans has not been established, and it is widely held

that fatty acids cannot be converted to glucose in humans directly. However, carbon-14 has been shown to end up in glucose when it is supplied in fatty acids. Despite these findings, it is considered unlikely that the 2-carbon acetyl-CoA derived from the oxidation of fatty acids would produce a net yield of glucose via the citric acid cycle - however, acetyl-CoA can be converted into pyruvate and lactate through the ketogenic pathway.Put simply, acetic acid (in the form of acetyl-CoA) is used to partially produce glucose; acetyl groups can only form part of the glucose molecules (not the 5th carbon atom) and require extra substrates (such as pyruvate) in order to form the rest of the glucose molecule. But a roundabout pathway does lead from acetyl-coA to pyruvate, via acetoacetate, acetone, acetol and then either propylene glycol or methylglyoxal.

Location

In mammals, gluconeogenesis is restricted to the liver, the kidney and possibly the intestine. However these organs use somewhat different gluconeogenic precursors. The liver uses primarily lactate, alanine and glycerol while the kidney uses lactate, glutamine and glycerol. Propionate is the principal substrate for gluconeogenesis in the ruminant liver, and the ruminant liver may make increased use of gluconeogenic amino acids, e.g. alanine, when glucose demand is increased. The capacity of liver cells to use lactate for gluconeogenesis declines from the preruminant stage to the ruminant stage in calves and lambs. In sheep kidney tissue, very high rates of gluconeogenesis from propionate have been observed. The intestine uses mostly glutamine and glycerol.

In all species, the formation of oxaloacetate from pyruvate and TCA cycle intermediates is restricted to the mitochondrion, and the enzymes that convert Phosphoenolpyruvic acid (PEP) to glucose are found in the cytosol. The location of the enzyme that links these two parts of gluconeogenesis by converting oxaloacetate to PEP, PEP carboxykinase, is variable by species: it can be found entirely within the mitochondria, entirely within the cytosol, or dispersed evenly between the two, as it is in humans. Transport of PEP across the mitochondrial membrane is accomplished by dedicated transport proteins; however no such proteins exist for oxaloacetate. Therefore, in species that lack intra-mitochondrial PEP carboxykinase, oxaloacetate must be converted into malate or aspartate, exported from the mitochondrion, and converted back into oxaloacetate in order to allow gluconeogenesis to continue.



Pathway

Gluconeogenesis is a pathway consisting of a series of eleven enzyme-catalyzed reactions. The pathway may begin in the mitochondria or cytoplasm, this being dependent on the substrate being used. Many of the reactions are the reversible steps found in glycolysis. Gluconeogenesis begins in the mitochondria with the formation of oxaloacetate by the carboxylation of pyruvate. This reaction also requires one molecule of ATP, and is catalyzed by pyruvate carboxylase. This enzyme is stimulated by high levels of acetyl-CoA (produced in -oxidation in the liver) and inhibited by high levels of ADP and glucose.

Oxaloacetate is reduced to malate using NADH, a step required for its transportation out of the mitochondria. Malate is oxidized to oxaloacetate using NAD^+ in the cytosol, where the remaining steps of gluconeogenesis take place. Oxaloacetate is decarboxylated and then phosphorylated to form phosphoenolpyruvate using the enzyme phosphoenolpyruvate carboxykinase. A molecule of GTP is hydrolyzed to GDP during this reaction. The next steps in the reaction are the same as reversed glycolysis. However, fructose-1,6-bisphosphatase converts fructose-1,6-bisphosphate to fructose 6-phosphate, using one water molecule and releasing one phosphate. This is also the rate-limiting step of gluconeogenesis. Glucose-6-phosphate is formed from fructose 6-phosphate by phosphoglucoisomerase. Glucose-6-phosphate can be used in other metabolic pathways or dephosphorylated to free glucose. Whereas free glucose can easily diffuse in and out of the cell, the phosphorylated form (glucose-6-phosphate) is locked in the cell, a mechanism by which intracellular glucose levels are controlled by cells. The final reaction of gluconeogenesis, the formation of glucose, occurs in the lumen of the endoplasmic reticulum, where glucose-6-phosphate is hydrolyzed by glucose-6-phosphatase to produce glucose. Glucose is shuttled into the cytoplasm by glucose transporters located in the endoplasmic reticulum's membrane.

RECIPROCAL CONTROL OF GLYCOLYSIS AND GLUCONEOGENESIS

Gluconeogenesis and glycolysis are coordinated so that within a cell one pathway is relatively inactive while the other is highly active. If both sets of reactions were highly active at the same time, the net result would be the hydrolysis of four nucleotide triphosphates (two ATP plus

two GTP) per reaction cycle. Both glycolysis and gluconeogenesis are highly exergonic under cellular conditions, and so there is no thermodynamic barrier to such simultaneous activity. However, the *amounts* and *activities* of the distinctive enzymes of each pathway are controlled so that both pathways are not highly active at the same time. The rate of glycolysis is also determined by the concentration of glucose, and the rate of gluconeogenesis by the concentrations of lactate and other precursors of glucose.

The interconversion of fructose 6-phosphate and fructose 1,6-bisphosphate is stringently controlled (Figure 16.30). As discussed in Section 16.2.1, AMP stimulates phosphofructokinase, whereas ATP and citrate inhibit it. Fructose 1,6-bisphosphatase, on the other hand, is inhibited by AMP and activated by citrate. A high level of AMP indicates that the energy charge is low and signals the need for ATP generation. Conversely, high levels of ATP and citrate indicate that the energy charge is high and that biosynthetic intermediates are abundant. Under these conditions, glycolysis is nearly switched off and gluconeogenesis is promoted.

hosphofructokinase and fructose 1,6-bisphosphatase are also reciprocally controlled by *fructose 2,6-bisphosphate in the liver* (Section 16.2.2). The level of F-2,6-BP is low during starvation and high in the fed state, because of the antagonistic effects of glucagon and insulin on the production and degradation of this signal molecule. *Fructose 2,6-bisphosphate strongly stimulates phosphofructokinase and inhibits fructose 1,6-bisphosphatase*. Hence, glycolysis is accelerated and gluconeogenesis is diminished in the fed state. During starvation, gluconeogenesis predominates because the level of F-2,6-BP is very low. Glucose formed by the liver under these conditions is essential for the viability of brain and muscle.

The interconversion of phosphoenolpyruvate and pyruvate also is precisely regulated. Recall that pyruvate kinase is controlled by allosteric effectors and by phosphorylation (Section 16.2.3). High levels of ATP and alanine, which signal that the energy charge is high and that building blocks are abundant, inhibit the enzyme in liver. Conversely, pyruvate carboxylase, which catalyzes the first step in gluconeogenesis from pyruvate, is activated by acetyl CoA and inhibited by ADP. Likewise, ADP inhibits phosphoenolpyruvate carboxykinase. Hence, gluconeogenesis is favored when the cell is rich in biosynthetic precursors and ATP.

The amounts and the activities of these essential enzymes also are regulated. The regulators in this case are hormones. Hormones affect gene expression primarily by changing the rate of transcription, as well as by regulating the degradation of mRNA. Insulin, which rises subsequent to eating, stimulates the expression of phosphofructokinase, pyruvate kinase, and the bifunctional enzyme that makes and degrades F-2,6-BP. Glucagon, which rises during starvation, inhibits the expression of these enzymes and stimulates instead the production of two key gluconeogenic enzymes, phosphoenolpyruvate carboxykinase and fructose 1,6-bisphosphatase. Transcriptional control in eukaryotes is much slower than allosteric control; it takes hours or days in contrast with seconds to minutes. The richness and complexity of hormonal control are graphically displayed by the promoter of the phosphoenolpyruvate carboxykinase gene, which contains regulatory sequences that respond to insulin, glucagon, glucocorticoids, and thyroid hormone

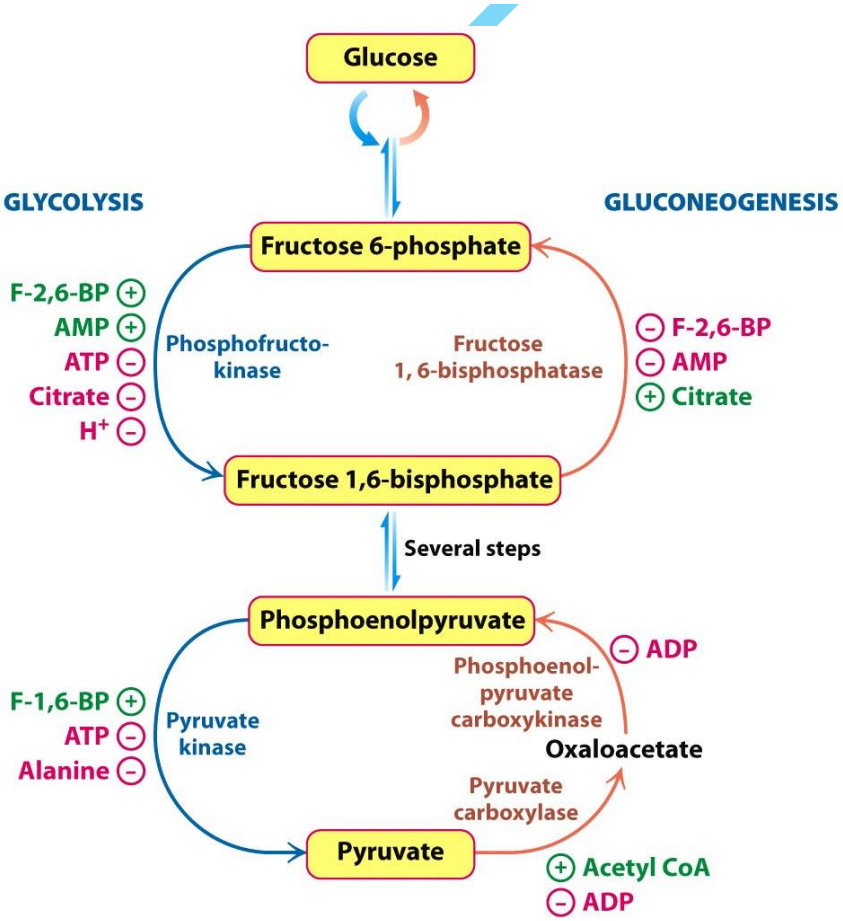


Figure 16.30
***Biochemistry*, Seventh Edition**

PENTOSE PHOSPHATE PATHWAY (HMP SHUNT)

The pentose phosphate pathway (also called the phosphogluconate pathway and the hexose monophosphate shunt) is a biochemical pathway parallel to glycolysis that generates NADPH and pentoses (5-carbon sugars). While it does involve oxidation of glucose, its primary role is anabolic rather than catabolic. There are two distinct phases in the pathway. The first is the oxidative phase, in which NADPH is generated, and the second is the non-oxidative synthesis of 5-carbon sugars. For most organisms, the pentose phosphate pathway takes place in the cytosol; in plants, most steps take place in plastids.

Similar to glycolysis, the pentose phosphate pathway appears to have a very ancient evolutionary origin. The reactions of this pathway are (mostly) enzyme catalysed in modern cells. They also occur however non-enzymatically under conditions that replicate those of the Archean ocean, and are then catalyzed by metal ions, ferrous iron Fe (II) in particular. The origins of the pathway could thus date back to the prebiotic world.

Pathway

The generation of reducing equivalents, in the form of NADPH, used in reductive biosynthesis reactions within cells (e.g. fatty acid synthesis). Production of ribose-5-phosphate (R5P), used in the synthesis of nucleotides and nucleic acids. Production of erythrose-4-phosphate (E4P), used in the synthesis of aromatic amino acids. Aromatic amino acids, in turn, are precursors for many biosynthetic pathways, including the lignin in wood.

Dietary pentose sugars derived from the digestion of nucleic acids may be metabolized through the pentose phosphate pathway, and the carbon skeletons of dietary carbohydrates may be converted into glycolytic/gluconeogenic intermediates. In mammals, the PPP occurs exclusively in the cytoplasm, and is found to be most active in the liver, mammary gland and adrenal cortex in the human. The PPP is one of the three main ways the body creates molecules with reducing power, accounting for approximately 60% of NADPH production in humans.

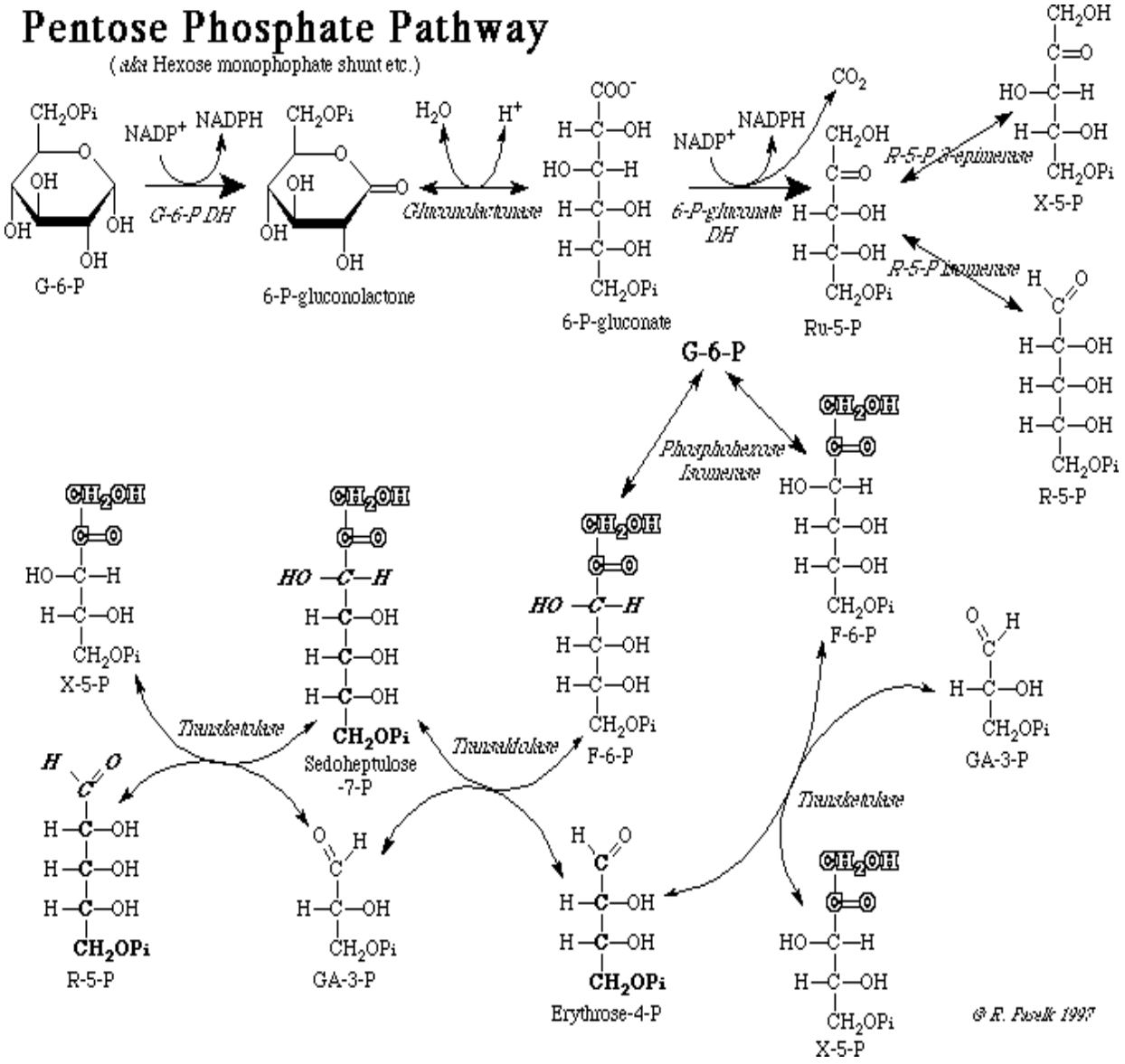
One of the uses of NADPH in the cell is to prevent oxidative stress. It reduces glutathione via glutathione reductase, which converts reactive H2O2 into H2O by glutathione peroxidase. If absent, the H_2O_2 would be converted to hydroxyl free radicals by Fenton chemistry, which can attack the cell. Erythrocytes, for example, generate a large amount of NADPH through the pentose phosphate pathway to use in the reduction of glutathione.Hydrogen peroxide is also

generated for phagocytes in a process often referred to as a respiratory burst.

Phases

Oxidative phase

In this phase, two molecules of NADP+ are reduced to NADPH, utilizing the energy from the conversion of glucose-6-phosphate into ribulose 5-phosphate.



Regulation

Glucose-6-phosphate dehydrogenase is the rate-controlling enzyme of this pathway. It is allosterically stimulated by $NADP^+$. The ratio of NADPH: $NADP^+$ is normally about 100:1 in

liver cytosol[citation needed]. This makes the cytosol a highly-reducing environment. An NADPH-utilizing pathway forms $NADP^+$, which stimulates Glucose-6-phosphate ehydrogenase to produce more NADPH. This step is also inhibited by acetyl CoA.

Erythrocytes and the pentose phosphate pathway

Several deficiencies in the level of activity of glucose-6-phosphate dehydrogenase have been observed to be associated with resistance to the malarial parasite Plasmodium falciparum among individuals of Mediterranean and African descent. The basis for this resistance may be a weakening of the red cell membrane (the erythrocyte is the host cell for the parasite) such that it cannot sustain the parasitic life cycle long enough for productive growth.

GLUCURONIC ACID PATHWAY

In liver, the **uronic acid pathway** catalyzes the conversion of glucose to glucuronic acid, ascorbic acid, and pentoses (Figure 20–4). It is also an alternative oxidative pathway for glucose, but—like the pentose phosphate pathway—it does not lead to the generation of ATP. Glucose 6-phosphate is isomerized to glucose 1-phosphate, which then reacts with uridine triphosphate (UTP) to form uridine diphosphate glucose (UDPGlc) in a reaction catalyzed by **UDPGlc pyrophosphorylase,** as occurs in glycogen synthesis (Chapter 18). UDPGlc is oxidized at carbon 6 by NAD-dependent **UDPGlc dehydrogenase** in a two-step reaction to yield UDP-glucuronate. UDP-glucuronate is the "active" form of glucuronate for reactions involving incorporation of glucuronic acid into proteoglycans or for reactions in which substrates such as steroid hormones, bilirubin, and a number of drugs are conjugated with glucuronate for excretion in urine or bile (Figure 32–14). Glucuronate is reduced to L-gulonate in an NADPH dependent reaction; L-gulonate is the direct precursor of **ascorbate** in those animals capable of synthesizing this vitamin. In humans and other primates as well as guinea pigs, ascorbic acid cannot be synthesized because of the absence of **L-gulonolactone oxidase.** L-Gulonate is metabolized ultimately to D-xylulose 5-phosphate, a constituent of the pentose phosphate pathway.

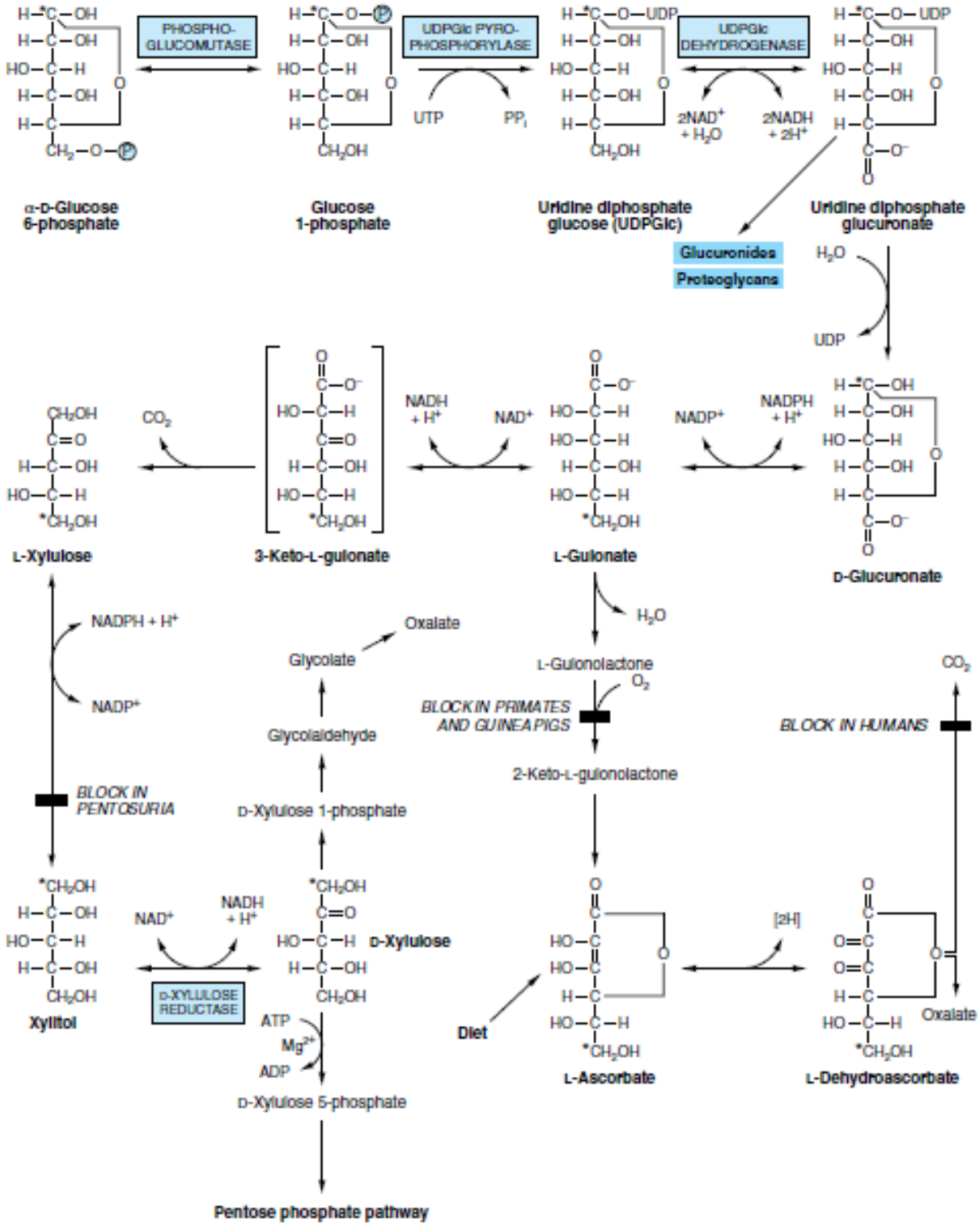


Figure 20–4. Uronic acid pathway. (Asterisk indicates the fate of carbon 1 of glucose; Ⓟ, $—PO_3^{2-}$.)

POSSIBLE QUESTIONS

PART A (1 mark)

Question number 1-20 (From given all possible 300 MCQs)

PART B (2 Marks)

1. Write a note on autotrophs
2. Brief about metabolic pathways
3. What do you mean by reducing power of the cell
4. Write a note on HMP
5. Explain the overview of intermediary metabolism?
6. Give an account on catabolism and anabolism?
7. What do you mean by amphibolic?
8. List the sources of gluconeogenesis
9. Write a short note on glycolysis
10. Give a brief note on gluconeogenesis
11. Brief about glycogenesis
12. Brief about glycogenolysis

PART C (6 Marks)

13. Explain in detail about the metabolism of glycolysis
14. Describe about reciprocal regulation of glycolysis
15. Explain the gluconeogenesis
16. Explain about pentose phosphate pathway and its importance
17. Explain reciprocal regulation of gluconeogenesis
18. Explain the importance and reactions of HMP shunt
19. Discuss in detail about glucuronic acid pathway and it's significance
20. Give a detail account on glycogenesis
21. Write in detail about glycogenolysis

Unit II

	Questions	Option 1	Option 2	Option 3	Option 4	Option 5	Option 6	Answer
1	Catabolism is	Breakdown of biological molecules	Synthesis of biological molecules	Conversion of biological molecules	Utilizing of biological molecules			Breakdown of biological molecules
2	Amphibolic is	Catabolism	Anabolism	Metabolism	Both catabolism and anabolism			Both catabolism and anabolism
3	Primary role of pentose phosphate pathway is	Catabolic	Anabolic	Both A and B	Amphibolic			Anabolic
4	Pentose phosphate pathway is parallel to	Glycolysis	Gluconeogenesis	Fermentation	Respiration			Glycolysis
5	Pentose phosphate pathway is also termed as	Glycolysis	Gluconeogenesis	Phosphogluconate pathway	Glycogenolysis			Phosphogluconate pathway
6	Uncoupling of mitochondrial oxidative phosphorylation	Allows continued mitochondrial ATP formation, but halts O2 consumption	Halts all mitochondrial metabolism	Halts mitochondrial ATP formation, but allows continued O2 consumption	Slows the conversion of glucose to pyruvate by glycolysis			Halts mitochondrial ATP formation, but allows continued O2 consumption
7	Which is not a metabolic intermediate used in amphibolic pathways?	Glyceraldehyde-3-phosphate		Acetyl CoA	Oxaloacetic acid			Fructose-1,6-bisphosphate
8	Complete oxidative breakdown of glucose results in ATP molecules	32	36	32	39			36
9	Which one of the following is a rate limiting enzyme of gluconeogenesis?	Hexokinase	Phsophofructokinase	Pyruvate carboxylase	Pyruvate kinase			Pyruvate carboxylase
10	Different enzymes that catalyze same reaction are called	isoenzymes	coenzymes	cofactors	isofactors			isoenzymes
11	Gluconeogenesis occurs in	Adipose tissue	Muscles	Kidneys	Brain			Kidneys
12	In glycolysis a net gain of two ATPs are generated by what process?	Chemiosmosis	ADP processing	Substrate level phosphorylation	Electron transport chain			Substrate level phosphorylation
13	The preparatory reaction breaks	Glucose into pyruvates	Pyruvates into glucose	Pyruvates into acetyl-coa and carbon dioxide	Pyruvates into acetyl-coa and water			Pyruvates into acetyl-coa and carbon dioxide
14	Embden-Meyerhof pathway referred as	Gluconeogenesis	Glycolysis	Citric acid	Glycogenesis			Glycolysis
15	In gluconeogenesis, glucose is generated by	Non carbohydrate carbon substrates	Carbohydrate carbon substrates	Sucrose	Yeast			Non carbohydrate carbon substrates
16	Gluconeogenesis is decreased by	Glucagon	Epinephrine	Glucocorticoids	Insulin			Insulin
17	Gluconeogenesis is often associated with	Ketosis	Hexoses	Pentoses	Aldolase			Ketosis
18	In vertebrates, gluconeogenesis mainly takes place in	Stomach	Liver	Heart	Intestine			Liver
19	The first two intermediates in the process of glycolysis are, respectively	Glucose 6-phosphate and glucose 1-phosphate	Glucose 1-phosphate and glucose 6-phosphate	Glucose 6-phosphate and fructose 6-phosphate	Glucose 1-phosphate and fructose 1-phosphate			Glucose 6-phosphate and fructose 6-phosphate
20	The name of the process in which glucose 6-phosphate is converted to glycogen is	Gluconeogenesis	Glycogenesis	Glycogenolysis	Glycolysis			Glycogenesis
21	Which of the following is a reactant in the first step of gluconeogenesis?	Carbon dioxide	GTP	Glucose	Phosphoenol pyruvate			Carbon dioxide
22	Which of the following metabolite integrates glucose and fatty acid metabolism?	Acetyl CoA	Pyruvate	Citrate	Lactate			Acetyl CoA
23	The formation of citrate from oxalo acetate and acetyl CoA is	Oxidation	Reduction	Condensation	Hydrolysis			Condensation
24	The carrier of the citric acid cycle is	Succinate	Fumarate	Malate	Oxaloacetate			Oxaloacetate
25	The Key enzymes in glycolysis are	Glucokinase	Glucokinase and Phosphofructokinase	Glucokinase, Phosphofructokinase and pyruvate kinase	Glucokinase, Phosphofructokinase and fructose -1-phosphatase			Glucokinase, Phosphofructokinase and pyruvate kinase
26	The complete oxidation of glucose occurs in	Glycolysis	HMP shunt	Glycolysis and TCA cycle	TCA cycle			Glycolysis and TCA cycle
27	TCA Cycle takes place in	Cytosol	Ribosomes	Mitochondria	Nucleus			Mitochondria
28	TCA Cycle is called as amphibolic pathway because it	Produces energy	Is catabolic and anabolic	Produces CO_2 and H_2O	Occurs in mitochondria			Is catabolic and anabolic
29	Von Gierke's disease is due to deficiency of enzyme	Glucose-6-phosphatase	Glucose -1-phosphatase	Fructose-6-phosphatase	Fructose-1-phosphatase			Glucose-6-phosphatase
30	Pentose provided by HMP shunt is used for	Energy production	Fatty acid production	Nucleic acid synthesis	Steroid synthesis			Nucleic acid synthesis
31	Essential pentosuria is due to deficiency of enzyme	Xylulose reductase	Xylitol dehydrogenase	Xylitol synthetase	Xylitol decarboxylase			Xylitol dehydrogenase
32	Rate limiting enzyme in glycogenolysis is	Phosphorylase	Phosphoglucomutase	Glucose 6 phosphatase	Fructose 1,6 phosphatase			Phosphorylase
33	Rate limiting enzyme in glycogenesis is	Glucokinase	Phophoglucomutase	UDPG phosphorylase	Glycogen synthetase			Glycogen synthetase
34	Tricarboxylic acid cycle to be continuous requires the regeneration of	Pyruvic acid	Oxaloacetic acid	α-oxoglutaric acid	Malic acid			Oxaloacetic acid
35	Where does the TCA cycle take place in bacteria?	Mitochondrial matrix	Cytoplasm	Mitochondrial membrane	Cytoplasmic membrane			Cytoplasm
36	In aerobic respiration when is the first molecule of carbon dioxide released?	During chemiosmosis	When pyruvic acid is reduced to lactic acid	During the conversion step when pyruvic acid is converted to acetyl-CoA	When glucose is phosphorylated in glycolysis			During the conversion step when pyruvic acid is converted to acetyl-CoA
37	One turn of the citric acid cycle produces	2 NADH, 2 $FADH_2$, 2 ATP	3 NADH, 1 $FADH_2$, 1 ATP	3 NADH, 2 $FADH_2$, 1 ATP	3 NADH, 1 $FADH_2$, 2 ATP			3 NADH, 1 $FADH_2$, 1 ATP
38	Intermediates of the citric acid cycle are replenished by a reaction converting pyruvate to	Oxaloacetate	Citrate	Alpha-ketoglutarate	Succinyl-coa			Oxaloacetate
39	End product of TCA cycle is	Citric acid	Pyruvic acid	Lactic acid	CO_2 and water			CO_2 and water
40	is the precursor for the	L-gulonate	L-alanine	Methionine	L-aspartic acid			L-gulonate
41	synthesis of ascorbic acid							
42	In the glyoxylate cycle, the sequential action of citrate lyase and isocitrate lyase converts acetyl CoA into glyoxylate and oxaloacetate into:	Malate	Aspartate	Pyruvate	Succinate			Succinate
43	ATP and NADH inhibit	Isocitrate hydrogenase	Isocitrate dehydrogenase	Pyruvate dehydrogenase	Pyruvate hydrogenase			Isocitrate dehydrogenase
44								
45	In hydration, fumarate is converted by fumarase to	L-Malate	D-Malate	A-Malate	C-Malate			L-Malate
46								
47	High levels of NADH will lower concentration of	Acetate	Dehydrogenase	Oxaloacetate	Carbonate			Oxaloacetate
48								
49	Number of enzyme catalyzed reactions in	12	13	11	10			11
50	gluconeogenesis are							
51	Which of the following processes requires UTP molecules?	Formation of glycogen from glucose 6-phosphate	Degradation of glycogen to glucose 6-phosphate	Formation of glucose 1-phosphate from glucose 6-phosphate	Degradation of glucose 1-phosphate from glucose 6-phosphate			Formation of glycogen from glucose 6-phosphate
52	Glycogen is converted to glucose in which of the following processes?	Gluconeogenesis	Glycogenesis	Glycogenolysis	Glycolysis			Glycogenolysis
53	Which of the following intermediates is not involved in glycolysis but is in gluconeogenesis?	Fructose 6-phosphate	Pyruvate	Oxaloacetate	Acetyl Coa			Oxaloacetate
54	In which of the following processes is glucose 6-phosphate the end product?	Glycogenesis	Glycogenolysis	Glycolysis	Citric acid cycle			Glycogenolysis
55	The compound glucose 1-phosphate is encountered in which of the following processes?	Glycogenesis and glycogenolysis	Glycolysis and gluconeogenesis	Glycogenesis and gluconeogenesis	Glycolysis and glycogenolysis			Glycogenesis and glycogenolysis
56	The compound oxaloacetate is an intermediate in the conversion of	Glycogen to glucose	Pyruvate to glucose	Pyruvate to acetyl coa	Glycogen to pyruvate			Pyruvate to glucose
57	In the human body, under aerobic conditions and anaerobic conditions, respectively, pyruvate is converted to	Lactate and ethanol	Lactate and acetyl Coa	Ethanol and lactate	Acetyl Coa and lactate			Acetyl Coa and lactate
58	Which of the following enzyme is not involved in HMP shunt?	Glyceraldehyde-3 phosphate-dehydrogenase	Glucose-6-phosphatedehydrogenase	Transketolase	Phosphogluconate dehydrogenase			Glyceraldehyde-3-phosphatedehydrogenase
59	Which of the following is a substrate for aldolase activity in Glycolytic pathway?	Glyceraldehyde-3-phosphate	Glucose-6-phosphate	Fructose-6-phosphate	Fructose1, 6-bisphosphate			Fructose1, 6-bisphosphate
60	An allosteric enzyme responsible for controlling the rate of T.C.A cycle is	Malate dehydrogenase	Isocitrate dehydrogenase	Fumarase	Aconitase			Isocitrate dehydrogenase

UNIT-III

Lipid metabolism: Fatty acid oxidation-α, β, ω oxidation. Biosynthesis of saturated and unsaturated fatty acids. Metabolism of cholesterol, triglycerides and ketone bodies.

B-Oxidation of fatty acids

The fatty acids in the body are mostly oxidized by β-oxidation. β-Oxidation may be defined as the oxidation of fatty acids on the p-carbon atom. This results in the sequential removal of a two carbon fragment, acetyl CoA.

Fatty acid oxidation -stages and tissues

The β-oxidation of fatty acids involves three stages

l. Activation of fatty acids occurring in the cytosol;

ll. Transport of fatty acids into mitochondria;

lll. β-Oxidation proper in the mitochondrial matrix.

Fatty acids are oxidized by most of the tissues in the body. However, brain, erythrocytes and adrenal medulla cannot utilize fatty acids for energy requirement.

I. Fatty acid activation

Fatty acids are activated to acyl CoA by thiokinases or acyl CoA synthetases. The reaction occurs in two steps and requires ATP, coenzyme A and Mg2+. Fatty acid reacts with ATP to form acyladenylate which then combines with coenzyme A to produce acyl CoA. In the activation, two high energy phosphates are utilized, since ATP is converted to pyrophosphate (PPi). The enzyme inorganic pyrophosphatase hydrolyses PPi to phosphate (Pi). The immediate elimination of PPi makes this reaction totally irreversible. Three different thiokinases, to activate long chain (10-20 carbon), medium chain (4-12 carbon) and short chain (< 4 carbon) fatty acids have been identified.

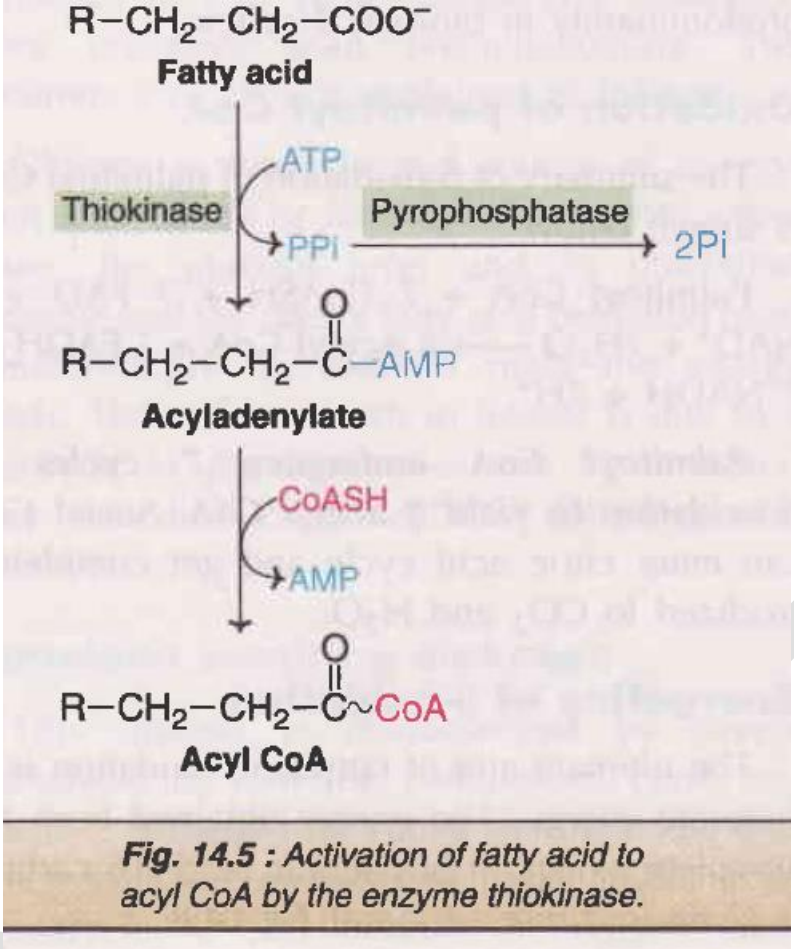


Fig. 14.5 : Activation of fatty acid to acyl CoA by the enzyme thiokinase.

II. Transport of acyl CoA into mitochondria

The inner mitochondrial membrane is impermeable to fatty acids. A specialized carnitine carrier system (carnitine shuttle) operates to transport activated fatty acids from cytosol to the mitochondria. This occurs in four steps,

1. Acyl group of acyl CoA is transferred to carnitine (B-hydroxy T-trimethyl aminobutyrate), catalysed by carnitine acyltransferase (present on the outer surface of inner mitochondrial membrane).
2. The acyl-carnitine is transported across the membrane to mitochondrial matrix by a specific carrier protein.
3. Carnitine acyl transferase II (found on the inner surface of inner mitochondrial membrane) converts acyl-carnitine to acyl CoA.
4. The carnitine released returns to cvtosol for reuse.

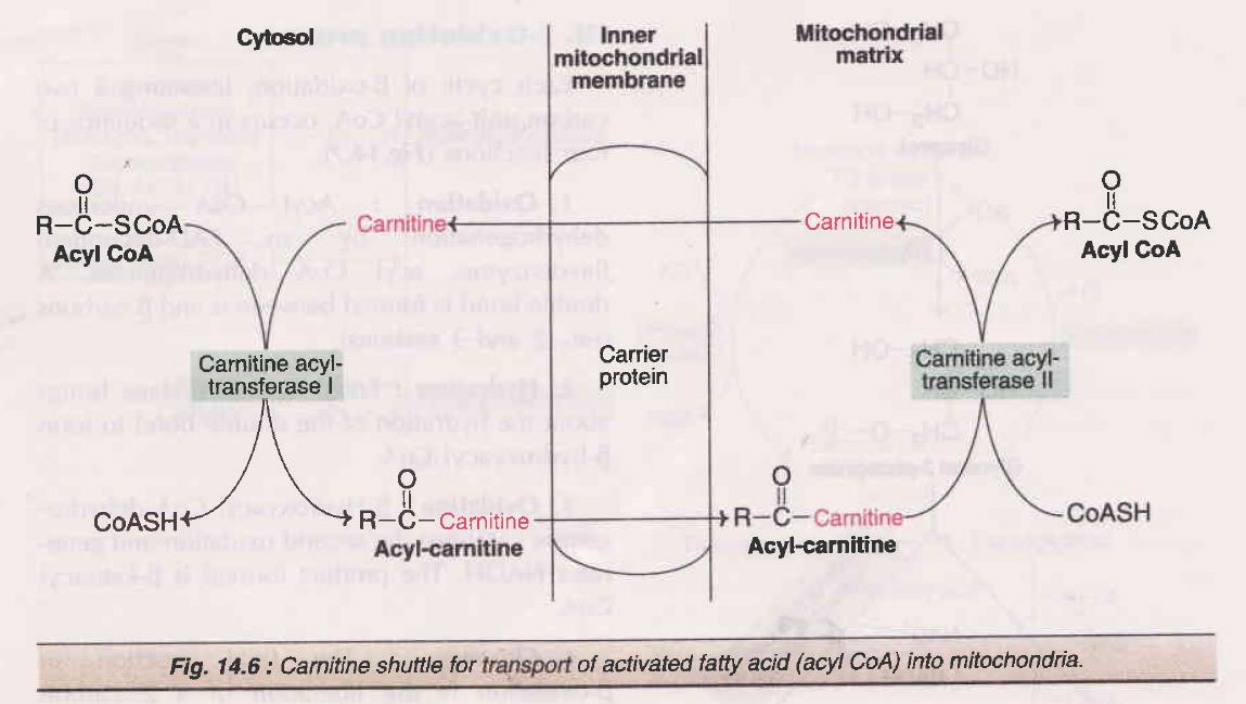


Fig. 14.6 : Carnitine shuttle for transport of activated fatty acid (acyl CoA) into mitochondria.

Inhibitor of carnitine shuttle : Carnitine acyl transferase I is inhibited by malonyl CoA, a key metabolite involved in fatty acid synthesis that occurs in cytosol (details given later). In other words, while the fatty acid synthesis is in progress (reflected by high concentration of malonyl CoA), their oxidation does not occur, since carnitine shuttle is impaired.

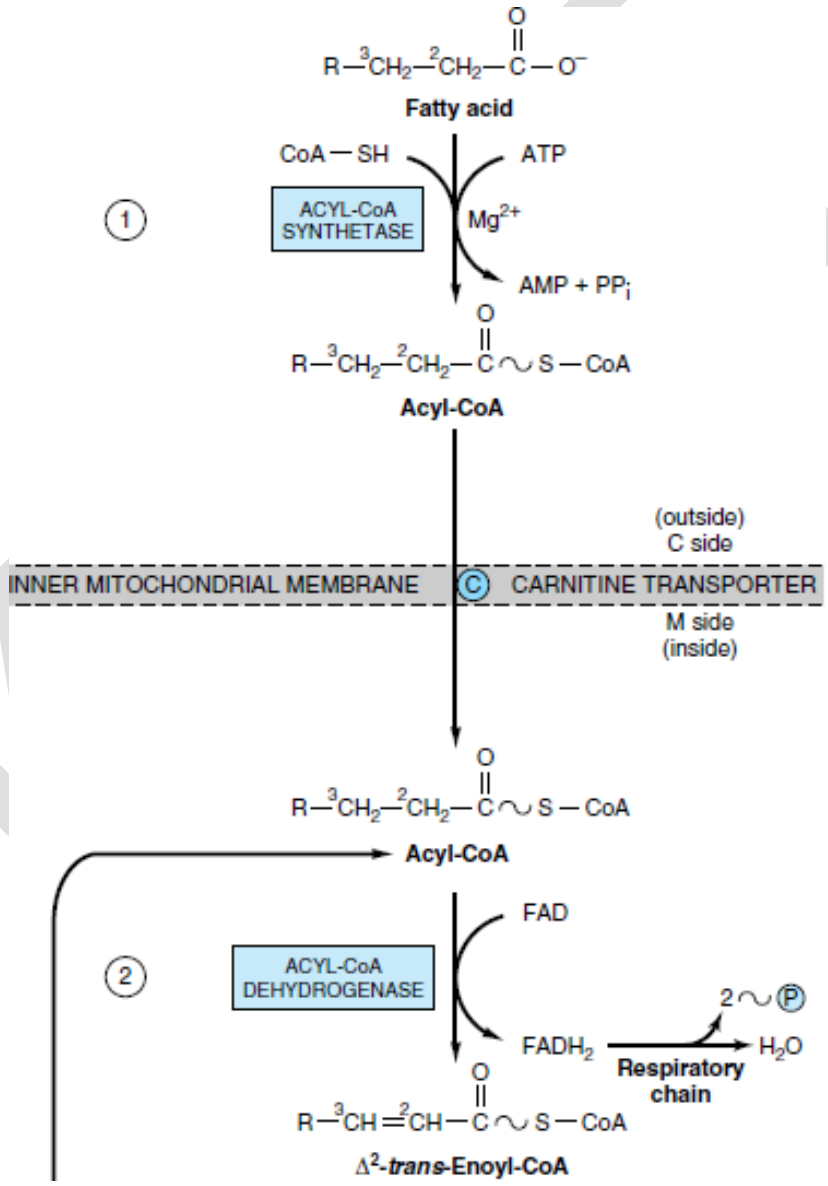
III. B- Oxidation proper

Each cycle of β-oxidation, liberating a two carbon unit-acetyl CoA, occurs in a sequence of four reactions.

1. Oxidation : Acyl CoA undergoes dehydrogenation by an FAD-dependent flavoenzyme, acyl CoA dehydrogenase. A double bond is formed between α and β carbons (i.e.,2 and 3 carbons).
2. Hydration : Enoyl CoA hydratase brings about the hydration of the double bond to form β-hydroxyacyl CoA.
3. Oxidation: β-Hydroxyacyl CoA dehydrogenase catalyses the second oxidation and generates NADH. The product formed is β-ketoacyl CoA.

4. Cleavage : The final reaction in p-oxidation is the liberation of a 2 carbon fragment, acetyl CoA from acyl CoA. This occurs by a thiolytic cleavage catalysed by β-ketoacyl CoA thiolase (or simply thiolase).

The new acyl CoA, containing two carbons less than the original, reenters the p-oxidation cycle. The process continues till the fatty acid is completely oxidized.



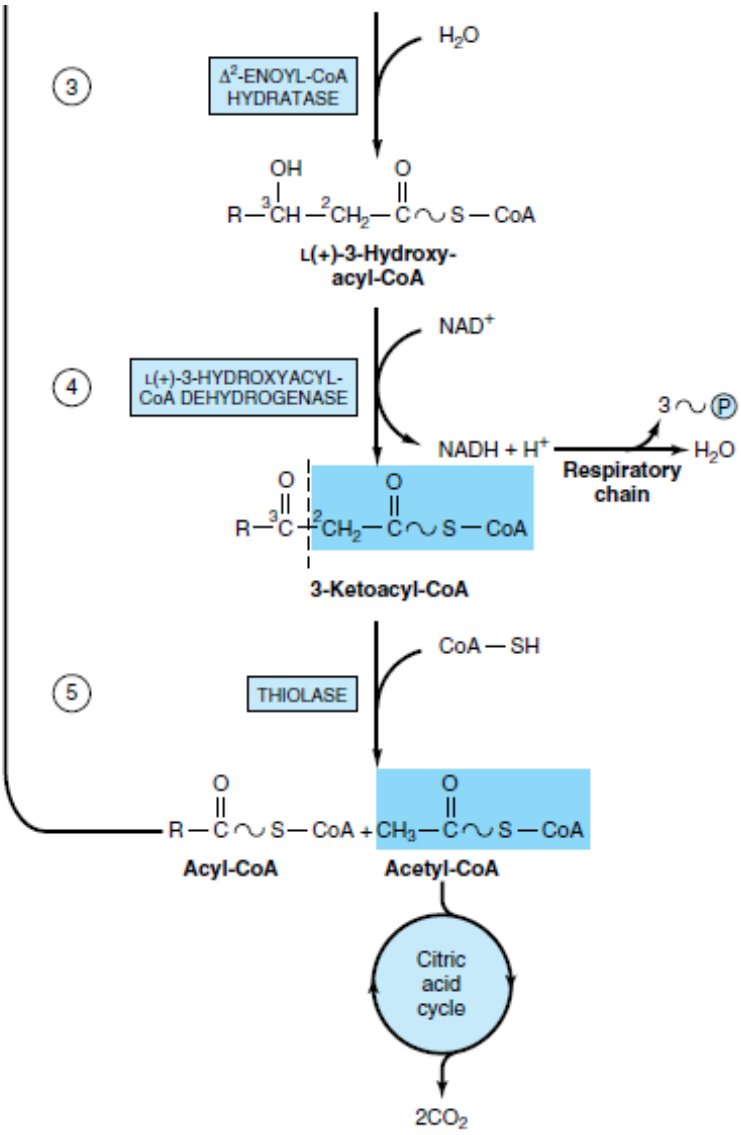


Fig. 14.7 : *β-Oxidation of fatty acids : Palmitoyl CoA (16 carbon) undergoes seven cycles to yield 8 acetyl CoA [I–Activation; II–Transport; III–β Oxidation proper— (1) Oxidation, (2) Hydration, (3) Oxidation and (4) Cleavage].*

Oxidation of palmitoyl CoA

The summary of β-oxidation of palmitoyl CoA is shown below Palmitoyl CoA + 7 CoASH + 7 FAD + 7 NAD+ + 7H2C- ---> 8 Acetyl CoA + 7 FADH2 + 7 NADH + 7H+ Palmitoyl CoA undergoes 7 cycles of β-oxidation to yield 8 acetyl CoA. Acetyl CoA can enter citric acid cycle and get completely oxidized to CO2 and H2O.

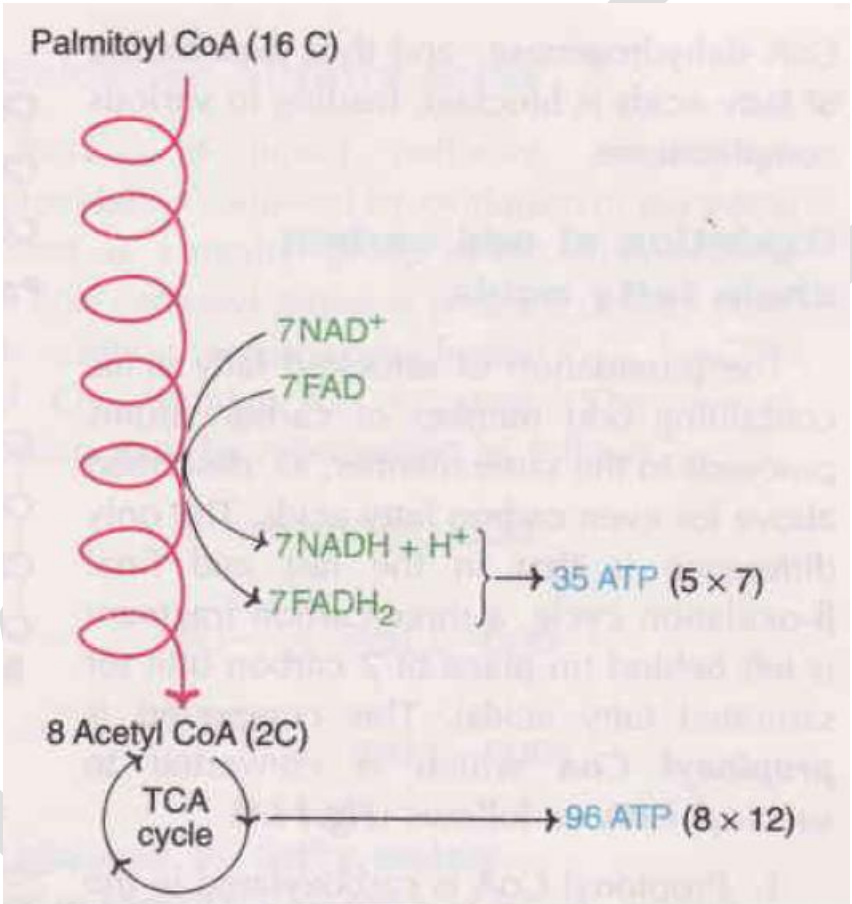


Fig. 14.8 : An overview of oxidation of palmitic acid.

Energetics of B-oxidation

The ultimate aim of fatty acid oxidation is to generate energy. The energy obtained from the complete oxidation of palmitic acid (16 carbon) is given in the below table.

The standard free energy of palmitate = 2,34O Cal.

The energy yield by its oxidation-129 ATP (129 x 7.3 Cal) = 940 Cal.

The efficiency of energy conservation by fatty acid oxidation=940/2340 x 100 = 40%.

TABLE 14.2 Energetics of palmitic acid oxidation

Mechanism	*ATP yield*
I. **β-Oxidation 7 cycles**	
7 $FADH_2$ [oxidized by electron transport chain (ETC), each $FADH_2$ gives 2 ATP]	14
7 NADH (oxidized by ETC, each NADH liberates 3 ATP)	21
II. **From 8 acetyl CoA**	
Oxidized by citric acid cycle, each acetyl CoA provides 12 ATP	96
Total energy from one mole of palmitoyl CoA	131
Energy utilized for activation (formation of palmitoyl CoA)	–2
Net yield of oxidation of one molecule of palmitate	129

SIDS-a disorder due to blockade in β-oxidation

The sudden infant death syndrome (SIDS) is an unexpected death of healthy infants, usually overnight. The real cause of SIDS is not known. It is now estimated that at least 10% of SIDS is due to deficiency of medium chain acyl CoA dehydrogenase. The enzyme defect has a frequency of 1 in 10,000 births and is, in fact, more prevalent than phenylketonuria. The occurrence of SIDS is explained as follows Glucose is the principal source of energy, soon after eating or feeding babies. After a few hours, the glucose level and its utilization decrease and the rate of fatty acid oxidation must simultaneously increase to meet the energy needs. The sudden death in

infants is due to a blockade in β-oxidation caused by a deficiency in medium chain acyl CoA dehydrogenase (MCAD).

Jamaican vomiting sickness

This disease is characterized bv severe hypoglycemia,v omiting,c onvulsions,c oma and death. lt is caused by eating unripe ackee fruit which contains an unusual toxic amino acid, hypoglycin A. This inhibits the enzyme acyl CoA dehydrogenase and thus p-oxidation of fatty acids is blocked, leading to various complications.

Oxidation of odd carbon chain fatty acids

The p-oxidation of saturated fatty acids containing odd number of carbon atoms proceeds in the same manner, as described above for even carbon fatty acids. The only difference is that in the last and final β-oxidation cycle, a three-carbon fragment is left behind (in place of 2 carbon unit for saturated fatty acids). This compound is propionyl CoA which is converted to succinyl CoA as follows

1. Propionyl CoA is carboxylated in the presence of ATP, CO2 and vitamin biofin to D-methylmalonyl CoA.
2. Methylmalonyl CoA racemase converts the methylmalonyl CoA to L-form. This reaction (D - + L) is essential or the entry of this compound into the metabolic reactions of the body.
3. The next enzyme, methylmalonyl CoA mutase, is dependant on vitamin 812 (deoxyadenosyl cobalamin). It catalyses the conversion of methylmalonyl CoA (a branched compound) to succinyl CoA (a straight chain compound), which can enter citric acid cycle.

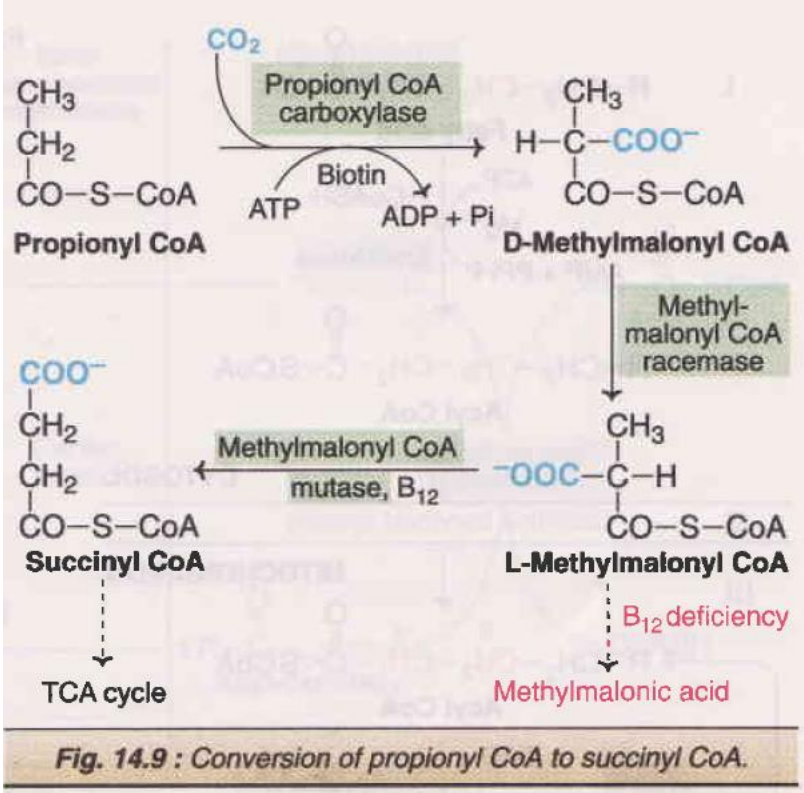


Fig. 14.9 : Conversion of propionyl CoA to succinyl CoA.

Methylmalonie acidemia

Two types of methylmalonic acidemias are known

1. Due to deficiency of vitamin B12;

2. Due to defect in the enzyme methylmalonyl CoA mutase.

In either case, there is an accumulation of methylmalonic acid in body, followed by its increased excretion in urine. This causes severe metabolic acidosis, damages the central neryous system and retards the growth. lt is often fatal in the early years of life.

Oxidation of unsaturated fatty acids

Due to the presence of double bonds, the unsaturated fatty acids are not reduced to the same extent as saturated fatty acids. Therefore, oxidation of unsaturated fatty acids, in general, provides less energy than that of saturated fatty acids. Most of the reactions involved in the oxidation of unsaturated fatty acids are the same as found in the p-oxidation of saturated fatty

acids. However, the presence of double bonds poses problem for p-oxidation to proceed. This is overcome by two additional enzymes-an isomerase and an epimerase.

α-Oxidation of fatty acids

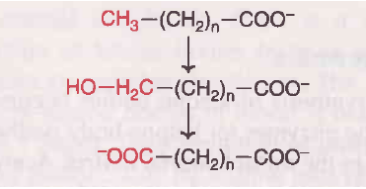
β-Oxidation is the most predominant pathway for fatty acid degradation. However, the removal of one carbon unit at a time by the oxidation of α-carbon atom of fatty acid is known. α-Oxidation does not involve the binding of fatty acid to coenzyme A and no energy is produced.

Refsum's disease is a rare but severe neurological disorder characterized by cerebral ataxia and peripheral neuropathy. The patients of this disease accumulate large quantities of an unusual fatty acid, phytanic acid. It is derived from phytol, a constituent of chlorophyll. Hence it is found mostly in plant foods. However, it I also present in milk lipids and animal fats. Phytanic acid cannot undergo B -oxidation due to the presence of a methyl group on carbon-3. This fatty acid undergoes initial α-oxidation (to remove c-carbon as carbon dioxide) and this is followed by β-oxidation.

Refsum's disease is caused by a defect in the α-oxidation due to the deficiency of the enzyme phytanic acid α-oxidase. The result is that phytanic acid cannot be converted to a compound that can be degraded by β-oxidation. The patients should not consume diets containing chlorophyll (i.e., green leafy vegetables).

ω-Oxidation of fatty ac;ds

This is a minor pathway. lt involves hydroxylation followed by oxidation of or-carbon present as a methyl group at the other end (at one end carboxyl group is present) of fatty acid. This reaction requires cytochrome P450, NADPH and O2, besides the enzymes. The overall reaction may be represented as follows.



BIOSYNTHESIS OF SATURATED FATTY ACIDS

De novo synthesis of fatty acids (lipogenesis) occurs in the cytosol

This system is present in many tissues, including liver, kidney, brain, lung, mammary gland, and adipose tissue. Its cofactor requirements include NADPH, ATP, Mn^{2+}, biotin, and HCO_3^- (as a source of CO_2). **Acetyl- CoA** is the immediate substrate, and **free palmitate** is the end product.

Production of Malonyl-CoA Is the Initial & Controlling Step in Fatty Acid Synthesis

Bicarbonate as a source of CO_2 is required in the initial reaction for the carboxylation of acetyl-CoA to **malonyl- CoA** in the presence of ATP and **acetyl-CoA carboxylase.** Acetyl-CoA carboxylase has a requirement for the vitamin **biotin** (Figure 21–1). The enzyme is a **multienzyme protein** containing a variable number of identical subunits, each containing biotin, biotin carboxylase, biotin carboxyl carrier protein, and transcarboxylase, as well as a regulatory allosteric site. The reaction takes place in two steps: (1) carboxylation of biotin involving ATP and (2) transfer of the carboxyl to acetyl-CoA to form malonyl-CoA.

The Fatty Acid Synthase Complex Is a Polypeptide Containing Seven Enzyme Activities

In bacteria and plants, the individual enzymes of the **fatty acid synthase** system are separate, and the acyl radicals are found in combination with a protein called the **acyl carrier protein (ACP).** However, in yeast, mammals, and birds, the synthase system is a multienzyme polypeptide complex that incorporates ACP,

which takes over the role of CoA. It contains the vitamin **pantothenic acid** in the form of 4′-phosphopantetheine. The use of one multienzyme functional unit has the advantages of achieving the effect of compartmentalization of the process within the cell without the erection of permeability barriers, and synthesis of all enzymes in the complex is coordinated since it is encoded by a single gene. In mammals, the fatty acid synthase complex is a dimer comprising two identical monomers, each containing all seven enzyme activities of fatty acid synthase on one polypeptide chain (Figure 21–2). Initially, a priming molecule of acetyl-CoA combines with a cysteine □SH group catalyzed by **acetyl transacylase**. Malonyl-CoA combines

with the adjacent □SH on the 4′-phosphopantetheine of ACP of the other monomer, catalyzed by **malonyl transacylase** (reaction 1b), to form **acetyl (acyl)-malonyl enzyme.** The acetyl group

attacks the methylene group of the malonyl residue, catalyzed by **3-ketoacyl synthase,** and liberates CO2, forming 3-ketoacyl enzyme (acetoacetyl enzyme) (reaction 2), freeing the cysteine SH group. Decarboxylation allows the reaction to go to completion, pulling the whole sequence of reactions in the forward direction. The 3-ketoacyl group is reduced, dehydrated, and reduced again (reactions 3, 4, 5) to form the corresponding saturated acyl-Senzyme. A new malonyl-CoA molecule combines with the □SH of 4′-phosphopantetheine, displacing the saturated acyl residue onto the free cysteine □SH group. The sequence of reactions is repeated six more times until a saturated 16-carbon acyl radical (palmityl) has been assembled. It is liberated from the enzyme complex by the activity of a seventh enzyme in the complex, **thioesterase** (deacylase). The free palmitate must be activated to acyl-CoA before it can proceed via any other metabolic pathway. Its usual fate is esterification into acylglycerols, chain elongation or desaturation, or esterification to cholesteryl ester. In mammary gland, there is a separate thioesterase specific for acyl residues of C8, C10, or C12, which are subsequently found in milk lipids.

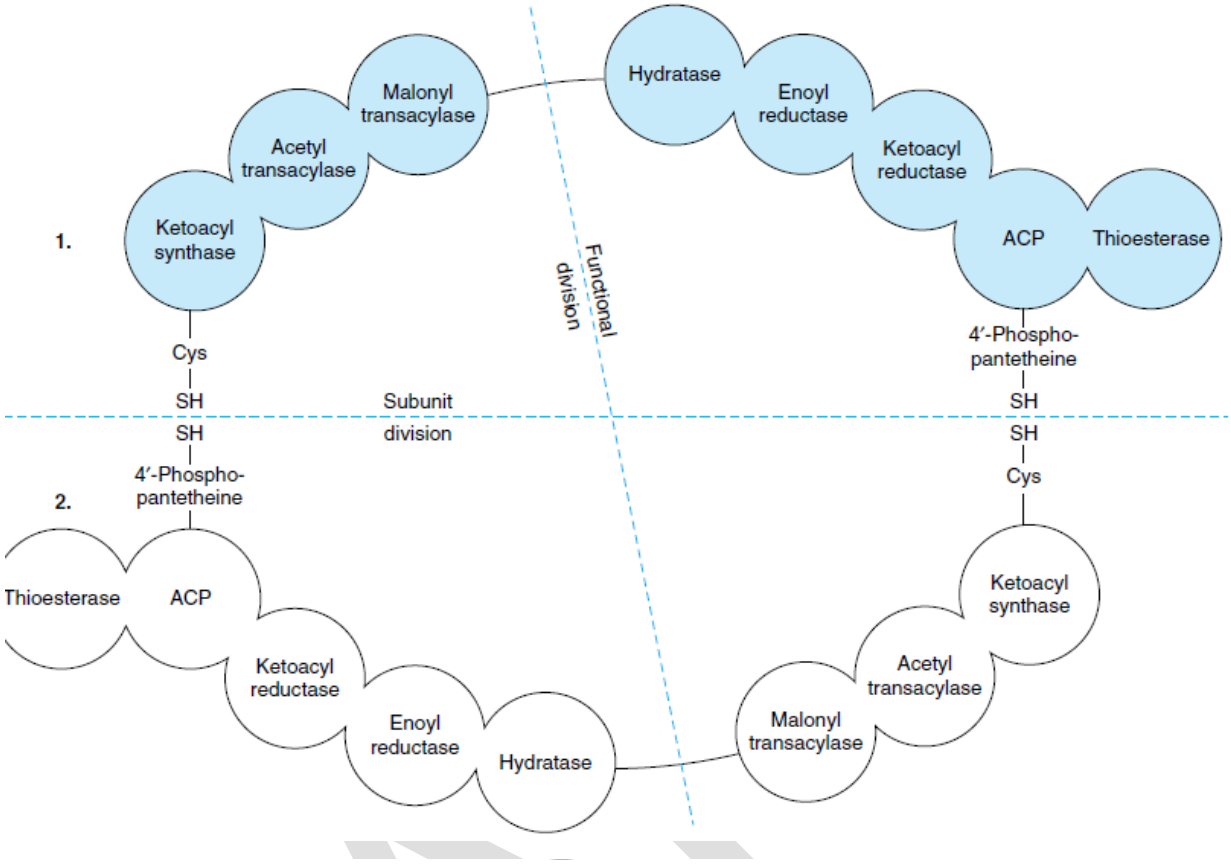


Figure 21–2. Fatty acid synthase multienzyme complex. The complex is a dimer of two identical polypeptide monomers, 1 and 2, each consisting of seven enzyme activities and the acyl carrier protein (ACP). (Cys—SH, cysteine thiol.) The —SH of the 4′-phosphopantetheine of one monomer is in close proximity to the —SH of the cysteine residue of the ketoacyl synthase of the other monomer, suggesting a "head-to-tail" arrangement of the two monomers. Though each monomer contains all the partial activities of the reaction sequence, the actual functional unit consists of one-half of one monomer interacting with the complementary half of the other. Thus, two acyl chains are produced simultaneously. The sequence of the enzymes in each monomer is based on Wakil.

The equation for the overall synthesis of palmitate from acetyl-CoA and malonyl-CoA is:

$$CH_2CO \cdot S \cdot CoA + 7HOOC \cdot CH_2CO \cdot S \cdot CoA + 14NADPH + 14H^+$$
$$\rightarrow CH_3(CH_2)_{14}COOH + 7CO_2 + 6H_2O + 8CoA \cdot SH + 14NADP^+$$

The acetyl-CoA used as a primer forms carbon atoms 15 and 16 of palmitate. The addition of all the subsequent C2 units is via malonyl-CoA. Propionyl- CoA acts as primer for the synthesis of long-chain fatty acids having an odd number of carbon atoms, found particularly in ruminant fat and milk.

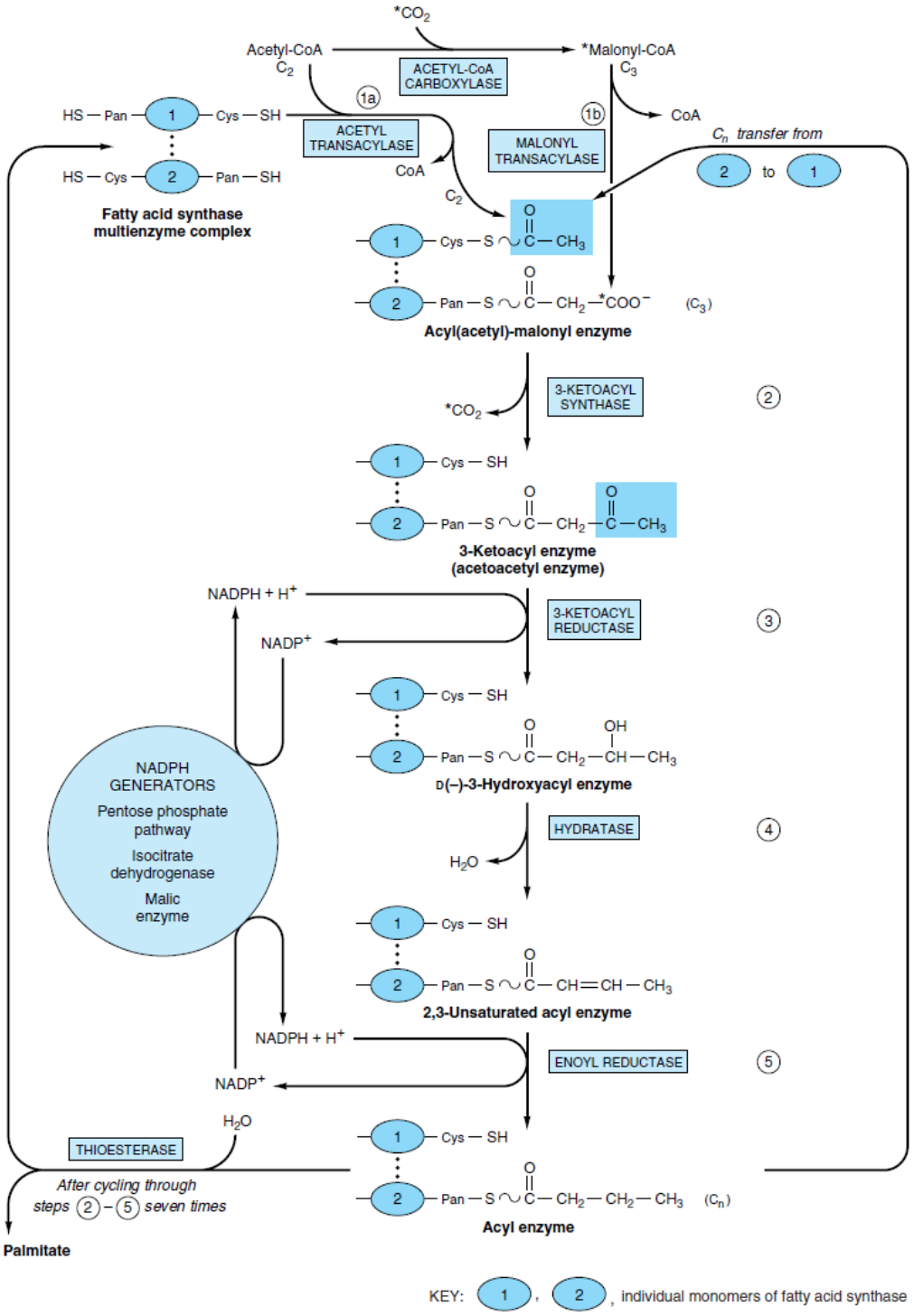


Figure 21–3. Biosynthesis of long-chain fatty acids. Details of how addition of a malonyl residue causes the acyl chain to grow by two carbon atoms. (Cys, cysteine residue; Pan, 4′-phosphopantetheine.) The blocks shown in dark blue contain initially a C_2 unit derived from acetyl-CoA (as illustrated) and subsequently the C_n unit formed in reaction 5.

Elongation of Fatty Acid Chains Occurs in the Endoplasmic Reticulum

This pathway (the "microsomal system") elongates saturated and unsaturated fatty acyl-CoAs (from C10 upward) by two carbons, using malonyl-CoA as acetyl donor and NADPH as reductant, and is catalyzed by the microsomal **fatty acid elongase** system of enzymes. Elongation of stearyl-CoA in brain increases rapidly during myelination in order to provide C22 and C24 fatty acids for sphingolipids.

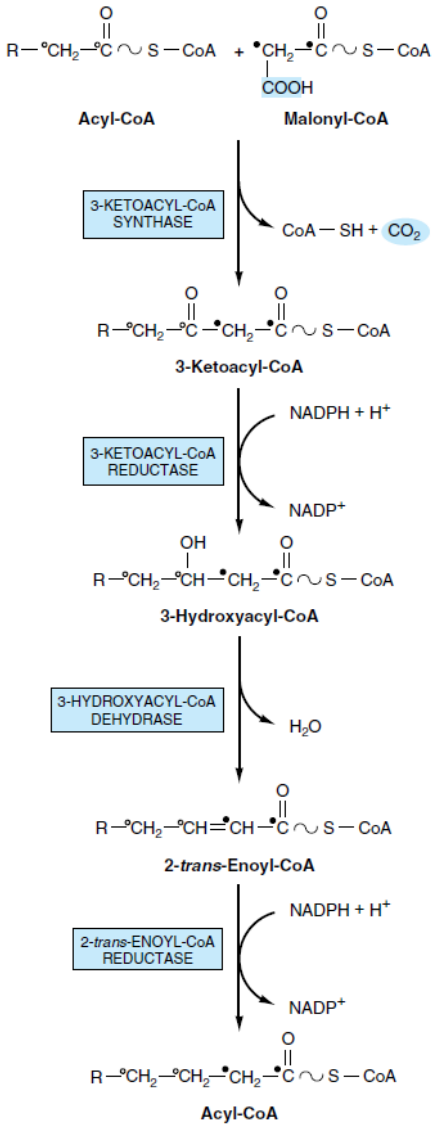
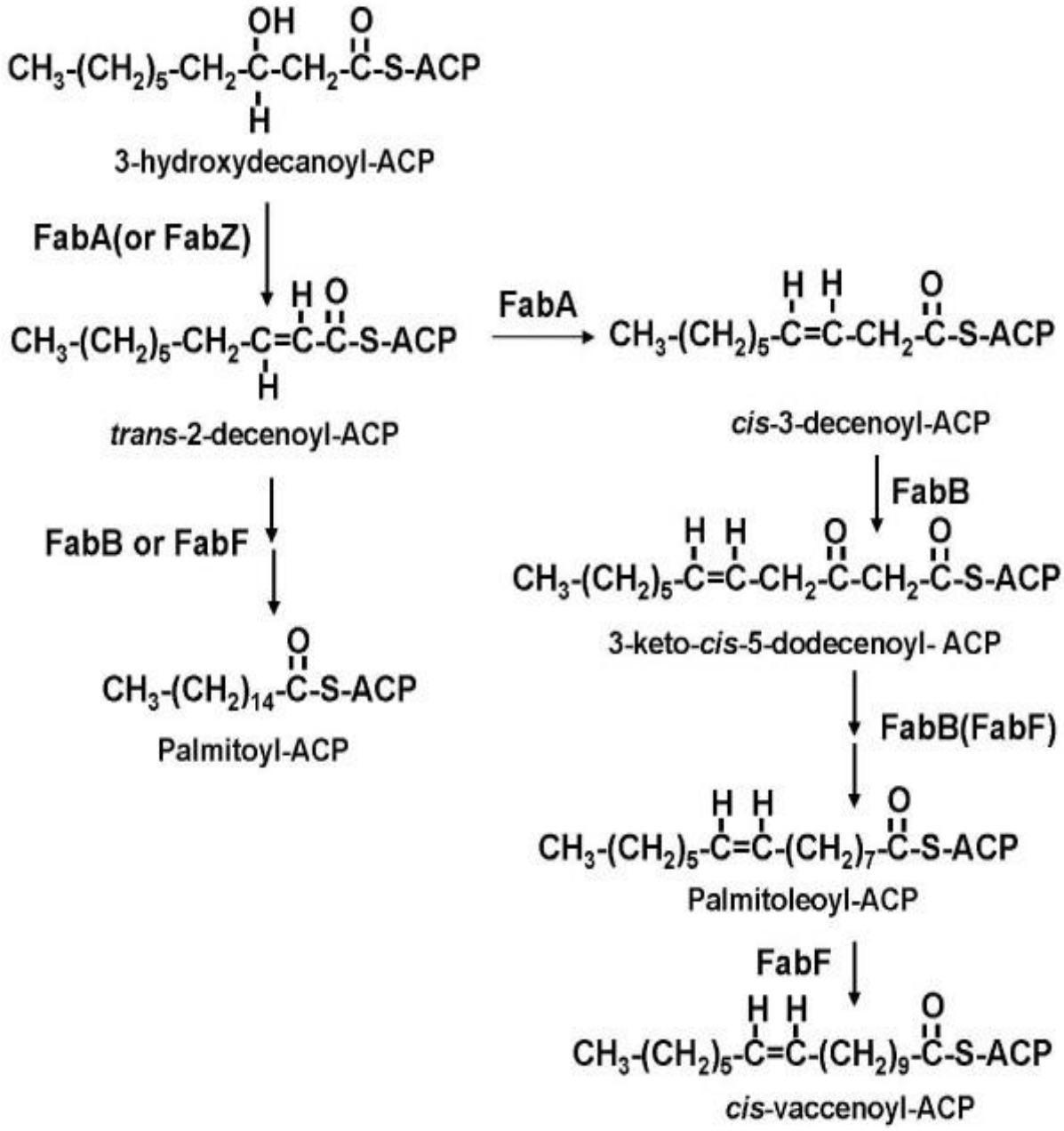


Figure 21–5. Microsomal elongase system for fatty acid chain elongation. NADH is also used by the reductases, but NADPH is preferred.

Biosynthesis of unsaturated fatty acid



THE NUTRITIONAL STATE REGULATES LIPOGENESIS

Excess carbohydrate is stored as fat in many animals in anticipation of periods of caloric deficiency such as starvation, hibernation, etc, and to provide energy for use between meals in animals, including humans, that take their food at spaced intervals. Lipogenesis converts surplus glucose and intermediates such as pyruvate, lactate, and acetyl-CoA to fat, assisting the anabolic phase of this feeding cycle. The nutritional state of the organism is the main factor regulating the rate of lipogenesis. Thus, the rate is high in the well-fed animal whose diet contains a high proportion of carbohydrate. It is depressed under conditions of restricted caloric intake, on a fat diet, or when there is a deficiency of insulin, as in diabetes mellitus. These latter conditions are associated with increased concentrations of plasma free fatty acids, and an inverse relationship has been demonstrated between hepatic lipogenesis and the concentration of serum-free fatty acids. Lipogenesis is increased when sucrose is fed instead of glucose because fructose bypasses the phosphofructokinase control point in glycolysis and floods the lipogenic pathway.

METABOLISM OF CHOLESTEROL

BIOMEDICAL IMPORTANCE

Cholesterol is present in tissues and in plasma either as free cholesterol or as a storage form, combined with a long-chain fatty acid as cholesteryl ester. In plasma, both forms are transported in lipoproteins (Chapter 25). Cholesterol is an amphipathic lipid and as such is an essential structural component of membranes and of

the outer layer of plasma lipoproteins. It is synthesized in many tissues from acetyl-CoA and is the precursor of all other steroids in the body such as corticosteroids, sex hormones, bile acids, and vitamin D. As a typical product of animal metabolism, cholesterol occurs in foods of animal origin such as egg yolk, meat, liver, and

brain. Plasma low-density lipoprotein (LDL) is the vehicle of uptake of cholesterol and cholesteryl ester into

many tissues. Free cholesterol is removed from tissues by plasma high-density lipoprotein (HDL) and transported to the liver, where it is eliminated from the body either unchanged or after conversion to bile acids in the process known as **reverse cholesterol transport.** Cholesterol is a major constituent of **gallstones.** However, its chief role in pathologic processes is as a factor

in the genesis of **atherosclerosis** of vital arteries, causing cerebrovascular, coronary, and peripheral vascular disease.

CHOLESTEROL IS DERIVED ABOUT EQUALLY FROM THE DIET & FROM BIOSYNTHESIS

A little more than half the cholesterol of the body arises by synthesis (about 700 mg/d), and the remainder is provided by the average diet. The liver and intestine account for approximately 10% each of total synthesis in

humans. Virtually all tissues containing nucleated cells are capable of cholesterol synthesis, which occurs in the endoplasmic reticulum and the cytosol.

Acetyl-CoA Is the Source of All Carbon Atoms in Cholesterol

The biosynthesis of cholesterol may be divided into five steps:

(1) Synthesis of mevalonate occurs from acetyl- CoA.

(2) Isoprenoid units are formed from mevalonate by loss of CO2.

(3) Six isoprenoid units condense to form squalene.

(4) Squalene cyclizes to give rise to the parent steroid, lanosterol.

(5) Cholesterol is formed from lanosterol.

Step 1—Biosynthesis of Mevalonate: HMG-CoA (3-hydroxy-3-methylglutaryl-CoA) is formed by the reactions used in mitochondria to synthesize ketone bodies (Figure 22–7). However, since cholesterol synthesis is extramitochondrial, the two pathways are distinct. Initially, two molecules of acetyl-CoA condense to form acetoacetyl-CoA catalyzed by cytosolic **thiolase.** Acetoacetyl-CoA condenses with a further molecule of acetyl-CoA catalyzed by **HMG-CoA synthase** to form HMG-CoA, which is reduced to **mevalonate** by NADPH catalyzed by **HMG-CoA reductase.** This is the principal regulatory step in the pathway of cholesterol synthesis and is the site of action of the most effective class of cholesterol-lowering drugs, the HMG-CoA reductase inhibitors (statins) (Figure 26–1).

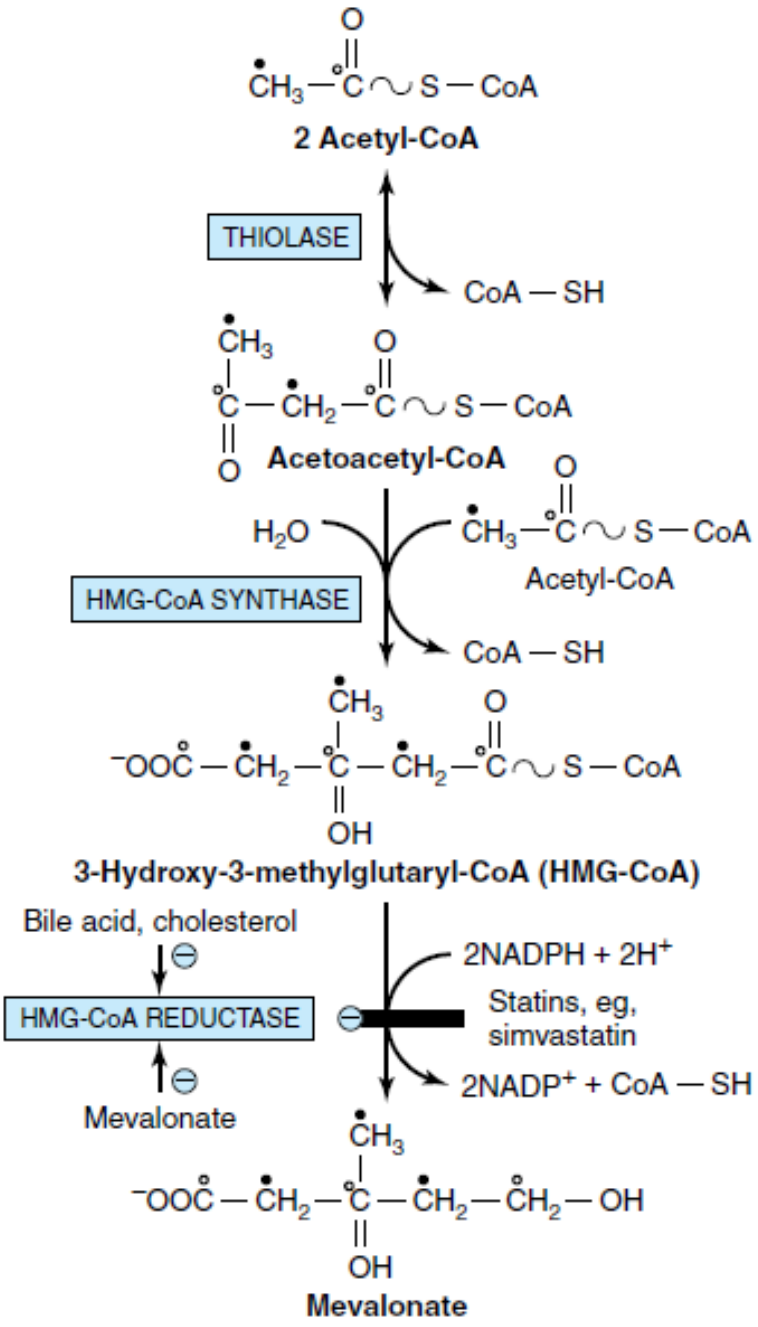


Figure 26–1. Biosynthesis of mevalonate. HMG-CoA reductase is inhibited by atorvastatin, pravastatin, and simvastatin. The open and solid circles indicate the fate of each of the carbons in the acetyl moiety of acetyl-CoA.

Step 2—Formation of Isoprenoid Units: Mevalonate is phosphorylated sequentially by ATP by three kinases, and after decarboxylation (Figure 26–2) the active isoprenoid unit, **isopentenyl diphosphate,** is formed.

Step 3—Six Isoprenoid Units Form Squalene:

Isopentenyl diphosphate is isomerized by a shift of the double bond to form **dimethylallyl diphosphate,** then condensed with another molecule of isopentenyl diphosphate to form the ten-carbon intermediate **geranyl diphosphate** (Figure 26–2). A further condensation with isopentenyl diphosphate forms **farnesyl diphosphate.** Two molecules of farnesyl diphosphate condense at the diphosphate end to form **squalene.** Initially, inorganic pyrophosphate is eliminated, forming presqualene diphosphate, which is then reduced by NADPH with elimination of a further inorganic pyrophosphate molecule.

Step 4—Formation of Lanosterol: Squalene can fold into a structure that closely resembles the steroid nucleus (Figure 26–3). Before ring closure occurs, squalene is converted to squalene 2,3-epoxide by a mixed. function oxidase in the endoplasmic reticulum, **squalene epoxidase.** The methyl group on C14 is transferred to C13 and that on C8 to C14 as cyclization occurs, catalysed by **oxidosqualene:lanosterol cyclase.**

Step 5—Formation of Cholesterol: The formation of cholesterol from **lanosterol** takes place in the

membranes of the endoplasmic reticulum and involves changes in the steroid nucleus and side chain (Figure 26–3). The methyl groups on C14 and C4 are removed to form 14-desmethyl lanosterol and then zymosterol.

The double bond at C8–C9 is subsequently moved to C5–C6 in two steps, forming **desmosterol.** Finally, the double bond of the side chain is reduced, producing cholesterol. The exact order in which the steps described actually take place is not known with certainty.

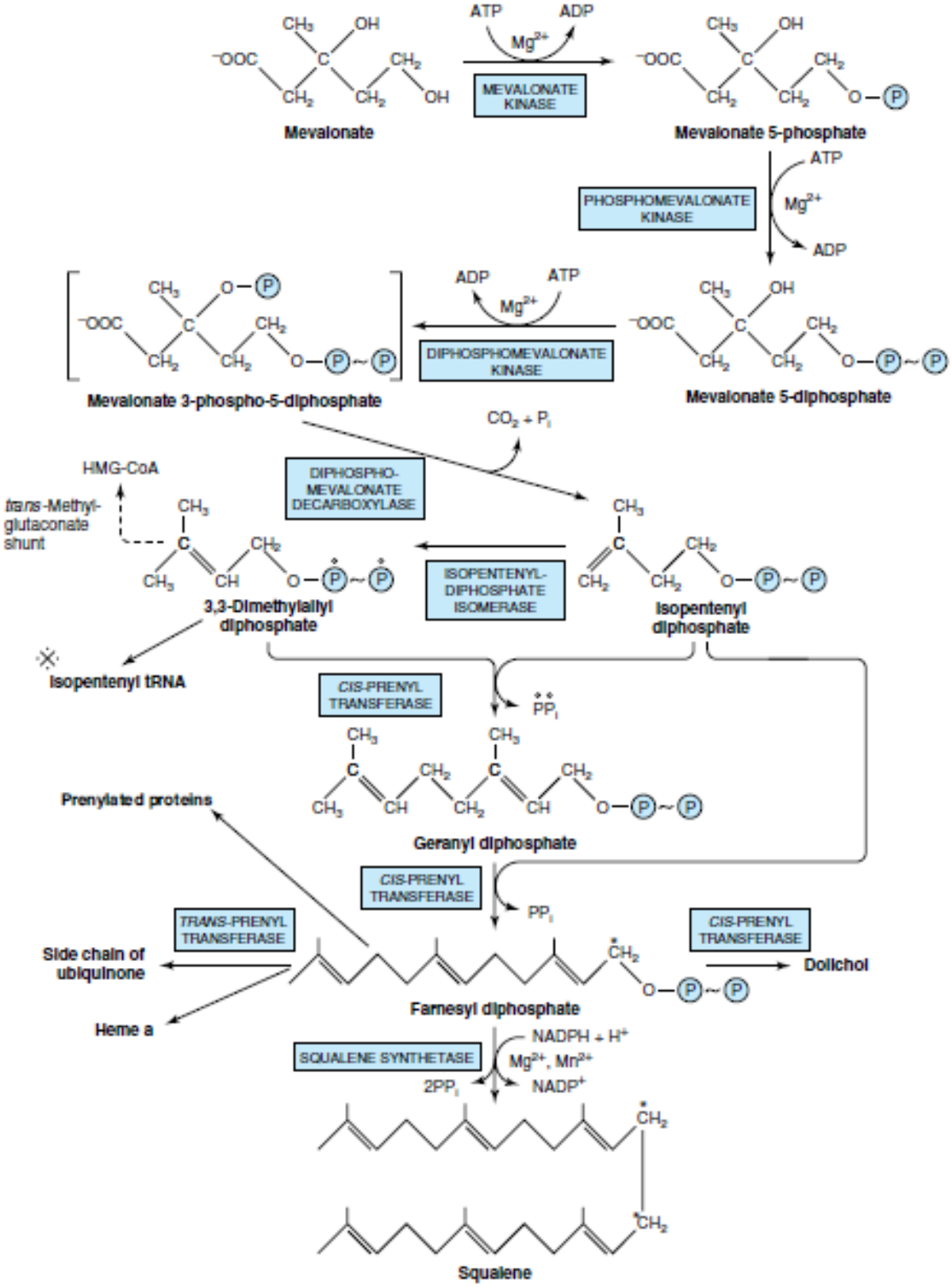

Mevalonate
ATP
ADP
Mg2+
MEVALONATE KINASE
Mevalonate 5-phosphate
PHOSPHOMEVALONATE KINASE
Mevalonate 5-diphosphate
DIPHOSPHOMEVALONATE KINASE
Mevalonate 3-phospho-5-diphosphate
CO2 + Pi
DIPHOSPHO-MEVALONATE DECARBOXYLASE
HMG-CoA
trans-Methyl-glutaconate shunt
3,3-Dimethylallyl diphosphate
ISOPENTENYL-DIPHOSPHATE ISOMERASE
Isopentenyl diphosphate
Isopentenyl tRNA
CIS-PRENYL TRANSFERASE
PPi
Geranyl diphosphate
Prenylated proteins
Farnesyl diphosphate
TRANS-PRENYL TRANSFERASE
Side chain of ubiquinone
Heme a
Dolichol
NADPH + H+
SQUALENE SYNTHETASE
Mg2+, Mn2+
2PPi
NADP+
Squalene

Figure 26–2. Biosynthesis of squalene, ubiquinone, dolichol, and other polyisoprene derivatives. (HMG, 3-hydroxy-3-methylglutaryl; ※, cytokinin.) A farnesyl residue is present in heme a of cytochrome oxidase. The carbon marked with asterisk becomes C_{11} or C_{12} in squalene. Squalene synthetase is a microsomal enzyme; all other enzymes indicated are soluble cytosolic proteins, and some are found in peroxisomes.

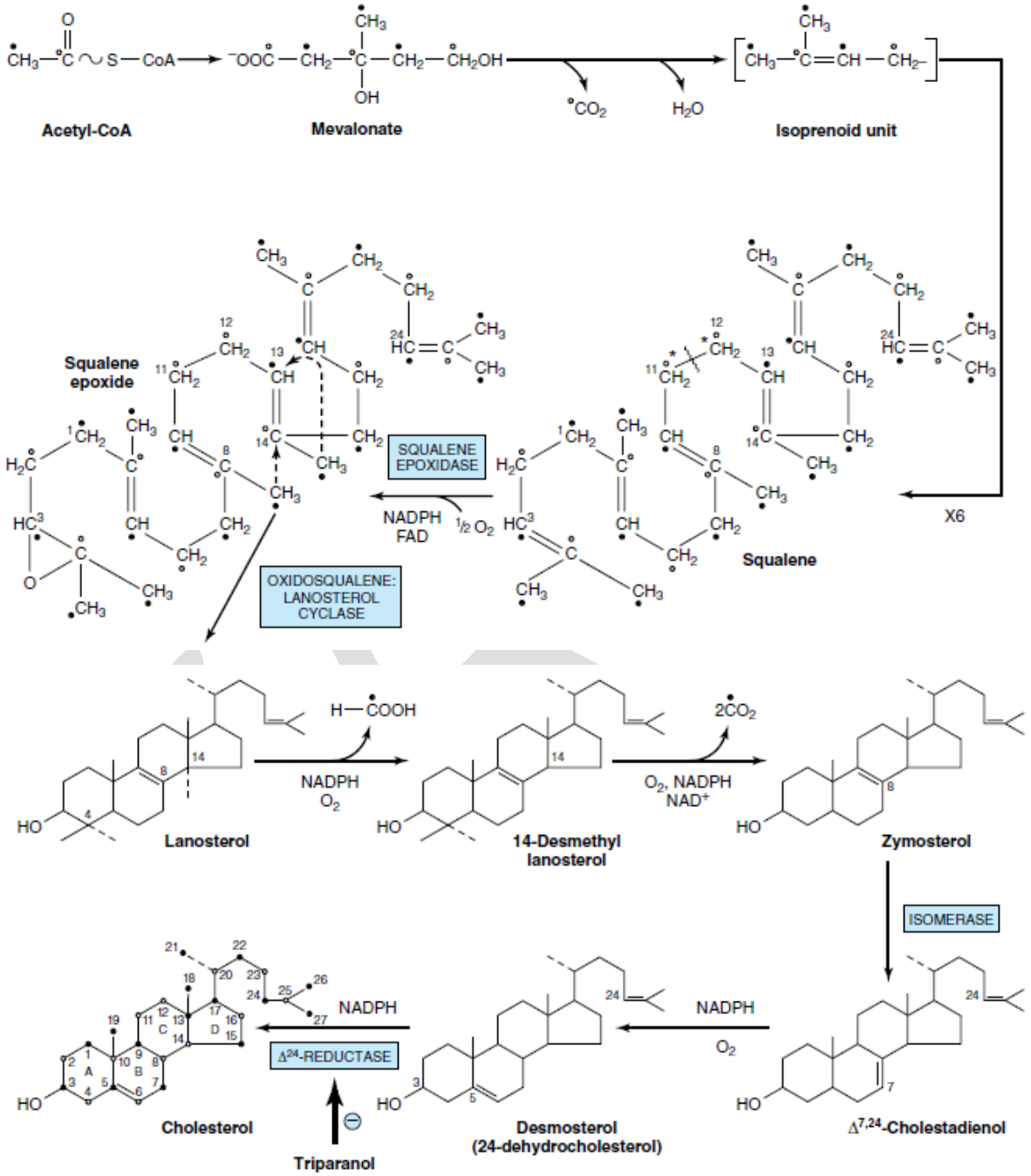


Figure 26–3. Biosynthesis of cholesterol. The numbered positions are those of the steroid nucleus and the open and solid circles indicate the fate of each of the carbons in the acetyl moiety of acetyl-CoA. Asterisks: Refer to labeling of squalene in Figure 26–2.

CHOLESTEROL SYNTHESIS IS CONTROLLED BY REGULATION OF HMG-CoA REDUCTASE

Regulation of cholesterol synthesis is exerted near the beginning of the pathway, at the HMG-CoA reductase step. The reduced synthesis of cholesterol in starving animals is accompanied by a decrease in the activity of the enzyme. However, it is only hepatic synthesis that is inhibited by dietary cholesterol. HMG-CoA reductase in liver is inhibited by mevalonate, the immediate product of the pathway, and by cholesterol, the main product. Cholesterol (or a metabolite, eg, oxygenated sterol) represses transcription of the HMG-CoA reductase gene and is also believed to influence translation. A **diurnal variation** occurs in both cholesterol synthesis and reductase activity. In addition to these mechanisms regulating the rate of protein synthesis, the enzyme activity is also modulated more rapidly by posttranslational modification (Figure 26–4). Insulin or thyroid hormone increases HMG-CoA reductase activity, whereas glucagon or glucocorticoids decrease it. Activity is reversibly modified by phosphorylation-dephosphorylation mechanisms, some of which may be cAMP-dependent and therefore immediately responsive to glucagon. Attempts to lower plasma cholesterol in humans by reducing the amount of cholesterol in the diet produce variable results. Generally, a decrease of 100 mg in dietary cholesterol causes a decrease of approximately 0.13 mmol/L of serum.

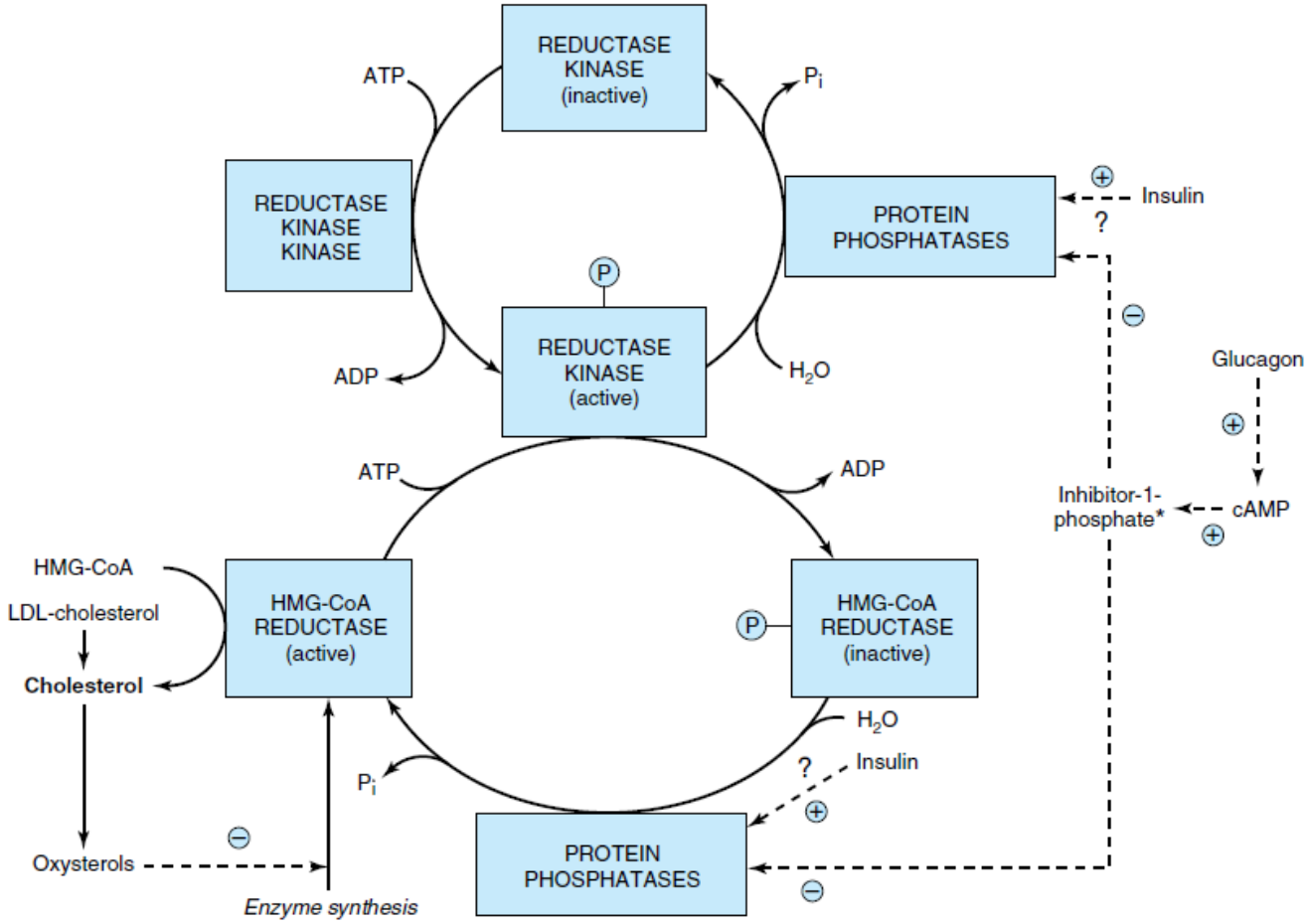


Figure 26–4. Possible mechanisms in the regulation of cholesterol synthesis by HMG-CoA reductase. Insulin has a dominant role compared with glucagon. Asterisk: See Figure 18–6.

CLINICAL ASPECTS

The Serum Cholesterol Is Correlated With the Incidence of Atherosclerosis & Coronary Heart Disease

While cholesterol is believed to be chiefly concerned in the relationship, other serum lipids such as triacylglycerols may also play a role. Atherosclerosis is characterized by the deposition of cholesterol and cholesteryl ester from the plasma lipoproteins into the artery wall. Diseases in which prolonged elevated levels of VLDL, IDL, chylomicron remnants, or LDL occur in the blood (eg, diabetes mellitus, lipid nephrosis, hypothyroidism, and other conditions of hyperlipidemia) are often accompanied by premature or more severe atherosclerosis. There is also an inverse relationship between HDL (HDL2) concentrations and coronary heart disease,

and some consider that the most predictive relationship is the **LDL:HDL cholesterol ratio.** This is consistent with the function of HDL in reverse cholesterol transport. Susceptibility to atherosclerosis varies widely among species, and humans are one of the few in which the disease can be induced by diets high in cholesterol.

Primary Disorders of the Plasma Lipoproteins (Dyslipoproteinemias) Are Inherited

Inherited defects in lipoprotein metabolism lead to the primary condition of either **hypo-** or **hyperlipoproteinemia** (Table 26–1). In addition, diseases such as diabetes mellitus, hypothyroidism, kidney disease (nephrotic syndrome), and atherosclerosis are associated with secondary abnormal lipoprotein patterns that are very similar to one or another of the primary inherited conditions. Virtually all of the primary conditions are due to a defect at a stage in lipoprotein formation, transport, or destruction (see Figures 25–4, 26–5, and 26–6). Not all of the abnormalities are harmful.

Triglycerides

Triglycerides or Triacylglycerol (TG) synthesis mostly occurs in Iiver and adipose tissue, and to a lesser extent in other tissues. Fatty acids and glycerol must be activated prior to the synthesis of triacylglycerols. Conversion of fatty acids to acyl CoA by thiokinase is already described.

Synthesis of glycerol 3 phosphate Two mechanisms are involved for the synthesis of glycerol 3-phosphate

1. In the liver, glycerol is activated by glycerol kinase. This enzyme is absent in adipose tissue.

2. In both liver and adipose tissue, glucose serves as a precursor for glycerol 3-phosphate. Dihydroxyacetone phosphate (DHAP) produced in glycolysis is reduced by glycerol 3-phosphate dehydrogenase to glycerol 3-phosphate.

Addition of acyl groups to form TG

Glycerol 3-phosphate acyltransferase catalyses the transfer of an acyl group to produce lysophosphatidic acid. DHAP can also accept acyl group, ultimately resulting in the formation of lysophosphatidic acid. Another acyl group is added to lysophosphatidic acid to form phosphatidic acid (1,2-diacyl glycerol phosphate). The enzyme phosphatase cleaves off phosphate of phosphatidic acid to produce diacylglycerol. Incorporation of another acyl group

finally results in synthesis of triacylglycerol (Fig.l4.1A. The three fatty acids found in triacylglycerol are not of the same type. A saturated fatty acid is usually present on carbon 1, an unsaturated fatty acid is found on carbon 2, and carbon 3 may have either.

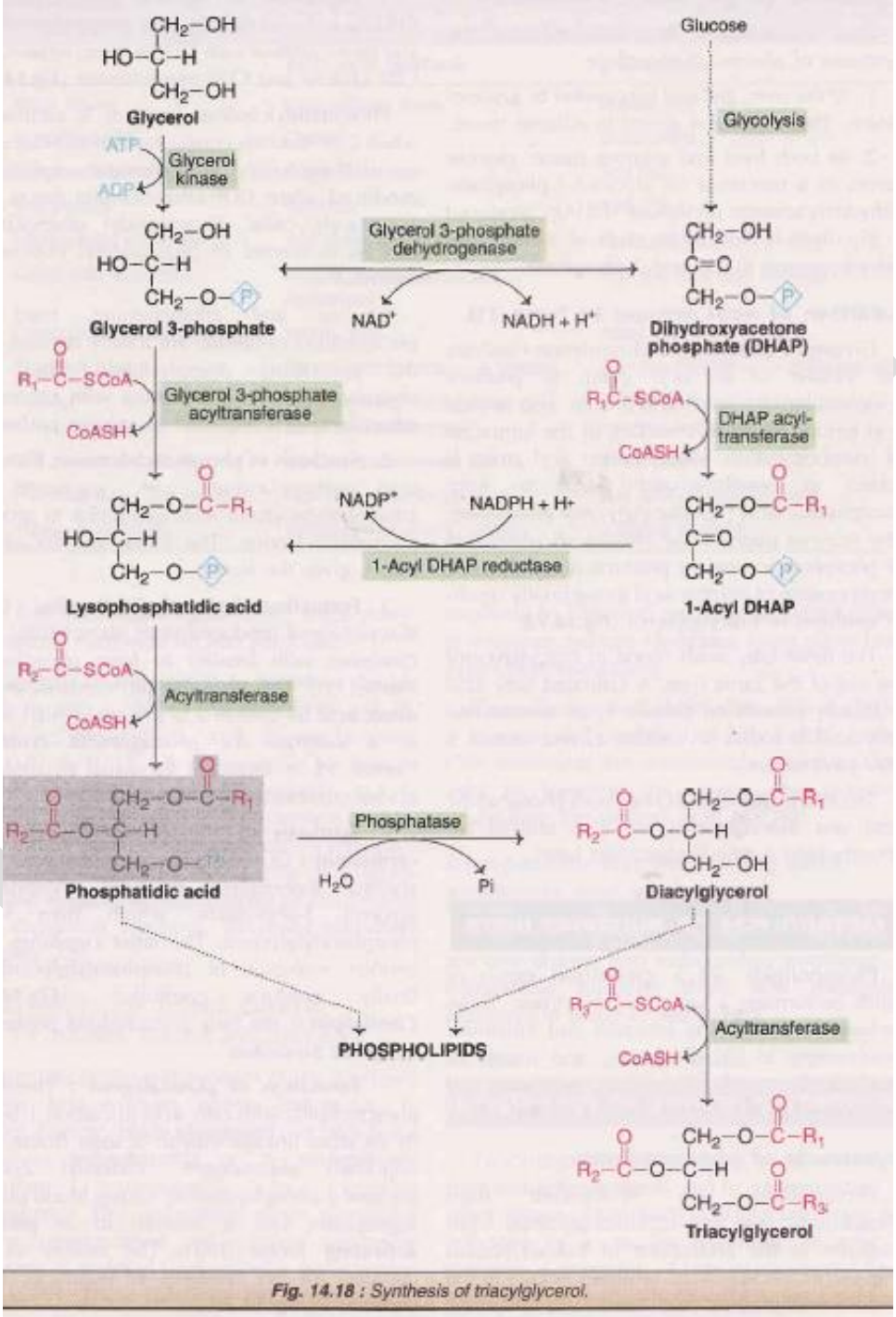


Fig. 14.18 : Synthesis of triacylglycerol.

Ketone Bodies

Under metabolic conditions associated with a high rate of fatty acid oxidation, the liver produces considerable quantities of **acetoacetate** and **D(_)-3-hydroxybutyrate** (β-hydroxybutyrate). Acetoacetate continually undergoes spontaneous decarboxylation to yield **acetone.** These three substances are collectively known as the **ketone bodies** (also called acetone bodies or [incorrectly*] "ketones") (Figure 22–5). Acetoacetate and 3-hydroxybutyrate are interconverted by the mitochondrial enzyme **D(_)-3-hydroxybutyrate dehydrogenase;** the equilibrium is controlled by the mitochondrial [NAD+]/ [NADH] ratio, ie, the **redox state.** The concentration of total ketone bodies in the blood of well-fed mammals does not normally exceed 0.2 mmol/L except in ruminants, where 3-hydroxybutyrate is formed continuously from butyric acid (a product of ruminal fermentation) in the rumen wall. In vivo, the liver appears to be the only organ in nonruminants to add significant quantities of ketone bodies to the blood. Extrahepatic tissues utilize them as respiratory substrates. The net flow of ketone bodies from the liver to the extrahepatic tissues results from active hepatic synthesis coupled with very low utilization. The reverse situation occurs in extrahepatic tissues (Figure 22–6).

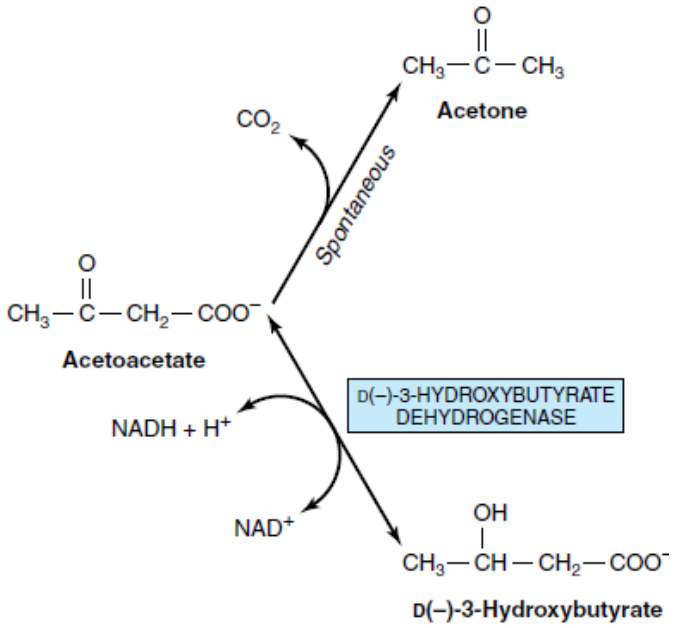
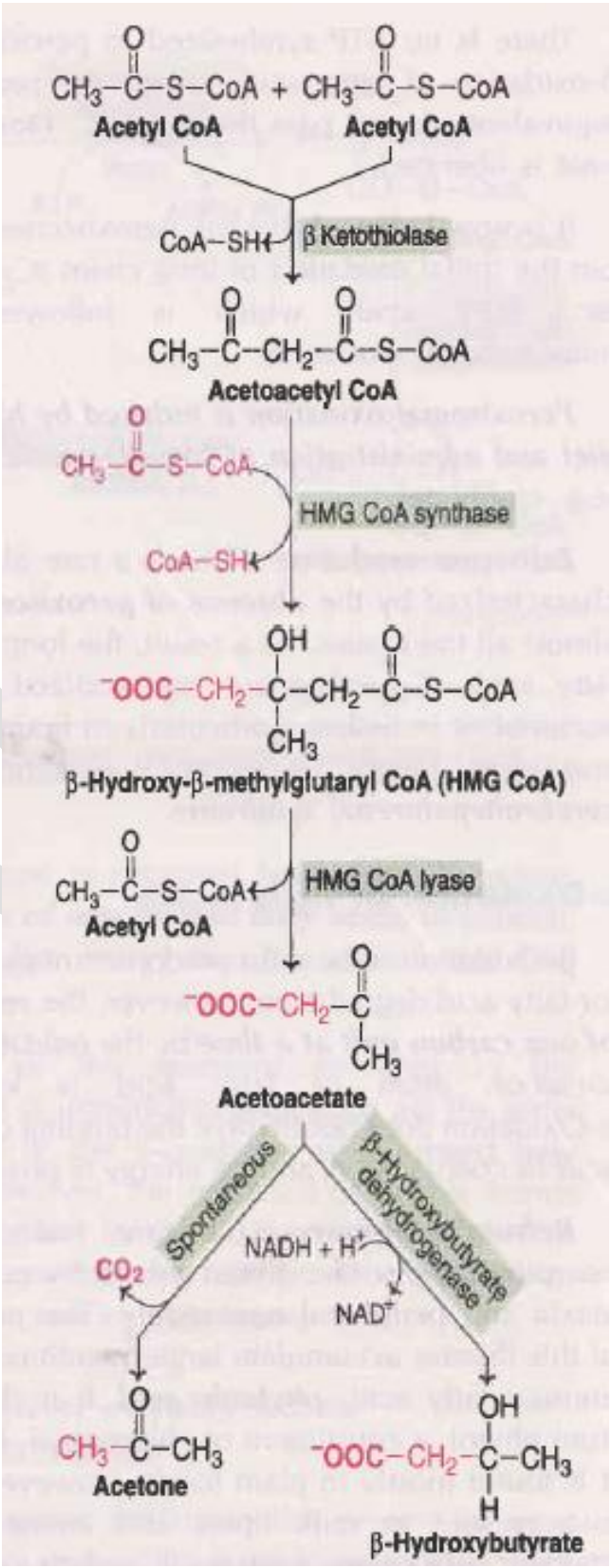


Figure 22–5. Interrelationships of the ketone bodies. D(–)-3-hydroxybutyrate dehydrogenase is a mitochondrial enzyme.

Figure. Synthesis of ketone bodies (Ketogenesis)



Utilization of ketone bodies

The ketone bodies, being water-soluble, are easily transported from the liver to various tissues. The two ketone bodies-acetoacetate and p-hydroxybutyrate serve as important sources of energy for the peripheral tissues such

as skeletal muscle, cardiac muscle/ renal cortex etc. The tissues which lack mitochondria (eg. erythrocytes) however, cannot utilize ketone bodies. The production of ketone bodies and their utilization become more significant when glucose is in short supply to the tissues, as observed in starvation, and diabetes mellitus.

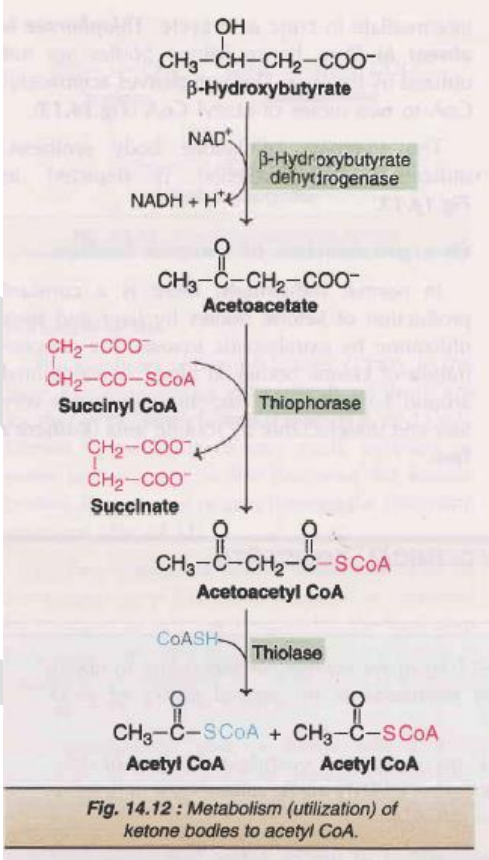


Fig. 14.12 : Metabolism (utilization) of ketone bodies to acetyl CoA.

During prolonged starvation, ketone bodies are the major fuel source for the brain and other parts of central nervous system. It should be noted that the ability of the brain to utilize fatty acids for energy is very limited. The ketone bodies can meet 50-70% of the brain's energy needs. This is an adaptation for the survival of the organism during the periods of food deprivation.

Reactions of ketone bodies: B-Hydroxybutyrate is first converted to acetoacetate (reversal of synthesis) and metabolized. Acetoacetate is activated to acetoacetyl CoA by a mitochondrial enzyme thiophorase (succinyl CoA acetoacetate CoA transferase. The coenzyme A is donated by succinyl CoA, an intermediate in citric acid cycle. Thiophorase is absent in liver, hence ketone bodies are not

utilized by the liver. Thiolase cleaves acetoacetyl CoA to two moles of acetyl CoA.

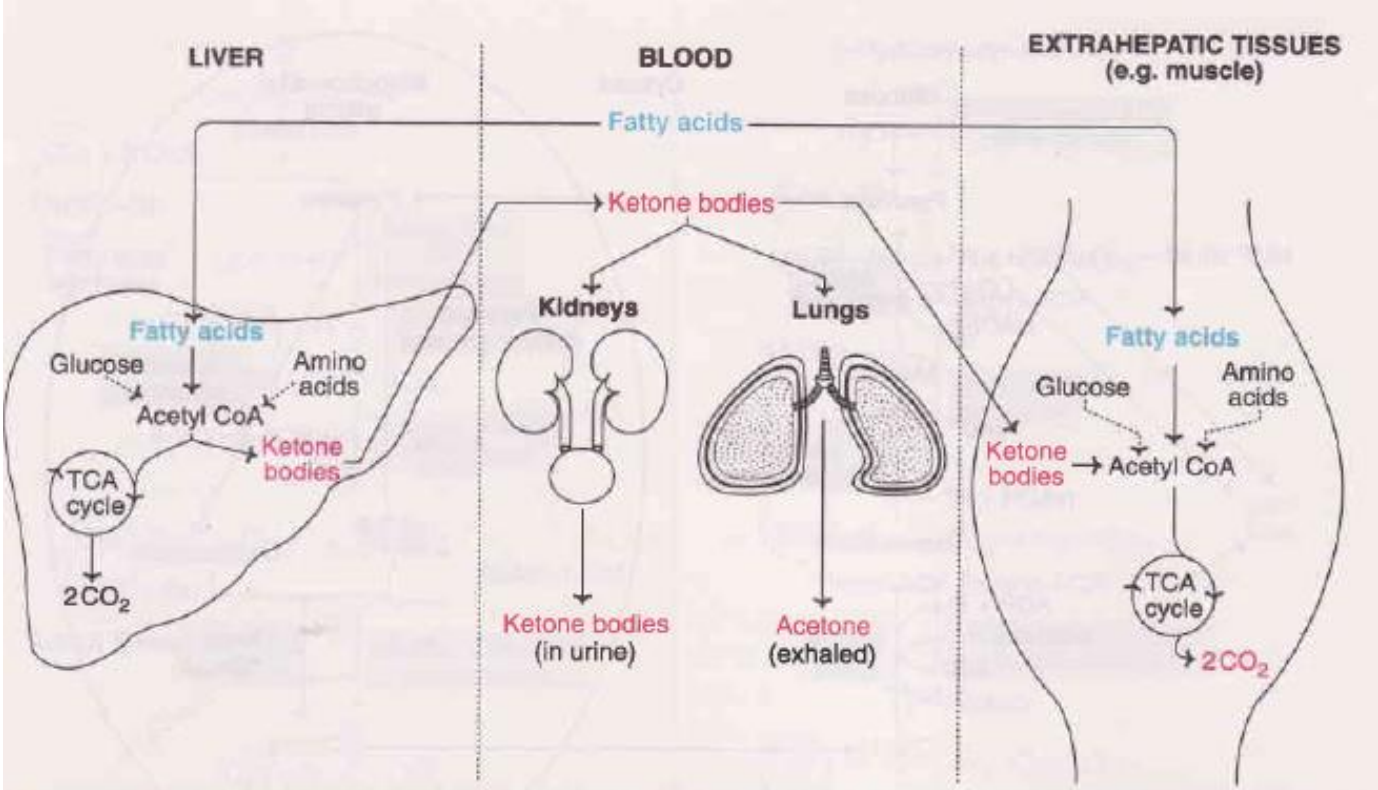


Fig. 14.13 : Summary of ketone body synthesis, utilization and excretion.

POSSIBLE QUESTIONS
UNIT I

PART A (1 mark)
Question number 1-20 (From given 300 all possible MCQs)

PART B (2 Marks)

1. Write a short note on saturated fatty acids

2. Give a brief note unsaturated fatty acid

3. Write a brief note on ketone bodies
4. Give notes on the importance of cholesterol in biological system
5. Write about α-oxidation of fatty acids
6. Write a short note on ω-oxidation of fatty acids

PART C (6 Marks)

7. Elaborate about β-oxidation of fatty acid pathway
8. Explain in detail about biosynthesis of unsaturated fatty acids
9. Explain in detail about biosynthesis of saturated fatty acids
10. Give a detail account on the metabolism of cholesterol
11. Discuss in detail about triglycerides metabolism
12. Write elaborately about Ketone bodies and ketogenesis and their metabolism

UNIT III								
S. No	**QUESTION**	**Option 1**	**Option 2**	**Option 3**	**Option 4**	**Option 5**	**Option 6**	**Answer**
1	β-oxidation of long chain fatty acids occours primarily in_____	Cytosol	Peroxisomes	Mitochondria	Golgi apparatus			**Mitochondria**
2	β-oxidation of palmitic acid produces a net synthesis of how many ATP molecules?	109	129	24	38			**129**
3	α-Oxidation of fatty acids occurs mainly in_____	Liver	Brain	Muscles	Adipose tissue			**Brain**
4	The enzyme involved ω-oxidation are located in	golgi complex	Cytoplasm	Endoplasmic recticulum	Mitochondria			**Endoplasmic recticulum**
5	The rate of fatty acid oxidation is increased by _____	Phospholipids	Glycolipids	Amino lipids	Spingolipids			**Phospholipids**
6	Which enzyme is involved in lipid digesion?	Elastase	lactase	Lipase	Lactate dehydrogenase			**Lipase**
7	Digesion of triglycerides requires	Bile salts	Bile pigments	Intrinsic factor	Bile acids			**Bile salts**
8	Absorption of fats occurs mainly in	Stomach	Duodenum	Jejunum	Ileum			**Jejunum**
9	Fatty acids are degraded mainly by	ω-oxidation	α- oxidation	β-oxidation	HMP shunt			**β-oxidation**
10	Majority of the absorbed fat appears in the form of	VLDL	LDL	HDL	Chylomicrones			**Chylomicrones**
11	The end product of fatty acid synthesis in mammals is	Arachidonic acid	Linoleic acid	Stearic acid	Palmitic acid			**Stearic acid**
12	The key regulatory enzyme of fatty acid synthesis is	Acyl coA synthetase	Acetyl coA carboxylase	Keto acyl synthase	Thioesterase			**Acetyl coA carboxylase**
13	NADPH required for fatty acid synthesis can be generated from	HMP shunt	Glycolysis	TCA cycle	Urea cycle			**HMP shunt**
14	Which of the following inhibits the acetylCoA carboxylase a rate limiting enzymes of carbohydrate metabolism?	Citrate	ATP	Malonyl CoA	Acyl CoA			**Acyl CoA**
15	Malonyl-CoA A is a direct inhibitor of which	Carnitine Acyl Transferase –I	Carnitine Acyl Transferase –II	Thiokinase	Acyl co A synthetase			**Carnitine Acyl Transferase –I**
16	enzyme of fatty acid oxidation_____							
17	Cholestrol is the precursor of	Steroid hormones	Vitamin A	Urea	Folic acid			**Steroid hormones**
18	The committed step in cholesterol biosynthesis	Formation of squaline	Formation of HMG CoA	Formation of mevalonic acid from HMG CoA	Cyclisation of squaline to lanodterol			**Formation of mevalonic acid from HMG CoA**
19	The principle building block of fatty acid is	Succynyl CoA	Acetyl CoA	Propionyl CoA	Acetoacetyl CoA			**Acetyl CoA**
20	Biosynthesis of fatty acid requires which vitamin?	Riboflavin	Pyridoxine	Thiamin	Pantothenic acid			**Pantothenic acid**
21	ACP is involved in the synthesis of	Phospholipids	Fatty acids	Glycogen	Triglycerides			**Fatty acids**
22	The main catabolic end product of cholesterol is _____	Acetyl CoA	Propionyl CoA	Coprosterol	Bile acids			**Bile acids**
23	The fattyacid synthase complex comprises two monomers, each containing_____	2 enzymes	5 enzymes	7 enzymes	10 enzymes			**7 enzymes**
24								
25	Bile acid are derived from	Cholesterol	Amino acids	Fatty acids	Bilirubin			**Cholesterol**
26	The major storage form of lipids is_____	Esterified cholesterol	Glycerophospholipids	Triglycerides	Sphingolipids			**Triglycerides**
27	The principal precursors of glycerophospholipids are	Phospholipids	Spingolipids	Diacylglycerols	Spingomyelins			**Diacylglycerols**
28	The important lipid involved in cell adhesion and cell recognition is	Phospholipids	Cholesterol	Glycospingolipids	Ceramide			**Glycospingolipids**
29	Acyl Carrier Protein contains the vitamin_____	Biotin	Lipoic acid	Pantothenic acid	Folic acid			**Pantothenic acid**
30	The starting material for the process of ketogenesis is_____	Acetyl CoA	Oxaloacetate	Pyruvate	Citrate			**Acetyl CoA**
31	Which among the following is the most complex sphingolipid_____	Cerebroside	Gangleoside	Globoside	Ceramide			**Gangleoside**
32	How many double bonds occur in Arachidonic acid_____	1	2	3	4			**4**
33	Which of the following is essential fatty acid_____	Linolenic acid	Arachidonic acid	Oleic acid	Palmitic acid			**Linolenic acid**
34	A genetic disorder caused by the accumulation of sphingomyelin in brain is called_____	Tay-Sach syndrome	Gout	Niemann-Pick Disease	Gauche's disease			**Niemann-Pick Disease**
35	Lipid molecule involved in the bio-signaling pathway that include membrane turnover and exocytosis is _____.	Phosphatidylinositol	Phosphatidyl glycerol	Myoinositol	Phosphatidyl glycerol and Myoinositol			**Phosphatidylinositol**
36	Most abundant membrane lipid in the biosphere is_____	Phospholipid	Galactolipid	Sphingolipid	Ether lipid			**Galactolipid**
37	What is the molecular formula of cholesterol?	$C_{27}H_{45}OH$	$C_{28}H_{47}OH$	$C_{29}H_{47}OH$	$C_{23}H_{41}OH$			**$C_{27}H_{45}OH$**
38	Enzymes for beta oxidation of fatty acids are located in_____	Mitochondria	Mitochondria and cytoplasm	Mitochondria and Golgi	Mitochondria and peroxisome			**Mitochondria**
39	Cerebroside may also classified as	Phospholipid	Sphingolipid	Aminolipid	Glycolipid			**Sphingolipid**
40	Glyco-sphingolipids are a combination of_____	Glycerol with two galactose residues	Ceramide with one or more sugar residues	Sphingosine with galactose and ceramide	Sphingosine with glucose			**Ceramide with one or more sugar residues**
41	Spingomyelins contain a complex amino alcohol named as	Serine	Lysolecithin	Spingosine	Glycol			**Spingosine**
42	The key regulatory enzyme of cholesterol	HMG- Co A synthase	HMG Co A lyase	HMG Co A reductase	Mevalonate kinase			**HMG Co A reductase**
43	synthesis is_____							
44	The enzyme 'Thiolase' catalyzes the conversion of_____	2 Acetyl co A to Acetoacetyl co A	Acetyl co A to Malonyl co A	Fatty acid to Fatty Acyl co A	Succinyl co A to succinate			**2 Acetyl co A to Acetoacetyl co A**
45	The enzyme involved in mammalian signal transduction is	Phospholipase A	Phospholipase B	Phospholipase C	Phospholipase D			**Phospholipase D**
46	In alpha oxidation which of the following products is released ?	Co A	CO_2	H_2O	Acetyl co A			**CO_2**
47	Which of the following is a break down product of odd chain fatty acids?	Acetyl co A only	Acetyl co A and Butyryl co A	Acetyl co A and Propionyl co A	Malonyl co A			**Acetyl co A and Propionyl co A**
48	All the 27 carbon atoms of cholesterol are derived from	Acetyl co A	Acetoacetyl co A	Propionyl co A	Succinyl co A			**Acetyl co A**
49	NADPH is synthesized by the action of which of the following enzymes?	Glucose-6-P dehydrogenase	Pyruvate dehydrogenase	Acetyl co A carboxylase	Lipoprotein lipase			**Glucose-6-P dehydrogenase**
50	How many carbons are removed from fatty acyl co A in one turn of β- oxidation spiral ?	1	2	3	4			**2**
51	What is the role of Thiolase in the β- oxidation of fatty acids?	Cleaves of Co A	Cleaves the bond between α- and β-carbons	Adds H_2O across the double bond	Generates NADH			**Cleaves the bond between α- and β- carbons**
52	The key enzyme for the utilization of ketone bodies is_____	Thiolase	Thiophorase	Thiokinase	Thioesterase			**Thiophorase**
53	High content of triglycerides are seen in_____	LDL	HDL	VLDL	Chylomicrones			**Chylomicrones**
54	Gangliosides are glycolipids occurring in_____	Liver	Brain	Kidney	Muscle			**Brain**
55	The prostaglandins are synthesized from _____	Aracadonic acid	Oleic acid	Linoleic acid	Linolenic acid			**Aracadonic acid**
56	Prostaglandins are liberated in the circulation by the stimulation of_____	Anterior pitutary glands	Posterior pitutary glands	Adrenal gland	Thyroid gland			**Adrenal gland**
57	The synthesis of prostaglandins is inhibited by_____	Aspirin	Arsenite	Fluoride	Cyanide			**Aspirin**
58	HDL is synthesized and secreted from_____	Pancrease	Liver	Kidney	Muscle			**Liver**
59	Fatty liver caused by_____	CH_3Cl	CCL_4	$MgSO_4$	CH_3COOH			**CCL_4**
60	Ketosis generally occurs in_____	Nephritis	Oedema	Infective hepatic disease	Coronary thrombosis			**Infective hepatic disease**

UNIT-IV

SYLLABUS

Protein metabolism: Ketogenic and Glucogenic amino acids. Degradation of proteins: Deamination, Transamination and Decarboxylation, Urea cycle.

The pathways of amino acid catabolism, taken together, normally account for only 10% to 15% of the human body's energy production; these pathways are not nearly as active as glycolysis and fatty acid oxidation. Flux through these catabolic routes also varies greatly, depending on the balance between requirements for biosynthetic processes and the availability of a particular amino acid. The 20 catabolic pathways converge to form only six major products, all of which enter the citric acid cycle (Fig. 18–15). From here the carbon skeletons are diverted to gluconeogenesis or ketogenesis or are completely oxidized to CO2 and H2O. All or part of the carbon skeletons of seven amino acids are ultimately broken down to acetyl-CoA. Five amino acids are converted to _-ketoglutarate, four to succinyl-CoA, two to fumarate, and two to oxaloacetate. Parts or all of six amino acids are converted to pyruvate, which can be converted to either acetyl-CoA or oxaloacetate. We later summarize the individual pathways for the 20 amino acids in flow diagrams, each leading to a specific point of entry into the citric acid cycle. In these diagrams the carbon atoms that enter the citric acid cycle are shown in color. Note that some amino acids appear more than once, reflecting different fates for different parts of their carbon skeletons. Rather than examining every step of every pathway in amino acid catabolism, we single out for special discussion some enzymatic reactions that are particularly noteworthy for their mechanisms or their medical significance.

KETOGENIC AND GLUCOGENIC AMINO ACIDS

Some Amino Acids Are Converted to Glucose, Others to Ketone Bodies

The seven amino acids that are degraded entirely or in part to acetoacetyl-CoA and/or acetyl-CoA—phenylalanine, tyrosine, isoleucine, leucine, tryptophan, threonine, and lysine—can yield ketone bodies in the liver, where acetoacetyl-CoA is converted to acetoacetate and then to acetone and _-hydroxybutyrate (see Fig. 17–18). These are the **ketogenic** amino acids (Fig. 18–

15). Their ability to form ketone bodies is particularly evident in uncontrolled diabetes mellitus, in which the liver produces large amounts of ketone bodies from both fatty acids and the ketogenic amino acids. The amino acids that are degraded to pyruvate, _- ketoglutarate, succinyl-CoA, fumarate, and/or oxaloacetate can be converted to glucose and glycogen by pathways described in Chapters 14 and 15. They are the **glucogenic** amino acids. The division between ketogenic and glucogenic amino acids is not sharp; five amino acids—tryptophan, phenylalanine, tyrosine, threonine, and isoleucine—are both ketogenic and glucogenic. Catabolism of amino acids is particularly critical to the survival of animals with high-protein diets or during starvation. Leucine is an exclusively ketogenic amino acid that is very common in proteins. Its degradation makes a substantial contribution to ketosis under starvation conditions.

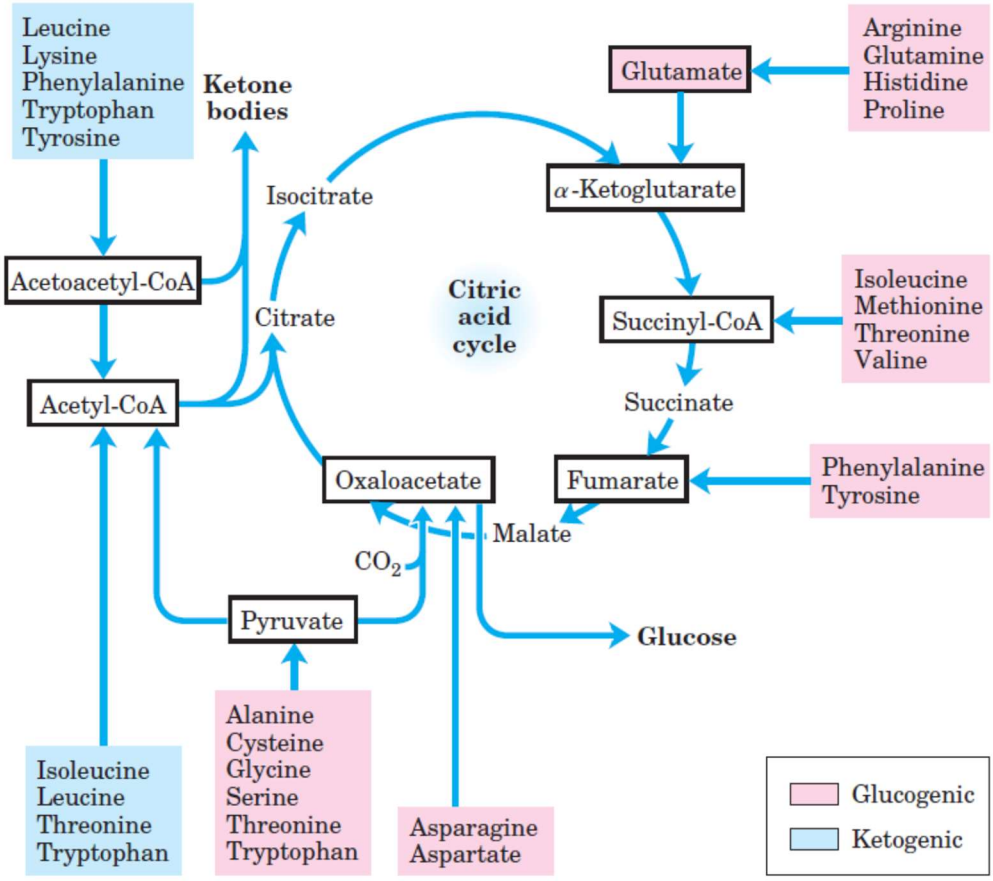


FIGURE 18–15 Summary of amino acid catabolism. Amino acids are grouped according to their major degradative end product. Some amino acids are listed more than once because different

parts of their carbon skeletons are degraded to different end products. The figure shows the most important catabolic pathways in vertebrates, but there are minor variations among vertebrate species. Threonine, for instance, is degraded via at least two different pathways, and the importance of a given pathway can vary with the organism and its metabolic conditions. The glucogenic and ketogenic amino acids are also delineated in the figure, by color shading. Notice that five of the amino acids are both glucogenic and ketogenic. The amino acids degraded to pyruvate are also potentially ketogenic. Only two amino acids, leucine and lysine, are exclusively ketogenic.

Degradation of proteins

The amino acids undergo certain common reactions like transamination followed by deamination for the liberation of ammonia. The amino group of the amino acids is utilized for the formation of urea which is an excretory end product of protein metabolism. The carbon skeleton of the amino acids is first converted to keto acids (by transamination) which meet one or more of the following fates

1. Utilized to generate energy.
2. Used for the synthesis of glucose.
3. Diverted for the formation of fat or ketone bodies.
4. Involved in the production of non-essential amino acids.

The details of general and specific metabolic reactions of amino acids are described in the following pages.

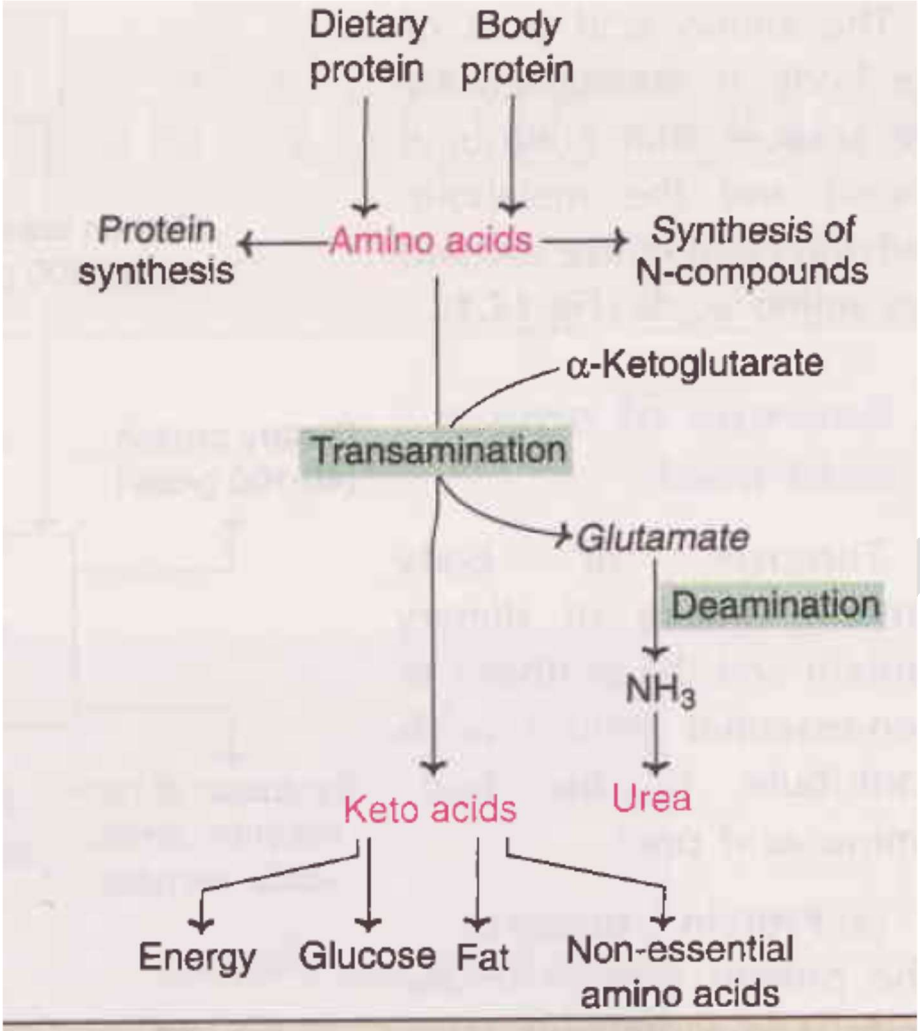


Fig. 15.2 : An overview of amino acid metabolism.

TRANSAMINATION

The transfer of an amino (- NH2) group from an amino acid to a keto acid is known as transamination. This process involves the interconversion of a pair of amino acids and a pair of keto acids, catalysed by a group of enzymes called transaminases (recently, aminotransferases).

Salient features of transamination

1. All transaminase require pyridoxal phosphate (PLP), a coenzyme derived from vitamin B6.

2. Specific transaminase exist for each pair of amino and keto acids. However, only two namely, aspartate transaminase and alanine transaminase-make a significant contribution for transamination.

3. There is no free NH3 liberated, only the transfer of amino group occurs.

4. Transamination is reversible

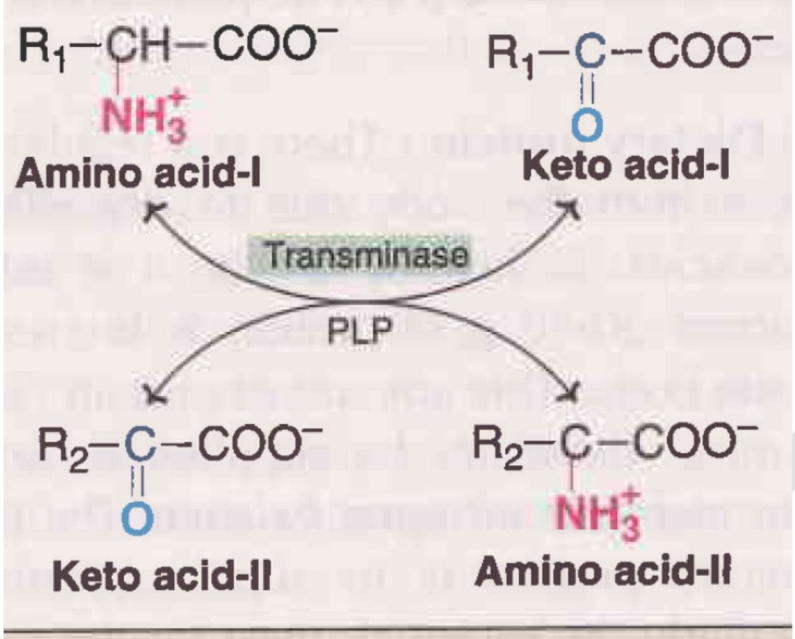


Fig. 15.3 : Transamination reaction.

5. Transamination is very important for the redistribution of amino groups and production of non' essential amino acids, as per the requirement of the cell. It involves both catabolism (degradation) and anabolism (synthesis) of amino acids.

6. Transamination diverts the excess amino acids towards energy generation.

7. The amino acids undergo transamination to finally concentrate nitrogen in glutamate. Glutamate is the only amino acid that undergoes oxidative deamination to a significant extent to liberate free NH3 for urea synthesis.

8. All amino acids except lysine, threonine, proline and hydroxyproline participate in transamination.

9. Transamination is not restricted to c-amino groups only. For instance, 6-amino group of ornithine is transaminated.

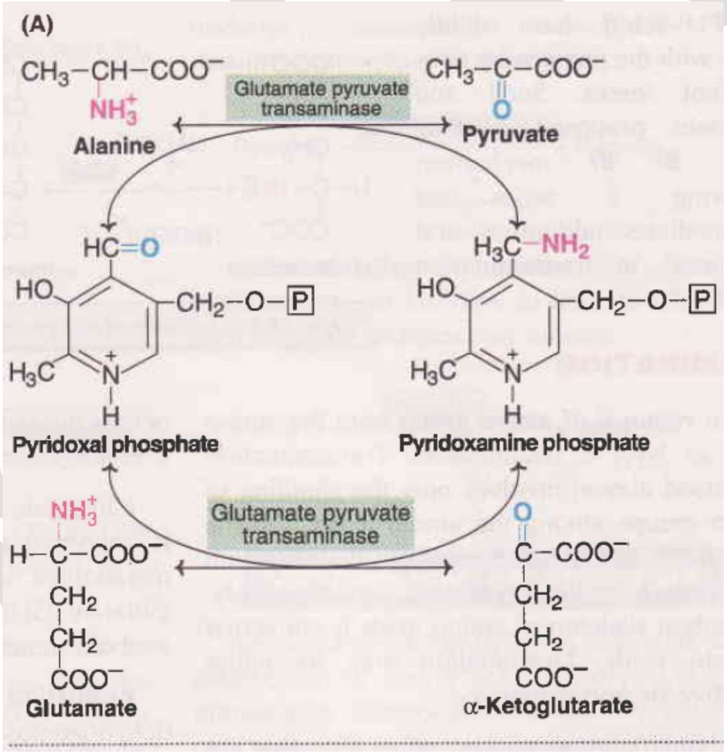
10. Serum transaminases are important for diagnostic and prognostic purpose.

Mechanism of transamination

Transamination occurs in two stages

1. Transfer of the amino group to the coenzyme pyridoxal phosphate (bound to the coenzyme) to form pyridoxamine phosphate.

2. The amino group of pyridoxamine phosphate is then transferred to a keto acid to produce a new amino acid and the enzyme with PLP is regenerated. All the transaminases require pyridoxal Phosphate (PLP), a derivative of vitamin 85. The aldehyde group of PLP is linked with e-amino group of lysine residue, at the active site of the enzyme forming a Schiff base (imine linkage). When an amino acid (substrate) comes in contact with the enzyme, it displaces lysine and a new Schiff base linkage is formed. The amino acid-PLP-Schiff base tightly binds with the enzyme by noncovalent forces. Snell and Braustein proposed a Ping Pong Bi Bi mechanism involving a series of intermediates (aldimines and ketimines) in transamination reaction.



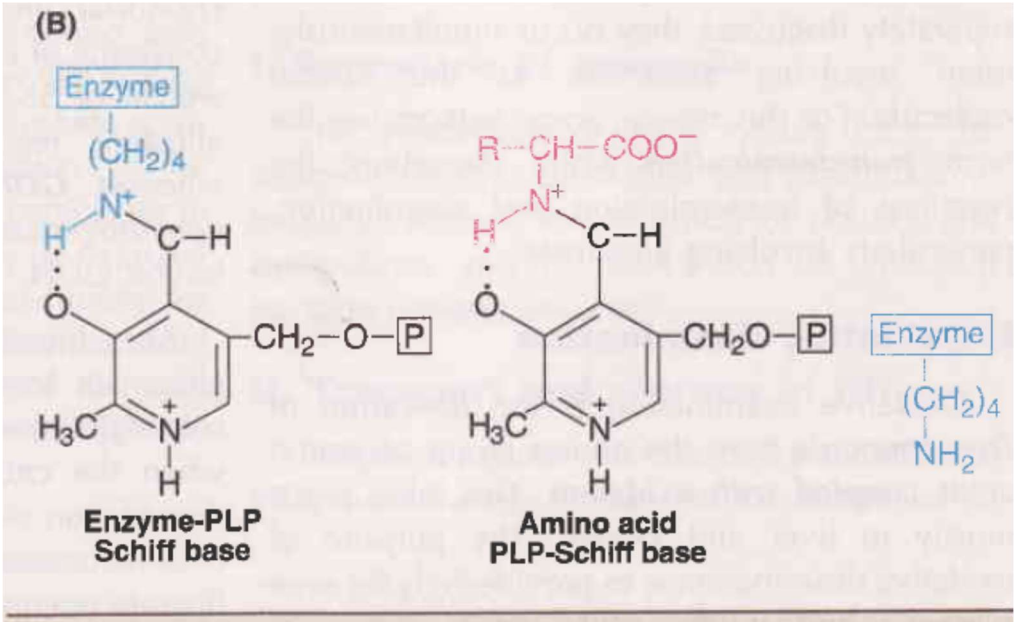


Fig. 15.4 : Mechanism of transamination—(A) Involvement of pyridoxal phosphate (PLP) in the transfer of amino group, (B) Formation of enzyme-PLP-Schiff base and amino acid-PLP-Schiff base. Note that when the amino acid binds, enzyme separates.

DEAMINATION

The removal of amino group from the amino acids as NH3 is deamination. Transamination (discussed above) involves only the shuffling of amino groups among the amino acids. On the Other hand, deamination results in the liberation of ammonia for urea synthesis. Simultaneously, the carbon skeleton of amino acids is converted to keto acids. Deamination may be either oxidative or non-oxidative.

1. Oxidative deamination

Oxidative deamination is the liberation of free ammonia from the amino group of amino acids coupled with oxidation. This takes place mostly in liver and kidney. The purpose of oxidative deamination is to provide NH3 for urea synthesis and c-keto acids for a variety of reactions, including energy generation.

Role of glutamate dehydrogenase: In the process of transamination the amino groups of most amino acids are transferred to a-ketoglutarate to produce glutamate. Thus, glutamate serves as a collection centre for amino groups in the biological system. Glutamate rapidly

undergoes oxidative deamination, catalysed by glutamate dehydrogenase (CDH) to liberate ammonia. This enzyme is unique in that it can utilize either NAD+ or NADP+ as a coenzvme. Conversion of glutamate to α-ketoglutarate occurs through the formation of an intermediate, α-iminoglutarate. Glutamate dehydrogenase catalysed reaction is important as it reversibly links up glutamate metabolism with TCA cycle through α-ketoglutarate G. DH is involved in both catabolic and anabolic reactions.

Regulation of GDH activity: Glutamate dehydrogenase is a zinc containing mitochondrial enzyme. It is a complex enzyme consisting of six identical units with a molecular weight of 56,000 each. CDH is controlled by allosteric regulation. GTP and ATP inhibit whereas GDP and ADP activate-glutamate dehydrogenase. Steroid and thyroid hormones inhibit GDH. After ingestion of a protein-rich meal, liver glutamate level is elevated. It is converted to α-ketoglutarate with liberation of NH3. Further, when the cellular energy levels are low, the degradation of glutamate is increased to provide a-ketoglutarate which enters TCA cycle to liberate energy.

Oxidative deamination by amino acid oxidases: L-Amino acid oxidase and D-amino acid oxidase are flavoproteins, possessing F MN and FAD, respectively. They act on the corresponding amino acids (L or D) to produce o-keto acids and NH3. In this reaction, oxygen is reduced lo H2O2, which is later decomposed by catalase. The activity of L-amino acid oxidase is much low while that of D-amino acid oxidase is high in tissues (mostly Liver and kidney). L - Amino acid oxidase does not act on glycine and dicarboxylic acids. This enzyme, due to its very low activity, does not appear to play any significant role in the amino acid metabolism.

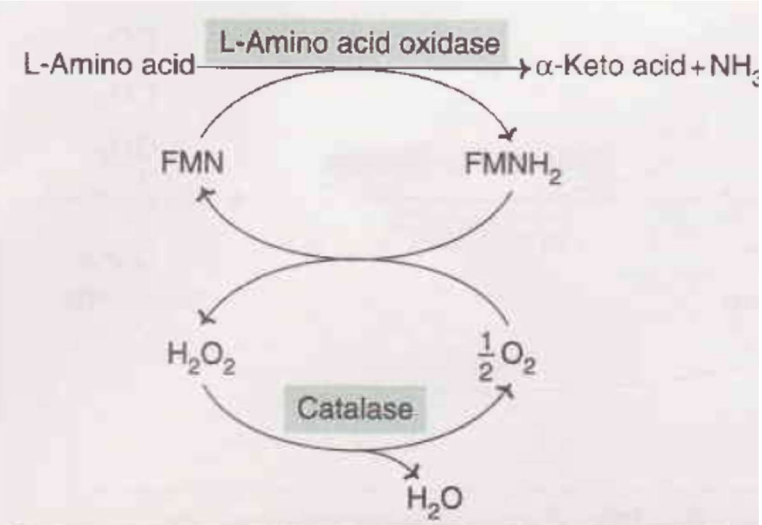


Fig. 15.6 : Oxidative deamination of amino acids.

Fate of D-amino acids: D-Amino acids are found in plants and microorganisms. They are, however, not present in the mammalian proteins. But D-amino acids are regularly taken in the diet and metabolized by the body. D-Amino acid oxidase converts them to the respective a-keto acids by oxidative deamination. The α-keto acids so produced undergo transamination to be converted to L-amino acids which participate in various metabolisms. Keto acids may be oxidized to generate energy or serve as precursors for glucose and fat synthesis. Thus, D-amino acid oxidase is important as it initiates the first step for the conversion of unnatural D-amino acids to L-amino acids in the body (Fig.l5.7).

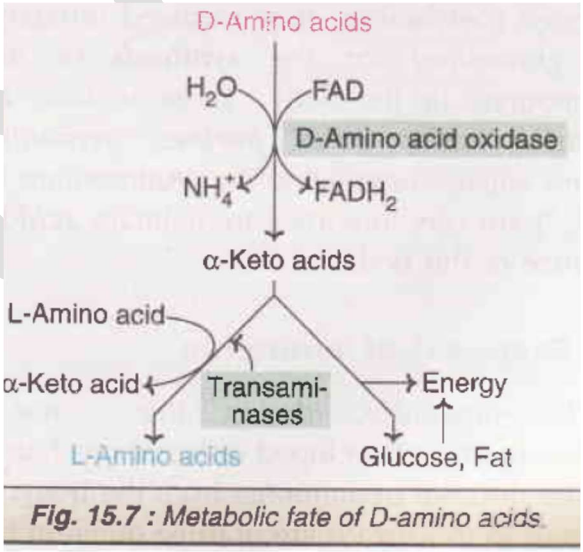
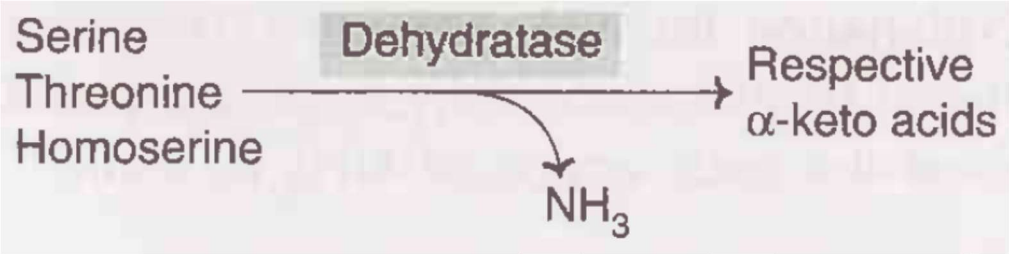


Fig. 15.7 : Metabolic fate of D-amino acids.

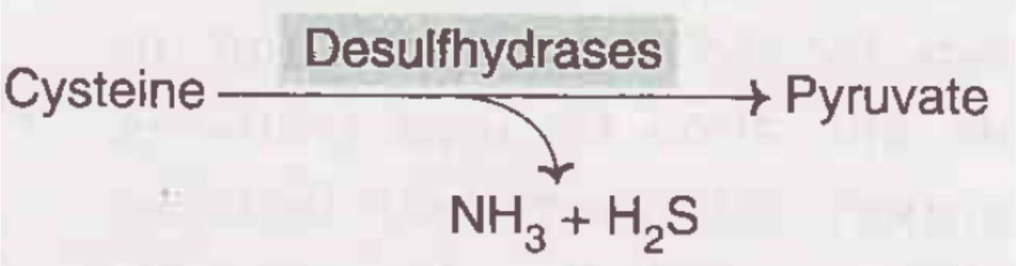
Non-Oxidative deamination

Some of the amino acids can be deaminated to liberate NH3 without undergoing oxidation

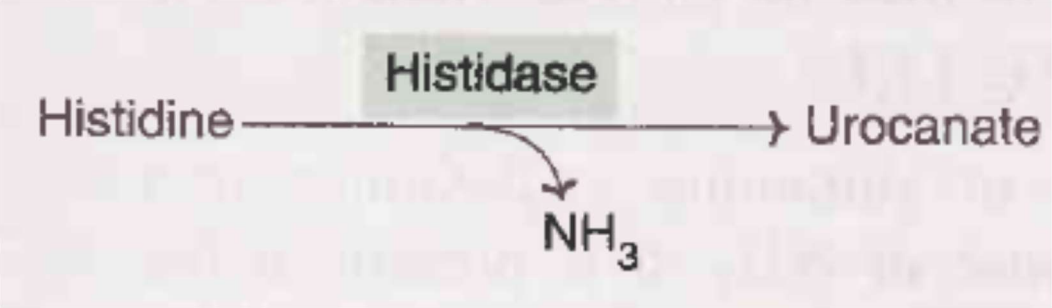
(a)**Amino acid dehydrases:** Serine, threonine and homoserine are the hydroxy amino acids. They undergo non-oxidative deamination catalysed by PLP-dependent dehydrases (dehydratases).



(b)**Amino acid desulfhydrases:** The sulfur amino acids, namely cysteine and homocysteine undergo deamination coupled with desulfhydration to give keto acids.



(c) Deamination of histidine: The enzyme histidase acts on histidine to liberate NH3 by a non-oxidative deamination process.



It Ammonia is constantly being liberated in the metabolism of amino acids (mostly) and other nitrogenous compounds. At the physiological pH, ammonia exist as ammonium (NH4+ ion).

1. **Formation of Ammonia**

The production of NH3 occurs from the amino acids (transamination and deamination), biogenic amines, amino group of purines and pyrimidines and by the action of intestinal bacteria (urease) on urea.

2. **Transport and storage of NH3**

Despite a regular and constant production of NH3 from various tissues, its concentration in is important for NH3 transport from muscle to liver by glucose-alanine cycle. Role of glutamine: Glutamine is a storehouse of NH3. It is present at the highest concentration (8 mg/dl in adults) in blood among the amino acids. Glutamine serves as a storage and transport form of NH3. Its synthesis mostly occurs in liver, brain and muscle. Ammonia is removed from the brain predominantly as glutamine. Glutamine is freely diffusible in tissues, hence easily transported. Glutamine synthetase (a mitochondrial enzyme) is responsible for the synthesis of glutamine from glutamate and ammonia. This reaction is unidirectional and requires ATP and Mg2+ ions. Glutamine can be deaminated by hydrolysis. To release ammonia by glutaminase an enzyme mostly found in kidney and intestinal cells.

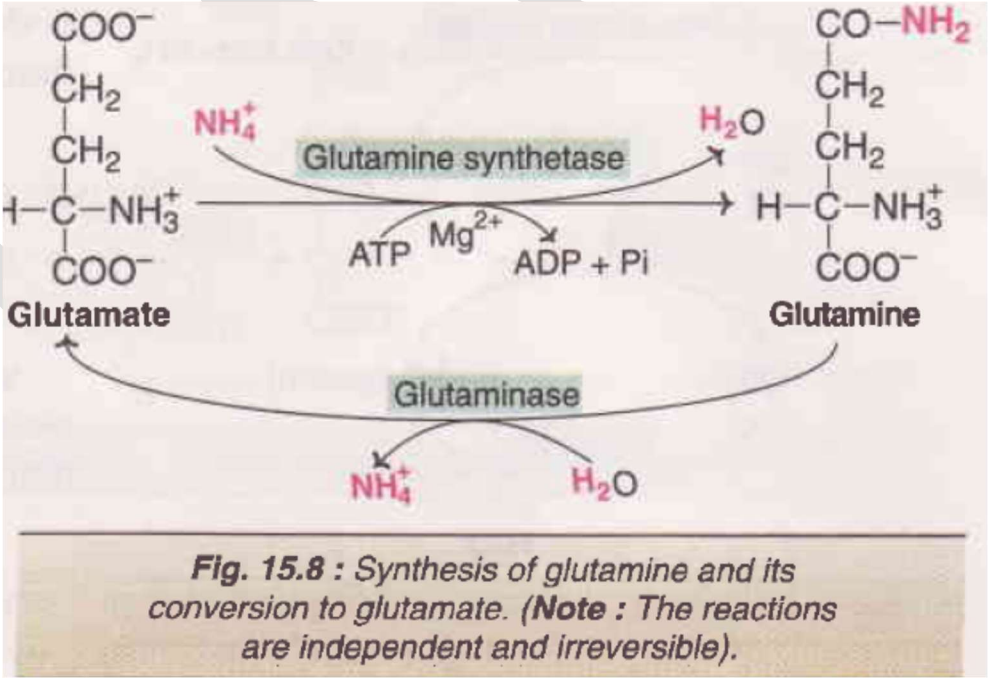


Fig. 15.8 : *Synthesis of glutamine and its conversion to glutamate. (**Note :** The reactions are independent and irreversible).*

Functions of ammonia is not just a waste product of nitrogen metabolism. It is involved (directly or via glutamine) for the synthesis of many compounds in the body. These include nonessential

amino acids, purines, pyrimidines, amino sugars, asparagine etc. Ammonium ions (NH4+) are very important to maintain acid-base balance of the body.

3. Functions of ammonia

The organisms, during the course of evolution, have developed different mechanisms for the disposal of ammonia from the body. The animals in this regard are of three different types

(a) Ammoniotelic: The aquatic animals dispose of NH3 into the surrounding water.

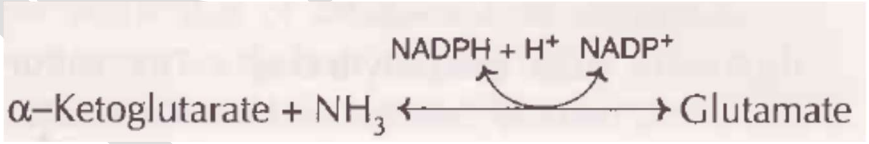
(b) Uricotelic: Ammonia is converted mostly to uric acid e.g. reptiles and birds.

(c) Ureotelic: The mammals including man convert NH3 to urea. Urea is a non-toxic and soluble compound, hence easily excreted.

4. Toxicity of ammonia

Even a marginal elevation in the blood ammonia concentration is harmful to the brain. Ammonia, when it accumulates in the body, results in slurring of speech and blurring of the vision and causes tremors. lt may lead to coma and, finally, death, if not corrected.

Hyperammonemia: Elevation in blood NH3 level may be genetic or acquired. Impairment in urea synthesis due to a defect in any one of the five enzymes is described in urea synthesis. All these disorders lead to hyperammonemia and cause mental retardation. The acquired hyperammonemia may be due to hepatitis, alcoholism etc. where the urea synthesis becomes defective, hence NH3 accumulates. Explanation for NH3 toxicity: The reaction catalysed by glutamate dehydrogenase probably explains the toxic effects of NH3 in brain.



Accumulation of NH3 shifts the equilibrium to the right with more glutamate formation, hence more utilization of α-ketoglutarate. α- Ketoglutarate is a key intermediate in TCA cycle and its depleted levels impair the TCA cycle. The net result is that production of energy (ATP) by the brain is reduced. The toxic effects of NHs on brain are, therefore, due to impairment in ATP formation.

Trapping and elimination of ammonia: When the plasma level of ammonia is highly elevated, intravenous administration of sodium benzoate and phenyllactate is done. These compounds can respectively condense with glycine and glutamate to form water soluble products that can be easily excreted. By this way, ammonia can be trapped and removed from the body. In some instances of toxic hyperammonemia, hemodialysis may become necessary.

Urea Cycle

Urea is the end product of protein metabolism (amino acid metabolism). The nitrogen of amino acids, converted to ammonia (as described above), is toxic to the body. It is converted to urea and detoxified. As such, urea accounts for 80-90% of the nitrogen containing substances excreted in urine.

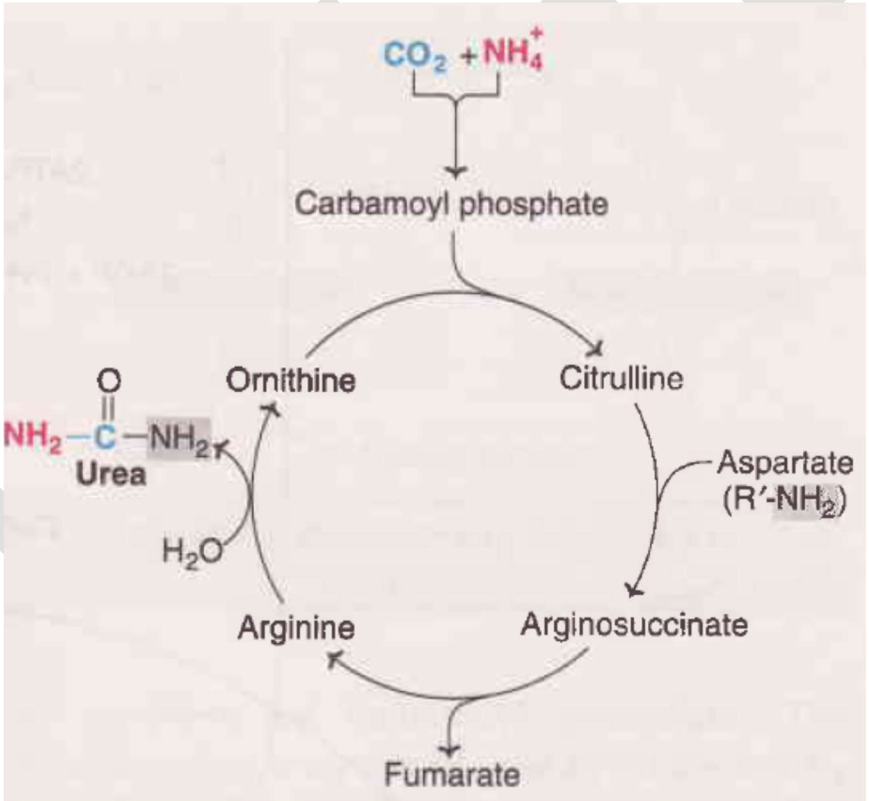


*Fig. 15.9 : Outline of urea cycle. (**Note :** In the synthesis of urea one amino group comes from ammonium ion while the other is from aspartate; carbon is derived from CO_2. This is represented in colours.)*

Urea is synthesized in liver and transported to kidneys for excretion in urine. Urea cycle is the first metabolic cycle that was elucidated by Hans Krebs and Kurt Henseleit (1932), hence it is known as Krebs-Henseleit cycle. The individual reactions, however w ere described in more detail later on by Ratner and Cohen. Urea has two amino (-NH) groups, one derived from NH3 and the other from aspartate. Carbon atom is supplied by CO2. Urea synthesis is a five-step cyclic process, with five distinct enzymes. The first two enzymes are present in mitochondria while the rest are localized in cytosol.

1. **Synthesis of carbamoyl phosphate:** Carbamoyl phosphate synthase 1 (CPS l) of mitochondria catalyses the condensation of NH4 ions with CO2 to form carbamoyl phosphate. This step consumes two ATP and is irreversible, and rate-limiting. CPS I requires N-acetylglutamate for its activity. Another enzyme, carbamoyl phosphate synthase II (CPS II)-involved in pyrimidine synthesis-is present in cytosol. It accepts amino group from glutamine and does not require N-acetylglutamate for its activity.

2. **Formation of citrulline:** Citrulline is synthesized from carbamoyl phosphate and ornithine by ornithine transcarbamoylase. Ornithine is regenerated and used in urea cycle. Therefore, its role is comparable to that of oxaloacetate in citric acid cycle. Ornithine and citrulline are basic amino acids. (They are never found in protein structure due to lack of codons). Citrulline produced in this reaction is transported to cytosol by a transporter system.

3. **Synthesis of arginosuccinate:** Arginosuccinate synthase condenses citrulline with aspartate to produce arginosuccinate. The second amino group of urea is incorporated in this reaction. This step requires ATP which is cleaved to AMP and pyrophosphate (PPi). The latter is immediately broken down to inorganic phosphate (Pi).

4. **Cleavage of arginosuccinate:** Arginosuccinase cleaves arginosuccinate to give arginine and fumarate. Arginine is the immediate precursor for urea. Fumarate liberated here provides a connecting link with TCA cycle, gluconeogenesis etc.

5. **Formation of urea:** Arginase is the fifth and final enzyme that cleaves arginine to yield urea and ornithine. Ornithine, so regenerated, enters mitochondria for its reuse in the urea cycle. Arginase is activated by Co2+ and Mn2+. Ornithine and lysine compete with arginine (competitive inhibition). Arginase is mostly found in the liver, while the rest of the enzymes

(four) of urea cycle are also present in other tissues. For this reason, arginine synthesis may occur to varying degrees in many tissues. But only the liver can ultimately produce urea.

Overall reactions and energetics

The urea cycle is irreversible and consumes 4 ATP. Two ATP are utilized for the synthesis of carbamoyl phosphate. One ATP is converted to AMP and PPi to produce arginosuccinate which equals to 2 ATP. Hence 4 ATP are actually consumed.

$NH_4^+ + CO_2 + Aspartate + 3ATP \rightarrow Urea + Fumarate + 2\ ADP + 2\ Pi + AMP + PPi$

Regulation of Urea cycle

The first reaction catalysed by carbamoyl phosphate synthase t (CPS l) is rate limiting reaction or committed step in urea synthesis. CPS I is allosterically activated by N-acetylglutamate (NAC). It is synthesized from glutamate and acetyl CoA by synthase and degraded by a hydrolase. The rate of urea synthesis in liver is correlated with the concentration of N-acetylglutamate. High concentrations of arginine increase NAG. The consumption of a protein-rich meal increases the level of NAG in liver, leading to enhanced urea synthesis. Carbamoyl phosphate synthase I and glutamate dehydrogenases are localized in the mitochondria. They coordinate with each other in the formation of NH1, and its utilization for the synthesis of carbamoyl phosphate. The remaining four enzymes of urea cycle are mostly controlled bv the concentration of their respective substrates.

Disposal of urea

Urea produced in the liver freely diffuses and is transported in blood to kidneys, and excreted. A small amount of urea enters the intestine where it is broken down to CO_2 and NH_3 by the bacterial enzyme urease. This ammonia is either lost in the feces or absorbed into the blood. In renal failure, the blood urea level is elevated (uremia), resulting in diffusion of more urea into intestine and its breakdown to NH_3. Hyperammonemia (increased blood NH_3) is commonly seen in patients of kidney failure. For these patients, oral administration of antibiotics (neomycin) to kill intestinal bacteria is advised.

Integration between urea cycle and TGA cycle

Urea cycle is linked with TCA cycle in three different ways. This is regarded as bicyclic integration between the two cycles.

1. The production of fumarate in urea cycle is the most important integrating point with TCA cycle. Fumarate is converted to malate and then to oxaloacetate in TCA cycle. Oxaloacetate undergoes transamination to produce aspartate which enters urea cycle. Here, it combines with citrulline to produce arginosuccinate. Oxaloacetate is an important metabolite which can combine with acetyl CoA to form citrate and get finally oxidized. Oxaloacetate can also serve as a precursor for the synthesis of glucose (gluconeogenesis).
2. ATP (12) are generated in the TCA cycle while ATP (4) are utilized for urea synthesis.
3. Citric acid cycle is an important metabolic pathway for the complete oxidation of various metabolites to CO2 and H2O. The CO2 liberated in TCA cycle (in the mitochondria) can be utilized in urea cycle.

Metabolic disorders of urea cycle

Metabolic defects associated with each of the five enzymes of urea cycle have been reported (Table 15.l). All the disorders invariably lead to a build-up in blood ammonia (hyperammonemia), leading to toxicity. Other metabolites of urea cycle also accumulate which, however, depends on the specific enzyme defect. The clinical symptoms associated with defect in urea cycle enzymes include vomiting, lethargy, irritability, ataxia and mental retardation.

TABLE 15.1 Metabolic defects in urea cycle

Defect	*Enzyme involved*
Hyperammonemia type I	Carbamoyl phosphate synthase I
Hyperammonemia type II	Ornithine transcarbamoylase
Citrullinemia	Arginosuccinate synthase
Arginosuccinic aciduria	Arginosuccinase
Hyperargininemia	Arginase

Blood urea-clinical importance

In healthy people, the normal blood urea concentration is 10-a0 mg/dl. Higher protein intake marginally increases blood urea level; however, this is well within normal range. About 15-30 g of urea (7-15 g nitrogen) is excreted in urine per day. Blood urea estimation is widely used as a screening test for the evaluation of kidney (renal) function. It is estimated in the laboratory either by urease method or diacetyl monoxime (DAM) procedure. Elevation in blood urea may be broadly classified into three categories.

1. Pre-renal: This is associated with increased protein breakdown, leading to a negative nitrogen balance, as observed after major surgery, prolonged fever, diabetic coma, thyrotoxicosis etc. In leukaemia and bleeding disorders also, blood urea is elevated.

2. Renal: In renal disorders like acute glomerulonephritis, chronic nephritis, nephrosclerosis, polycystic kidney, blood urea is increased.

3. Post-renal: Whenever there is an obstruction in the urinary tract (e.g. tumours, stones, enlargement of prostate gland etc.),blood urea is elevated. This is due to increased reabsorption of urea from the renal tubules.

The term uremia is used to indicate increased blood urea levels due to renal failure. Azotemia reflects a condition with elevation in blood urea/or other nitrogen metabolites which may or may not be associated with renal diseases.

Non-protein nitrogen (NPN)

As is obvious from the name, the term NPN refers to all the nitrogen-containing substances other than proteins. These include urea (most abundant), creatinine, creatine, uric acid, peptides, amino acids etc. In healthy persons, NPN concentration in blood is 20-4O mg/dl. The molecular weight of urea is 60 and about half of it (28) is contributed by the two nitrogen atoms. Thus, if blood urea concentration is 60 mg, then about half of it-28 mg-is blood urea nitrogen (BU N). Therefore,

BUN = ½ NPN

NPN = 2 BUN

In some countries, estimations of BUN or NPN are used rather than blood urea for assessing kidney function.

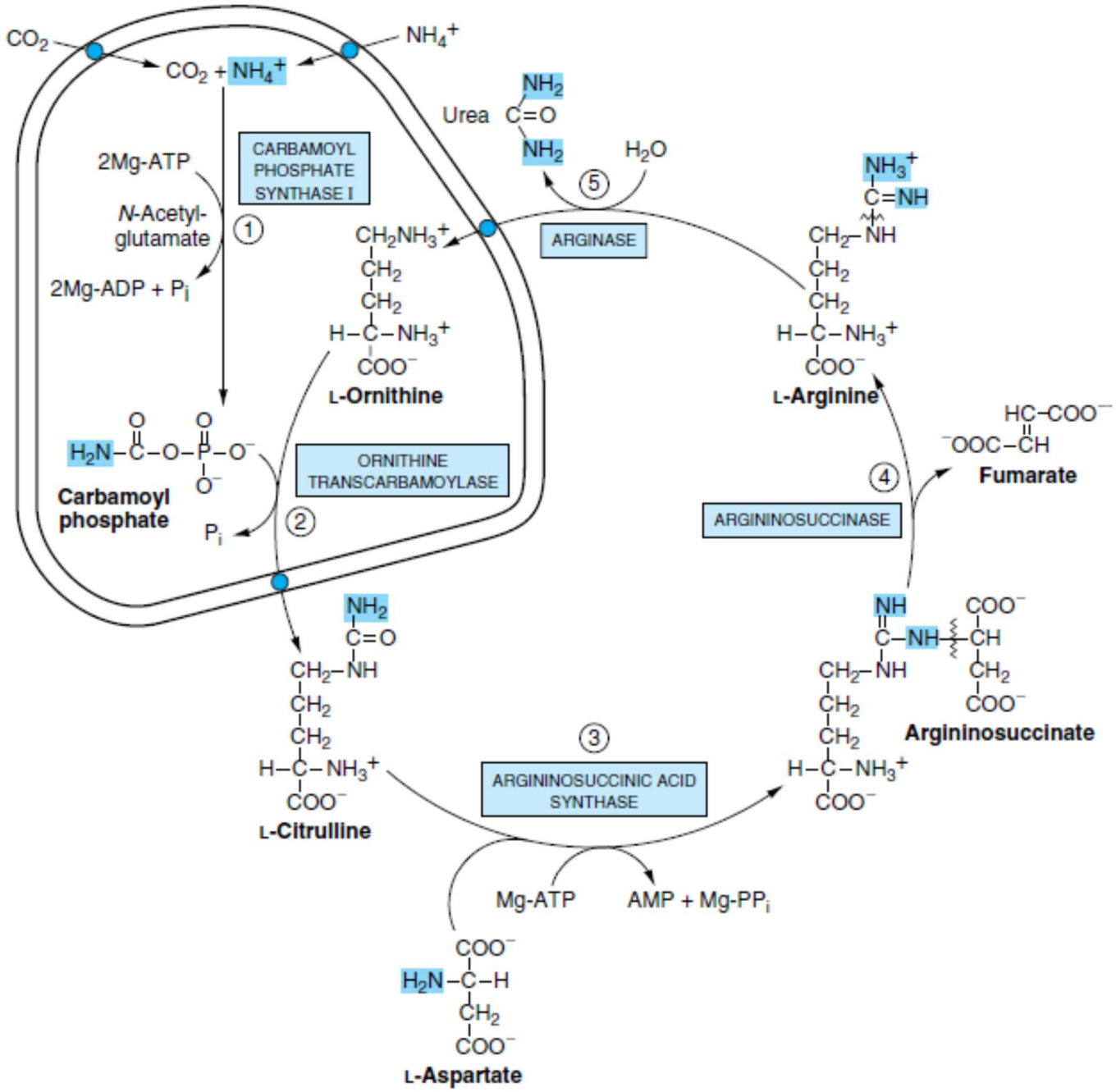


Figure 29–9. Reactions and intermediates of urea biosynthesis. The nitrogen-containing groups that contribute to the formation of urea are shaded. Reactions ① and ② occur in the matrix of liver mitochondria and reactions ③, ④, and ⑤ in liver cytosol. CO_2 (as bicarbonate), ammonium ion, ornithine, and citrulline enter the mitochondrial matrix via specific carriers (see heavy dots) present in the inner membrane of liver mitochondria.

POSSIBLE QUESTIONS

UNIT V

PART A (1 mark)

Question number 1-20 (From given 300 all possible MCQs)

PART B (2 Marks)

1. Write a short note ketogenic amino acid
2. Write a brief note on glucogenic amino acid
3. Explain briefly about deamination of proteins
4. Write notes on transamination of proteins
5. Give short note on deamination proteins

PART C (6 Marks)

1. Elaborate about the different types of protein degradation
2. Discuss in detail about urea cycle

UNIT IV	QUESTION	Option 1	Option 2	Option 3	Option 4			Answer
1	In which form the nitrogen is incorporated into an amino acid?	Nitrite	Glutamate	Nitrate	Ammonium ion			**Ammonium ion**
2	Transamination reaction in amino acid synthesis is catalyzed by enzyme _____	Nitric oxide synthase	Decarboxylase	Aminotransferase	Glutamate decarboxylase			**Nitric oxide synthase**
3	Intermediates of which of the following metabolic pathway have not been used in the synthesis of amino acids?	Glycolysis	Fatty acid biosynthesis	Citric acid cycle	Pentose phosphate pathway			**Fatty acid biosynthesis**
4	Name the amino acid which does not take part in transamination during amino acid catabolism	Proline	Threonine	Lysine	Serine			**Serine**
5	Which of these is a hereditary disease caused due to an error in amino acid metabolism?	Homocystinuria	Albinism	Phenylketonuria	Branched-chain ketoaciduria			**Phenylketonuria**
6	In deamination, amino acid is converted into	aldol acid	keto acid	hydrochloric acid	carboxylic acid			**keto acid**
7	Process of breakdown of amino acids to α keto acids is called	cisamination	amination	transamination	Racemization			**transamination**
8	Function of proteins is to	transport oxygen to hemoglobin	catalyze biochemical reactions	regulate reactions	all of above			**all of above**
9	Which of the following statements about protein synthesis is correct?	All the information in DNA codes for proteins	The mRNA formed by transcription of a region of DNA only contains information for the protein to be synthesised.	Both strands of DNA are transcribed to form mRNA	The RNA formed by transcription of DNA undergoes splicing and further modifications to form mRNA			**The RNA formed by transcription of DNA undergoes splicing and further modifications to form mRNA**
10	Which of the following statements about proteolysis is correct?	The rate of tissue protein catabolism is more or less constant throughout the day	All tissue proteins undergo catabolism at more or less the same rate.	All proteins that are to be catabolised are tagged with the peptide ubiquitin	Lysosomal enzymes provide the only mechanism for tissue protein catabolism			**The rate of tissue protein catabolism is more or less constant throughout the day**
11	Which of the following will result in an increase in the rate of tissue protein synthesis?	Increased glucagon secretion in the fasting state	Increased insulin secretion in the fed state	Increased availability of lysine in the fed state.	Secretion of cortisol under conditions of stress			**Increased insulin secretion in the fed state**
12	Which of the following amino acids cannot provide a substrate for gluconeogenesis?	Leucine	Tryptophan	Histidine	isoleucine			**Leucine**
13	Which of the following is the main nitrogenous compound in urine?	Uric acid	Ammonia	Urea	Creatinine			**Urea**
14	Which of the following statements concerning transamination is correct?	Only non-essential (dispensable) amino acids undergo transamination	Transamination is an irreversible reaction in amino acid catabolism.	Transaminases require a coenzyme derived from vitamin B12	Transaminases require a coenzyme derived from vitamin B6			**Transaminases require a coenzyme derived from vitamin B6**
15	Which of the following statements about the metabolic demand for amino acids is correct?	The metabolic demand is supplied only from the diet,	The metabolic demand is to maintain protein turnover	The metabolic demand involves consumption of amino-acids by a number of irreversible pathways and these are either obligatory or adaptive	The metabolic demand is a fixed amount			**The metabolic demand involves consumption of amino-acids by a number of irreversible pathways and these are either obligatory or adaptive**
16	Which of the following statements about the protein:energy ratio of the requirement is correct?	Infants and children need the most protein dense food	Children are most susceptible to protein deficient diets	Young adults need the most protein dense food	The elderly need the most protein dense food			**The elderly need the most protein dense food**
17	The ionisable groups of amino acids atleast	1	2	3	4			**2**
18	The ionisable groups of amino acids exist almost entirely as the conjugated base at	6.6	6.8	7.2	7.4			**7.4**
19	The melting point of amino acid is above	100^0C	180^0C	200^0C	220^0C			**200^0C**
20	Amino acids are insoluble in	Lactic acid	choloroform	ethanol	benzene			**benzone**
21	the reagent for the detection of amino acid is	Molisch's reagent	Dicholoro phenol indo phenol	ninhydrin	none			**ninhydrin**
22	Which among the following is an essential amino acid	Cysteine	leucine	tyrosine	Aspartic acid			**leucine**
23	The neutral amino acid is	leucine	lysine	proline	serine			**leucine**
24	Which amino acid is a lipotropic factor?	Lysine	leucine	tryptophan	methionine			**methionine**
25	The basic amino acid	Glycine	Histidine	proline	serine			**Histidine**
26	Which among the following is a nutritionally essential amino acid for man?	Alanine	Glycine	tyrosine	tryptophan			**tryptophan**
27	All amino acids are optically active except	Glycine	serine	threonine	tryptophan			**Glycine**
28	Which one of the following amino acid does not contain sulphur	methionine	cystine	Lysine	Cysteine			**Lysine**
29	Arginine has a	Thiol group	Guanidine group	phenolic group	imidazole group			**Guanidine group**
30	Formaldehyde reacts with amino acid	Carboxyl group	amino group	hydroxyl group	methyl group			**amino group**
31	Ninhydrin reaction is specific to	Amino & carboxylic acid	carboxylic acid	Aldehydes	Ketones			**Amino & carboxylic acid**
32	Serotonin is derived in the body from the following amino acid	Phenyl alanine	histidine	tryptophan	Serine			**tryptophan**
33	The amino acid which contains a indole group is	Histidine	arginine	attulin	glycine			**Tryptophan**
34	The amino acid which contains a guanidine group is	Histidine	arginine	attulin	attulin			**arginine**
35	The amino acid which synthesises many hormones	Valine	alanine	Phenylalanine	alanine			**alanine**
36	Sakaguchi reaction is answered by	cystine	ornithine	arginine	arginine			**arginine**
37	The isoelecrtic point of an amino acid depends on its	optical rotation	dissociation constant	diffusion coeffient	diffusion coeffient			**optical rotation**
38	The amino acids exist as zwitter ions when they are in	solid state	acidic solution	alkaline solution	alkaline solutio			**solid state**
39	Amino acids are	absorbed into portal circulation	absorbed into into lymph	excreted to the extent of 50%	excreted to the extent of 50%			**are absorbed into portal circulation**
40	An amino acid which contains a disulfide bond is	lysine	methionine	homocysteine	homocysteine			**Cystine**
41	Amino acids are insoluble in	Acetic acid	Chloroform	Ethanol	Ethanol			**Benzene**
42	Owing to the opposite reactions depending on the acidity or alkalinity of the solution, the amino acid sare called	Amphibolic	Ampholytic	Both	None			**Ampholytic**
43	This protein has a pigment as the prosthetic group	heme	haemoglobin	hematin	Collagen			**haemoglobin**
44	Out of 200 different amino acids found in nature the number of aminoacids present in protein	20	25	43	30			**20**
45	At iso electric pH amino acids exist as	anion	cation	zwitter ion	anion and cation			**zwitter ion**
46	Ampholytes have	only positive charges	only negative charges	both positive & negative charges	none			**all options**
47	Histidine is degraded to a-ketoglutarate and is described as a	gluco amino acid	glucogenic amino acid	ketogenic amino acid	keto-gluco amino acid			**glucogenic amino acid**
48	Which of the following amino acids is considered as both ketogenic and glucogenic?	Tyrosine	Valine	Lysine	tryptophan			**Tyrosine**
49	A glucogenic amino acid is one which is degraded to	keto-sugars	acetyl CoA	acetoacetyl CoA	pyruvate or citric acid cycle intermediates			**pyruvate or citric acid cycle intermediates**
50	Which of the following is the best described glucogenic amino acid?	Lysine	Tryptophan	Valine	Glysine			**Lysine**
51	A person with phenylketonuria cannot convert	phenylalanine to tyrosine	phenylalanine to isoleucine	phenol into ketones	phenylalanine to lysine			**phenylalanine to tyrosine**
52	Oxidative deamination is the conversion of an amino	group from an amino acid to a keto acid	acid to a carboxylic acid plus ammonia	acid to a keto acid plus ammonia	group from an amino acid to a carboxylic acid			**acid to a keto acid plus ammonia**
53	An example of a transamination process is	glutamate = hexanoic acid + NH3	aspartate + hexanoic acid = glutamate + oxaloacetate	aspartate + a ketoglutarate = glutamate + oxaloacetate	glutamate = a-ketoglutarate + NH3			**aspartate + a ketoglutarate = glutamate + oxaloacetate**
54	Transamination is the process where	carboxyl group is transferred from amino acid	a-amino group is removed from the amino acid	polymerisation of amino acid takes place	acid to a carboxylic acid plus ammonia			**a-amino group is removed from the amino acid**
55	The most toxic compounds is	Tyrosine	phenylpyruvate	lysine	phenylalanine			**phenylpyruvate**
56	A person with phenylketonuria is advised not to consume which of the following products?	Glycine containing foods	Fat containing food	Aspartame	Glucose			**Aspartame**
57	Tyrosine is degraded to acetoacetyl CoA and fumarate and is described as a	glucogenic amino acid	ketogenic amino acid	ketogenic and glucogenic amino acid	keto-gluco amino acid			**ketogenic and glucogenic amino acid**
58	Transaminase enzymes are present in	Liver	pancreas	kidney	intestine			**Liver**
59	Which of the following vitamins provides the coenzyme for oxidative decarboxylation of pyruvate?	Folate	Niacin	Thiamine	Riboflavin			**Thiamine**
60	The amino acid substitution of Val for Glu in Hemoglobin S results in aggregation of the protein because of - - - - interactions between molecules.	Covalent bond	disulfide bond	hydrogen bond	hydrophobic			**hydrophobic**

UNIT-IV

Nucleic acid Metabolism and Biological oxidation: Biosynthesis and degradation of purine and pyrimidine nucleotides. Mitochondrial Electron Transport Chain: electron carriers, sites of ATP production, inhibitors of ETC, oxidative phosphorylation: structure of ATPase complex, chemiosmotic theory, inhibitors of oxidative phosphorylation and uncouplers, Mitochondrial shuttle system.

PURINES, PYRIMIDINES, NUCLEOSIDES, & NUCLEOTIDES

Purines and pyrimidines are nitrogen-containing heterocycles, cyclic compounds whose rings contain both carbon and other elements (hetero atoms). Note that the smaller pyrimidine has the *longer* name and the larger purine the *shorter* name and that their six-atom rings are numbered in opposite directions (Figure 33–1). The planar character of purines and pyrimidines facilitates their close association, or "stacking," which stabilizes double-stranded DNA (Chapter 36). The oxo and amino groups of purines and pyrimidines exhibit keto-enol and amine-imine tautomerism (Figure 33–2), but physiologic conditions strongly favor the amino and oxo forms.

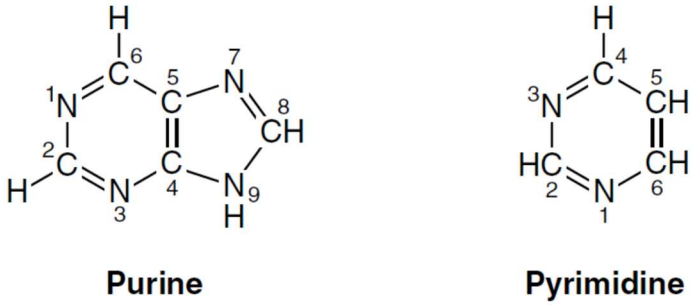


Figure 33–1. Purine and pyrimidine. The atoms are numbered according to the international system.

Nucleosides & Nucleotides

Nucleo**sides** are derivatives of purines and pyrimidines that have a sugar linked to a ring nitrogen. Numerals with a prime (eg, 2′ or 3′) distinguish atoms of the sugar from those of the heterocyclic base. The sugar in **ribonucleosides** is D-ribose, and in **deoxyribonucleosides** it is 2-deoxy-D-ribose. The sugar is linked to the heterocyclic base via a _**-N-glycosidic bond,** almost always to N-1 of a pyrimidine or to N-9 of a purine.

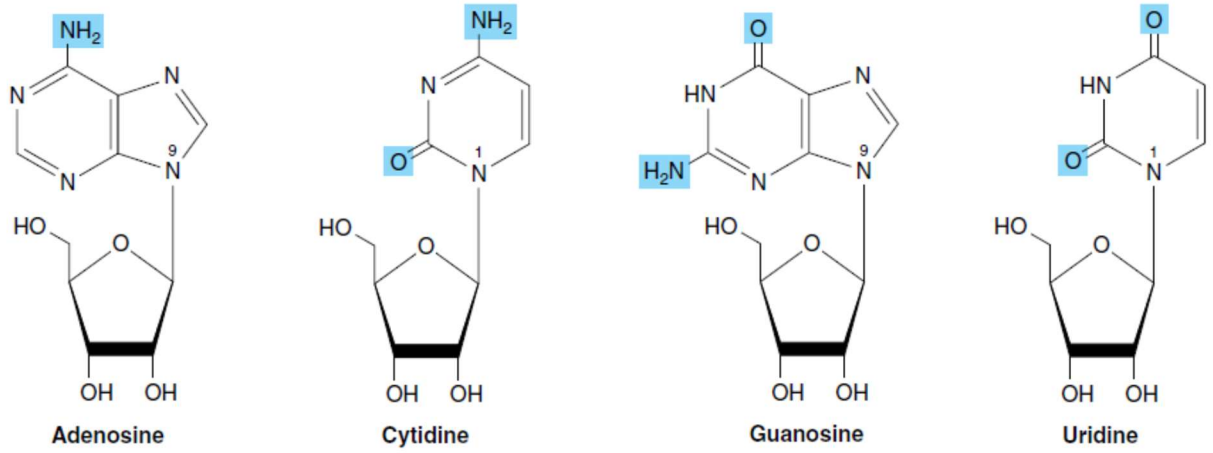


Figure 33–3. Ribonucleosides, drawn as the syn conformers.

Mononucleo**tides** are nucleo**sides** with a phosphoryl group esterified to a hydroxyl group of the sugar. The 3′- and 5′-nucleo**tides** are nucleo**sides** with a phosphoryl group on the 3′- or 5′-hydroxyl group of the sugar, respectively. Since most nucleotides are 5′-, the prefix "5′-" is usually omitted when naming them. UMP and dAMP thus represent nucleotides with a phosphoryl group on C-5 of the pentose. Additional phosphoryl groups linked by **acid anhydride bonds** to the phosphoryl group of a mononucleotide form nucleoside **diphosphates** and **triphosphates** (Figure 33–4). Steric hindrance by the base restricts rotation about sthe β-N-glycosidic bond of nucleosides and nucleotides. Both therefore exist as syn or anti conformers (Figure 33–5). While both conformers occur in nature, anti conformers predominate. Table 33–1 lists the major purines and pyrimidines and their nucleoside and nucleotide derivatives. Single-letter abbreviations are used to identify adenine (A), guanine (G), cytosine (C), thymine (T), and uracil (U), whether free or present in nucleosides or nucleotides. The prefix "d" (deoxy) indicates that the sugar is 2′-deoxy-D-ribose (eg, dGTP).

BIOSYNTHESIS OF PURINE NUCLEOTIDES

Purine and pyrimidine nucleotides are synthesized in vivo at rates consistent with physiologic need. Intracellular mechanisms sense and regulate the pool sizes of nucleotide triphosphates (NTPs), which rise during growth or tissue regeneration when cells are rapidly dividing. Early investigations of nucleotide biosynthesis employed birds, and later ones used *Escherichia coli.* Isotopic precursors fed to pigeons established the source of each atom of a purine base (Figure

34–1) and initiated study of the intermediates of purine biosynthesis. Three processes contribute to purine nucleotide biosynthesis. These are, in order of decreasing importance:

(1) synthesis from amphibolic intermediates (synthesis de novo),

(2) phosphoribosylation of purines, and

(3) phosphorylation of purine nucleosides.

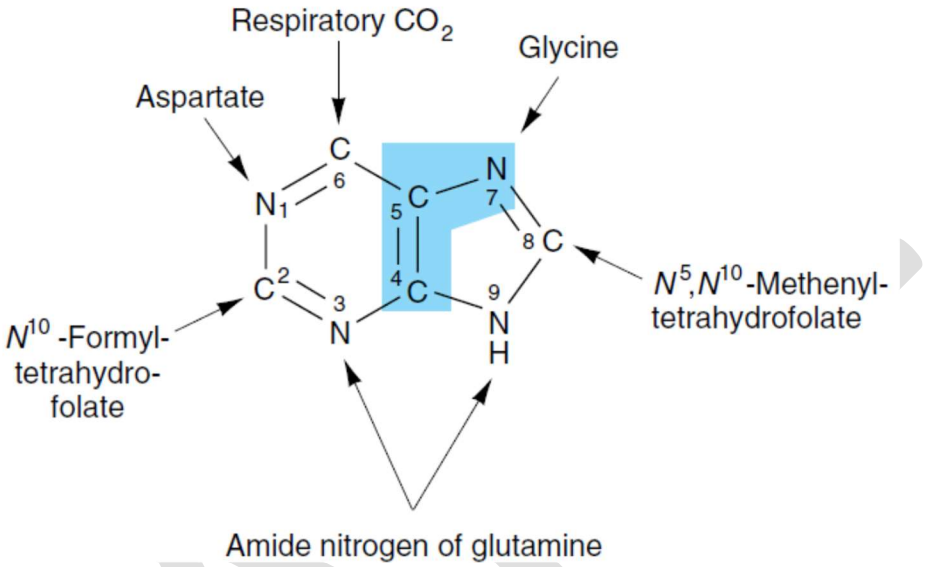


Figure 34–1. Sources of the nitrogen and carbon atoms of the purine ring. Atoms 4, 5, and 7 (shaded) derive from glycine.

INOSINE MONOPHOSPHATE (IMP) IS SYNTHESIZED FROM AMPHIBOLIC INTERMEDIATES

Figure 34–2 illustrates the intermediates and reactions for conversion of α-D-ribose 5-phosphate to inosine monophosphate (IMP). Separate branches then lead to AMP and GMP (Figure 34–3). Subsequent phosphoryl transfer from ATP converts AMP and GMP to ADP and GDP. Conversion of GDP to GTP involves a second phosphoryl transfer from ATP, whereas conversion of ADP to ATP is achieved primarily by oxidative phosphorylation (see Chapter 12).

Multifunctional Catalysts Participate in Purine Nucleotide Biosynthesis

In prokaryotes, each reaction of Figure 34–2 is catalysed by a different polypeptide. By contrast, in eukaryotes, the enzymes are polypeptides with multiple catalytic activities whose adjacent catalytic sites facilitate channeling of intermediates between sites. Three distinct multifunctional

enzymes catalyze reactions 3, 4, and 6, reactions 7 and 8, and reactions 10 and 11 of Figure 34–2.

Antifolate Drugs or Glutamine Analogs Block Purine Nucleotide Biosynthesis

The carbons added in reactions 4 and 5 of Figure 34–2 are contributed by derivatives of tetrahydrofolate. Purine deficiency states, which are rare in humans, generally reflect a deficiency of folic acid. Compounds that inhibit formation of tetrahydrofolates and therefore block purine synthesis have been used in cancer chemotherapy. Inhibitory compounds and the reactions they inhibit include azaserine (reaction 5, Figure 34–2), diazanorleucine (reaction 2), 6-mercaptopurine (reactions 13 and 14), and mycophenolic acid (reaction 14).

"SALVAGE REACTIONS" CONVERT PURINES & THEIR NUCLEOSIDES TO MONONUCLEOTIDES

Conversion of purines, their ribonucleo**sides**, and their deoxyribonucleo**sides** to mononucleo**tides** involves socalled "salvage reactions" that require far less energy than de novo synthesis. The more important mechanism involves phosphoribosylation by PRPP (structure II, Figure 34–2) of a free purine (Pu) to form a purine 5′-mononucleotide (Pu-RP).

$$Pu + PR - PP \rightarrow PRP + PP_i$$

Two phosphoribosyl transferases then convert adenine to AMP and hypoxanthine and guanine to IMP or GMP (Figure 34–4). A second salvage mechanism involves phosphoryl transfer from ATP to a purine ribonucleo**side** (PuR):

$$PuR + ATP \rightarrow PuR - P + ADP$$

Adenosine kinase catalyzes phosphorylation of adenosine and deoxyadenosine to AMP and dAMP, and deoxycytidine kinase phosphorylates deoxycytidine and 2′-deoxyguanosine to dCMP and dGMP. Liver, the major site of purine nucleotide biosynthesis, provides purines and purine nucleosides for salvage and utilization by tissues incapable of their biosynthesis. For example, human brain has a low level of PRPP amidotransferase (reaction 2, Figure 34–2) and hence depends in part on exogenous purines. Erythrocytes and polymorphonuclear leukocytes cannot synthesize 5-phosphoribosylamine (structure III, Figure 34–2) and therefore utilize exogenous purines to form nucleotides.

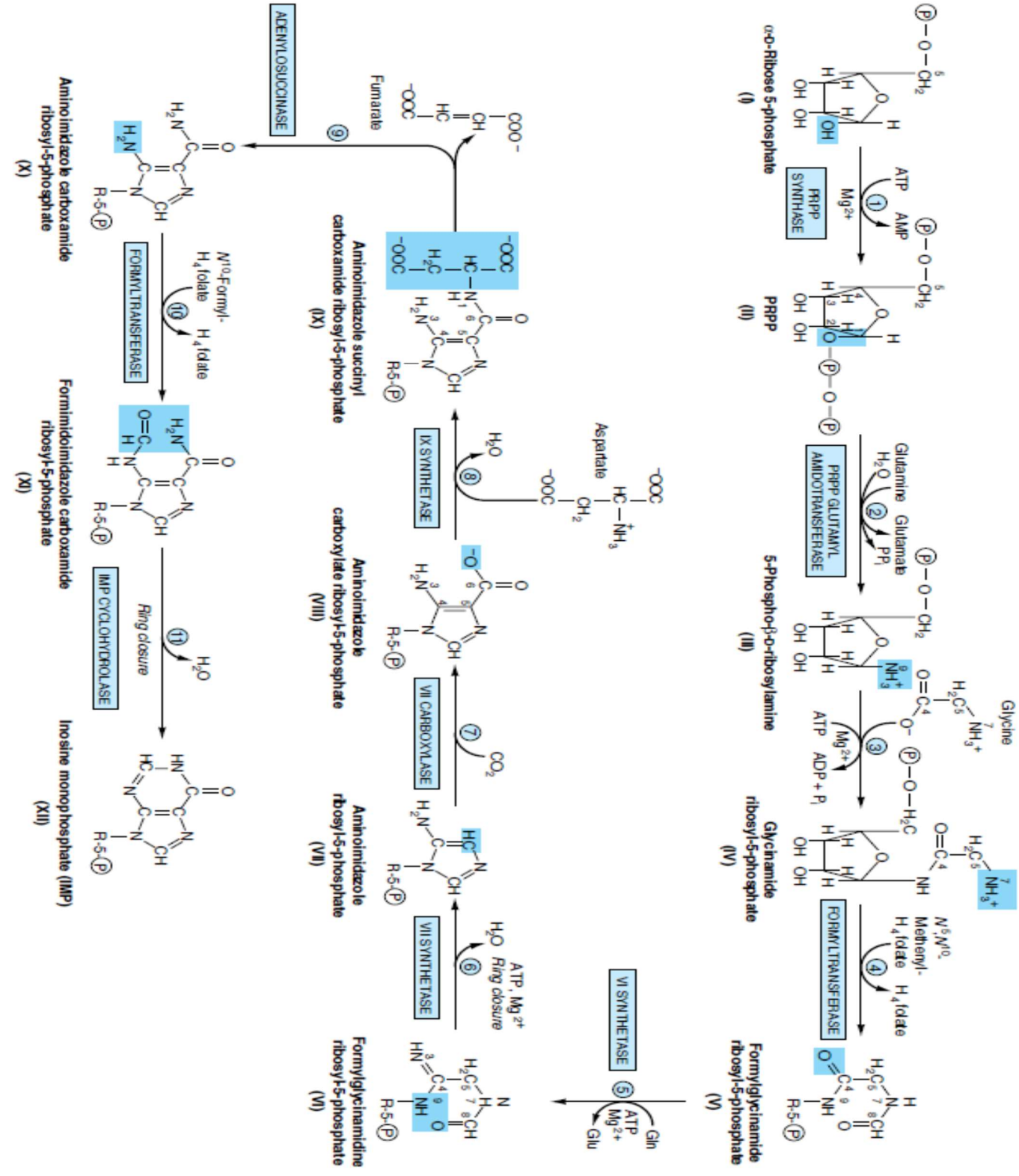


Figure 34–2. Purine biosynthesis from ribose 5-phosphate and ATP. See text for explanations. (Ⓟ, PO_3^{2-} or PO_2^-.)

AMP & GMP Feedback-Regulate PRPP Glutamyl Amidotransferase

Since biosynthesis of IMP consumes glycine, glutamine, tetrahydrofolate derivatives, aspartate, and ATP, it is advantageous to regulate purine biosynthesis. The major determinant of the rate of de novo purine nucleotide biosynthesis is the concentration of PRPP, whose pool size depends on its rates of synthesis, utilization, and degradation. The rate of PRPP synthesis depends on the availability of ribose 5-phosphate and on the activity of PRPP synthase, an enzyme sensitive to feedback inhibition by AMP, ADP, GMP, and GDP.

AMP & GMP Feedback-Regulate Their Formation From IMP

Two mechanisms regulate conversion of IMP to GMP and AMP. AMP and GMP feedback-inhibit adenylosuccinate synthase and IMP dehydrogenase (reactions 12 and 14, Figure 34–3), respectively. Furthermore, conversion of IMP to adenylosuccinate en route to AMP requires GTP, and conversion of xanthinylate (XMP) to GMP requires ATP. This cross-regulation between the pathways of IMP metabolism thus serves to decrease synthesis of one purine nucleotide when there is a deficiency of the other nucleotide. AMP and GMP also inhibit hypoxanthine-guanine phosphoribosyltransferase, which converts hypoxanthine and guanine to IMP and GMP (Figure 34–4), and GMP feedback- inhibits PRPP glutamyl amidotransferase (reaction 2, Figure 34–2).

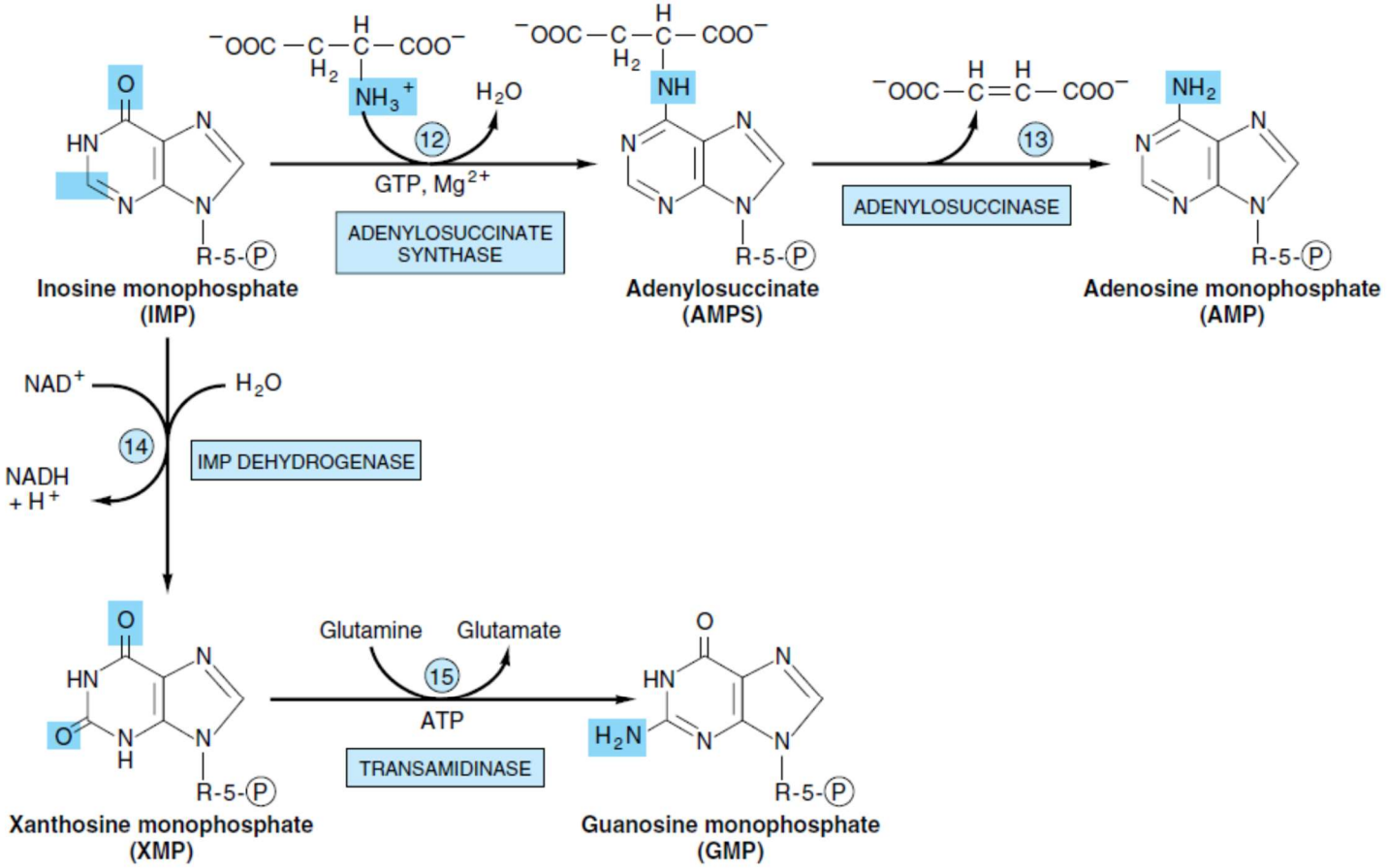


Figure 34–3. Conversion of IMP to AMP and GMP.

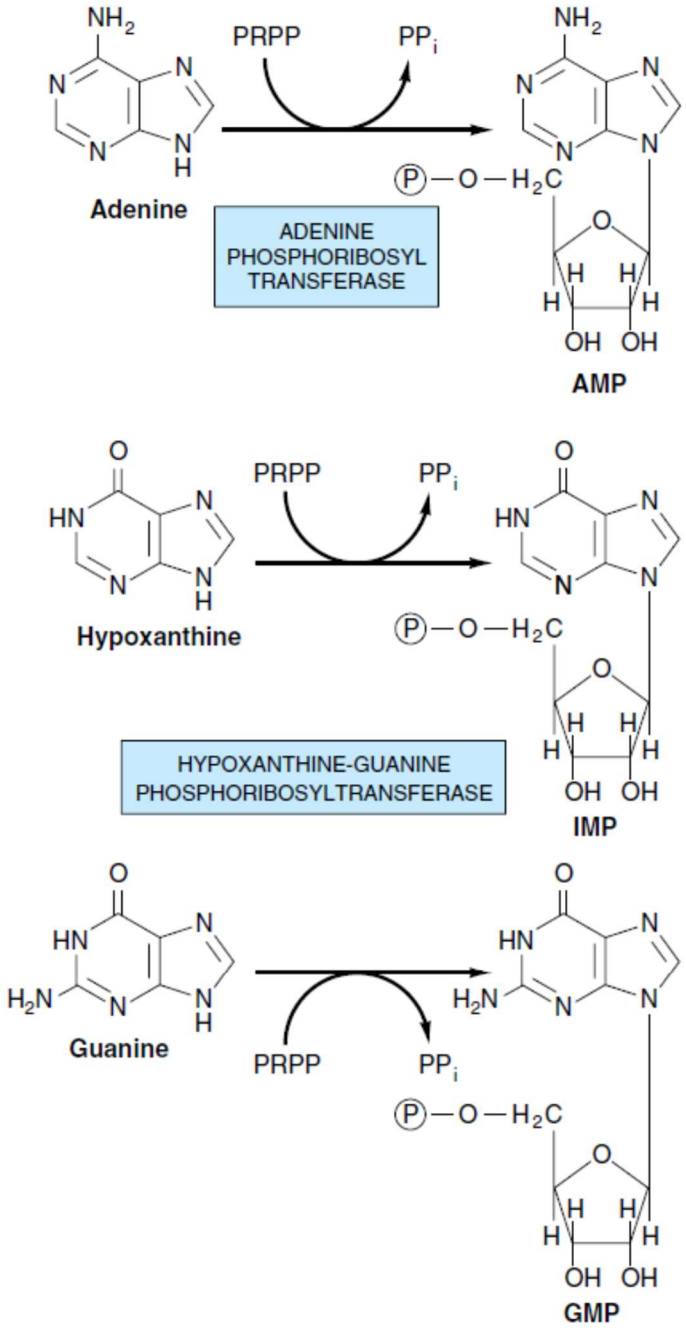
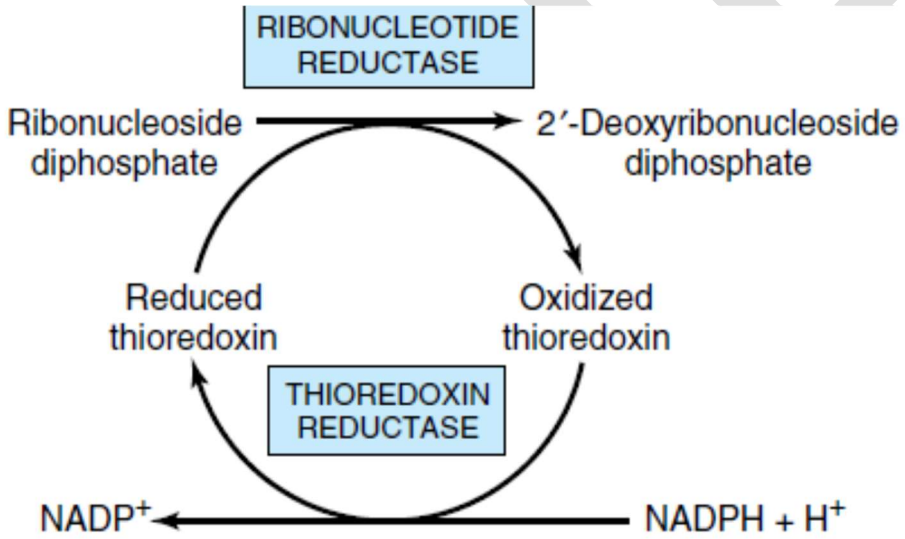


Figure 34–4. Phosphoribosylation of adenine, hypoxanthine, and guanine to form AMP, IMP, and GMP, respectively.

REDUCTION OF RIBONUCLEOSIDE DIPHOSPHATES FORMS DEOXYRIBONUCLEOSIDE DIPHOSPHATES

Reduction of the 2′-hydroxyl of purine and pyrimidine ribonucleotides, catalyzed by the **ribonucleotide reductase complex** (Figure 34–5), forms deoxyribonucleoside diphosphates (dNDPs). The enzyme complex is active only when cells are actively synthesizing DNA. Reduction requires thioredoxin, thioredoxin reductase, and NADPH. The immediate reductant, reduced thioredoxin, is produced by NADPH:thioredoxin reductase (Figure 34–5). Reduction of ribonucleoside diphosphates (NDPs) to deoxyribonucleoside diphosphates (dNDPs) is subject to complex regulatory controls that achieve balanced production of deoxyribonucleotides for synthesis of DNA (Figure 34–6).



BIOSYNTHESIS OF PYRIMIDINE NUCLEOTIDES

Figure 34–7 summarizes the roles of the intermediates and enzymes of pyrimidine nucleotide biosynthesis. The catalyst for the initial reaction is *cytosolic* carbamoyl phosphate synthase II, a different enzyme from the *mitochondrial* carbamoyl phosphate synthase I of urea synthesis (Figure 29–9). Compartmentation thus provides two independent pools of carbamoyl phosphate. PRPP, an early participant in purine nucleotide synthesis (Figure 34–2), is a much later participant in pyrimidine biosynthesis.

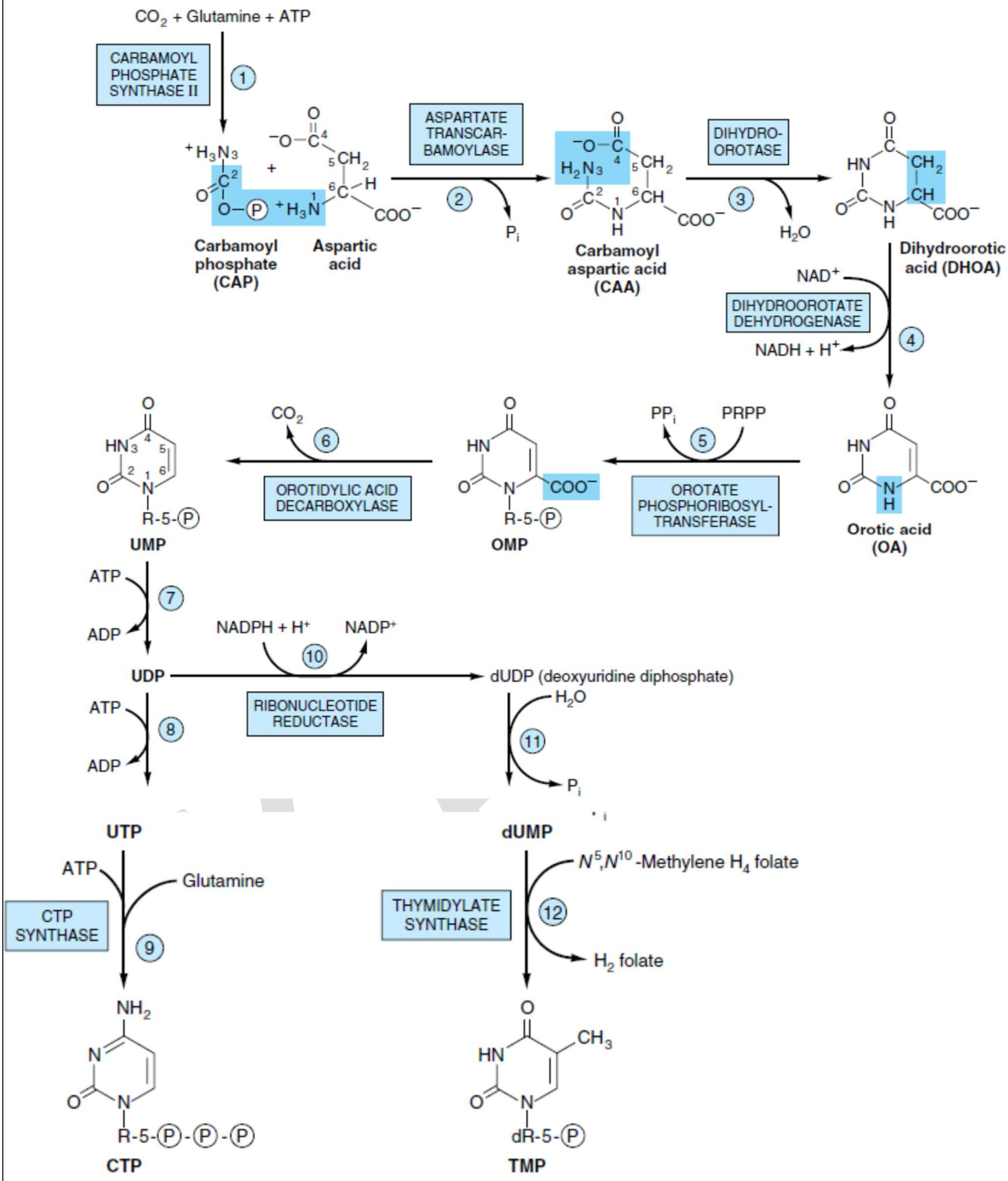


Figure 34–7. The biosynthetic pathway for pyrimidine nucleotides.

Multifunctional Proteins Catalyze the Early Reactions of Pyrimidine Biosynthesis

Five of the first six enzyme activities of pyrimidine biosynthesis reside on multifunctional polypeptides. One such polypeptide catalyzes the first three reactions of Figure 34–2 and ensures efficient channeling of carbamoyl phosphate to pyrimidine biosynthesis. A second bifunctional enzyme catalyzes reactions 5 and 6.

THE DEOXYRIBONUCLEOSIDES OF URACIL & CYTOSINE ARE SALVAGED

While mammalian cells reutilize few free pyrimidines, "salvage reactions" convert the ribonucleosides uridine and cytidine and the deoxyribonucleosides thymidine and deoxycytidine to their respective nucleotides. ATPdependent phosphoryltransferases (kinases) catalyze the phosphorylation of the nucleoside diphosphates 2′-deoxycytidine, 2′-deoxyguanosine, and 2′-deoxyadenosine to their corresponding nucleoside triphosphates. In addition, orotate phosphoribosyltransferase (reaction 5, Figure 34–7), an enzyme of pyrimidine nucleotide synthesis, salvages orotic acid by converting it to orotidine monophosphate (OMP).

Methotrexate Blocks Reduction of Dihydrofolate

Reaction 12 of Figure 34–7 is the only reaction of pyrimidine nucleotide biosynthesis that requires a tetrahydrofolate derivative. The methylene group of *N*5,*N*10-methylene-tetrahydrofolate is reduced to the methyl group that is transferred, and tetrahydrofolate is oxidized to dihydroch34 For further pyrimidine synthesis to occur, dihydrofolate must be reduced back to tetrahydrofolate, a reaction catalyzed by dihydrofolate reductase. Dividing cells, which must generate TMP and dihydrofolate, thus are especially sensitive to inhibitors of dihydrofolate reductase such as the anticancer drug **methotrexate.**

Certain Pyrimidine Analogs Are Substrates for Enzymes of Pyrimidine Nucleotide Biosynthesis

Orotate phosphoribosyltransferase (reaction 5, Figure 34–7) converts the drug **allopurinol** (Figure 33–12) to a nucleotide in which the ribosyl phosphate is attached to N-1 of the pyrimidine ring. The anticancer drug **5-fluorouracil** (Figure 33–12) is also phosphoribosylated by orotate phosphoribosyl transferase.

REGULATION OF PYRIMIDINE NUCLEOTIDE BIOSYNTHESIS

Gene Expression & Enzyme Activity Both Are Regulated

The activities of the first and second enzymes of pyrimidine nucleotide biosynthesis are controlled by allosteric regulation. Carbamoyl phosphate synthase II (reaction 1, Figure 34–7) is inhibited by UTP and purine nucleotides but activated by PRPP. Aspartate transcarbamoylase (reaction 2, Figure 34–7) is inhibited by CTP but activated by ATP. In addition, the first three and the last two enzymes of the pathway are regulated by coordinate repression and derepression.

Purine & Pyrimidine Nucleotide Biosynthesis Are Coordinately Regulated

Purine and pyrimidine biosynthesis parallel one another mole for mole, suggesting coordinated control of their biosynthesis. Several sites of cross-regulation characterize purine and pyrimidine nucleotide biosynthesis. The PRPP synthase reaction (reaction 1, Figure 34–2), which forms a precursor essential for both processes, isfeedback-inhibited by both purine and pyrimidine nucleotides.

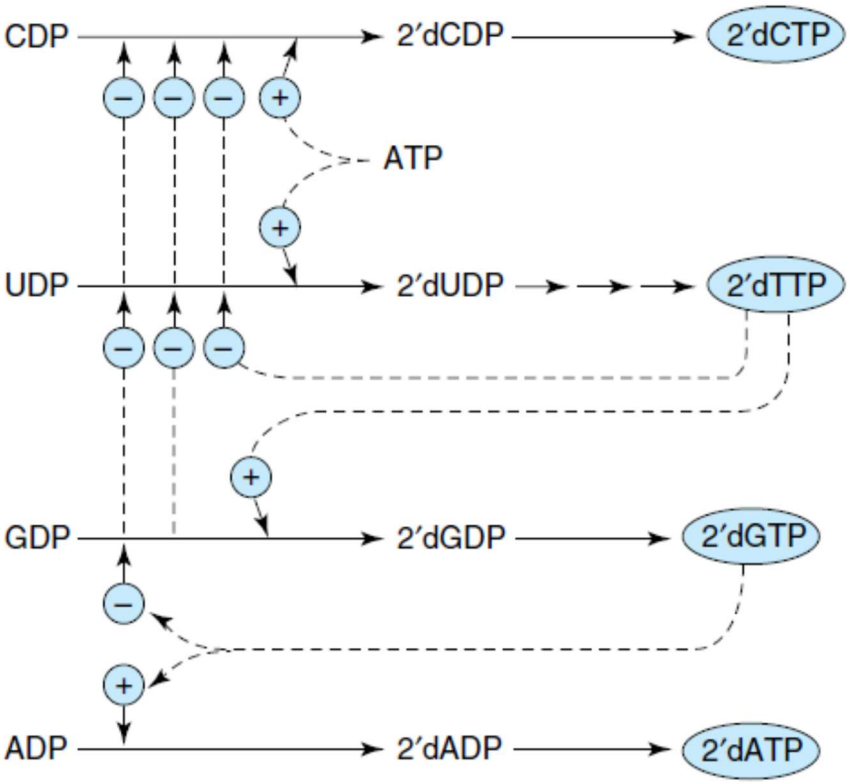
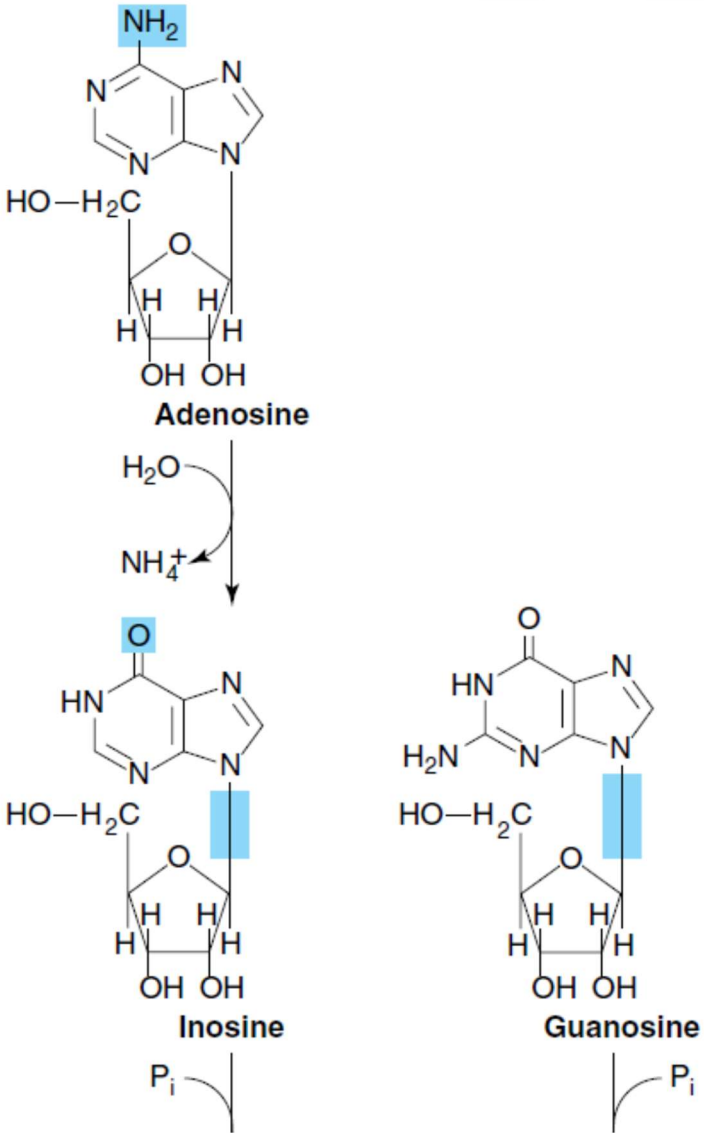


Figure 34–6. Regulation of the reduction of purine and pyrimidine ribonucleotides to their respective 2′-deoxyribonucleotides. Solid lines represent chemical flow. Broken lines show negative (_–) or positive (_+) feedback regulation.

HUMANS CATABOLIZE PURINES TO URIC ACID

Humans convert adenosine and guanosine to uric acid (Figure 34–8). Adenosine is first converted to inosine by adenosine deaminase. In mammals other than higher primates, uricase converts uric acid to the watersoluble product allantoin. However, since humans lack uricase, the end product of purine catabolism in humans is uric acid.



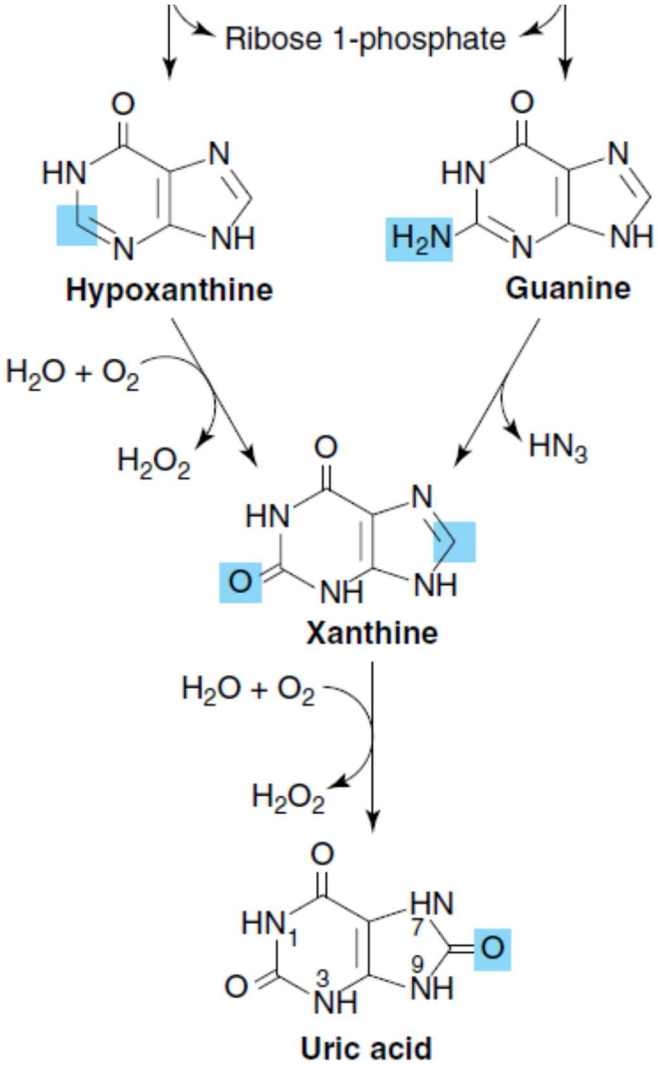


Figure 34-8. Catabolic pathway of purine nucleotides

GOUT IS A METABOLIC DISORDER OF PURINE CATABOLISM

Various genetic defects in PRPP synthetase (reaction 1, Figure 34–2) present clinically as gout. Each defect— eg, an elevated Vmax, increased affinity for ribose 5- phosphate, or resistance to feedback inhibition—results in overproduction and overexcretion of purine catabolites. When serum urate levels exceed the solubility limit, sodium urate crystalizes in soft tissues and joints and causes an inflammatory reaction, **gouty arthritis.** However, most cases of gout reflect abnormalities in renal handling of uric acid.

OTHER DISORDERS OF PURINE CATABOLISM

While purine deficiency states are rare in human subjects, there are numerous genetic disorders of purine catabolism. **Hyperuricemias** may be differentiated based on whether patients excrete normal or excessive quantities of total urates. Some hyperuricemias reflect specific enzyme defects. Others are secondary to diseases such as cancer or psoriasis that enhance tissue turnover.

Lesch-Nyhan Syndrome

Lesch-Nyhan syndrome, an overproduction hyperuricemia characterized by frequent episodes of uric acid lithiasis and a bizarre syndrome of self-mutilation, reflects a defect in **hypoxanthine-guanine phosphoribosyl transferase,** an enzyme of purine salvage (Figure 34–4). The accompanying rise in intracellular PRPP results in purine overproduction. Mutations that decrease or abolish hypoxanthine-guanine phosphoribosyltransferase activity include deletions, frameshift mutations, base substitutions, and aberrant mRNA splicing.

Von Gierke's Disease

Purine overproduction and hyperuricemia in von Gierke's disease **(glucose-6-phosphatase deficiency)** occurs secondary to enhanced generation of the PRPP precursor ribose 5-phosphate. An associated lactic acidosis elevates the renal threshold for urate, elevating total body urates.

Hypouricemia

Hypouricemia and increased excretion of hypoxanthine and xanthine are associated with **xanthine oxidase deficiency** due to a genetic defect or to severe liver damage. Patients with a severe enzyme deficiency may exhibit xanthinuria and xanthine lithiasis.

Adenosine Deaminase & Purine Nucleoside Phosphorylase Deficiency

Adenosine deaminase deficiency is associated with an immunodeficiency disease in which both thymusderived lymphocytes (T cells) and bone marrow-derived lymphocytes (B cells) are sparse and dysfunctional.

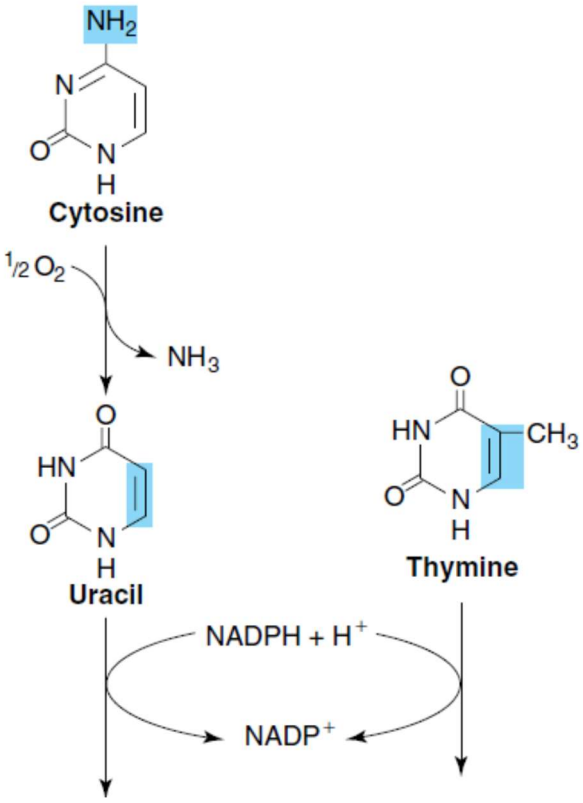
Purine nucleoside phosphorylase deficiency is associated with a severe deficiency of T cells but apparently normal B cell function. Immune dysfunctions appear to result from accumulation of dGTP and dATP, which inhibit ribonucleotide reductase and thereby deplete cells of DNA precursors.

CATABOLISM OF PYRIMIDINES PRODUCES WATER-SOLUBLE METABOLITES

Unlike the end products of purine catabolism, those of pyrimidine catabolism are highly water-soluble: CO2, NH3, β-alanine, and β-aminoisobutyrate (Figure 34–9). Excretion of β-aminoisobutyrate increases in leukemia and severe x-ray radiation exposure due to increased destruction of DNA. However, many persons of Chinese or Japanese ancestry routinely excrete β-aminoisobutyrate. Humans probably transaminate β-aminoisobutyrate to methylmalonate semialdehyde, which then forms succinyl-CoA (Figure 19–2).

Pseudouridine Is Excreted Unchanged

Since no human enzyme catalyzes hydrolysis or phosphorolysis of pseudouridine, this unusual nucleoside is excreted unchanged in the urine of normal subjects.



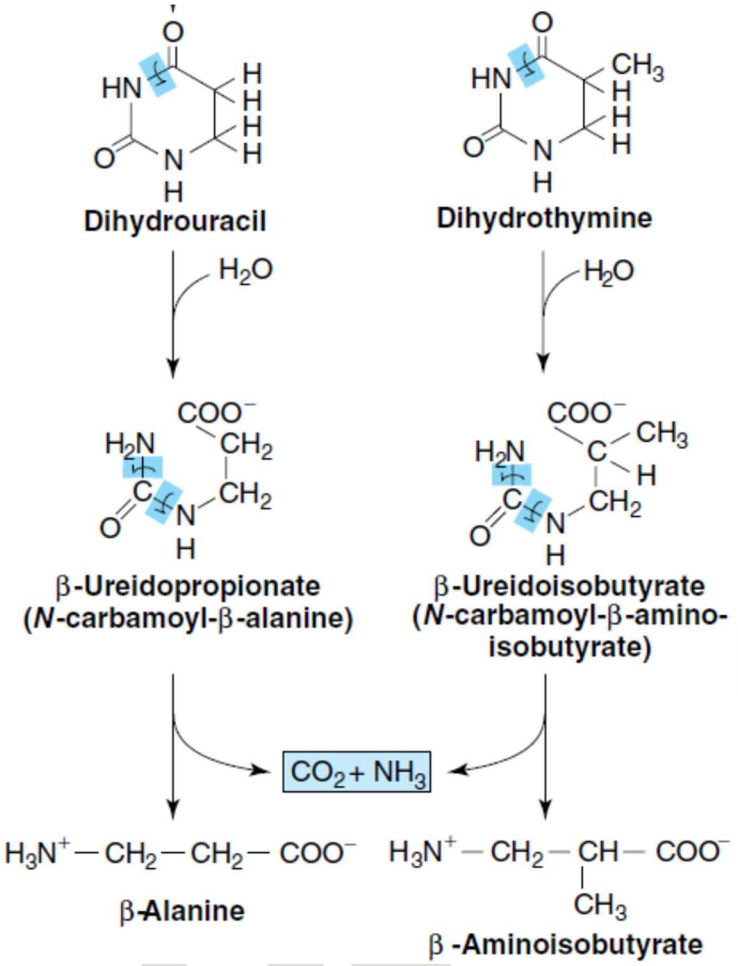


Figure 34-9. Catabolic pathway of pyrimidine nucleotides

OVERPRODUCTION OF PYRIMIDINE CATABOLITES IS ONLY RARELY ASSOCIATED WITH CLINICALLY SIGNIFICANT ABNORMALITIES

Since the end products of pyrimidine catabolism are highly water-soluble, pyrimidine overproduction results in few clinical signs or symptoms. In hyperuricemia associated with severe overproduction of PRPP, there is overproduction of pyrimidine nucleotides and increased excretion of β-alanine. Since *N*5,*N*10-methylene- tetrahydrofolate is required for thymidylate synthesis, disorders of folate and vitamin B12 metabolism result in deficiencies of TMP.

Orotic Acidurias

The orotic aciduria that accompanies **Reye's syndrome** probably is a consequence of the inability of severely damaged mitochondria to utilize carbamoyl phosphate, which then becomes available for cytosolic overproduction of orotic acid. **Type I orotic aciduria** reflects a deficiency of both orotate phosphoribosyltransferase and orotidylate decarboxylase (reactions 5 and 6, Figure 34–7); the rarer **type II orotic aciduria** is due to a deficiency only of orotidylate decarboxylase (reaction 6, Figure 34–7).

Deficiency of a Urea Cycle Enzyme Results in Excretion of Pyrimidine Precursors

Increased excretion of orotic acid, uracil, and uridine accompanies a deficiency in liver mitochondrial ornithine transcarbamoylase (reaction 2, Figure 29–9). Excess carbamoyl phosphate exits to the cytosol, where it stimulates pyrimidine nucleotide biosynthesis. The resulting mild **orotic aciduria** is increased by highnitrogen foods.

Drugs May Precipitate Orotic Aciduria

Allopurinol (Figure 33–12), an alternative substrate for orotate phosphoribosyltransferase (reaction 5, Figure 34–7), competes with orotic acid. The resulting nucleotide product also inhibits orotidylate decarboxylase (reaction 6, Figure 34–7), resulting in **orotic aciduria** and **orotidinuria.** 6-Azauridine, following conversion to 6-azauridylate, also competitively inhibits orotidylate decarboxylase (reaction 6, Figure 34–7), enhancing excretion of orotic acid and orotidine.

OXIDATIVE PHOSPHORYLATION

Electron-Transfer Reactions in Mitochondria

The discovery in 1948 by Eugene Kennedy and Albert Lehninger that mitochondria are the site of oxidative phosphorylation in eukaryotes marked the beginning of the modern phase of studies

in biological energy transductions. Mitochondria, like gramnegative bacteria, have two membranes (Fig. 19–1). The outer mitochondrial membrane is readily permeable to small molecules (*M*r _5,000) and ions, which move freely through transmembrane channels formed by a family of integral membrane proteins called porins. The inner membrane is impermeable to most small molecules and ions, including protons (H_); the only species that cross this membrane do so through specific transporters. The inner membrane bears the components of the respiratory chain and the ATP synthase. The mitochondrial matrix, enclosed by the inner membrane, contains the pyruvate dehydrogenase complex and the enzymes of the citric acid cycle, the fatty acid *β*-oxidation pathway, and the pathways of amino acid oxidation—all the pathways of fuel oxidation except glycolysis, which takes place in the cytosol. The selectively permeable inner membrane segregates the intermediates and enzymes of cytosolic metabolic pathways from those of metabolic processes occurring in the matrix. However, specific transporters carry pyruvate, fatty acids, and amino acids or their α-keto derivatives into the matrix for access to the machinery of the citric acid cycle. ADP and Pi are specifically transported into the matrix as newly synthesized ATP is transported out.

UNIVERSAL ELECTRON ACCEPTORS

Oxidative phosphorylation begins with the entry of electrons into the respiratory chain. Most of these electrons arise from the action of dehydrogenases that collect electrons from catabolic pathways and funnel them into universal electron acceptors—nicotinamide nucleotides (NAD_ or NADP_) or flavin nucleotides (FMN or FAD).

Nicotinamide nucleotide–linked dehydrogenases

catalyze reversible reactions of the following general types:

$$\text{Reduced substrate} + \text{NAD}^+ \rightleftharpoons \text{oxidized substrate} + \text{NADH} + \text{H}^+$$

$$\text{Reduced substrate} + \text{NADP}^+ \rightleftharpoons \text{oxidized substrate} + \text{NADPH} + \text{H}^+$$

Most dehydrogenases that act in catabolism are specific for NAD_ as electron acceptor (Table 19–1). Some are in the cytosol, others are in mitochondria, and still others have mitochondrial and cytosolic isozymes. NAD-linked dehydrogenases remove two hydrogen atoms from their substrates. One of these is transferred as a hydride ion (**:**H_) to NAD_; the other is released as H_ in the medium (see Fig. 13–15). NADH and NADPH are water-soluble electron carriers that associate *reversibly* with dehydrogenases. NADH carries electrons from catabolic reactions to their point of entry into the respiratory chain, the NADH dehydrogenase complex described below. NADPH generally supplies electrons to anabolic reactions. Cells maintain separate pools of NADPH and NADH, with different redox potentials. This is accomplished by holding the ratios of [reduced form]/[oxidized form] relatively high for NADPH and relatively low for NADH. Neither NADH nor NADPH can cross the inner mitochondrial membrane, but the electrons they carry can be shuttled across indirectly, as we shall see.

Flavoproteins contain a very tightly, sometimes covalently, bound flavin nucleotide, either FMN or FAD (see Fig. 13–18). The oxidized flavin nucleotide can accept either one electron (yielding the semiquinone form) or two (yielding FADH2 or FMNH2). Electron transfer occurs because the flavoprotein has a higher reduction potential than the compound oxidized. The standard reduction potential of a flavin nucleotide, unlike that of NAD or NADP, depends on the protein with which it is associated. Local interactions with functional groups in the protein distort the electron orbitals in the flavin ring, changing the relative stabilities of oxidized and reduced forms. The relevant standard reduction potential is therefore that of the particular flavoprotein, not that of isolated FAD or FMN. The flavin nucleotide should be considered part of the flavoprotein's active site rather than a reactant or product in the electrontransfer reaction. Because flavoproteins can participate in either one- or two-electron transfers, they can serve as intermediates between reactions in which two electrons are donated (as in dehydrogenations) and those in which only one electron is accepted (as in the reduction of a quinone to a hydroquinone, described below).

Electrons Carriers

The mitochondrial respiratory chain consists of a series of sequentially acting electron carriers, most of which are integral proteins with prosthetic groups capable of accepting and donating either one or two electrons. Three types of electron transfers occur in oxidative phosphorylation: (1) direct transfer of electrons, as in the reduction of Fe3_ to Fe2_;

(2) transfer as a hydrogen atom (H_ _ *e*_); and (3) transfer as a hydride ion (**:**H_), which bears two electrons. The term **reducing equivalent** is used to designate a single electron equivalent transferred in an oxidation-reduction reaction. In addition to NAD and flavoproteins, three other types of electron-carrying molecules function in the respiratory chain: a hydrophobic quinone (ubiquinone) and two different types of iron-containing proteins (cytochromes and iron-sulfur proteins). **Ubiquinone** (also called **coenzyme Q,** or simply **Q**) is a lipid-soluble benzoquinone with a long isoprenoid side chain (Fig. 19–2). The closely related compounds plastoquinone (of plant chloroplasts) and menaquinone (of bacteria) play roles analogous to that of ubiquinone, carrying electrons in membrane-associated electron-transfer chains. Ubiquinone can accept one electron to become the semiquinone radical (_QH) or two electrons to form ubiquinol (QH2) (Fig. 19–2) and, like flavoprotein carriers, it can act at the junction between a two-electron donor and a one-electron acceptor. Because ubiquinone is both small and hydrophobic, it is freely diffusible within the lipid bilayer of the inner mitochondrial membrane and can shuttle reducing equivalents between other, less mobile electron carriers in the membrane. And because it carries both electrons and protons, it plays a central role in coupling electron flow to proton movement.

The **cytochromes** are proteins with characteristic strong absorption of visible light, due to their ironcontaining heme prosthetic groups (Fig. 19–3). Mitochondria contain three classes of cytochromes, designated *a, b,* and *c,* which are distinguished by differences in their light-absorption spectra. Each type of cytochrome in its reduced (Fe2_) state has three absorption bands in the visible range (Fig. 19–4). The longestwavelength band is near 600 nm in type *a* cytochromes, near 560 nm in type *b,* and near 550 nm in type *c.* To distinguish among closely related cytochromes of one type, the exact absorption maximum is sometimes used in the names, as in cytochrome *b*562. The heme cofactors of *a* and *b* cytochromes are tightly, but not covalently, bound to their associated proteins; the hemes of *c*-type cytochromes are covalently

attached through Cys residues (Fig. 19–3). As with the flavoproteins, the standard reduction potential of the heme iron atom of a cytochrome depends on its interaction with protein side chains and is therefore different for each cytochrome. The cytochromes of type *a* and *b* and some of type *c* are integral proteins of the inner mitochondrial membrane. One striking exception is the cytochrome *c* of mitochondria, a soluble protein that associates through electrostatic interactions with the outer surface of the inner membrane. We encountered cytochrome *c* in earlier discussions of protein structure (see Fig. 4–18).

In **iron-sulfur proteins,** first discovered by Helmut Beinert, the iron is present not in heme but in association with inorganic sulfur atoms or with the sulfur atoms of Cys residues in the protein, or both. These iron-sulfur (Fe-S) centers range from simple structures with a single Fe atom coordinated to four Cys OSH groups to more complex Fe-S centers with two or four Fe atoms.

Rieske iron-sulfur proteins (named after their discoverer, John S. Rieske) are a variation on this theme, in which one Fe atom is coordinated to two His residues rather than two Cys residues. All iron-sulfur proteins participate in one-electron transfers in which one iron atom of the iron-sulfur cluster is oxidized or reduced. At least eight Fe-S proteins function in mitochondrial electron transfer. The reduction potential of Fe-S proteins varies from _0.65 V to _0.45 V, depending on the microenvironment of the iron within the protein. In the overall reaction catalyzed by the mitochondrial respiratory chain, electrons move from NADH, succinate, or some other primary electron donor through flavoproteins, ubiquinone, iron-sulfur proteins, and cytochromes, and finally to O2. A look at the methods used to determine the sequence in which the carriers act is instructive, as the same general approaches have been used to study other electron-transfer chains, such as those of chloroplasts. First, the standard reduction potentials of the individual electron carriers have been determined experimentally (Table 19–2). We would expect the carriers to function in order of increasing reduction potential, because electrons tend to flow spontaneously from carriers of lower $E_$ to carriers of higher $E_$. The order of carriers deduced by this method is NADH → Q → cytochrome *b* → cytochrome *c*1 → cytochrome *c* → cytochrome *a* → cytochrome *a*3 → O2. Note, however, that the order of standard reduction potentials is not necessarily the same as the order of *actual* reduction potentials under cellular

conditions, which depend on the concentration of reduced and oxidized forms (p. 510). A second method for determining the sequence of electron carriers involves reducing the entire chain of carriers experimentally by providing an electron source but no electron acceptor (no O2). When O2 is suddenly introduced into the system, the rate at which each electron carrier becomes oxidized (measured spectroscopically) reveals the order in which the carriers function. The carrier nearest O2 (at the end of the chain) gives up its electrons first, the second carrier from the end is oxidized next, and so on. Such experiments have confirmed the sequence deduced from standard reduction potentials. In a final confirmation, agents that inhibit the flow of electrons through the chain have been used in combination with measurements of the degree of oxidation of each carrier. In the presence of O2 and an electron donor, carriers that function before the inhibited step become fully reduced, and those that function after this step are completely oxidized (Fig. 19–6). By using several inhibitors that block different steps in the chain, investigators have determined the entire sequence; it is the same as deduced in the first two approaches.

ELECTRON CARRIERS FUNCTION IN MULTIENZYME COMPLEXES

The electron carriers of the respiratory chain are organized into membrane-embedded supramolecular complexes that can be physically separated. Gentle treatment of the inner mitochondrial membrane with detergents allows the resolution of four unique electroncarrier complexes, each capable of catalyzing electron transfer through a portion of the chain (Table 19–3; Fig.19–7). Complexes I and II catalyze electron transfer to ubiquinone from two different electron donors: NADH (Complex I) and succinate (Complex II). Complex III carries electrons from reduced ubiquinone to cytochrome *c,* and Complex IV completes the sequence by transferring electrons from cytochrome *c* to O2. We now look in more detail at the structure and function of each complex of the mitochondrial respiratory chain.

COMPLEX I:

NADH to Ubiquinone illustrates the relationship between Complexes I and II and ubiquinone. **Complex I,** also called **NADH:ubiquinone oxidoreductase** or **NADH dehydrogenase,** is a large enzyme composed of 42 different polypeptide chains, including an FMN-containing

flavoprotein and at least six ironsulfur centers. High-resolution electron microscopy shows Complex I to be L-shaped, with one arm of the L in the membrane and the other extending into the matrix. Complex I catalyzes two simultaneous and obligately coupled processes:

(1) the exergonic transfer to ubiquinone of a hydride ion from NADH and a proton from the matrix, expressed by

$$NADH + H^+ + Q \longrightarrow NAD^+ + QH_2$$

and

(2) the endergonic transfer of four protons from thematrix to the intermembrane space. Complex I is therefore a proton pump driven by the energy of electron transfer, and the reaction it catalyzes is **vectorial:** it moves protons in a specific direction from one location (the matrix, which becomes negatively charged with the departure of protons) to another (the intermembrane space, which becomes positively charged). To emphasize the vectorial nature of the process, the overall reaction is often written with subscripts that indicate the location of the protons: P for the positive side of the inner membrane (the intermembrane space), N for the negative side (the matrix):

$$NADH + 5H^+_N + Q \longrightarrow NAD^+ + QH_2 + 4H^+_P$$

Amytal (a barbiturate drug), rotenone (a plant product commonly used as an insecticide), and piericidin A (an antibiotic) inhibit electron flow from the Fe-S centers of Complex I to ubiquinone (Table 19–4) and therefore block the overall process of oxidative phosphorylation. Ubiquinol (QH2, the fully reduced form; Fig. 19–2) diffuses in the inner mitochondrial membrane from Complex I to Complex III, where it is oxidized to Q in a process that also involves the outward movement of H+.

COMPLEX II: Succinate to Ubiquinone We encountered **Complex II** in Chapter 16 as **succinate dehydrogenase,** the only membrane-bound enzyme in the citric acid cycle (p. 612). Although smaller and simpler than Complex I, it contains five prosthetic groups of two types and four different protein subunits (Fig. 19–10). Subunits C and D are integral membrane proteins,

each with three transmembrane helices. They contain a heme group, heme *b,* and a binding site for ubiquinone, the final electron acceptor in the reaction catalyzed by Complex II. Subunits A and B extend into the matrix (or the cytosol of a bacterium); they contain three 2Fe-2S centers, bound FAD, and a binding site for the substrate, succinate. The path of electron transfer from the succinate-binding site to FAD, then through the Fe-S centers to the Q-binding site, is more than 40 Å long, but none of the individual electron-transfer distances exceeds about 11 Å—a reasonable distance for rapid electron transfer.

Clinical aspaect: The heme *b* of Complex II is apparently not in the direct path of electron transfer; it may serve instead to reduce the frequency with which electrons "leak" out of the system, moving from succinate to molecular oxygen to produce the **reactive oxygen species (ROS)** hydrogen peroxide (H2O2) and the **superoxide radical** ($\cdot O_2^-$) described in Section 19.5. Humans with point mutations in Complex II subunits near heme *b* or the quinone-binding site suffer from hereditary paraganglioma. This inherited condition is characterized by benign tumors of the head and neck, commonly in the carotid body, an organ that senses O2 levels in the blood. These mutations result in greater production of ROS and perhaps greater tissue damage during succinate oxidation. ■

Other substrates for mitochondrial dehydrogenases pass electrons into the respiratory chain at the level of ubiquinone, but not through Complex II. The first step in the _ oxidation of fatty acyl–CoA, catalyzed by the flavoprotein **acyl-CoA dehydrogenase** (see Fig. 17–8), involves transfer of electrons from the substrate to the FAD of the dehydrogenase, then to electron-transferring flavoprotein (ETF), which in turn passes its electrons to **ETF: ubiquinone oxidoreductase**. This enzyme transfers electrons into the respiratory chain by reducing ubiquinone. Glycerol 3-phosphate, formed either from glycerol released by triacylglycerol breakdown or by the reduction of dihydroxyacetone phosphate from glycolysis, is oxidized by **glycerol 3-phosphate dehydrogenase** (see Fig. 17–4). This enzyme is a flavoprotein located on the outer face of the inner mitochondrial membrane, and like succinate dehydrogenase and acyl-CoA dehydrogenase it channels electrons into the respiratory chain by reducing ubiquinone. The effect of each of these electron-transferring enzymes is to contribute to the pool of reduced ubiquinone. QH2 from all these reactions is reoxidized by Complex III.

COMPLEX III: Ubiquinone to Cytochrome c The next respiratory complex, **Complex III,** also called **cytochrome *bc*1 complex** or **ubiquinone:cytochrome *c* oxidoreductase,** couples the transfer of electrons from ubiquinol (QH2) to cytochrome *c* with the vectorial transport of protons from the matrix to the intermembrane space. The determination of the complete structure of this huge complex (Fig. 19–11) and of Complex IV (below) by x-ray crystallography, achieved between 1995 and 1998, were landmarks in the study of mitochondrial electron transfer, providing the structural framework to integrate the many biochemical observations on the functions of the respiratory complexes. Based on the structure of Complex III and detailed biochemical studies of the redox reactions, a reasonable model has been proposed for the passage of electrons and protons through the complex. The net equation for the redox reactions of this **Q cycle** is

$$QH_2 + 2 \text{ cyt } c_1(\text{oxidized}) + 2H^+_N \longrightarrow Q + 2 \text{ cyt } c_1(\text{reduced}) + 4H^+_P$$

The Q cycle accommodates the switch between the twoelectron carrier ubiquinone and the one-electron carriers— cytochromes *b*562, *b*566, *c*1, and *c*—and explains the measured stoichiometry of four protons translocated per pair of electrons passing through the Complex III to cytochrome *c.* Although the path of electrons through this segment of the respiratory chain is complicated, the net effect of the transfer is simple: QH2 is oxidized to Q and two molecules of cytochrome *c* are reduced. Cytochrome *c* (see Fig. 4–18) is a soluble protein of the intermembrane space. After its single heme accepts an electron from Complex III, cytochrome *c* moves to Complex IV to donate the electron to a binuclear copper center.

COMPLEX IV: Cytochrome c to O2 In the final step of the respiratory chain, **Complex IV,** also called **cytochrome oxidase,** carries electrons from cytochrome *c* to molecular oxygen, reducing it to H2O. Complex IV is a large enzyme (13 subunits; *M*r 204,000) of the inner mitochondrial membrane. Bacteria contain a form that is much simpler, with only three or four subunits, but still capable of catalyzing both electron transfer and proton pumping. Comparison of the

mitochondrial and bacterial complexes suggests that three subunits are critical to the function (Fig. 19–13). Mitochondrial subunit II contains two Cu ions complexed with the OSH groups of two Cys residues in a binuclear center (CuA; Fig. 19–13b) that resembles the 2Fe-2S centers of iron-sulfur proteins. Subunit I contains two heme groups, designated *a* and *a*3, and another copper ion (CuB). Heme *a*3 and CuB form a second binuclear center that accepts electrons from heme *a* and transfers them to O2 bound to heme *a*3. Electron transfer through Complex IV is from cytochrome *c* to the CuA center, to heme *a,* to the heme *a*3–CuB center, and finally to O2. For every four electrons passing through this complex, the enzyme consumes four "substrate" H_ from the matrix (N side) in converting O2 to 2H2O. It also uses the energy of this redox reaction to pump one proton outward into the intermembrane space (P side) for each electron that passes through, adding to the electrochemical potential produced by redox-driven proton transport through Complexes I and III. The overall reaction catalyzed by Complex IV is

$$4\ \text{Cyt}\ c\ (\text{reduced}) + 8\text{H}^+_\text{N} + \text{O}_2 \longrightarrow 4\ \text{cyt}\ c\ (\text{oxidized}) + 4\text{H}^+_\text{P} + 2\text{H}_2\text{O}$$

This four-electron reduction of O2 involves redox centers that carry only one electron at a time, and it must occur without the release of incompletely reduced intermediates such as hydrogen peroxide or hydroxyl free radicals—very reactive species that would damage cellular components. The intermediates remain tightly bound to the complex until completely converted to water.

ATP Synthesis

How is a concentration gradient of protons transformed into ATP? We have seen that electron transfer releases, and the proton-motive force conserves, more than enough free energy (about 200 kJ) per "mole" of electron pairs to drive the formation of a mole of ATP, which requires about 50 kJ (see Box 13–1). Mitochondrial oxidative phosphorylation therefore poses no thermodynamic problem. But what is the chemical mechanism that couples proton flux with phosphorylation?

STRUCTURE OF ATPase COMPLEX

Mitochondrial **ATP synthase** is an F-type ATPase similar in structure and mechanism to the ATP synthases of chloroplasts and eubacteria. This large enzyme complex of the inner mitochondrial membrane catalyzes the formation of ATP from ADP and Pi, accompanied by the flow of protons from the P to the N side of the membrane (Eqn 19–10). ATP synthase, also called Complex V, has two distinct components: F1, a peripheral membrane protein, and Fo (*o* denoting oligomycin-sensitive), which is integral to the membrane. F1, the first factor recognized as essential for oxidative phosphorylation, was identified and purified by Efraim Racker and his colleagues in the early 1960s.

In the laboratory, small membrane vesicles formed from inner mitochondrial membranes carry out ATP synthesis coupled to electron transfer. When F1 is gently extracted, the "stripped" vesicles still contain intact respiratory chains and the Fo portion of ATP synthase. The vesicles can catalyze electron transfer from NADH to O2 but cannot produce a proton gradient: Fo has a proton pore through which protons leak as fast as they are pumped by electron transfer, and without a proton gradient the F1-depleted vesicles cannot make ATP. Isolated F1 catalyzes ATP hydrolysis (the reversal of synthesis) and was therefore originally called **F1ATPase.** When purified F1 is added back to the depleted vesicles, it reassociates with Fo, plugging its proton pore and restoring the membrane's capacity to couple electron transfer and ATP synthesis.

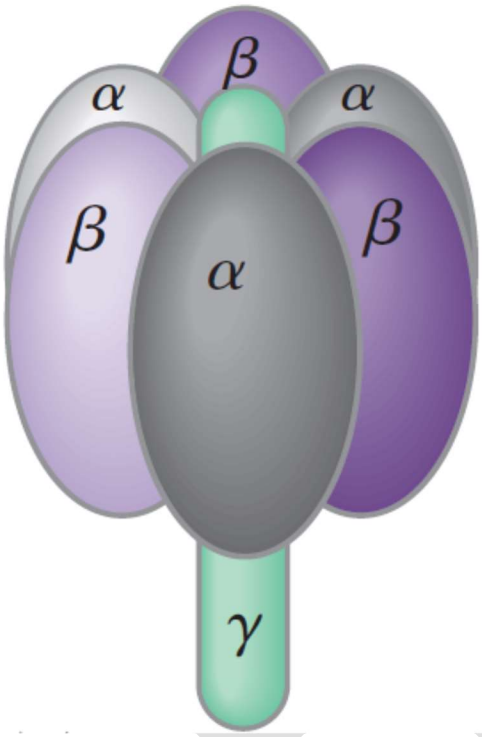


FIGURE 19–23 Mitochondrial ATP synthase complex. (a) Structure of the F1 complex, deduced from crystallographic and biochemical studies. In F1, three _ and three _ subunits are arranged like the segments of an orange, with alternating _ (shades of gray) and _ (shades of purple) subunits around a central shaft, the _ subunit (green).

CHEMIOSMOTIC MODEL

The **chemiosmotic model,** proposed by Peter Mitchell, is the paradigm for this mechanism. According to the model (Fig. 19–17), the electrochemical energy inherent in the difference in proton concentration and separation of charge across the inner mitochondrial membrane—the proton-motive force—drives the synthesis of ATP as protons flow passively back into the matrix through a proton pore associated with **ATP synthase.** To emphasize this crucial role of the protonmotive force, the equation for ATP synthesis is sometimes written

$$ADP + P_i + nH^+_P \longrightarrow ATP + H_2O + nH^+_N$$

Mitchell used "chemiosmotic" to describe enzymatic reactions that involve, simultaneously, a chemical reaction and a transport process. The operational definition of "coupling" is shown in

Figure 19–18. When isolated mitochondria are suspended in a buffer containing ADP, Pi, and an oxidizable substrate such as succinate, three easily measured processes occur: (1) the substrate is oxidized (succinate yields fumarate), (2) O2 is consumed, and (3) ATP is synthesized. Oxygen consumption and ATP synthesis depend on the presence of an oxidizable substrate (succinate in this case) as well as ADP and Pi.

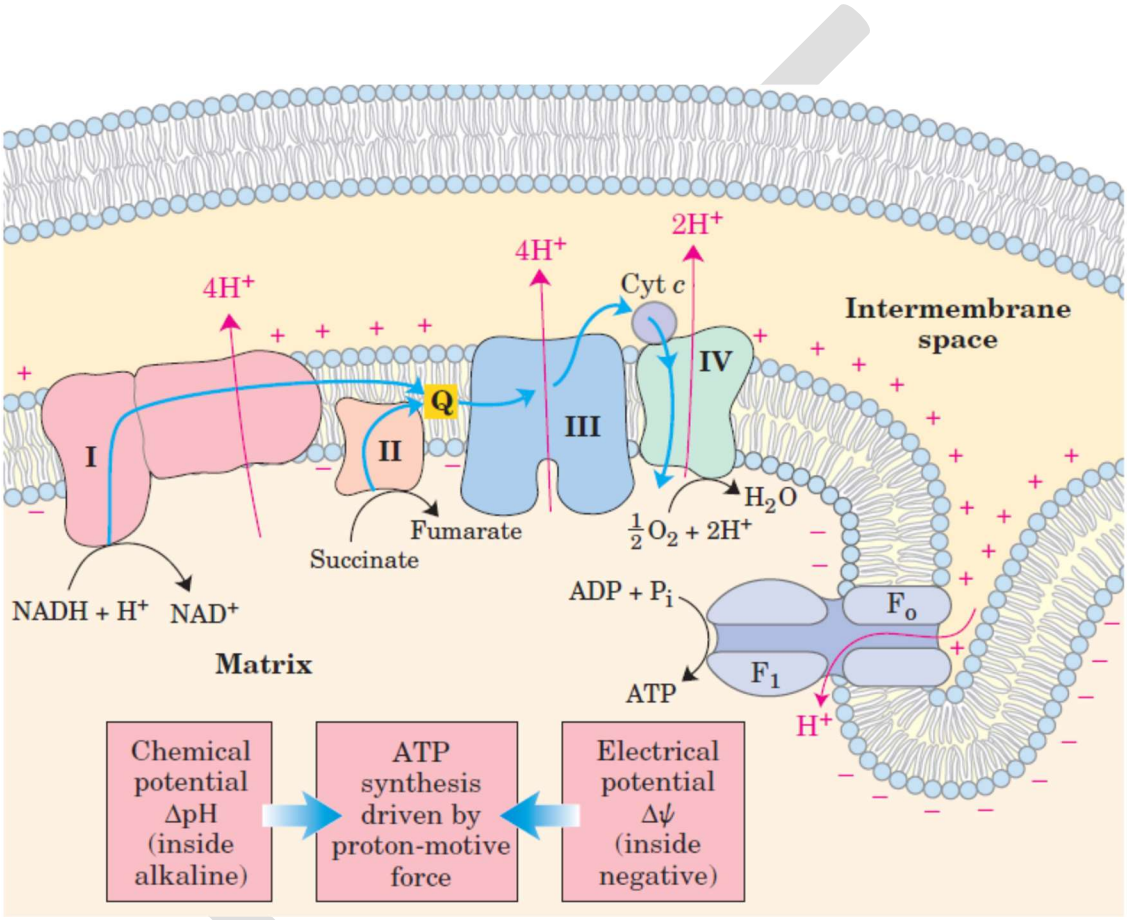


FIGURE 19–17 Chemiosmotic model. In this simple representation of the chemiosmotic theory applied to mitochondria, electrons from NADH and other oxidizable substrates pass through a chain of carriers arranged asymmetrically in the inner membrane. Electron flow is accompanied by proton transfer across the membrane, producing both a chemical gradient (ΔpH) and an electrical gradient (ΔΨ). The inner mitochondrial membrane is impermeable to protons; protons can reenter the matrix only through proton-specific channels (Fo). The proton-motive force that

drives protons back into the matrix provides the energy for ATP synthesis, catalyzed by the F1 complex associated with Fo.

INHIBITORS OF ATP SYNTHESIS

Since the energy of substrate oxidation drives ATP synthesis in mitochondria, we would expect inhibitors of the passage of electrons to O2 (such as cyanide, carbon monoxide, and antimycin A) to block ATP synthesis (Fig. 19–18a). More surprising is the finding that the converse is also true: inhibition of ATP synthesis blocks electron transfer in intact mitochondria. This obligatory coupling can be demonstrated in isolated mitochondria by providing O2 and oxidizable substrates, but not ADP (Fig. 19–18b). Under these conditions, no ATP synthesis can occur and electron transfer to O2 does not proceed. Coupling of oxidation and phosphorylation can also be demonstrated using oligomycin or venturicidin, toxic antibiotics that bind to the ATP synthase in mitochondria. These compounds are potent inhibitors of both ATP synthesis *and* the transfer of electrons through the chain of carriers to O2. Because oligomycin is known to interact not directly with the electron carriers but with ATP synthase, it follows that electron transfer and ATP synthesis are obligately coupled; neither reaction occurs without the other. Chemiosmotic theory readily explains the dependence of electron transfer on ATP synthesis in mitochondria. When the flow of protons into the matrix through the proton channel of ATP synthase is blocked (with oligomycin, for example), no path exists for the return of protons to the matrix, and the continued extrusion of protons driven by the activity of the respiratory chain generates a large proton gradient. The proton-motive force builds up until the cost (free energy) of pumping protons out of the matrix against this gradient equals or exceeds the energy released by the transfer of electrons from NADH to O2. At this point electron flow must stop; the free energy for the overall process of electron flow coupled to proton pumping becomes zero, and the system is at equilibrium.

INHIBITORS OF OXIDATIVE PHOSPHORYLATION AND UNCOUPLERS

We have already encountered ATP synthase as an ATPdriven proton pump, catalyzing the reverse of ATP synthesis. When a cell is ischemic (deprived of oxygen), as in a heart attack or stroke, electron transfer to oxygen ceases, and so does the pumping of protons. The proton-motive force soon collapses. Under these conditions, the ATP synthase could operate in reverse,

hydrolyzing ATP to pump protons outward and causing a disastrous drop in ATP levels. This is prevented by a small (84 amino acids) protein inhibitor, IF1, which simultaneously binds to two ATP synthase molecules, inhibiting their ATPase activity. IF1 is inhibitory only in its dimeric form, which is favored at pH lower than 6.5. In a cell starved for oxygen, the main source of ATP becomes glycolysis, and the pyruvic or lactic acid thus formed lowers the pH in the cytosol and the mitochondrial matrix. This favors IF1 dimerization, leading to inhibition of the ATPase activity of ATP synthase, thereby preventing wasteful hydrolysis of ATP. When aerobic metabolism resumes, production of pyruvic acid slows, the pH of the cytosol rises, the IF1 dimer is destabilized, and the inhibition of ATP synthase is lifted.

UNCOUPLED MITOCHONDRIA IN BROWN FAT PRODUCE HEAT

There is a remarkable and instructive exception to the general rule that respiration slows when the ATP supply is adequate. Most newborn mammals, including humans, have a type of adipose tissue called **brown fat** in which fuel oxidation serves not to produce ATP but to generate heat to keep the newborn warm. This specialized adipose tissue is brown because of the presence of large numbers of mitochondria and thus large amounts of cytochromes, whose heme groups are strong absorbers of visible light. The mitochondria of brown fat are like those of other mammalian cells in all respects, except that they have a unique protein in their inner membrane. **Thermogenin,** also called the **uncoupling protein**, provides a path for protons to return to the matrix without passing through the FoF1 complex.

POSSIBLE QUESTIONS

UNIT V

PART A (1 mark)

Question number 1-20 (From given 300 all possible MCQs)

PART B (2 Marks)

1. Write a short note on purine nucleotides with examples.
2. Write a brief note on the structure of pyrimidine nucleotides.
3. Give brief notes on ETC.
4. Define Oxidative phosphorylation
5. Explain briefly about ATPase complex with a neat diagram
6. Write notes on Chemiosmotic theory

PART C (6 Marks)

1. Elaborate about the biosynthesis of purine nucleotides
2. Explain in detail the biosynthesis of pyrimidine nucleotides
3. Explain in detail about degradation of purine and pyrimidine nucleotides
4. Give a detail account on ETC and electron carriers.

Unit V								
S.No	**QUESTION**	**Option 1**	**Option 2**	**Option 3**	**Option 4**	**Option 5**	**Option 6**	**Answer**
1	Identify the purine base of nucleic acids in the following	Cytosine	Thymine	Uracil	Adenine			**Adenine**
2	Which of the following are not the components of RNA?	Cytosine	Thymine	Uracil	Adenine			**Thymine**
3	Which of the following statements is true?	Sugar component of a nucleotide is ribose	Sugar component of a nucleotide is deoxyribose	The bases in nucleotides are attached to a pentose sugar moiety by a glycosidic linkage	The sugar molecule of the nucleotide is in L-configuration			**The bases in nucleotides are attached to a pentose sugar moiety by a glycosidic linkage**
4	What is the composition of nucleoside?	a sugar + a phosphate	a base + a sugar	a base + a phosphate	a base + a sugar + phosphate			**a base + a sugar**
5	What is the composition of nucleotide?	a sugar + a phosphate	a base + a sugar	a base + a phosphate	a base + a sugar + phosphate			**a base + a sugar + phosphate**
6	Group of adjacent nucleotides are joined by	Phosphodiester bond	Peptide bond	Ionic bond	Covalent bond			**Phosphodiester bond**
7	The sugar molecule in a nucleotide is	Pentose	Hexose	Tetrose	Triose			**Pentose**
8	Building blocks of nucleic acids are	Nucleotides	Nucleosides	Amino acids	Histones			**Nucleotides**
9	Which macromolecule is not abundantly found though being of critical importance for biological mechanism?	Proteins	Lipids	Nucleic acid	Polysaccharides			**Nucleic acid**
10	What is the most common electron carrier in biological systems?	FAD	Coenzyme A	NAD	NADP			**NAD**
11	What is not a compound in the electron transport system?	NADH dehydrogenase	Flavoproteins	NADPH dehydrogenase	Coenzyme Q			**NADPH dehydrogenase**
12	The coupling of ATP synthesis to electron transport is known as	Oxidative phosphorylation	Chemiosmosis	ATP synthesis	Proton motive force			**Oxidative phosphorylation**
13	Which of the following drives the synthesis of ATP by ATP synthase in oxidative phosphorylation?	Distribution of electric potential across a membrane	Distribution of Cytochrome oxidase	Distribution of NADH	Distribution of FADH			**Distribution of electric potential across a membrane**
14	Which of the following is a coenzyme?	FAD	Ca^{2+}	Mg^{2+}	CO_2			**FAD**
15	An exergonic reaction is one in which	Electrons are added to a molecule	Electrons are removed from a molecule	The products have more free energy than the reactants	The reactants have more free energy than the products			**The reactants have more free energy than the products**
16	The main endergonic reaction that is driven by most of the body's exergonic reactions is the	Oxidation of $FADH_2$	Synthesis of ATP	Reduction of NAD	Hydrolysis of ATP			**Synthesis of ATP**
17	The "universal energy carrier" is	FAD	$FADH_2$	Glucose	Adenosine triphosphate			**Adenosine triphosphate**
18	If molecule A accepts electrons from molecule B, molecule A is	Reduced agent	A reducing agent	An oxidizing agent	An exergonic agent			**An oxidizing agent**
19	Any oxidation reaction must be coupled to	The synthesis of ATP	The availability of oxygen	An exergonic reaction	A reduction reaction			**A reduction reaction**
20	If a molecule accepts a hydrogen atom, it becomes	Hydrolyzed	Dehydrated	Oxidized	Reduced			**Reduced**
21	Nicotinamide adenine dinucleotide (NAD) is	A vitamin	An oxidizing agent	A reducing agent	A coenzyme			**A coenzyme**
22	In the electron transport chain, the hydrogen ions enter the inner compartment of mitochondria through special channels formed by	ATP synthase	Coenzyme A	Acetyl CoA	Oxygen			**ATP synthase**
23	Which process produces both NADH and FADH2?	The citric acid cycle	Glycolysis	The electron transport system	Fermentation			**The citric acid cycle**
24	Among the RNA's which of the following is very unstable	rRNA	tRNA	mRNA	5SRNA			**mRNA**
25	Ribose is linked with purine by	C_1 to N_1	C_1 to N_9	C_5 to N_9	C_1 to N_7			**C_1 to N_9**
26	mRNA has complementary sequence of	C-DNA	t-RNA	RNA	r-RNA			**t-RNA**
27	The higher percentage of RNA found is	Nucleus	Mitochondria	microsomes	golgibodies			**Nucleus**
28	RNA is easily hydrolysed by base due to	presence of OH group in position 2 of the ribose	Differences in the bases	Low molecular weight	none			**presence of OH group in position 2 of the ribose**
29	The enzymes of ETC belong to the following classes except	Oxidases	Dehydrogenases	Peroxidases	Reductases			**Peroxidases**
30	Which of the electron carriers is soluble and mobile	CoQ	Cytochrome c	Cytochrome a	Cytochrome b			**CoQ**
31	The final electron acceptor in lactic acid fermentation is:	NAD^+	Pyruvate	O_2	Lactic acid			**Pyruvate**
32	The concentration of ATP in living mammalian cells in mm is near	0.2	0.3	0.4	1			**1**
33	Enzymes catalyzing electron transport are present mainly in the	Ribosomes	Endoplasmic reticulum	Lysosomes	Inner mitochondrial membrane			**Inner mitochondrial membrane**
34	The power house of the cell is	Nucleus	Cell membrane	Mitochondria	Lysosomes			**Mitochondria**
35	Oxidation is a	Gain of the electron	Removal of an electron	Gain of an oxygen	Removal of an oxygen			**Removal of an electron**
36	Reduction is a	Removal of an oxygen	Gain of an oxygen	Gain of the electron	Removal of an electron			**Gain of the electron**
37	Electron transport and phosphorylation can be uncoupled by compounds that increase the permeability of the inner mitochondrial membrane to	Electrons	Protons	Uncouplers	Couplers			**Protons**
38	The function of an electron in the electron transport chain is	To transfer energy from complex II to complex I	To pump hydrogen ions using complex II	To use its free energy to pump protons against their concentration gradient	To combine with phosphate when ATP is synthesized			**To use its free energy to pump protons against their concentration gradient**
39	In aerobic cellular respiration, which generates more ATP?	Substrate-level phosphorylation	Chemiosmosis	Both generate the same amount of ATP	Neither generates any ATP			**Chemiosmosis**
40	Which of the following is a coenzyme associated with cellular respiration?	NAD^+	O_2	FAD	NAD and FAD			**NAD and FAD**
41	The final electron acceptor in the electron transport system is _____	$FADH_2$	O_2	Coenzyme Q	Cytochrome b			**O_2**
42	Oxidative phosphorylation occurs in	Mitochondria	Nucleus	Cell Membrane	Ribosomes			**Mitochondria**
43	Electrons in electron transport chains reduces oxygen in to	Carbondioxide	Water	Carbonmonoxide	Ozone			**Water**
44	Process of flow of electrons generated by oxidation of NADH and FADH2 is called	Phosphorylation	Electron transport chain	Dehydrogenation	Oxidative phosphorylation			**Electron transport chain**
45	Electron transport chain consists of how many complexes	7	5	3	4			**4**
46	Major components of electron transport chain are arranged in order of redox potential which is	Decreasing	Increasing	Variable	Alternatively increasing and decreasing			**Increasing**
47	Cytochromes are enzymes which function as electron transfer agent in	Hydrolysis	Conjugation reaction	Transamination	Oxidation and reduction			**Oxidation and reduction**
48	Guanosine nucletide is held by the cytosine nucletide by the number of hydrogen bonds	1	2	3	4			**2**
49	Within single turn of DNA the number of base pairs exists	4	6	8	10			**10**
50	Each turn of DNA structure has a pitch in nm of	1.4	2.4	3.4	4.4			**3.4**
51	The double stranded DNA molecule loses its viscosity upon	Denaturation	Filteration	sedimentation	concentration			**Denaturation**
52	Z-DNA was discovered by	Watson&Crick	Hoogsten	Chargaff	Chargaff			**Hoogsten**
53	The base sequence at the end of all tRNA is	CCA	CAA	CCC	CCC			**CCA**
54	Deoxy ribose has no oxygen in	5' position	2' position	3' position	4' position			**2' position**
55	When pancreatic deoxyribonuclease attacks DNA, the product obtained is	3' phosphonucleotide	5' phosphonucleotide	5'3' phosphodiesters	2'3' phosphodiesters			**5'3' phosphodiesters**
56	6-Amino purine is	Guanine	adenine	cytosine	adenine			**adenine**
57	The chemical name of 2-amino –6- oxy purine	Adenine	xanthine	guanine	Cytosine			**guanine**
58	N7-methyl guanine has been found more recently in the nucleic acids of the cells of	Bacteria	yeast	mammals	plant			**mammals**
59	Hypoxanyhine and ribose contitute	Adenosine	inosine	guanosine	cytidine			**inosine**
60	The most abundant intracellular free nucletide	ATP	FAD	NAD+	NADP+			**ATP**