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BASICS OF BIOCHEMISTRY

Manual for the Medical Faculty for International students
(in English)

Edited by professor V.V. Lelevich

ОСНОВЫ БИОХИМИИ

пособие для студентов факультета иностранных учащихся
(с английским языком обучения)

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Reviewers:

This issue contains information that will be useful for studying biochemistry.

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ABBREVIATIONS

ACAT – acyl-CoA cholesterol acyltransferase
ACP – acyl carrier protein
ACTH – adrenocorticotropic hormone
ADH – antidiuretic hormone (vasopressin)
ALT – alanine aminotransferase
AST – aspartate aminotransferase
cAMP – cyclic AMP
CNS – central nervous system
Co A – coenzyme A
Co Q – coenzyme Q (ubiquinone)
DNA – deoxyribonucleic acid
Dopa – 3,4-dihydroxyphenylalanine
ECF – extracellular fluid
EFA – essential fatty acids
ETC – electron transport chain
FAD – flavin adenine dinucleotide
FFA – free fatty acids
FMN – flavin mononucleotide
GABA – γ-aminobutyric acid
GAGs – glycosainoglycans
GH – growth hormone
GSH – glutathione
HDL – high-dencity lipoproteins
HMG-CoA – 3-hydroxy-3-methylglutaryl-CoA
IDL – intermediate-density lipoproteins
ICF – intracellular fluid
IMP – inosine monophosphate
LCAT – lecithin: cholesterol acyltransferase
LDH – lactate dehydrogenase
LDL – low-density lipoproteins
mRNA – messenger RNA
NAD^+ - nicotinamide adenine dinucleotide

$NADP^+$ - nicotinamide adenine dinucleotide phosphate
PAPS – adenosine 3'-phosphate-5'-phosphosulfate
PCR – polymerase chain reaction
PUFAs – polyunsaturated fatty acids
RNA – ribonucleic acid
ROS – reactive oxygen species
rRNA – ribosomal RNA
TAG - triacylgycerol
TCA – tricarboxylic acid cycle
tRNA – transfer RNA
VLDL – very low-density lipoproteins

CHAPTER 1

INTRODUCTION TO BIOCHEMISTRY

Biochemistry is the science of the chemical constituents of living cells and of the reactions and processes they undergo. The term "biochemistry" itself is derived from a combination of *biology* and *chemistry*. In 1877 German physician, chemist and physiologist Felix Hoppe-Seyler used the term as a synonym for physiological chemistry in the first issue of Journal of Physiological Chemistry.

Biochemistry is the chemistry of living cell. Life depends on various biochemical reactions, that is why biochemistry has become the basic language of all biological sciences. Controlling information flow through biochemical signalling and the flow of chemical energy through metabolism, biochemical processes give rise to the incredible complexity and diversity of life. Biochemistry mostly deals with the structures and functions of cellular components such as proteins, carbohydrates, lipids, nucleic acids and other biomolecules as well as with their conversions. Over the last 50 years biochemistry has become so successful at explaining living processes, that now almost all areas of the life sciences from botany to medicine are engaged in biochemical research. Today the main focus of pure biochemistry lies in understanding how biological molecules give rise to the processes that occur within living cells which in turn relates greatly to the study and understanding of whole organisms.

Biochemistry is a field of science concerned with the study of:

- chemical properties of the compounds constitutive of the living organism (**static biochemistry**);
- their conversions (**dynamic biochemistry**);
- relation of these conversions to the activity of organs and tissues (**functional biochemistry**).

The major objective of biochemistry is complete understanding of all the chemical processes associated with living cells at the molecular level.

Biochemistry is concerned with the entire spectrum of life forms, from relatively simple viruses and bacteria to complex human beings. Depending on the **object of study** biochemistry is divided into:

- biochemistry of humans and animals
- biochemistry of plants
- biochemistry of microorganisms

The following research objects are used in biochemical studies:

1. Whole organism.
2. Isolated perfused organ.
3. Tissue slice.
4. Body fluids.
5. Whole cells.
6. Homogenates.
7. Isolated cell organelles.
8. Enzymes
9. Purified metabolites.
10. Isolated genes.

Researchers in biochemistry use specific techniques native to biochemistry, but increasingly combine these with techniques and ideas developed in the fields of genetics, molecular biology and biophysics.

Methods used in biochemistry

- <u>for separating and purifying of biomolecules</u>
  salt fractionation
  chromatography
  electrophoresis
  ultracentrifugation

- <u>for determining biomolecular structure</u>
  elemental analysis
  spectroscopy
  mass spectrometry
  X-ray crystallography
  use of hydrolysis and enzymes to degrade the biomolecules

- <u>for determining substances concentrations</u>
  spectrophotometry
  colourimetry

History of biochemistry

I period: ancient time – 15th century. In this period people used biochemical processes to make bread, cheese, wine, though the essence of these processes was unknown to them.

II period: 15th century– the first half of the 19th century. In this period German physician Paracelsus (1493-1541) put forward the concept of a close relationship between chemistry and medicine: chemical reactions formed the basis of vital activity and the cause of any disease is a disturbance of the natural course of chemical processes within the organism.The first controlled experiments in human metabolism were published by Santorio Santorio in 1614 in his book *"Ars de statica medicina"*. This book describes how he weighed himself before and after eating, sleeping, working, sex, fasting, drinking, and excreting. He found that most of the food he took in was lost through what he called "insensible perspiration". Russian scientist Lomonosov (1711-1765) formulated the law of conservation of mass. French chemist Lavoisier (1743-1794) proposed that in respiration of living organism oxygen is consumed and carbon dioxide is evolved. Russian chemist Kirchhoff (1764-1833) described in 1814 enzymatic process of starch saccharization by the action of an extract from the germinated barleycorn. By the 1850s, other enzymes were discovered: salivary amylase, pepsin in gastric juice, trypsin of pancreatic juice.

III period: the second half of the 19th century – first half of the 20th century. In this period French scientist Pasteur (1821-1895) performed studies of fermentation with participation of living yeast cells. German chemist Buchner in 1897 provided an evidence for the ability of a cell-free yeast juice to produce alcoholic fermentation. For his works on cell-free fermentation Buchner was awarded a Nobel Prize in chemistry in 1907.

In the second half of the 19th century special chairs of medical, or physiological, chemistry were instituted at the medical departments of many European universities.

IV period: 1950s – present time. Advent of biochemistry, development of new methodologic principles and techniques such as chromatography, X-ray diffraction, nuclear magnetic resonance spectroscopy, radioisotopic labelling, electron microscopy ,and molecular dynamics simulations. These techniques allowed for the discovery and detailed analysis of many molecules and metabolic pathways of the cell,

such as glycolysis and the Krebs cycle (citric acid cycle). In 1960, the biochemist Robert K. Crane revealed his discovery of the sodium-glucose cotransport as the mechanism for intestinal glucose absorption. This was the very first proposal of a coupling between the fluxes of ions and a substrate that has been seen as sparking a revolution in biology.

Today, the findings of biochemistry are used in many areas, from genetics to molecular biology and from agriculture to medicine.

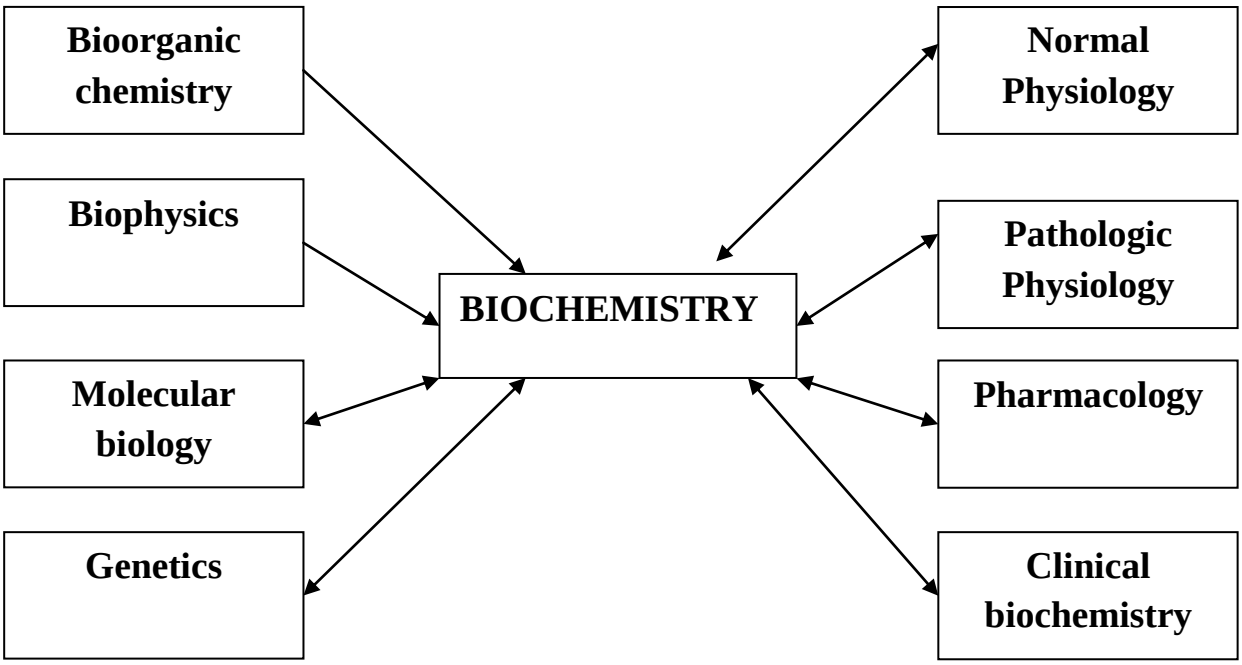


Figure 1.1 – Relations of biochemistry with other disciplines

The study of human biochemistry will open your eyes to how the body works as a chemical system. From a physician's point of view, biochemistry not only describes how the system works, but also provides a foundation for understanding how to improve its operation, how to diagnose problems and, where possible, how to remedy them. To understand links between nutrients, metabolism, health and disease, is one of the most important reasons to study biochemistry. Knowing biochemistry helps understand current therapies, which include recombinant proteins, such as human insulin or erythropoietin synthesized by bacteria. It helps understand the action of new drugs. In future, therapies will possibly involve gene rather than organ transplants. Pharmacogenomics and nutritional genomics will create a basis for designer treatments, customized to an individual's genetic makeup.

CHAPTER 2

PROTEINS

Proteins are the high-molecular nitrogen-containing organic compounds whose molecules are built up of amino acid residues. They are the primary structural and functional polymers in living systems.

History of protein study (main events)

1838 – G.Mulder	proteins were first described
1838 – J.Berzelius	proposed term "protein"
1888 – A.Danilevsky	hypothesis of proteins structure, proposed existence of peptide bonds
1925-1930 –T.Svedberg	developed sedimentation analysis
1951 – L.Pauling& R. Corey	prediction of regular protein secondary structure
1952 – K.Linderstrom-Lang	investigation of protein folding and structure mediated by hydrophobic interactions.
1953 – F.Sanger	determined the amino acid sequence of insulin
1958 – J. Kendrew	determined structure of myoglobin
1959 – M.Perutz	determined structure of hemoglobin

AMINO ACIDS

Amino acids are organic acids with at least a minimum of one of its hydrogen atoms in the carbon chains substituted by an amino group. There are over 300 naturally occurring amino acids. In the human organism are present over 60 amino acids and its derivatives. Amino acids can be divided into two groups: proteinogenous (constituents of proteins, 20 amino acids) and nonproteinogenous, which are not constituents of proteins.

There are three classifications of amino acids:

1. **Structural**, based on chemical structure of its side chain (-R or side radical).
2. **Electrochemical**, based on the acid-base properties of amino acids.

3. **Biological**, based on the essentiality of amino acids for the human organism.

The carbon skeletons of many amino acids may be derived from metabolites in central pathways, allowing the biosynthesis of some amino acids in humans. Amino acids that can be synthesized in this way are therefore not required in the diet (**non-essential** amino acids) whereas amino acids having carbon skeletons that cannot be derived from normal human metabolism must be supplied in the diet (essential amino acids). Valine, leucine, isoleucine, threonine, lysine, methionine, phenylalanine and tryptophan are absolutely **essential** for humans. Arginine and histidine are partially essential.

Proteins may contain residues created by modification of common residues already incorporated into a polypeptide. Among these uncommon amino acids are 4-hydroxyproline, a derivative of proline, and 5-hydroxylysine, derived from lysine. The former is found in plant cell wall proteins, and both are found in collagen. 6-N-Methyllysine is a constituent of myosin, γ-carboxyglutamate, found in the blood-clotting protein prothrombin.

In addition to 20 L-amino acids that take part in protein synthesis (proteinogenic), recently several new amino acids have been described. Among them is selenocysteine, which is present in the active center of some enzymes.

Some 300 additional amino acids have been found in cells. They have a variety of functions but are not constituents of proteins. For example, ornithine and citrulline are key metabolites in the biosynthesis of arginine and in the urea cycle.

PEPTIDES

Peptides are short polymers of amino acids. When a few amino acids (from 2 to 10) are joined, the structure is called an oligopeptide. When many (from 11 to 50) amino acids are joined, the product is called a polypeptide. Proteins may have from 51 to thousands of amino acid residues. The terms “protein” and “polypeptide” are sometimes used as synonyms.

Peptides exhibit a number of specific functions. Even the smallest peptides can have biologically important effects. By functions peptides can be classified to:

1. regulatory peptides (glutathione, angiotensin, bradykinin);
2. peptides with hormonal activity (oxytocin, vasopressin);
3. peptides involved in digestion (gastrin, secretin);
4. neuropeptides (endorphins, tachykinins, neuropeptide Y);
5. alkaloids (ergotamine, pandamine);
6. antibiotics (bacitracin, gramicidin A, B, C);
7. toxines (amanitin, microcystin, nodularin).

Many peptides are potentially useful as pharmacologic agents, and their production is of considerable commercial importance. There are three ways to obtain a peptide:

- purification from tissue, the task often made difficult by the vanishingly low concentrations of some peptides;
- genetic engineering;
- short proteins and peptides (up to about 100 residues) can be chemically synthesized. The peptide is built up, one amino acid residue at a time, while remaining tethered to a solid support.

PROTEINS

Biological functions of proteins

1. Structural.
2. Nutritive (reserve).
3. Catalytic.
4. Hormonal.
5. Receptive.
6. Transport.
7. Contractile.
8. Protective.
9. Hemostatic.
10. Electro-osmotic.
11. Energo-transformative.
12. Toxigenic.
13. Antitoxic.

In the whole human body the proteins account for **45%** of dry mass.

Table 2.1. – **Protein content of the organs and tissues**

	Dry tissue mass	Fresh tissue mass
Muscle	80%	18-23%
Lung	82%	14-15%
Spleen	84%	17-18%
Kidney	72%	16-17%
Liver	57%	18-19%

Physico-chemical properties of proteins

- high molecular weight;
- shape and size of molecules;
- high viscosity in solution;
- inability to penetrate semipermeable membranes
- pronounced swelling ability;
- optical activity;
- carge;
- mobility in electric field;
- low osmotic and high oncotic pressures;
- solubility;
- ability to absorhb UV light at 280 nm;
- amphoteric;
- denaturation.

Denaturation of proteins. Destruction of the three-dimensional structure of a protein molecule with loss of its specific properties (solubility, electrophoretic mobility, biological activity and other) is called denaturation.

Most proteins can be denatured:

- by heat, which affects the weak interactions in a protein;
- by extremes of pH;
- by certain miscible organic solvents such as alcohol or acetone;
- by certain solutes such as urea and guanidine hydrochloride;
- by detergents;
- by heavy metal salts.

Molecular weight of proteins varies from 6,000 Da to over 1,000,000 Da. For determination of molecular weight are used:

- sedimentation analysis;
- gel chromatography;
- gel electrophoresis.

Purification of proteins

The aim of protein purification is to isolate one particular protein from all the others in the starting material.

Table 2.2. – **Protein purification stages**

STAGE		COMMENTS
I	Selecton of a protein source	The source of a protein is generally tissue or microbial cells.
II	Homogenization and solubilization	Disintegration of samples resulting in breakdown of the cell structure and releasing their proteins into a solution called a homogenate (or crude extract). If necessary, differential centrifugation can be used to prepare subcellular fractions or isolate specific organelles.
III	Fractionation (separation)	The extract is subjected to treatments that separate the proteins into different fractions based on a property such as size or charge.
	- salting-out	The solubility of proteins is lowered in the presence of some salts. Addition of certain salts in the right amount can selectively precipitate some proteins, while others remain in solution. The proteins thus precipitated are removed from those remaining in solution by low-speed centrifugation.
	- chromatography	The most powerful methods for fractionating proteins which takes advantage of differences in protein charge,

		size, binding affinity, and other properties. A porous solid material with appropriate chemical properties (the stationary phase) is held in a column, and a buffered solution (the mobile phase) migrates through it. The protein, dissolved in the same buffered solution is layered on the top of the column. The protein then percolates through the solid matrix as an ever-expanding band within the larger mobile phase. Individual proteins migrate faster or more slowly through the column, depending on their properties.
	- electrophoresis	The technique is based on the migration of charged proteins in an electric field. Its advantage is that proteins can be visualized as well as separated, permitting a researcher to estimate quickly the number of different proteins in a mixture or the degree of purity of a particular protein preparation.
IV	Dialysis	The procedure that separates proteins from small solutes.The partially purified extract is placed in a bag/tube made of a semipermeable membrane. When this is suspended in a large volume of buffered solution, the membrane allows the exchange of salt and buffer but not proteins. Thus dialysis retains large proteins within the membranous bag/tube while allowing the concentration of other solutes in the protein preparation to change until they come into equilibrium with the solution outside the membrane.

The structure of proteins

The **primary structure** is the sequence of amino acid residues in the polypeptide chaine. By convention, the sequence is written from left to right, beginning with N-terminal amino acid. Each type of protein has a unique amino acid sequence. Amino acid sequence plays a fundamental role in determining the three-dimensional structure of the protein, and ultimately its function.

Figure. 2.1 – Primary structure of proteins

Relationship between amino acid sequence and biological function:

- proteins with different functions always have different amino acid sequences;
- if the primary structure is altered, the function of the protein may also be changed;
- functionally similar proteins from different species, often have similar amino acid sequences.

Main steps in determination of primary structure

1. Determination of amino acid composition (hydrolysis, automated amino acid analyzer).
2. Identifying of N- and C-terminal amino acids.
3. The protein is cleaved into a set of specific fragments by chemical or enzymatic methods. It is necessary to generate several sets of peptides using more than one method of cleavage.
4. Each fragment is purified, then sequenced.
5. The order in which the fragments appear in the original protein is determined (sequences overlap).

Methods for determination of N-terminal amino acids:

1. **Sanger's** method (with 1-fluoro-2,4-dinitrobenzene**)**.
2. **Edman** degradation procedure (with phenylisothiocyanate; carried out on a machine, called sequenator).
3. Reaction with **dansyl chloride**.

4. Use of **aminopeptidase**.

Methods for determination of C-terminal amino acids:

1. **Akabori's** method (based on hydrazinolysis of polypeptides).
2. Use of **carboxypeptidase**.
3. Reaction with **sodium borohydride** ($NaBH_4$).

A protein sequence can also be deduced from the nucleotide sequence of its corresponding gene in DNA.

Secondary structure is the three-dimensional form of local segments of proteins. Secondary structure is stabilized by hydrogen and disulfide bonds. The most prominent types of secondary structure are the α-helix and β-sheet (Fig.2.2).

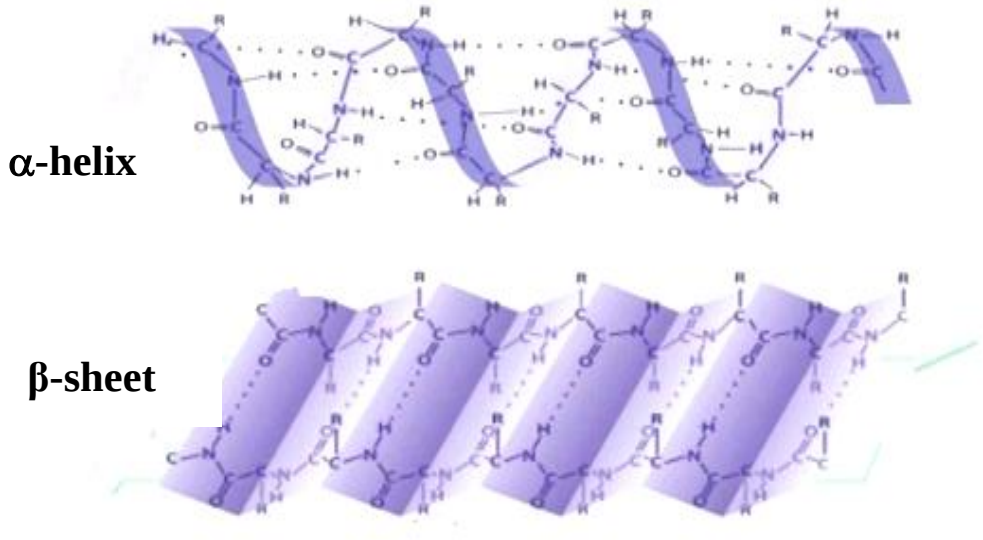


Figure. 2.2 – Secondary structure of proteins

Supersecondary structures, also called motifs or simply folds, are particularly stable arrangements of several elements of secondary structure and the connections between them. Examples: β-barrel, α-αcorner, zinc finger, leucine zipper.

Tertiary structure is the complete three-dimensional structure of a polypeptide chain. Tertiary structure is stabilized by ionic, hydrogen, covalent (disulfide) bonds, hydrophobic interactions, and Van der Waal forces (Fig.2.3).

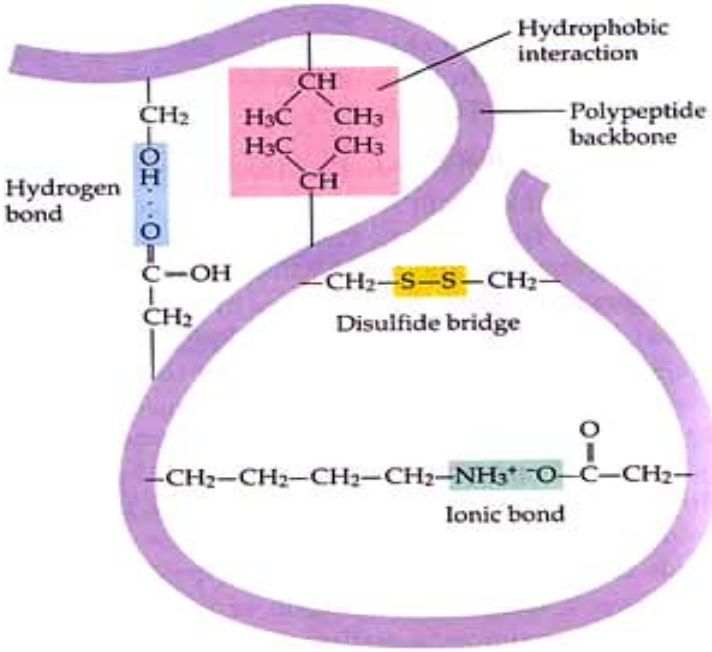


Figure. 2.3 – Types of bonds stabilizing the tertiary structure of protein

There are two general classes of proteins based on tertiary structure: fibrous and globular.

- Fibrous proteins, which serve mainly structural roles, have simple repeating elements of secondary structure.
- Globular proteins have more complicated tertiary structures, often containing several types of secondary structure in the same polypeptide chain.

Methods for determining the three-dimensional structure of a protein:

1. X-Ray Diffraction.
2. Nuclear Magnetic Resonance.

Quaternary structure results from interactions between two or more polypeptide subunits. Some multisubunit (multimeric) proteins have a repeated unit consisting of a single subunit or a group of subunits referred to as a protomer. Quaternary structure is stabilized by the same kinds of bonds as the tertiary structure.

Multimeric proteins can have from two to hundreds of subunits. A multimer with just a few subunits is often called an oligomer. Most multimers have identical subunits or repeating groups of nonidentical subunits, usually in symmetric arrangements.

Biological role of quaternary structure:

- the binding of small molecules may affect the interaction between subunits, causing large changes in the protein's activity in response to small changes in the concentration of substrate or regulatory molecules;
- separate subunits can take part in separate but related functions, such as catalysis and regulation;
- some associations, such as the fibrous proteins and the coat proteins of viruses, serve primarily structural roles;
- some very large protein assemblies are the sites of complex, multistep reactions.

Protein folding

All proteins begin their existence on a ribosome as a linear sequence of amino acid residues. This polypeptide must fold during and following synthesis to take up its native conformation (Fig.2.4). Folding for many proteins is facilitated by the action of specialized proteins. **Chaperones** are a group of proteins that assist in protein folding. They have the ability to prevent non-specific aggregation by binding to non-native proteins. There are several families of chaperones and each possesses different functions. Some chaperone systems work as foldases: they support the folding of proteins in an ATP-dependent manner. Other chaperones work as holdases: they bind folding intermediates to prevent their aggregation. The example of chaperon proteins are the "heat shock proteins" (Hsps).

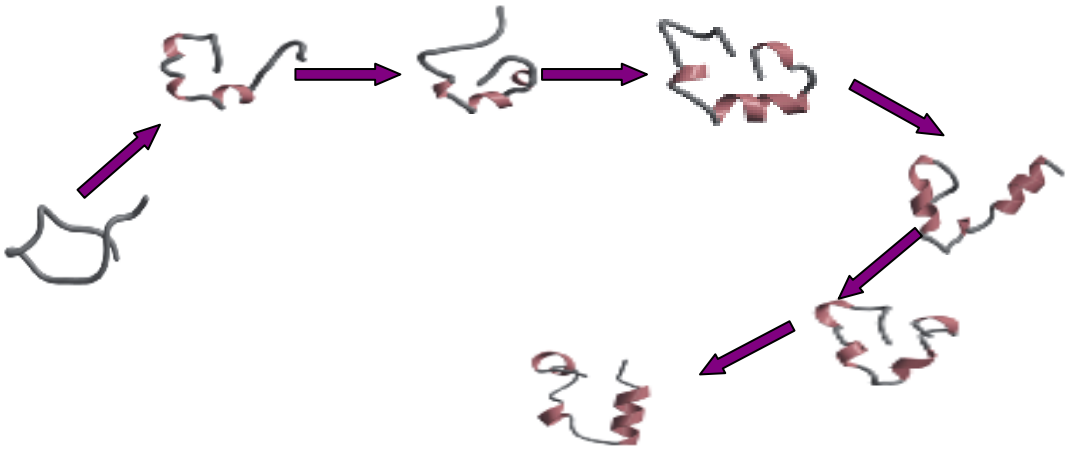


Figure 2.4 – Simulated folding pathway

Proteins in any of their functional, folded conformations are called native proteins. A misfolded protein appears to be the causative agent of a number of diseases. Accumulation of misfolded proteins can cause disease,

known as **amyloid diseases**. The most prevalent one is Alzheimer's disease. Parkinson's disease and Huntington's disease have similar amyloid origins. Protein aggregation diseases are not exclusive to the central nervous system; they can also appear in peripheral tissues. Such diseases include type 2 diabetes, inherited cataracts, some forms of atherosclerosis, hemodialysis-related disorders, and short-chain amyloidosis. All these diseases have in common the expression of a protein outside its normal context, leading to an irreversible change into a sticky conformation rich in beta sheets that make the protein molecules interact with each other.

Simple and conjugated proteins

Depending on their composition proteins can be classified as simple and conjugated. Simple proteins are composed of amino acid residues only. Conjugated proteins are composed of a simple protein and a non-protein moiety.

Simple proteins

Protamines – proteins of a small molecular size, display pronounced basic properties due to the occurrence of large amounts of arginine, readily soluble in water.

Histones – proteins of a small molecular size, with basic properties (major constituents – arginine and lysine), play an important role in the metabolic control of genome activity.

Prolamines and **Glutelins** – proteins of vegetal origin, soluble in 60-80% ethanol solution.

Albumins and **Globulins** – widely occur in animal organs and tissues.

Conjugated proteins

The classification of conjugated proteins is based on the chemical nature of their non-protein components.

Chromoproteins. These are proteins in combination with a proshetic group that is a pigment. The examples are the respiratory pigments hemoglobin and hemocyanin, visual purple or rhodopsin found in the rods of the eye, flavoproteins and cytochromes. In this group are present:

- hemoproteins;
  (containing heme as prostetic group);
  includes hemoglobin, myoglobin;
- flavoproteins;

(FMN, FAD as prostetic group);
constituents of oxidoreductases.

Lipoproteins. These are proteins conjugated with lipids. There are five types of lipoproteins:

high density lipoproteins (HDL);
intermediate density lipoproteins (IDL);
low density lipoproteins (LDL);
very low density lipoproteins (VLDL);
chylomicrons.

Glycoproteins. These are proteins conjugated with carbohydrates. Glycoproteins are of two main categories, intracellular and secretory. Intracellular glycoproteins are present in cell membranes and have an important role in membrane interaction and recognition. Secretory glycoproteins are: plasma glycoproteins, secreted by the liver, thyroglobulin, secreted by the thyroid gland, immunoglobins, secreted by plasma cells, ovoalbumins, secreted by the oviduct in the hen, ribonuclease, theenzyme which breaks down RNA, and deoxyribonuclease, which breaks down DNA.

Phosphoproteins are proteins in combination with a phosphate-containing radical other than a nucleic acid or a phospholipid. The examples of phosphoproteins are casein of milk and ovovitelline in eggs.

Nucleoproteins are proteins in combination with nucleic acids. There are two types of nucleoproteins:

- deoxyribonucleoproteins (chromatin);
- ribonucleoproteins (ribosomes).

Metalloproteins are proteins conjugated to metal ion(s) which are not part of the prosthetic group. They include ceruloplasmin, an enzyme with oxidase activity that may transport copper in plasma, siderophilin, ferritin, hemosiderin. Functions of metalloproteins: transport, depot, structural and enzymatic functions.

Protein functioning

Protein function often entails interactions with other molecules. A molecule bound by a protein is called a ligand. The site to which it binds is called the binding site. Binding site is complementary to the ligand in size, shape, charge, and hydrophobic or hydrophilic character. A ligand may be any kind of molecule, including another protein.

Interaction protein – ligand is specific: the protein can discriminate among the thousands of different molecules in its environment and selectively bind only one or a few. The given protein may have separate binding sites for several different ligands.

Proteins may undergo conformational changes when a ligand binds, a process called induced fit. In a multi-subunit protein, the binding of a ligand to one subunit may affect ligand binding to other subunits. Interactions between ligands and proteins may be regulated, usually through specific interactions with one or more additional ligands. These other ligands may cause conformational changes in the protein that affect the binding of the first ligand.

Protein-ligand interactions can be described quantitatively.

A quantitative description of this interaction is therefore a central part of many biochemical investigations.

In general, the reversible binding of a protein (P) to a ligand (L) can be described by a simple equilibrium expression:

$$P + L = PL$$

The reaction is characterized by an equilibrium constant, K_a (an association constant).

CHAPTER 3

ENZYMES. MECHANISM OF ACTION OF ENZYMES

Enzymes are called specific proteins that are part of all cells and tissues of living organisms which serve as biological catalysts.

Common properties of enzymes and inorganic catalysts:

1. Not consumed in the reaction.
2. Exert their effects at low concentrations.
3. Do not affect the value of the equilibrium constant of the reaction.
4. Their action is subject to the law of mass action.

Differences between enzymes and inorganic catalysts:

1. Thermolability of enzymes.
2. The dependence of enzyme activity on pH.
3. Specificity of action of enzymes.
4. Rate of enzymatic reactions is subject to certain kinetic regularities.
5. Enzyme activity depends on the action of regulators - activators and inhibitors.
6. Several enzymes in the formation of tertiary and quaternary structures are subjected to postsynthetic modification.
7. Size of enzyme molecules is usually much larger than their substrates.

The structure of the enzyme molecules

By the structure all enzymes can be simple or complex proteins. The enzyme is a complex protein called **holoenzyme**. The protein part of the enzyme is called **apoenzyme**, non-protein part is called **cofactor**. There are two types of cofactors:

1. Prosthetic group – strongly associated with the apoenzyme, often by covalent bonds.

2. Coenzyme – non-protein part is easily separated from the apoenzyme. Coenzymes are often vitamin derivatives.

Coenzymes include the following compounds:

- Vitamin derivatives;
- Hemes that are parts of cytochromes, catalases, peroxidases, guanylatecyclase, NO-synthase and the enzyme prosthetic group are nucleotides;
- Donors and acceptors phosphoric acid residue;
- Ubiquinone or coenzyme Q, involved in the transfer of electrons and

protons in the chain of tissue respiration;

- Phoshoadenosylphosphosulfat (PAPS) involved in the transport of sulfate;

- Glutathione involved in redox reactions.

Table 3.1. – **Coenzyme functions of vitamins**

Vitamin	***Coenzyme***	***Enzyme***
B_1 thiamine	Thiamin diphosphate	Transketolase, pyruvatedehydrogenase
B_2 riboflavin	FMN, FAD	Flavin-dependent dehydrogenases
B_3 pantothenic acid	coenzyme A (CoA)	Reaction of acylation
B_6 pyridoxine	Pyridoxal-phosphate	Aminotransferases
PP nicotinamide	NAD, NADP	NAD (NADP)-dependent dehydrogenase
Folic acid	Tetrahydrofolic acid	Transferring one-carbon groups

Metal ions as cofactors

More than 25% of all enzymes exhibit full catalytic activity of metal ions needs. Consider their role in enzymatic catalysis.

Role in joining metal substrate in the active site of the enzyme. The metal ions act as stabilizers of the substrate molecule, the active center of the enzyme and protein conformation of the enzyme molecule, namely the tertiary and quaternary structures.

Metal ions are stabilizers of substrate molecule. For some enzymes, a substrate is the complex attached to the metal ion. For example, in most kinases ATP should combine with Mg^{2+} and form Mg^{2+}-ATP complex. In this case, the Mg^{2+} ions do not interact directly with the enzyme but are involved in the stabilization of ATP molecules and neutralizing the negative charge of the substrate, which facilitates its connection to the active center of the enzyme.

Schematically, cofactor role in the interaction of enzyme and substrate may be present as a complex

ES-Me,

where E - the enzyme, S - substrate Me - metal ion.

Metal ions are the active center of the enzyme stabilizers. In some cases, metal ions serve as a "bridge" between the enzyme and the substrate. They act as stabilizers of the active center, facilitating accession to the substrate and chemical reaction. In some instances, the metal ion may facilitate adherence of the coenzyme. The above functions are performed by metals such as Mg^{2+}, Mn^{2+}, Zn^{2+}, Co^{2+}, Mo^{2+}. In the absence of metal, these enzymes have no activity. Such enzymes are called "metalloenzymes". Metalloenzymes include, for example, the enzyme pyruvate kinase.

The role of metals in the stabilization of the enzyme structure. Metal ions ensure the preservation of the secondary, tertiary, quaternary structure of the enzyme molecule. Such enzymes in the absence of metal ions are capable of chemical catalysis, however, they are unstable. Their activity is reduced or even disappears completely for small changes in pH, temperature, and other minor changes in the external environment. Thus, metal ions function as stabilizers of the protein molecule.

Sometimes alkaline earth metal ions are involved in stabilizing the secondary and tertiary structures. Thus, to maintain the tertiary structures pyruvate kinase requires ions K^{+}.

To stabilize the quaternary structure of the alcohol dehydrogenase, which catalyzes the reaction of oxidation of ethanol, Zn^{2+} ions are required.

The role of metals in enzyme catalysis

Not less important role in the implementation of the metal ions plays enzymatic catalysis.

Participation of metal in electrophilic catalysis. Most often this function is performed by metal ions with variable valence, having a free d-orbital and act as electrophiles. This is, primarily, metals such as Zn^{2+}, Fe^{2+}, Mn^{2+}, Cu^{2+}. Alkali metal ions such as Na^{+} and K^{+}, do not possess this property. During catalysis electrophilic metal ions are often involved in the stabilization of intermediates.

Participation of metals in redox reactions. Metal ions with variable valency may also participate in electron transfer. For example, in cytochromes (heme-containing proteins), the iron ion can bind and release one electron. Due to this property cytochromes are involved in redox reactions.

The enzyme active site

Part of the enzyme molecule which specifically interacts with the substrate is called an active center. **Active site** is a unique combination of amino acid residues in the enzyme molecule, providing direct interaction with its substrate molecule and participating directly in the act of catalysis. In complex enzymes cofactor is a part of the active center.

Properties of the active sites of enzymes:

1. On the active site accounts for a relatively small portion of the total enzyme.
2. Active center is in the form of a narrow recess or gap in the globule enzyme.
3. Active site – a three-dimensional formation, which is involved in the formation of functional groups – linearly spaced apart amino acids.
4. Substrates are relatively weakly bound to the active site.
5. The specificity of binding of the substrate depends on a well-defined arrangement of atoms and functional groups in the active center.

In some regulatory enzymes there is another center called allosteric (regulatory) center. It is spatially separated from the active center.

Allosteric center is the portion of the enzyme molecule, which normally binds certain small molecules (allosteric regulators), molecules which are not similar in structure to the substrate. Accession of allosteric regulator to the center results in a change of the tertiary and quaternary structure of the enzyme molecule and accordingly, the conformation of the active site, resulting in reduced or increased enzymatic activity.

The mechanism of enzymes action

Any enzymatic reaction includes the following stages (Fig.3.1):

$$E + S \rightleftharpoons [ES] \rightleftharpoons E + P$$

where E - enzyme, S - substrate, [ES] - enzyme - substrate complex, P - product.

The mechanism of enzymes action can be considered from two perspectives: in terms of changing the energy of chemical reactions as well as in terms of events in the active site.

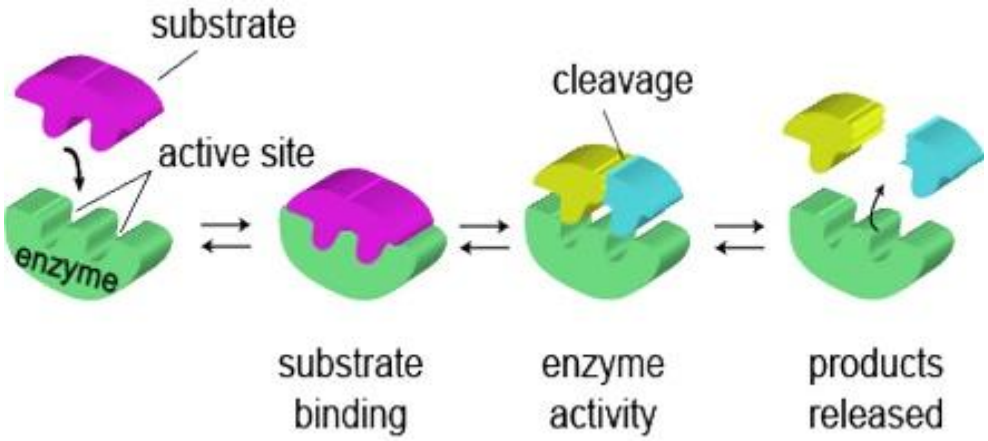


Figure 3.1 – Mechanism of catalysis

Energy changes in chemical reactions

Any chemical reactions taking place, are subject to two basic laws of thermodynamics: energy conservation law and the law of entropy. Under these laws, the total energy of a chemical system and its environment remains constant, while the chemical system tends to reduce order (increasing entropy). To understand the chemical reaction energy is not enough to know the energy balance of incoming and outgoing of the reactants. It is necessary to take into account changes in the energy of the chemical reaction process and the role of this enzyme in the process dynamics.

The larger molecules have an energy level higher than E_a (activation energy), the higher the rate of a chemical reaction. Heat can increase the rate of a chemical reaction. It increases the energy of the reacting molecules. However, living organisms detrimental high temperatures, so the cell to accelerate chemical reactions using enzymes. Enzymes provide high-speed reactions under optimal conditions in the cell, **by lowering E_a**. Thus, enzymes reduce the energy barrier height, thereby increasing the amount of reactive molecules and, consequently, increases the reaction rate (Fig.3.2).

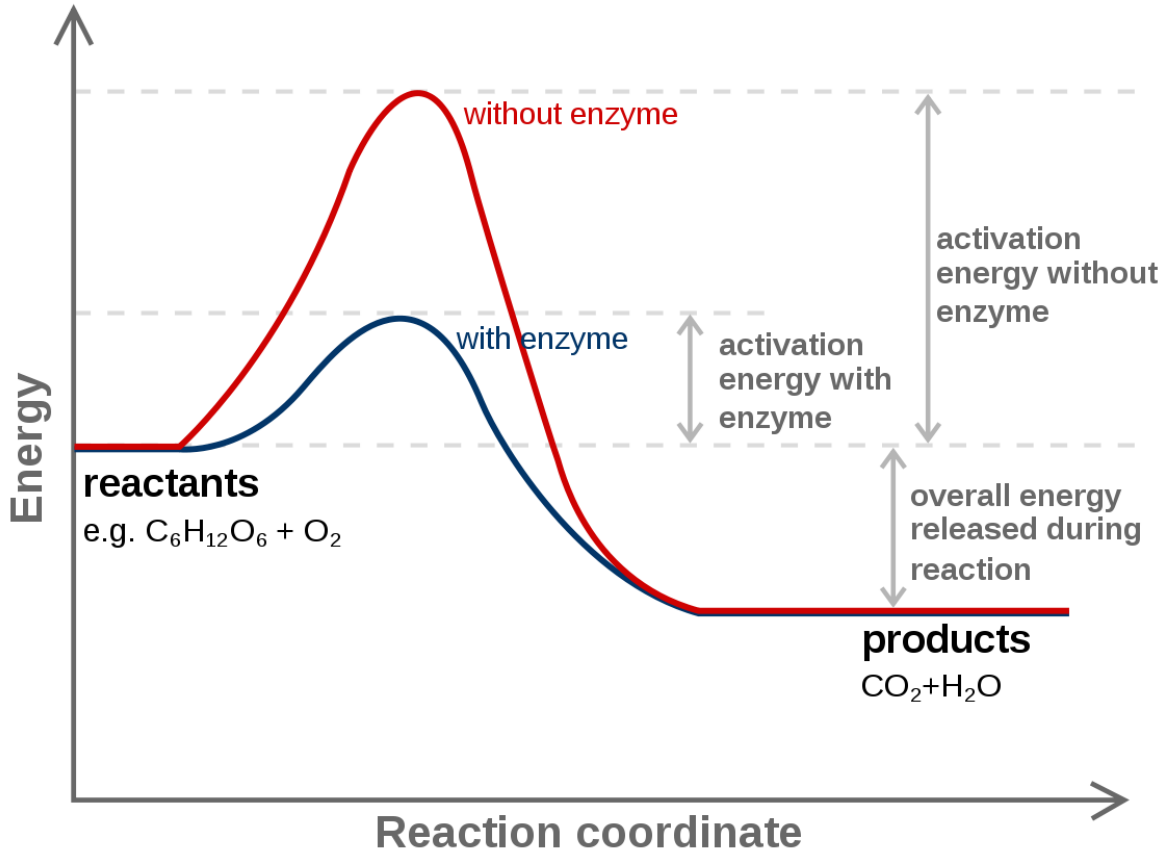


Figure 3.2 – The mechanism of enzymes action

The role of the active center in enzymatic catalysis

Investigations have shown that the molecule is an enzyme, usually much larger than the substrate molecule, undergoes chemical conversion by this enzyme. In contact with the substrate it enters only a small portion of the enzyme molecule, typically from 5 to 10 amino acid residues forming the active site of the enzyme. The role of other residues is to provide proper conformation of the enzyme molecule for optimum chemical reaction.

The active site at all stages of enzymatic catalysis can't be regarded as a passive site for substrate binding. This is a complex molecular "machine" using a variety of chemical mechanisms that facilitate the transformation of substrate to product.

In the active site of the enzyme substrates are arranged in such a way, allowing to participate in the reaction; functional groups of the substrates were in close proximity to each other. This property is called the effect of the active center of convergence and orientation of the reagents. Such an orderly arrangement of substrates causes a decrease in entropy and,

consequently, lower activation energy (E_a), which determines the catalytic efficiency of enzymes.

The active site of the enzyme promotes destabilization of the interatomic bonds in the molecule of the substrate, which facilitates the chemical reaction and product formation. This property is called the active site of the ***effect of deformation*** of the substrate.

Molecular mechanisms of enzymatic catalysis

Mechanisms of enzymatic catalysis are determined by the role of the functional groups in the active center of the enzyme reaction, a chemical conversion of the substrate into the product. There are two main mechanisms of enzymatic catalysis: ***acid-base catalysis and covalent catalysis***.

Acid-base catalysis

The concept of acid-base catalysis explains enzymatic activity involving chemical reaction of acid groups (proton donors) and / or basic groups (proton acceptors). Acid-base catalysis is a frequent phenomenon. Amino acid residues belonging to the active center have functional groups that exhibit properties of both acids and bases.

Such amino acids as Cys, Tyr, Ser, Lys, Glu, Asp, and His participate in the acid-base catalysis most often. Radicals of these amino acids in the protonated form are acids (proton donor), while in the deprotonated form are bases (proton acceptor). Due to this property of the functional groups enzyme active center becomes unique biological catalysts, in contrast to the non-biological catalysts, capable of exhibiting either acidic or basic properties.

Covalent catalysis

In covalent catalysis based on nucleophilic or electrophilic attack groups of the active center of the enzyme molecules form a covalent bond between the substrate and cofactor. The action of serine proteases such as trypsin, chymotrypsin, and thrombin - example of covalent catalysis mechanism, when covalent bond is formed between the substrate and the amino acid residue serine active site of the enzyme. The term "serine protease" is associated with the fact that the amino acid residue serine is included in the active center of these enzymes directly involved in catalysis.

Consider the mechanism of covalent catalysis by chymotrypsin example, performing the hydrolysis of peptide bonds in the digestion of

proteins in the duodenum. Substrates of chymotrypsin are peptides containing amino acids with aromatic and cyclic hydrophobic radicals (Phe, Tyr, Trp), indicating that the hydrophobic forces are involved in the formation of enzyme-substrate complex.

Specificity of enzyme action

Enzymes have a higher specificity of action than inorganic catalysts. There exists specificity towards the type of chemical reaction catalyzed by the enzyme and specificity relative to the substrate. There are two kinds of specificity for each characteristic of the enzyme.

Specificity with respect to the substrate is enzyme's substrate preference for a particular structure in comparison with other substrates. There are 4 types of substrate specificity of enzymes:

1. **Absolute specificity** is the ability of the enzyme to catalyze the conversion of only one substrate. For example, glucokinase phosphorylates only glucose, arginase cleaves only the arginine, urease – urea.

2. **Relative specificity** is the ability of the enzyme to catalyze the conversion of several substrates having the same type of bond. For example, lipase cleaves the ester bond in any triacylglycerols.

3. **Relative group specificity**. The enzyme catalyzes the conversion of several substrates having one type of bond, but requires the presence of certain functional groups. For example, all proteolytic enzymes cleave the peptide bond, but pepsin breaks the bond only between aromatic amino acids. Chymotrypsin cleaves the bonds between amino acids with carboxyl groups.

4. **Stereochemical specificity** (stereospecificity) is the property of the enzyme to convert only one stereoisomer. For example, bacterial aspartate decarboxylase catalyzes the decarboxylation of L-spartate only and doesn’t act on the D-aspartic acid.

Specificity with respect to the reaction

Each enzyme catalyzes the reaction of one or a group of the same type of reactions. Often, the same chemical compound acts as a substrate for different enzymes, each of which is specific to the catalytic reaction leading to the formation of different products. Specificity for the type of reaction is the basis of a uniform classification of enzymes.

CHAPTER 4

REGULATION OF THE ENZYME ACTIVITY MEDICAL ENZYMOLOGY

Methods for regulation of enzyme activity:

1. Changing the amount of enzymes;
2. Changes in catalytic efficiency of the enzyme;
3. Changing the reaction conditions.

Regulation of the amount of enzymes

The number of enzyme molecules in the cell is determined by the ratio of two processes – the speed of protein synthesis and degradation of the enzyme molecule.

In the cells there are two types of enzymes;

1. ***Constitutive enzymes*** are essential components of the cell synthesized at a constant rate in constant amounts.

2. Formation of ***adaptive enzyme*s** depends on certain conditions. There are inducible and repressible enzymes among them.

Inducible ones are usually catabolic enzymes with function. Their formation may be caused or accelerated by the substrate of the enzyme. Repressible enzymes are usually included in anabolic reaction. Inhibitor (repressor) may be the end product of the enzymatic reaction.

Changing the catalytic efficiency of enzymes

Influence of activators and inhibitors on the enzyme activity.

Activators can increase enzymatic activity by the following mechanisms:

- form the active site of the enzyme;
- facilitate the formation of enzyme-substrate complex;
- stabilize the native structure of the enzyme;
- protect the functional groups of the active center.

Classification of enzyme inhibitors:

1. Nonspecific;
2. Specific: a) irreversible;
   b) reversible:
      - competitive;
      - noncompetitive.

Nonspecific inhibitors cause denaturation of the enzyme – are the

strong acid or alkali, salts of heavy metals. Their action is not connected with the mechanism of enzyme catalysis.

Irreversible inhibition

Irreversible inhibition was observed in the case of the formation of stable covalent bonds between the inhibitor and the enzyme molecule. Most often enzyme active site is exposed to modification. As a result, the enzyme can't perform a catalytic function.

Irreversible inhibitors include heavy metals, such as mercury (Hg^{2+}), silver (Ag^{+}) and arsenic (As^{3+}), which in low concentrations block sulfhydryl groups of the active center, wherein the substrate may be subjected to chemical conversion.

Diisopropylfluorophosphate (DFP) reacts specifically with only one of the many serine (Ser) residues in the active center of the enzyme. Ser residue is capable of reacting with the DFP that has identical or very similar amino acid environment. DFF relate to specific irreversible inhibitor "serine-enzymes", as it forms a covalent bond with the hydroxyl group of serine in the active center and plays a key role in catalysis.

Monoiodinacetic acid, p-chloromercuribenzoate easily react with SH-groups of cysteine residues of proteins. These inhibitors are not considered to be specific, since they react with all free SH-groups of proteins, and are called nonspecific inhibitors. If the SH-groups are involved directly in catalysis, with the help of these inhibitors it appears possible to identify the role of SH-groups of the enzyme catalysis.

Irreversible enzyme inhibitors as drugs

The example of drug action is based on the irreversible inhibition of enzymes – a widely used drug ***aspirine***. Non-steroidal anti-inflammatory drug aspirin provides pharmacological action through inhibition of the cyclooxygenase enzyme catalyzing the reaction of formation of prostaglandins from arachidonic acid. The chemical reaction aspirin acetyl residue is attached to the free terminal OH-group of serine cyclooxygenase. This causes a decrease in prostaglandin formation, like a reaction products that have a wide spectrum of biological functions.

Reversible inhibition

Reversible inhibitors are bound to the enzyme by weak non-covalent bonds and under certain conditions can be easily separated from the enzyme. Reversible inhibitors are competitive and non-competitive.

Competitive inhibition

Competitive inhibition includes reversible decrease in the rate of enzymatic reactions caused by the inhibitor binding to the active site of the enzyme and prevents the formation of enzyme-substrate complex. This type of inhibition is observed when the inhibitor – a structural analogue of the substrate, occurs as a result of competition of the inhibitor molecules in the substrate and in the active center of the enzyme. In this case, the enzyme is reacted with either the substrate- or inhibitor-enzyme complexes forming substrate- (ES) or an enzyme-inhibitor complex (EI). In the formation of the enzyme-inhibitor complex (EI) reaction product is formed.

A classic example of competitive inhibition is inhibition activity of succinate dehydrogenase by malonic acid. Malonic acid is a structural analog of succinate (the presence of two carboxyl groups), and can also interact with the active site of succinate dehydrogenase. However, cleavage of two hydrogen atoms from malonic acid is impossible, hence, the reaction rate decreases.

Drugs as competitive inhibitors

Many drugs exert their therapeutic effect by a mechanism of competitive inhibition. For example, quaternary ammonium compounds inhibit acetylcholine esterase, which catalyzes the hydrolysis of acetylcholine on choline and acetic acid.

When adding inhibitors, activity of acetylcholine esterase decreases the concentration of acetylcholine increases, which is accompanied by increased nerve impulse conduction. Cholinesterase inhibitors are used in the treatment of muscular dystrophy. Effective anticholine esterase drugs are **neostigmine, endrofony** etc.

Antimetabolites as drugs

Competitive inhibitors of the enzymes used in medical practice are called antimetabolites. These compounds having the structural analogues of natural substrates, cause a competitive inhibition of enzymes, on the one hand, and, on the other, can be used with the same enzymes as the pseudosubstrate which leads to abnormal synthesis products. Abnormal products have no functional activity and lead to decrease of the certain metabolic pathways speed.

Such drugs include **sulfanilamide drugs** (analogs of p-aminobenzoic acid), used for treatment of infectious diseases, nucleotide analogs in the treatment of cancer (6-mercaptopurine, 5-fluorouracil).

Noncompetitive inhibition

Noncompetitive inhibition of the enzymatic reaction is called the process wherein the inhibitor interacts with the enzyme at a site different from the active center. Noncompetitive inhibitors *are not* structural analogues of the substrate.

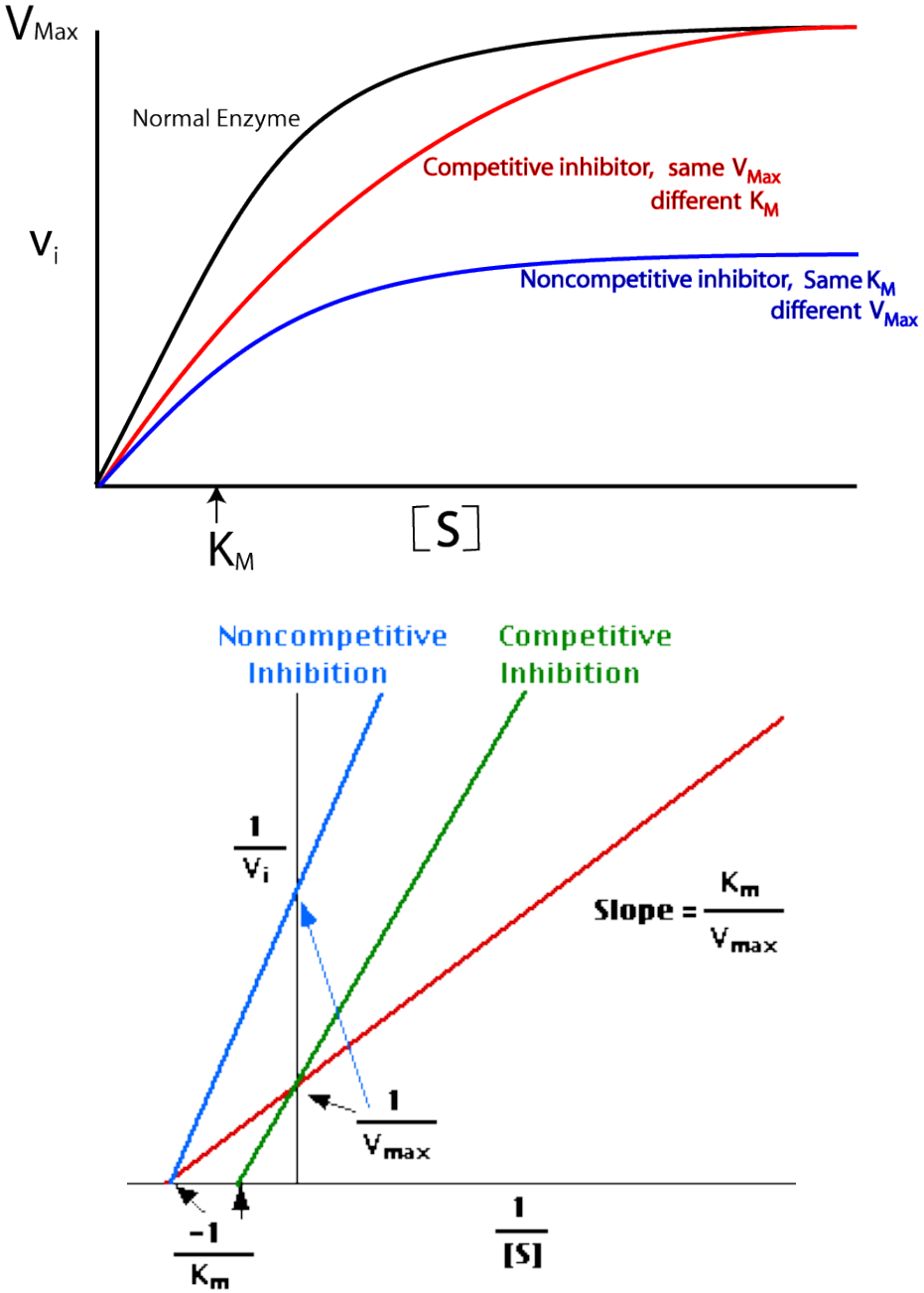


Figure 4.1 – Inhibition of enzymes

Noncompetitive inhibitor may bind either to the enzyme or to the enzyme-substrate complex to form an inactive complex. Accession of noncompetitive inhibitor alters the conformation of the enzyme molecule in such a manner that the substrate is disrupted interaction with the active site

of the enzyme, which leads to a decrease in the rate of enzymatic reaction.

Allosteric regulation

Allosteric enzymes are enzymes which activity is regulated not only by the number of substrate molecules, but also by other substances called effectors.

The role of allosteric enzymes in the metabolism of the cell. Allosteric enzymes play an important role in metabolism, as they are extremely quick to react to the slightest changes in the internal state of the cell. Allosteric regulation is important in the following situations:

• in anabolic processes. Inhibition of the final product of the metabolic pathway and activation of the initial metabolites allow the regulation of the synthesis of these compounds;

• in catabolic processes. In case of ATP accumulation in a cell, metabolic pathways of ATP formation are inhibited. Substrates at a cost of storing backup reaction nutrients;

• in coordination of anabolic and catabolic pathways. ATP and ADP are allosteric effectors acting as antagonists;

• in coordination of parallel and interconnected reactions in metabolic pathways (for example, the synthesis of purine and pyrimidine nucleotides that are used for synthesis of nucleic acids). Thus, the end products of one pathway could be another allosteric effector pathway.

Features of the structure and functioning of allosteric enzymes:

• usually oligomeric proteins are composed of several protomers or have a domain structure;

• they have an allosteric center, spatially remote from the catalytic active center;

• effectors are covalently attached to the enzyme in the allosteric (regulatory) centers;

• allosteric centers, as well as the catalyst, can exhibit different specificity with respect to the ligands: it can be absolute and the group. Some allosteric enzymes have several points, some of which are specific to activators, others are inhibitors;

• protomer, which is an allosteric center – regulatory protomer, unlike catalytic protomer containing the active center in which a chemical reaction takes place;

• allosteric enzymes have the ability to cooperativity: allosteric effector interaction with allosteric center is consistent cooperative

conformational change of all subunits, resulting in a change in the conformation of the active site and change the affinity of the enzyme to the substrate, which reduces or increases the catalytic activity of the enzyme;

• regulation of allosteric enzymes is reversible: dissociating effector from regulatory subunit restores the original catalytic activity of the enzyme;

• allosteric enzymes catalyze key reactions of this metabolic pathway.

Regulation of the catalytic activity of enzymes by protein-protein interactions. Some enzymes alter its catalytic activity as a result of protein-protein interactions. There are 2 mechanism of enzyme activation via protein-protein interactions:

• activation of enzymes as a result of merger of regulatory proteins;

• changes in the catalytic activity of enzymes due to the association or dissociation of the enzyme protomers.

Regulation of the catalytic activity of enzymes by phosphorylation/dephosphorylation. In biological systems the most frequently encountered mechanism of regulation of enzyme activity is covalent modification of the amino acid residues. Rapid and widespread method of chemical modification of enzymes is phosphorylation/dephosphorylation. Modifications involve OH groups of the enzyme. Phosphorylation by protein is catalyzed by enzymes kinases and dephosphorylation is catalyzed by phosphotases. Attaching a phosphoric acid residue leads to a change in the conformation of the active site and its catalytic activity. Thus the result can be two-fold: one when the enzymes are activated by phosphorylation, while others become less active.

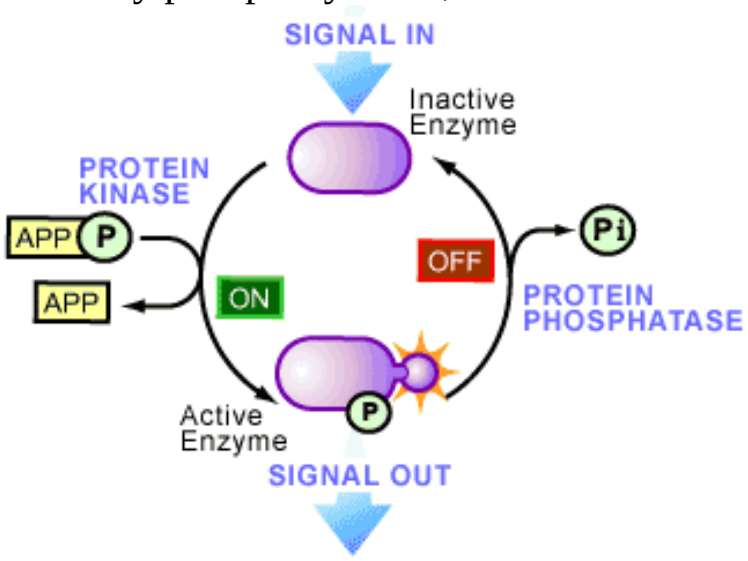


Figure 4.2 – Regulation of the activity of enzymes by phosphorylation/dephosphorylation.

Regulation of the catalytic activity of enzymes by partial (limited) proteolysis.

Certain enzymes that operate outside the cells (in the digestive tract or blood plasma), are synthesized as inactive precursors and are activated only as a result of the hydrolysis of one or more specific peptide bonds, which leads to cleavage of protein precursor molecules. As a result, the remainder of the protein molecule occurs conformational rearrangement and forms the active center of the enzyme (trypsinogen– trypsin).

MEDICAL ENZYMOLOGY

Blood plasma enzymes

By origin, blood plasma enzymes can be divided into 3 groups.

1. **Secretory** enzymes of plasma. They are formed in the liver, but exert their action in the blood. These include enzymes of blood coagulation – prothrombin, proaccelerin, proconvertin, ceruloplasmin, and cholinesterase.

2. **Excretory** enzymes enter the blood from various secrets: duodenal juice, saliva, etc. They include amylase, lipase.

3. **Cellular** enzymes are released into the blood when cells or tissues are damaged or destroyed.

Table 4.1. – **Organ-specific enzymes (isozymes)**

Enzyme (isozyme)	***Organ (system)***
LDH_1, LDH_2	heart
LDH_3	lungs
LDH_4, LDH_5	liver, muscle
amylase	pancreas
ALT	liver
AST	heart
acidphosphatase	prostate
alkalinephosphatase	bone

Enzymopathies

The reason for many diseases is dysfunction of enzymes in the cell (enzymopathies). Acquired enzymopathies are mostly proteinopathies, apparently observed in all diseases.

Primary enzymopathy is the inherited lack of enzymes, mainly in an autosomal recessive manner. Heterozygotes often have no phenotypic abnormalities. Primary enzymopathies are commonly referred to as metabolic diseases, as there is a violation of certain metabolic pathways. Thus the development of the disease can occur in one of the following pattern. Consider the schematic diagram of a metabolic pathway:

$$A \xrightarrow{E_1} B \xrightarrow{E_2} C \xrightarrow{E_3} D \xrightarrow{E_4} P$$

Substance A by successive enzymatic reactions is converted into a product P. In hereditary deficiency of an enzyme, such as the enzyme E3, violations of various metabolic pathways are possible:

Disturbances of final products formation. Lack of end product of this pathway in the absence of alternative synthetic routes can lead to the development of clinical symptoms characteristic of the disease.

Clinical manifestations. For example, in case of **albinism** disrupted synthesis in melanocytes pigment (melanin) occurs. Melanin is a pigment affecting coloration of the skin, hair, iris, retinal epithelium. In albinism weak pigmentation of the skin is observed as well as blond hair and reddish iris because of translucent capillaries. Manifestation is associated with albinism lack of tyrosine hydroxylase enzyme (tyrosinase) – one of the enzymes catalyzing the metabolic pathway for the formation of melanin.

Accumulation of precursor substrates. In enzyme deficiency certain substances and their precursor compounds are accumulated.

Clinical manifestations. Alcaptonuria (homogentisinuria) is a well-known disease in which oxidation of homogentisinic acid in tissues is impaired (alcapton–intermediate metabolite of tyrosine catabolism). In these patients, there is a lack of the enzyme oxidation alcapton – dioxygenase alcapton leading to the development of the disease. As a result, homogentisic acid concentration increases and it excretes into the urine. In the presence of oxygen acid converts into a black substrate – alkapton. Therefore, the urine of these patients turns black in the air. Alkapton is also formed in biological fluids, settling in tissues, skin, tendons, and joints. In case of significant accumulation of alkapton in the joints their mobility becomes violated.

Violation of formation of the final products and the accumulation of precursor substrates. Such situation is observed in diseases when both the product and lack of accumulation of the initial substrate cause clinical manifestations.

Clinical manifestations. For example, in people with **Gierke disease** (glycogen storage disease type I) a decrease in blood glucose (hypoglycemia) is observed between food intake. This occurs due to disturbances of glycogen breakdown in the liver due to enzyme deficiency glucose-6-phosphatase. Simultaneously, hepatomegaly is observed in these people because glycogen is not cleaved in their liver.

APPLICATION OF ENZYMES IN MEDICINE

Enzymes are widely used in medicine for diagnostic and therapeutic purposes.

Furthermore, enzymes are used as specific reagents for the determination of various substances. Thus, the glucose oxidase is used for the quantitative determination of glucose in urine and blood. The enzyme urease is used for the determination of the amount of urea in blood and urine. Using of various appropriate substrates detects dehydrogenases such as pyruvate, lactate, ethanol, etc.

Use of enzymes in diagnostics

The diagnosis of diseases (or syndromes) is based on the activity of the enzyme in human biological fluids. The main principles are:

• damage of the cells leads to the increase of the intracellular enzymes concentration of the damaged cells in the blood or other biological fluids (e.g., urine);

• the amount of released enzymes is enough to detect it;

• a number of enzymes takes precedence or absolute localization in certain organs (organ-specific enzymes);

• there are differences in the intracellular localization of the enzyme.

The use of enzymes as drugs

The use of enzymes as therapeutic agents has many limitations because of their high immunogenicity. Nevertheless enzymotherapy actively develops in the following directions:

• replacement therapy – the use of enzymes in the event of failure;

• elements of the complex therapy – the use of enzymes in combination with other therapies.

Replacement enzyme therapy is effective in treatment of gastrointestinal diseases associated with lack of secretion of digestive juice. For example, pepsin is used in ahilia, hypo-and an- acidic gastritis. Deficiency of pancreatic enzymes also can be largely offset by the ingestion of drugs containing the main pancreatic enzymes (festal, enzistal, mezim forte, etc.).

As additional therapeutic agents, enzymes are used in a number of diseases. Proteolytic enzymes (trypsin, chymotrypsin) are used for local treatment of septic wounds and destroy proteins of dead cells, remove blood clots or viscous secrets with inflammatory airway diseases. Enzyme preparations are widely applied in thrombosis and thromboembolism. For this purpose such drugs as fibrinolisin, streptoliase, streptodecase, urokinase are used.

The enzyme hyaluronidase (lidase) catalyzes cleavage of hyaluronic acid, e.g. subcutaneously and intramuscularly use of lidase for scars resorption after burns or operations (hyaluronic acid forms a crosslinking connective tissue).

Enzyme preparations are used in oncology. Asparaginase catalyzes the reaction of asparagine catabolism, and is applied for the treatment of leukemia.

Prerequisite antileukemic action of asparaginase was the discovery that the leukemic cells are not able to synthesize asparagine. When asparagine is destroyed due to asparaginase administration, the leukemic cells die which leads to slower progression of the disease.

Immobilized enzymes are enzymes associated with the solid support or placed in a polymer capsule. For enzyme immobilization two main approaches are used:

1. Chemical modification of the enzyme.
2. Physical isolation of the enzyme in an inert material.

Immobilized enzymes are most often used in lipid capsules (liposomes), which easily pass through the membrane and provide the necessary effects within the cell. Benefits of immobilized enzymes are:

1. They are easily separated from the reaction medium, which allows using the enzyme again. The product is not contaminated with the enzyme.
2. Enzymatic process can be carried out continuously.

3. Increased stability of the enzyme.

Immobilized enzymes can be used for analytical and preparative purposes. There are several types of devices, where the immobilized enzymes are used in analytical purposes – enzyme electrodes, automatic analyzers, the test system, etc.

Preparative use of immobilized enzymes in industry:

1. Preparation of L-amino acids using aminoacylase.
2. Preparation of high concentreted fructose syrup, using glucose isomerase.
3. Processing of milk.

CHAPTER 5

STRUCTURE AND FUNCTION OF NUCLEIC ACIDS

Nucleic acids are long polymers made from repeating units called nucleotides. A nucleotide consists of:

- a nitrogenous base (purine or pyrimidine),
- a pentose sugar,
- one or more phosphate groups.

History of nucleic acids study

1869 – F.Meischer	nucleic acids were isolated from the nucleus
1943 – O.Avery	DNA carries information on heredity
1949 – E.Chargaff	regularity of the formation of the DNA structure
1953 – J.Watson & F.Crick	discovery of the structure of DNA
1958 – A.Kornberg	purification and characterization of DNA polymerase from *E.coli*
1960 – F.Jacob & J.Monod	the operon model of gene regulation
1966 – M.Nirenberg, S.Ochoa, G.Khorana	Genetic Code
1972-1973 – H.Boyer, S.Cohen, P.Berg	DNA cloning
1990-2003	Human Genome Project

Cellular nucleic acids exist in two forms, deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). Approximately 90% of the nucleic acid within cells is RNA, and the remainder is DNA. DNA is the repository of genetic information within the cell.

Chemical components of nucleic acids

DNA: adenine, guanine, cytosine, thymine, deoxyribose, phosphoric acid.
RNA: adenine, guanine, cytosine, uracil, ribose, phosphoric acid.

Nucleotides of both DNA and RNA are covalently linked through phosphate-group “bridges”, in which the 3'-hydroxyl group of one nucleotide unit is joined to the 5'-phosphate group of the next nucleotide, creating a phosphodiester linkage. These asymmetric bonds mean a strand

of nucleic acid has a direction. The asymmetric ends of nucleic acid strand are called the 5′ (five prime) and 3′ (three prime) ends, with the 5' end having a terminal phosphate group and the 3' end a terminal hydroxyl group. By convention, nucleic acid is read from the 5' to 3' end.

Table 5.1. – **Differences between RNA and DNA**

	RNA	**DNA**
Content	Ribose Uracil	Deoxyribose Thymine
Location	Cytoplasm	Nucleus
Structure	Irregular	Regular
Function	Transfer of information	Storage of information

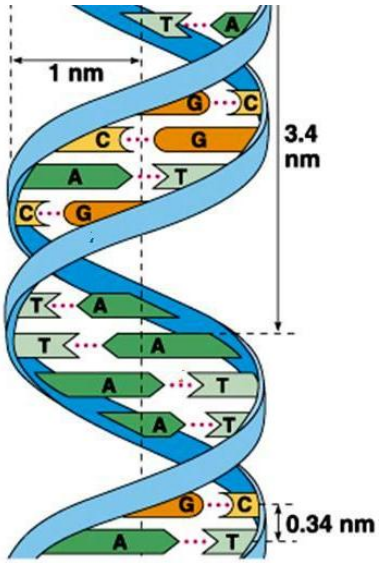
Structure and function of DNA

DNA contains and stores the genetic information.

- It is the source of information for the synthesis of all proteins of the cell and organism.
- Provides the information inherited by daughter cell.

DNA has primary, secondary, and tertiary structures. The primary structure of DNA is a linear sequence of mononucleotides in a polynucleotide chain. Erwin Chargaff and his colleagues found that in all cellular DNAs, regardless of the species, the number of adenosine residues is equal to the number of thymidine residues (A = T), and the number of guanosine residues is equal to the number of cytidine residues (G = C). The sum of the purine residues equals the sum of the pyrimidine residues: A + G = T + C. These quantitative relationships, sometimes called “Chargaff’s rules”, were a key to establishing the three-dimensional structure of DNA.

The secondary structure of DNA is presented by two strands, wound around each other in a right-handed, helical structure with the base pairs in the middle and the deoxyribosylphosphate chains on the outside (Fig.5.1). The orientation of the DNA strands is antiparallel (i.e. the strands run in opposite directions). The nucleotide bases on each strand interact with the nucleotide bases on the other strand to form base pairs (adenine with thymine, guanine with cytosine).



The DNA double helix is stabilized primarily by two forces:

- hydrogen bonds between complementary nucleobases;
- base-stacking interactions among the aromatic nucleobases.

Figure. 5.1 – Secondary structure of DNA

Tertiary structure of DNA is different in prokaryotic and eukaryotic cells. In prokaryotes, there are different types of supercoiling. In eukaryotes, DNA is complexed with RNA and an approximately equal mass of protein. These DNA-RNA-protein complexes are termed **chromatin**.

Functions of chromatin:

- to package DNA into a smaller volume to fit in the cell,
- to strengthen the DNA to allow mitosis,
- to prevent DNA damage,
- control of gene expression and DNA replication.

There are four levels of chromatin organization (Fig.5.2).

The histone proteins associate into a complex termed a **nucleosome (1)**. Each of these complexes contains two molecules each of H2A, H2B, H3, and H4, and one molecule of H1. The nucleosome protein complex is encircled with about 200 base pairs of DNA that form two coils around the nucleosome core. The H1 protein associates with the outside of the nucleosome core to stabilize the complex. The nucleosome particles themselves are also organized into other, more tightly packed structures, termed 30-nm **chromatin filaments (fibers)**. These filaments are constructed by winding the nucleosome particles into a spring-shaped **solenoid (2)** structure with about six nucleosomes per turn. The solenoid is stabilized by head-to-tail associations of the H1 histones. Nucleosomes can be organized into 30 nm fibers *in vitro*, but this structure has not been found in cells. Higher-order folding of chromosomes involves attachment to a

chromosomal scaffold, notably topoisomerase II and SMC proteins. In interphase chromosomes chromatin fibers are organized into **loops (3),** anchored in a scaffold. The DNA in a loop may contain a set of related genes. The final level of chromatin organization **is metaphase chromosome (4)**.

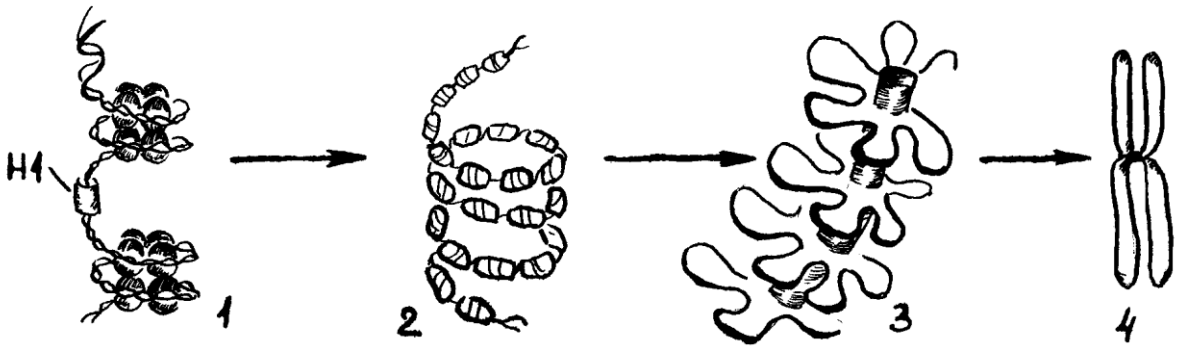


Fig. 5.2 – Levels of chromatin organization

Individual chromosomes are constrained within nuclear subdomains called territories. Histone H1,topoisomerase II, and SMC proteins (structural maintenance of chromosomes) play organizational roles in chromosomes. The SMC proteins, principally cohesins and condensins, have important roles in keeping the chromosomes organized during each stage of the cell cycle.

RNAs

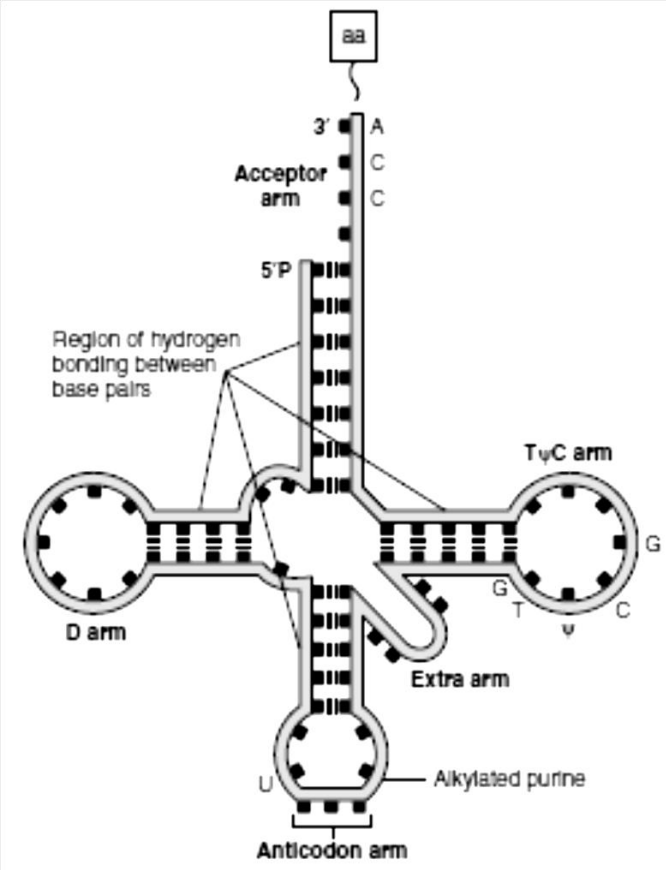
The RNAs produced by prokaryotic and eukaryotic cells are single-stranded molecules. Even though most RNAs are single stranded, they exhibit extensive secondary structures. Single RNA strands can fold into hairpins, double-stranded regions, or complex loops that are important to RNAs function. These secondary structures are the product of intramolecular base pairing that occurs between complementary nucleotides within a single RNA molecule. All eukaryotic cells have two major classes of RNA, protein coding RNAs (messenger RNAs) and non-protein coding RNAs (ribosomal, long noncoding, transfer, small nuclear, micro and silencing RNAs). Each class has a distinctive size and function. The messenger, transfer, and ribosomal RNAs are involved in protein synthesis. The other RNAs are participate in either RNA processing or modulation of gene expression.

Messenger RNAs (mRNAs) represent the most heterogeneous class. They are:

- messenger conveying the information from the gene to the protein synthesizing machinery;
- template for protein synthesis.

All eukaryotic mRNAs contain a methylated guanine nucleotide "cap" at their 5' end. The function of this structure is two-fold; it is essential for ribosomal binding and it protects the mRNA from attack by 5' exonucleases. The 3' end is modified by the addition of a number of adenine residues - known as a polyA tail. The number of adenine residues added to a particular transcript can vary from as few as 30 to more than 300 residues. This structure protects the mRNA from attack by 3' exonucleases, increasing its half-life.

Transfer RNAs (tRNAs) transfer a specific amino acid to a growing polypeptide chain at the ribosomal site of protein synthesis during translation. They function as adapter molecules that translate the information stored in the mRNA nucleotide sequence to the amino acid sequence of proteins. Length of molecules is from 65 to 110 nucleotides.
They exhibit extensive secondary structure and contain several ribonucleotides that differ from the usual four by a variety of modifications.



All tRNAs have a similar folded structure, with four distinctive loops (arms), that have been described as clover leafs (Fig.5.3). The D loop contains several modified bases, including methylated cytosine and dihydrouridine, for which the loop is named. The anticodon loop is the structure responsible for recognition of the complementary codon of an mRNA molecule. A variable loop exists in most tRNAs, but its function is unknown. Finally, there is a TψC loop, which is named for the presence in this loop of the modified base, pseudouridine.

Figure 5.3 – Structure of tRNA

Another prominent structure found in all tRNA molecules is the acceptor stem.

This structure is formed by base pairing between the nucleotides found at the 5' and 3' ends of the tRNA. The last three bases found at the extreme 3' end remain unpaired, and always have the same sequence: 5'-CCA-3'. The 3' end of the acceptor stem is the point at which an amino acid is attached via an ester bond between the 3'-hydroxyl group of the adenosine and the carboxyl group of an amino acid.

Ribosomal RNAs (rRNAs) are the structural and catalytic component of the ribosomes. rRNA from eukaryotes consists of four different sizes of RNA (5S, 5.8S, 18S, 28S). These RNAs interact with each other, and with proteins, to form a ribosome that provides the basic machinery on which protein synthesis takes place.

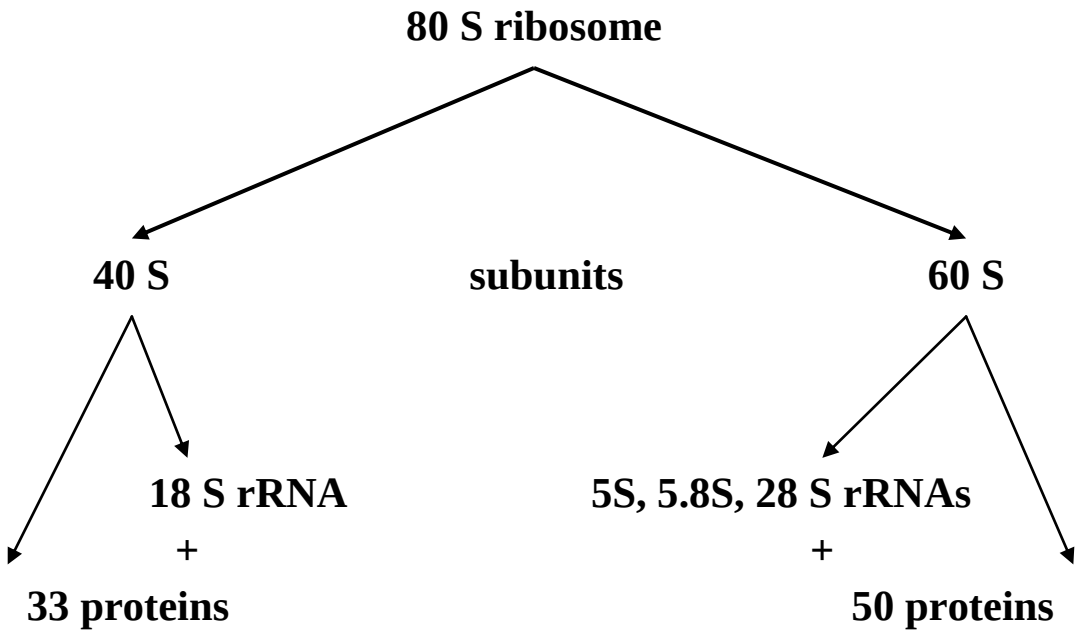


Figure. 5.4 – Structure of eukaryotic ribosomes

Denaturation of DNA

Because the DNA strands are complementary and are held together only by noncovalent forces, they can be separated into individual strands. This strand separation or **denaturation** of DNA is commonly induced by heating or decreasing the salt concentration. Denaturation of DNA is accompanied by increasing of optical density – a phenomenon referred as hyperchromicity. The dissociation is reversible, and when physiologic

temperature or salt concentration is achieved, the interactions between the complementary nucleotide sequences reassociate or reanneal to reform their original base pairs. This process is referred as **hybridization**.

The ability of two complementary DNA strands to pair with one another can be used to detect similar DNA sequences in two different species or within the genome of a single species. For example, if duplex DNAs isolated from human cells and from mouse cells are completely denatured by heating, then mixed some strands of the mouse DNA will associate with human DNA strands to yield hybrid duplexes, in which segments of a mouse DNA strand form base-paired regions with segments of a human DNA strand. The closer the evolutionary relationship between two species, the more extensively their DNAs will hybridize. The hybridization of DNA strands from different sources forms the basis for a powerful set of techniques essential to the practice of modern molecular genetics.

A specific DNA sequence or gene can be detected in the presence of many other sequences, if one already has an appropriate complementary DNA strand (usually labeled in some way) to hybridize with. The complementary DNA can be from a different species or from the same species, or it can be synthesized chemically in the laboratory.

Hybridization techniques can be varied to detect a specific RNA rather than DNA. The isolation and identification of specific genes and RNAs relies on these hybridization techniques. Applications of this technology make possible the identification of an individual on the basis of a single hair left at the scene of a crime or the prediction of the onset of a disease decades before symptoms appear.

CHAPTER 6

BIOSYNTHESIS OF NUCLEIC ACIDS

The central dogma of molecular biology comprises the three major processes in the cellular utilization of genetic information.

- the first is **replication**, the copying of parental DNA to form daughter DNA molecules with identical nucleotide sequences;
- the second is **transcription**, the process by which parts of the genetic message encoded in DNA are copied precisely into RNA;
- the third is **translation**, whereby the genetic message encoded in messenger RNA is translated on the ribosomes into a polypeptide with a particular sequence of amino acids.

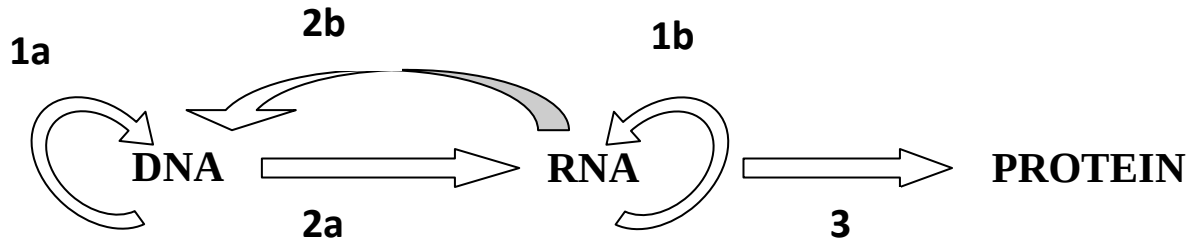


Figure 6.1 – Ways of transport of genetic information

1a - replication of DNA; 1b - replication of RNA; 2a – transcription; 2b - reverse transcription; 3 – translation.

Biosynthesis of DNA (replication)

Biosynthesis of DNA occurs during the S phase of the cell cycle.

DNA replication is semiconservative: each DNA strand serves as a template for the synthesis of a new strand, producing two new DNA molecules, each with one new strand and one old strand.

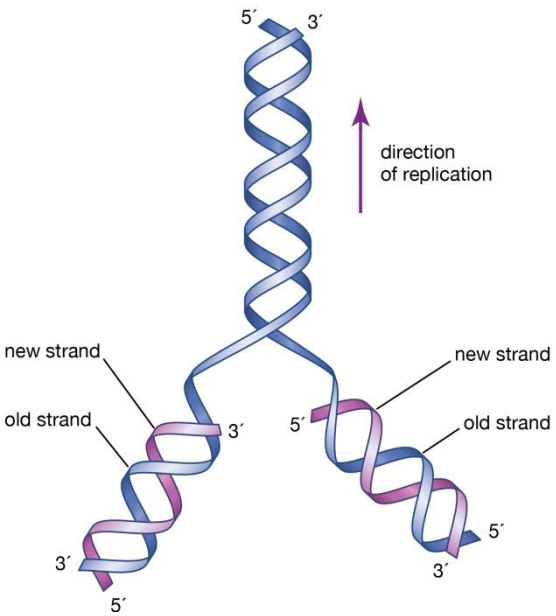


Figure 6.2 – Semiconservative replication of DNA

Commitment to replication requires the synthesis and activity of S-phase cyclin-cyclin dependent kinase complexes which help to activate replication by binding to and phosphorylating several proteins in the pre-replicative complexes. The site at which DNA replication is initiated is termed the "**origin of replication**". Eukaryotes have many origins of replication. The key event in the initiation of replication in all eukaryotes is the loading of the replicative helicase. It is loaded onto the DNA by protein complex, called **origin recognition complex** (ORC).

Major steps of DNA replication

1. Identification of the origins of replication.
2. Denaturation of double strand DNA to provide single strand DNA template.
3. Formation of the replication fork, synthesis of RNA primer.
4. Initiation of DNA synthesis and elongation.
5. Formation of replication bubbles with ligation of the newly synthesized DNA segments.
6. Reconstitution of chromatin structure.

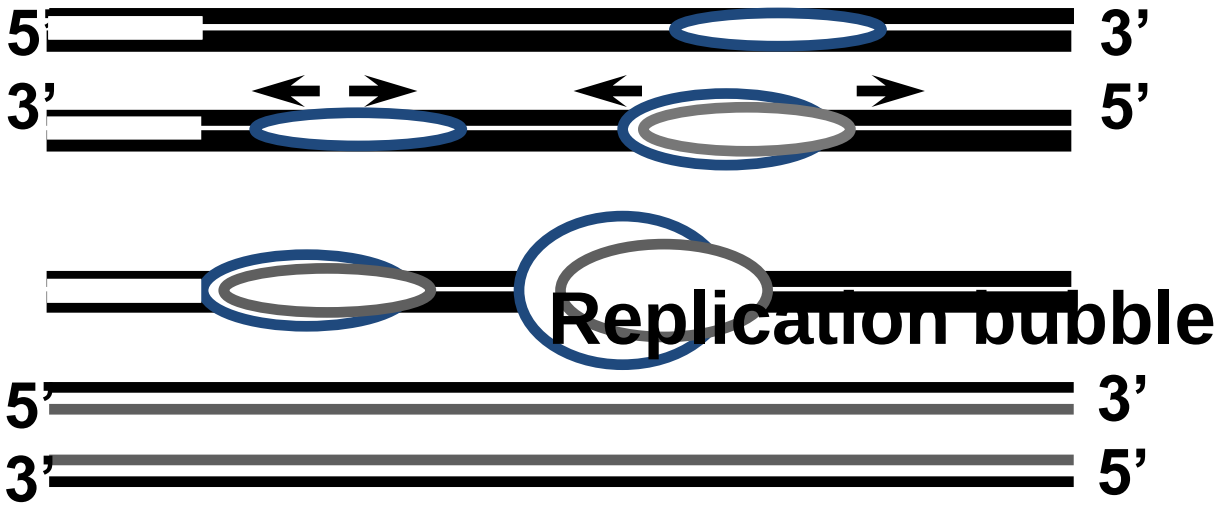


Figure 6.3 – General scheme of replication

One or both ends of the replication bubble are dynamic points, termed replication fork, where parent DNA is being unwound and the separated strands are replicated. A new strand of DNA is always synthesized in the 5'→3' direction, with the free 3'-OH as the point at which the DNA is elongated. Because the two DNA strands are antiparallel, the strand serving as the template is read from its 3' end toward its 5' end.

The **DNA polymerase α** synthesizes RNA oligonucleotides complementary to parental DNA strands (Fig.6.4). These oligonucleotides serve as **primers** for DNA synthesis. Because of the unidirectional synthetic activity of the polymerase and the antiparallel nature of the two strands, the synthesis of DNA along the two strands is different. The two daughter strands being synthesized are termed the **leading strand** and the **lagging strand**. In the leading strand, synthesis proceeds in the same direction as replication fork movement. In the lagging strand, synthesis proceeds in the direction opposite to the direction of fork movement. Because DNA synthesis adds new nucleotides only at the 3'-end of the elongating DNA strand, the lagging strand is synthesized in small fragments, termed **Okazaki fragments.** Synthesis of each Okazaki fragment begins with primer synthesis (by DNA polymerase α).

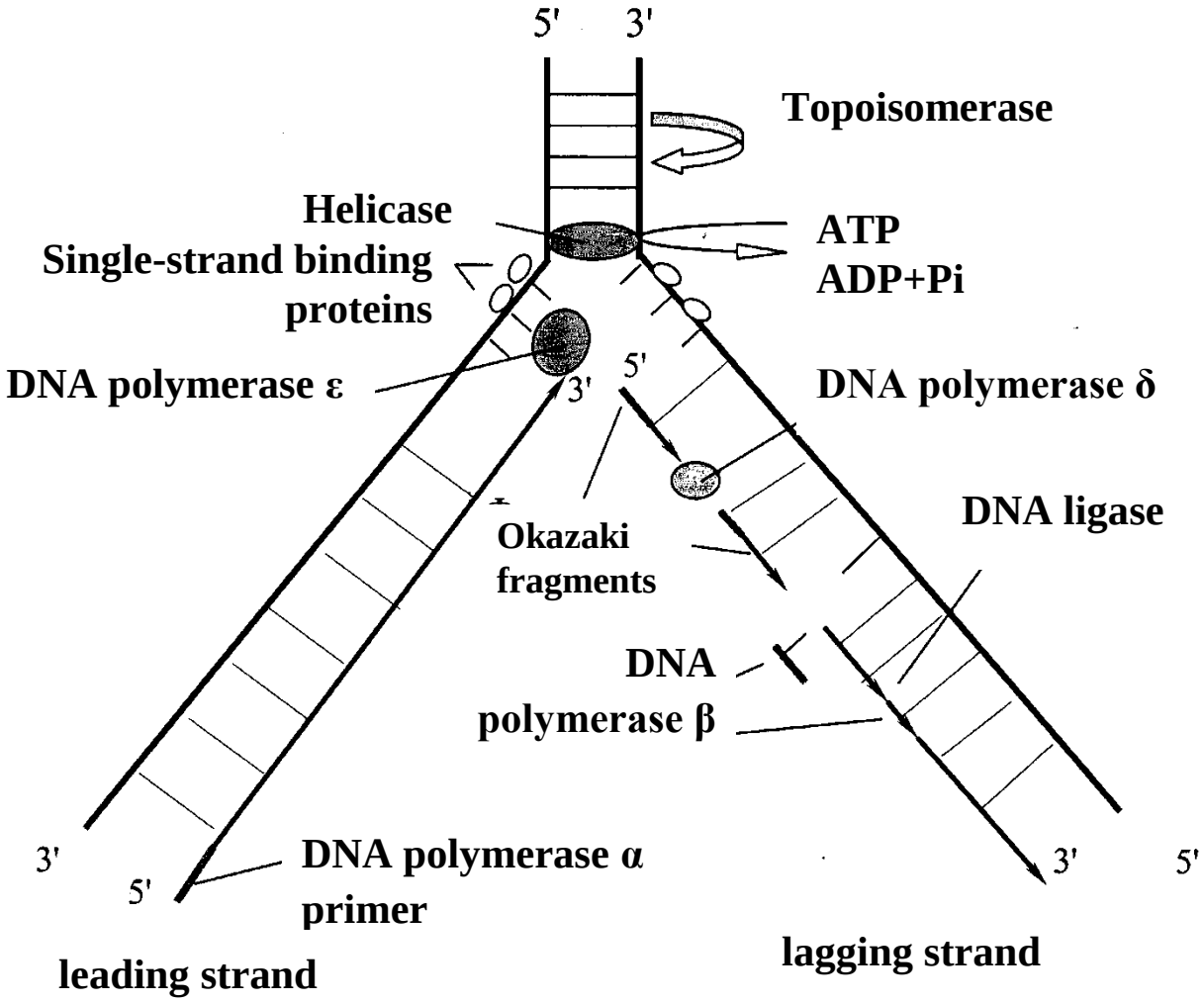


Figure 6.4 – Replication fork

DNA polymerase ε binds to the primer and template strand and synthesizes the leading strand, **DNA polymerase δ –** the lagging-strand DNA fragments. **DNA polymerase β** removes the RNA primers from Okazaki fragments and replaces it with DNA. Finally, **DNA ligase** joins the lagging-strand DNA fragments to form a continuous strand.

Biosynthesis of RNA (transcription)

Transcription is more selective than replication. Only particular genes or groups of genes are transcribed, and some portions of the DNA genome are never transcribed. The sum of all the RNA molecules produced in a cell under a given set of conditions is called the cellular transcriptome.

The two complementary DNA strands have different roles in transcription. The strand that serves as template for RNA synthesis is called the template strand. The DNA strand complementary to the template, the nontemplate strand, or coding strand, is identical in base sequence to the RNA transcribed from the gene,

The enzymes responsible for the synthesis of RNA, using DNA as a template, are called **RNA polymerases**. All RNAs are synthesized by these enzymes in a direction that is 5' to 3'. This polarity of synthesis dictates that the DNA strand used as a template is read in the 3' to 5' direction (Fig. 6.5). DNA-dependent RNA polymerase uses the DNA template strand to synthesize the complementary RNA strand, adding each new nucleotide onto the 3' end of the growing chain. Unlike DNA polymerase, RNA polymerase does not require a primer to initiate synthesis. Initiation occurs when RNA polymerase binds at specific DNA sequences called **promoters**.

Types of DNA-dependent RNA polymerases:

- **RNA polymerase I** synthesizes the rRNAs;
- **RNA polymerase II** synthesizes mRNA, lncRNAs, miRNAs, snRNAs;
- **RNA polymerase III** synthesizes tRNAs and 5S rRNAs.

Transcription is a cyclical process that involves RNA chain **initiation, elongation** and **termination**.

Initiation involves the interaction of the RNA polymerase with specific site on the DNA template strands, known as **promoter** (Fig. 6.5). Promoters are located in front of the gene that is to be transcribed. Once RNA polymerase has bound to a promoter, it begins the process of selecting the appropriate complementary ribonucleotide and forming phosphodiester bridges between this nucleotide and the nascent chain, in a process called **elongation.** For elongation to occur, the double-stranded DNA must be

continually unwound, so that the template strand is accessible to the RNA polymerase. Finally, **termination** involves the dissociation of the RNA polymerase from the DNA template. This step requires either the presence of termination site with specific RNA secondary structure or the action of specific protein factor.

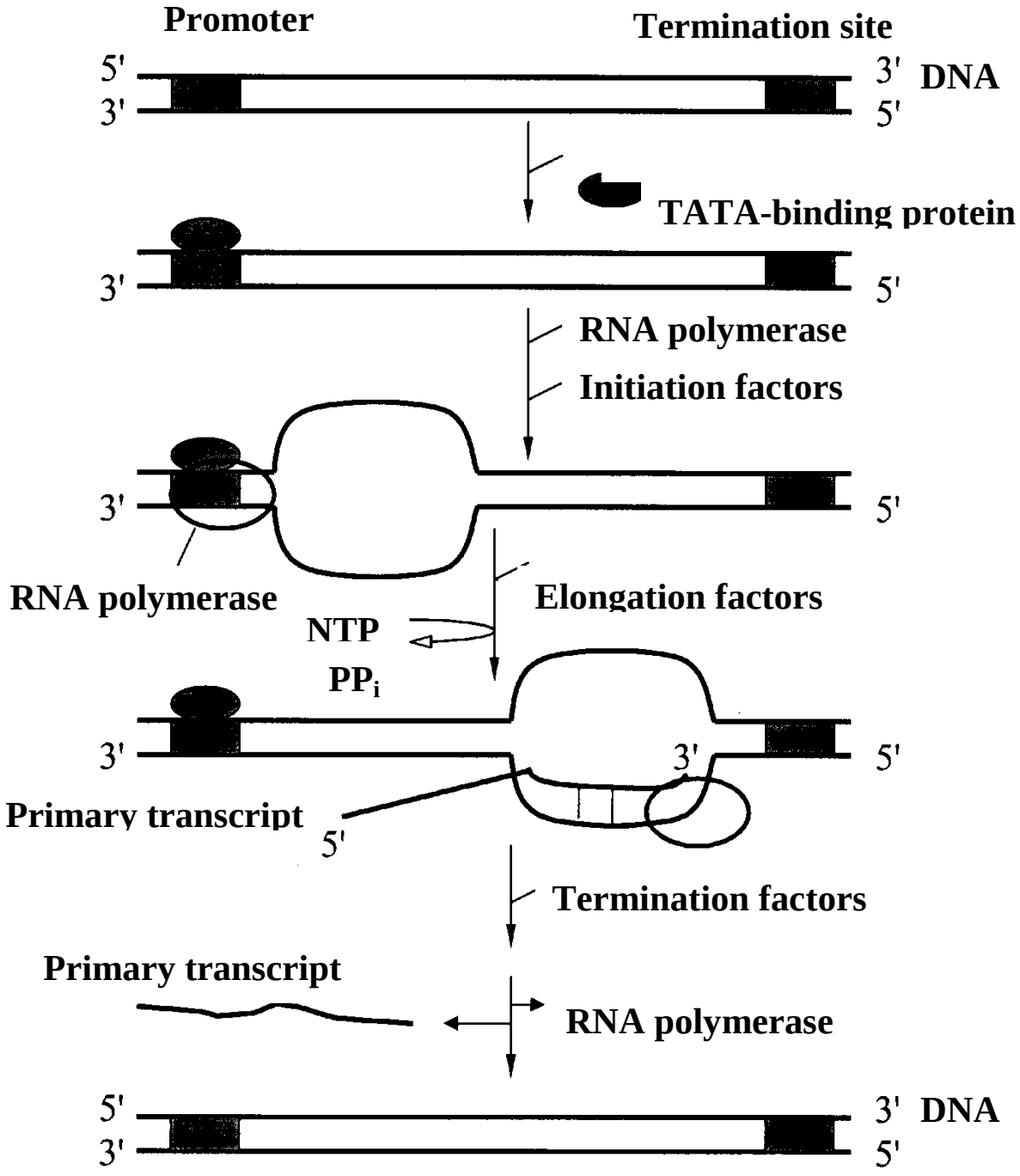


Figure 6.5 – Scheme of transcription

RNA processing

The newly synthesized RNA molecules are called a **primary transcript**. They usually processed before they become functional.

Most eukaryotic **mRNAs** contain specific structures that are added to their 5' and 3' ends after transcription has occurred. A 7-methyl guanine residue (**cap**) is added to the 5' end of mRNAs. A poly(A) tail of varying length (100-300 residues) is added to the 3' end of mRNAs. The A residues are not encoded by the DNA but added by the action of poly(A) polymerase using ATP as a substrate. Both the 5' and 3' ends of eukaryotic mRNAs are modified first, prior to further processing. After that, the sequences called **introns** (intravening sequences) are removed from the primary transcript and the remaining segments, termed **exons** (expressed sequences), are ligated to form a functional RNA. This process is called **splicing**. Some eukaryotic mRNA transcripts can be processed in more than one way to produce different mRNAs and thus different polypeptides. Much of the variability in processing is the result of **alternative splicing**, in which a particular exon may or may not be incorporated into the mature mRNA transcript. Alternative splicing occurs in more than 95% of human genes.

Processing of **transfer RNAs.** Endoribonucleases cleave phosphodiester bonds within the primary transcript to release individual RNAs; exoribonucleases remove excess nucleotides from the 5' and 3' ends of these RNAs, until a molecule of the correct size is produced. The sequence 5'-CCA-3' is added to the 3' end of tRNAs. The final type of tRNA processing is the modification of some bases by methylation, deamination, or reduction.

The eukaryotic **rRNAs** are synthesized as a single RNA transcript with a size of 45S. This large primary transcript is processed into 28S, 18S, 5.8S rRNAs.

Regulation of transcription in eukaryotes

Regulation of transcription in prokaryotes is described by the Jacob and Monod operon model. Operons contain promoter regions were proteins bind and facilitate or inhibit the binding of RNA polymerase. Transcription in eukaryotes is regulated in the similar manner, but with more complex mechanisms.

The promoter sequence acts as a basic recognition unit, signaling that there is a gene that can be transcribed. Promoter also plays an important role in determining that RNA is synthesized at the right time in the right

cell. The structure of promoters varies from gene to gene, but there are a number of key sequence elements that can be identified within the promoter. These elements may be present in varying combinations, some elements being present in one gene but absent in another. Most promoters possess a sequence known as the TATA box. This sequence appears to be very important in the process of transcription, as nucleotide substitutions that disrupt the TATA box result in a marked reduction in the efficiency of transcription.

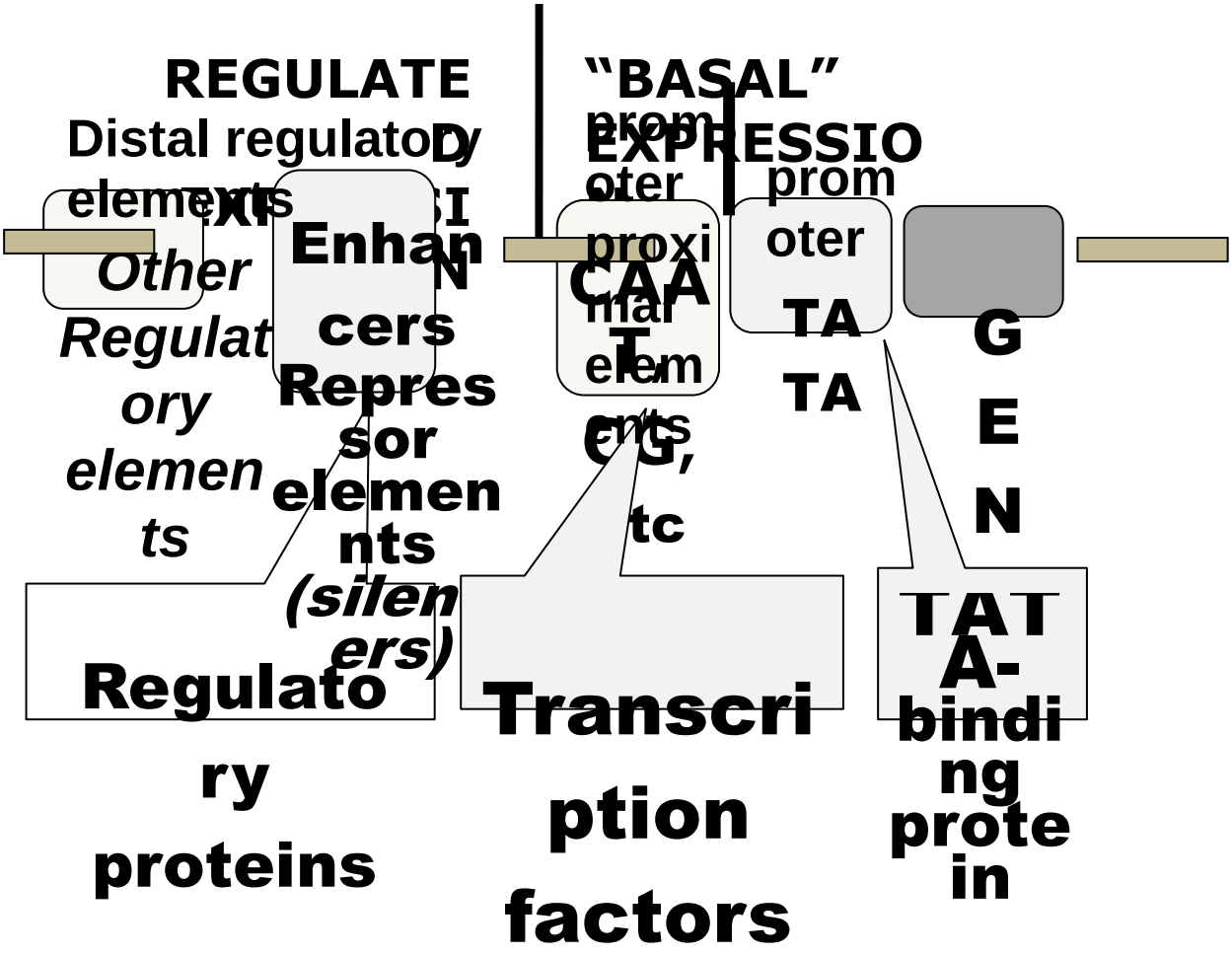


Figure 6.6 – Transcription control regions

In addition to the TATA box, other promoter elements have been described. For example, the CAAT box is often found upstream of the TATA box. As in the case of the TATA box, it may be more important for its ability to increase the strength of the promoter signal rather than in controlling tissue- or time-specific expression of the gene. Another commonly noted promoter element is the GC box.

Promoters found in eukaryotic cells are complicated. In addition to the sequence elements required for basal expression (TATA box, CAAT box, GC box), they have additional sequences that are responsible for regulating the rate of initiation of transcription. These sequence elements are known as either **enhancers** or **silencers**, depending on the effect they have on transcription. They can be located at great distances either upstream or downstream of the start of transcription. Promoters with these types of sequence elements exert their effect on transcription by acting as the binding site for a variety of proteins known as trans-acting factors. The type of trans-acting factor that binds to these sequence elements will determine whether the rate of transcription is increased or decreased.

The transcription of the gene is initiated and regulated by a number of different sequence-specific DNA-binding proteins, known as transcription factors. These factors bind to specific nucleotide sequences and bring about differential expression of the gene.

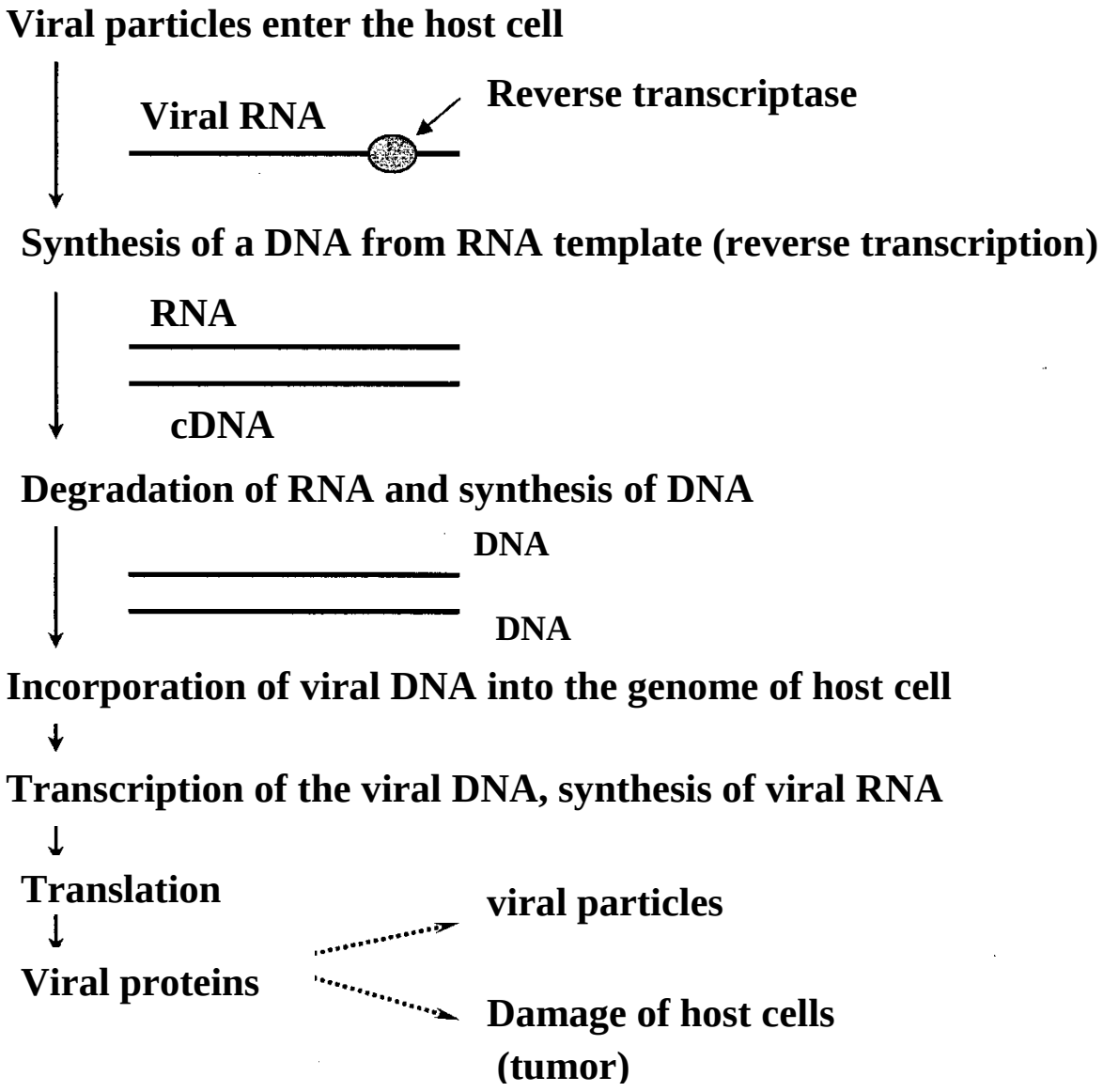


Figure 6.7 – Scheme of reverse transcription

Response elements are nucleotide sequences that allow specific stimuli, such as steroid hormones, cyclic AMP, or insulin-like growth factor-1, to control gene expression. Response elements are often part of promoters or enhancers where they function as binding sites for particular transcription factors.

Reverse transcription

Certain RNA viruses that infect animal cells carry within the viral particle an RNA-dependent DNA polymerase called **reverse transcriptase**. On infection, the viral RNA and the enzyme enter the host cell. The reverse transcriptase first catalyzes the synthesis of a DNA strand complementary to the viral RNA (Fig. 6.7), then degrades the RNA strand of the viral

RNA-DNA hybrid and replaces it with DNA. The resulting duplex DNA often becomes incorporated into the genome of the eukaryotic host cell. These integrated viral genes can be activated and transcribed, and the gene products – viral proteins and RNA –packaged as new viruses. The RNA viruses that contain reverse transcriptases are known as retroviruses. Most retroviruses do not kill their host cells but remain integrated in the cellular DNA, replicating when the cell divides. Some retroviruses can cause cancer and AIDS.

Each reverse transcriptase is most active with the RNA of its own virus, but each can be used experimentally to make DNA complementary to a variety of RNAs. Reverse transcriptases have become important reagents in the study of DNA-RNA relationships and in DNA cloning techniques. They make possible the synthesis of DNA complementary to an mRNA template, and synthetic DNA prepared in this manner, called complementary DNA (cDNA), can be used to clone cellular genes.

CHAPTER 7

BIOSYNTHESIS OF PROTEIN

Protein synthesis is the culmination of the transfer of genetic information from DNA to proteins. In this transfer, information must be translated from the four-nucleotide-language of DNA and RNA to the twenty-amino acids-language of proteins. The **genetic code**, in which three nucleotides in mRNA (codon) specify an amino acid, represents the translation dictionary of the two languages.

Features of the genetic code:

- triplet – each codon consist of a sequence of 3 nucleotides;
- degenerate – multiple codons decode the same amino acid;
- unambiguous – given specific codon indicate only a single amino acid;
- non overlapping – code not involved any overlap of codons;
- not punctuated – no punctuation between codons, message (mRNA) is read in continuing sequence;
- universal - standard genetic code is universal in all species, with some minor deviations in mitochondria and a few single-celled organisms;
- presence of sense and nonsense codons - the codon AUG signals initiation of translation, in addition to coding for Met residues, the triplets UAA, UAG, and UGA are signals for termination,(also called stop codons or nonsense codons);
- the message is read in direction 5′→3′.

Stages of protein synthesis:

I. activation of amino acids;

II. initiation;

III. elongation;

IV. termination and ribosome recycling;

V. folding and posttranslational processing.

I	Activation of amino acids

Amino acids are activated and attached to their corresponding tRNAs by highly specific enzymes aminoacyl-tRNA synthetases. Each aminoacyl-tRNA synthetase recognizes a particular amino acid and the tRNAs specific for that amino acid. An amino acid first reacts with ATP, forming an enzyme-aminoacyl-AMP complex. The aminoacyl-AMP then forms an ester with 2′ or 3′ hydroxyl of tRNA.

The aminoacyl-tRNA synthetases have the ability not only to discriminate between amino acids before they are attached to the appropriate tRNA, but also to remove amino acids that are attached to the wrong tRNA.

Once an amino acid is attached to a tRNA, insertion of the amino acid into the growing polypeptide chain depends only on the codon-anticodon interaction. In other words, the tRNA acts as adapter between the codons in mRNA and amino acid sequence in the protein.

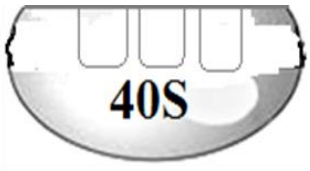
II	Initiation of translation

Initiationof protein synthesis takes place when a ribosome (both large and small subunit) has assembled on the mRNA and the P site is occupied by a methionyl-tRNA (met-tRNA) molecule. This complex is formed by the action of proteins known as initiation factors (eIF). Eukaryotic cells have at least 12 initiation factors. They help to promote the association of the small ribosomal subunit with the mRNA and met-tRNA. The small ribosomal subunit (40S) binds to the7-methylguanine cap structure of mRNA (Fig. 7.1) The subunit moves down the mRNA until the first AUG codon is encountered. Then the initiating met-tRNA base-pairs with this codon. After that the large ribosomal subunit (60S) joins the complex and protein synthesis is ready to begin.

Ribosomes possess specific sites at which tRNAs bind. These sites are known as the aminoacyl, or **A site**, and the peptidyl, or **P site**.

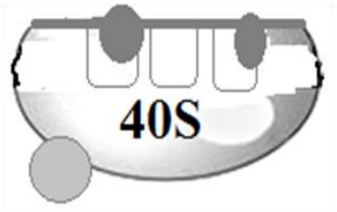
The assembly of the initiation complex is driven by the hydrolysis of guanosine triphosphate (GTP), and the movement of this complex down the mRNA is driven by the hydrolysis of ATP.

+ eIF1, eIF3, eIF1A



The small ribosomal subunit (40S) binds to the initiation factors.
(1, 3, 1A).

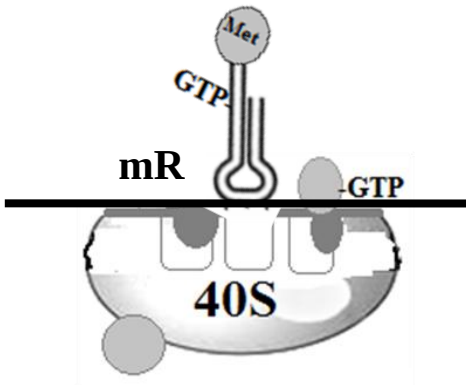
+ GTP-eIF2, Met-tRNA, GTP-eIF5



The initiator Met-tRNA is bound by the initiation factor eIF2, which also has bound GTP. This ternary complex binds to the 40S ribosomal subunit, along with GTP-eIF5. This creates a 43S preinitiation complex.

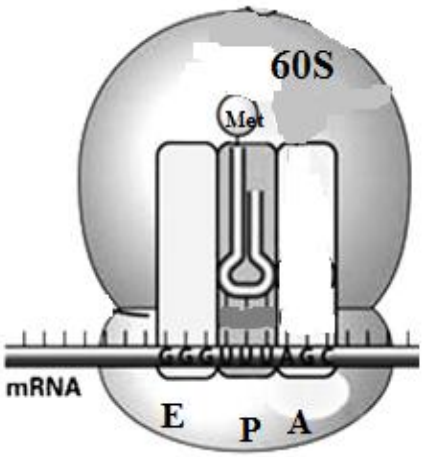
+mRNA-eIF4F

The mRNA binds to the eIF4F complex, which mediates its association with the 43S preinitiation complex.



Addition of the mRNA and its associated factors creates a 48S complex. This complex scans the bound mRNA, starting at the 5′ cap, until an AUG codon is encountered.

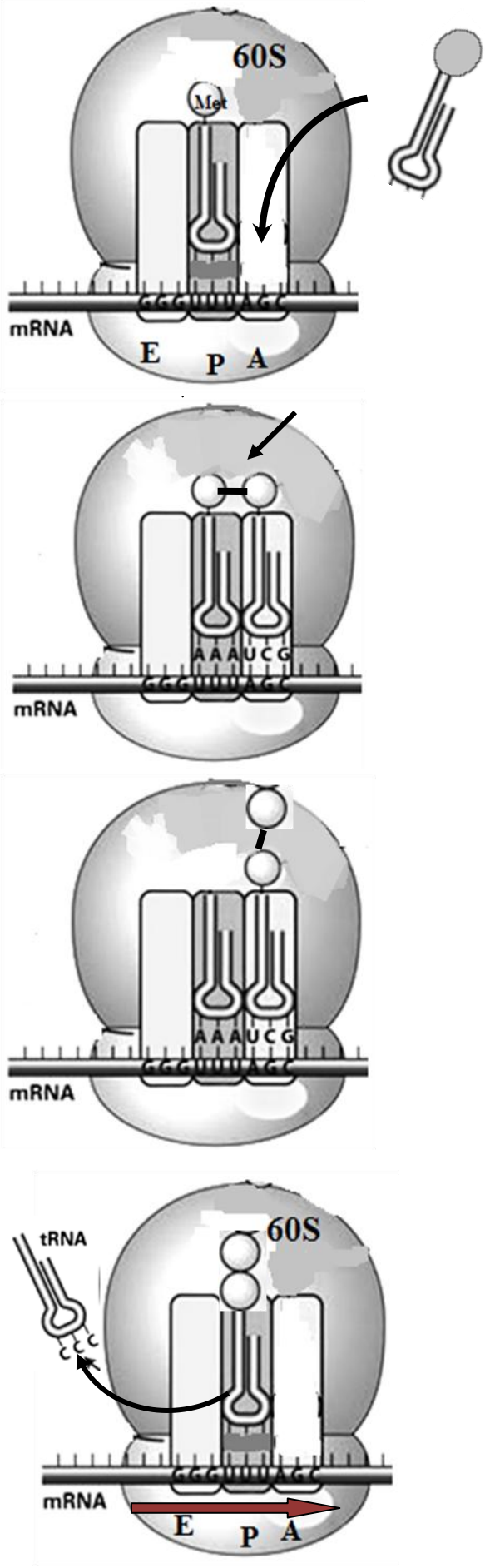
+60S



Once the initiating AUG site is encountered, the 60S ribosomal subunit associates with the complex. Ribosomes possess specific sites at which tRNAs bind. These sites are known as the aminoacyl, or **A site**, the peptidyl, or **P site**, and exit or **E site.** The assembly of the initiation complex is driven by the hydrolysis of GTP.

Figure 7.1 – Initiation of translation

III	Elongation



Elongation begins with the binding of an aminoacyl-tRNA to the A site of the ribosome The binding of the aminoacyl-tRNA in the A site requires the energy of GTP hydrolysis.

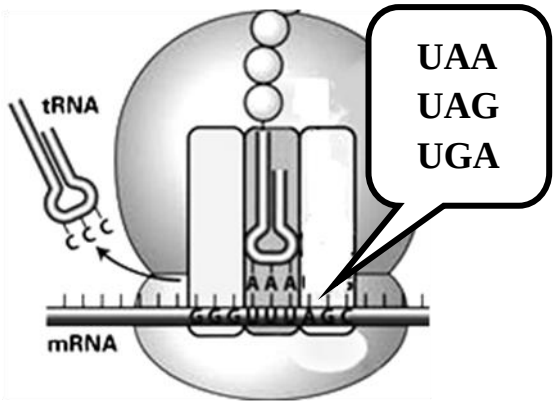
Once the correct aminoacyl-tRNA molecule has been delivered to the A site of the ribosome, peptidyltransferase catalyzes the formation of a peptide bond between the amino acid in the A site and the amino acid at the end of the growing peptide chain in the P site.

The tRNA-peptide chain is now transiently bound to the A site.

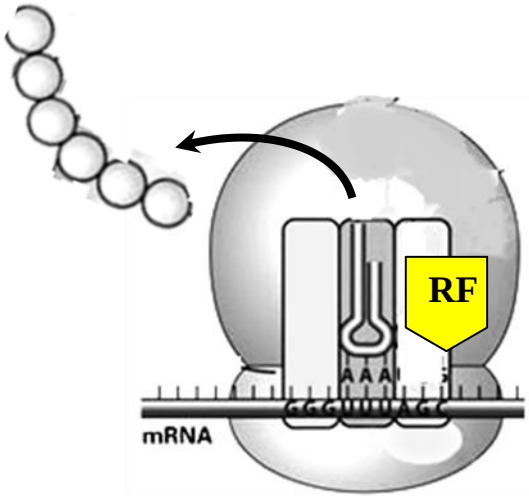
The ribosome is then moved one codon down, and the nascent peptide chain at the A site moves to the P site. This step called **translocation**.
The whole process recycles for addition of the next amino acid.

Figure. 7.2 – Elongation

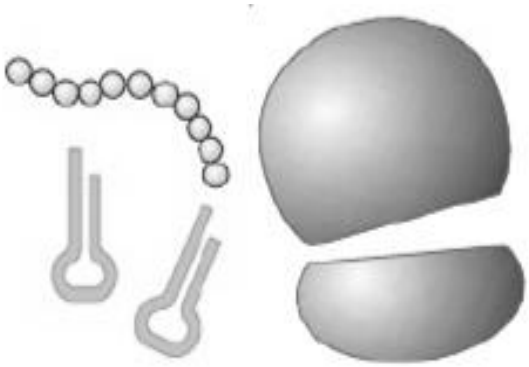
IV	Termination of translation



Termination of protein synthesis is accomplished when the A site of the ribosome reaches one of the stop-codons of the mRNA (UAA, UAG, and UGA).



Protein factors called releasing factors (RF) recognize these codons, and cause the protein that is attached to the last tRNA molecule in the P site to be released. This process is an energy-dependent reaction catalyzed by the hydrolysis of GTP.



After release of the newly synthesized protein, the ribosomal subunits, tRNA, and mRNA dissociate from each other and another cycle can be repeated.

Figure 7.3 – Termination of translation.

V	Folding and posttranslational processing (Post-translational modification of proteins)

For a newly synthesized protein to become functionally active it must be **folded** into a unique three-dimensional structure. Newly synthesized proteins achieve their native structures with the help of a class of proteins called **chaperones**.

Many proteins must be altered by chemical modification before they become biologically active; collectively, these alterations are known as post-translational modifications. They may include amino-terminal modification, modification of individual amino acids, proteolytic processing, and formation of disulfide bridges. One of the common amino-terminal modifications of eukaryotic cells is the removal of the amino-terminal methionine, residue that initiates protein synthesis. Amino acids within the protein can also be altered. Some amino acids, such as lysine, will be modified by the addition of a methyl group; others, such as cysteine, can have isoprenyl groups or other lipids added to their side chains, to facilitate protein binding to membranes. Cysteine residues also form specific disulfide bridges that are important to the structural integrity of the protein. Finally, many proteins are synthesized as preproproteins and proproteins that must be proteolytically cleaved for them to be active.

Regulation of protein synthesis

Regulation of protein synthesis in eukaryotes can occur at the next levels:

- changes in genes;
- transcriptional level (Chapter 6);
- processing and transport of mRNA;
- half-life of mRNA (some mRNAs are degraded more rapidly than others);
- level of translation (For example, iron status regulates translation of an iron carrier-protein);
- post-translational processing of protein.

Regulation of protein synthesis may result from **changes in genes**. Genes may be amplified or may be lost from cell so that functional proteins are extensively produced or cannot be produced.

Segments of DNA may move from one location to another on the genome, associating with each other in various ways so that different proteins are produced. Modification of bases in DNA affects the transcriptional activity of a gene.

Other factors that affect the conversion of gene to protein include:

- access of the gene to the transcriptional apparatus,
- enzymatic modification of histones and nucleotides
- factors that effected alternative splicing,

- post-transcriptional editing of pre-mRNA
- restricted expression of biallelic genes.

The promoters of some genes may not be readily accessible to transcription factors. Condensed chromatin is usually not a good template for transcription, and in many cases it is necessary for chromatin remodeling to occur before transcription proceeds. Histone packaging, nucleosome stability and the accessibility of DNA is controlled by reversible acetylation and deacetylation of lysine residues in the amino terminal regions of the core histones.

When the transcription of a gene is completed, the pre-mRNA undergoes a process whereby the introns in the RNA are removed, to produce an mRNA molecule that is smaller than the pre-RNA, but contains all the necessary information to allow the gene to be translated into the protein product. For many genes, the pre-mRNA can be spliced in several alternate ways. This process may provide sufficient diversity to explain individual uniqueness, despite similarities in the gene complement of a species. Thus, by **alternative splicing**, a particular exon or exons may be spliced out on some but not all occasions. Since many genes have a lot of exons, some pre-mRNAs can eventually give rise to many different versions of the mRNA and of the final protein. The proteins may differ by only a few amino acids, or may have major differences; they often have different biological roles.

Editing of RNA at the post-transcriptional level involves the enzyme-mediated alteration of RNA in the cell nucleus before translation. This process may involve the **insertion, deletion, or substitution of nucleotides** in the RNA molecule. Like alternative splicing, the substitution of one nucleotide for another can result in tissue-specific differences in transcripts.

The normal complement of human chromosomes comprises 22 pairs of autosomes and two sex chromosomes. Therefore there are 23 pairs of chromosomes, each of which has genes that are present in both chromosomes: they are biallelic. Under normal circumstances, both genes are expressed without preference being given to either allele of the gene – that is, both the paternal and the maternal copies of the gene can be expressed, unless there is a mutation in one allele that prevents this from occurring.

Inhibitors of template synthesis

Protein synthesis is a central function in cellular physiology and is the primary target of many naturally occurring antibiotics and toxins.

Table 7.1. – **Antibiotics and their targets**

Antibiotic	Target, mechanism of action
Actinomycin D	DNA coding chain, inhibition of transcription
Rifampicin	Bacterial RNA polymerase, prevents initiation of RNA synthesis
Streptolydigin	Bacterial RNA polymerase, prevents elongation of RNA chains
Tetracycline	Bacterial ribosome-A site, prevents binding of aminoacyl-tRNAsto the A site
Streptomycin	Bacterial 50S ribosome subunit, causes misreading of mRNA
Erythromycin	Bacterial 50S ribosome subunit, prevents translocation
Chloramphenicol	Bacterial ribosome-inhibition of peptidyl transferase activity
Cycloheximide	Eukaryotic 80S ribosome, inhibits peptidyltransferase in the 60S ribosomal subunit

CHAPTER 8

PRINCIPLES OF MOLECULAR BIOLOGY

Molecular biology is the science that strives to understand the chemical and physical basis of biological specificity and variation, especially with regard to the structure, replication, and expression of genes, and to the structure, interaction, and physiological function of gene products. Hand in hand with the development of our understanding of DNA, genes, and their functions has been the explosion of technology for the clinical analysis of DNA and RNA. That's why an understanding of the basic principles of the methods and examples of some commonly used applications will become essential to diagnostic services providing genetic analysis in the clinical setting. Particularly important to molecular biology techniques is a set of enzymes.

Restriction endonucleases (restriction enzymes) comprise a group of enzymes that cleave double stranded DNA. These enzymes are sequence-specific and each enzyme acts at a limited number of sites in DNA called 'recognition' or 'cutting' sites. If DNA is digested by a restriction enzyme, the resulting digested DNA will be reduced to fragments of varying sizes depending on how many cutting sites for that restriction enzyme are present in the DNA. It is important to note that each enzyme will cut DNA into a unique set of fragments. Some restriction endonucleases make staggered cuts on the two DNA strands, leaving two to four nucleotides of one strand unpaired at each resulting end. These unpaired strands are referred to as sticky ends, because they can base-pair with each other or with complementary sticky ends of other DNA fragments. Other restriction endonucleases cleave both strands of DNA at the opposing phosphodiester bonds, leaving no unpaired bases on the ends, often called blunt ends. Sticky ends are particularly useful in constructing hybrid or chimeric DNA molecules.

DNA ligases can join the DNA fragment to a suitable cloning vector to link the DNA molecules together.

Hybridization of nucleic acids

Hybridization is a fundamental feature of DNA technology. It is a process by which a piece of DNA or RNA of known nucleotide sequence, which can range in size, is used to identify a region or fragment of DNA

containing complementary sequences. The first piece of DNA or RNA is called a probe. Probe DNA will form complementary base pairings with another strand of DNA, often termed the target, if the two strands are complementary, and a sufficient number of hydrogen bonds is formed. Probes must have a label to be identified. There are many ways in which probes can be labeled, but they fall into two categories, either isotopic, i.e. involving radioactive atoms, or nonisotopic, e.g. end-labeling probes with fluorescent tags or small ligand molecules.

There are several ways in which hybridization can be used in the study of DNA, which exploit either the stringency of the hybridization of probe to target or the utility of restriction enzymes for detecting variations in nucleotide sequences.

DNA fingerprinting (also called DNA typing or DNA profiling) is based on sequence polymorphisms, slight sequence differences between individuals. Each difference from the prototype human genome sequence occurs in some fraction of the human population; every individual has some differences. Some of the sequence changes affect recognition sites for restriction enzymes, resulting in variation in the size of DNA fragments produced by digestion with a particular restriction enzyme. These variations are restriction fragment length polymorphisms.

Restriction fragment length polymorphisms (RFLPs). Restriction enzymes cleave DNA at specific recognition sites; if these sites are altered by mutation or polymorphism, the size of DNA fragments on a blot will differ. If the recognition sequence is disrupted, either by a pathologic change in the DNA sequence resulting in a disease (a mutation), or a naturally occurring variation in the DNA sequence unaccompanied by disease (a polymorphism), the results of probing a Southern blot of DNA digested by a restriction enzyme may differ. Such differences in DNA sequence may lead to the creation of new restriction sites or the abolition of existing sites, and result in DNA fragments of different lengths - pattern differences known as restriction fragment length polymorphisms (RFLPs) (Fig. 8.1). Such RFLPs can be used either to identify disease-causing mutations or to study variation in noncoding DNA.

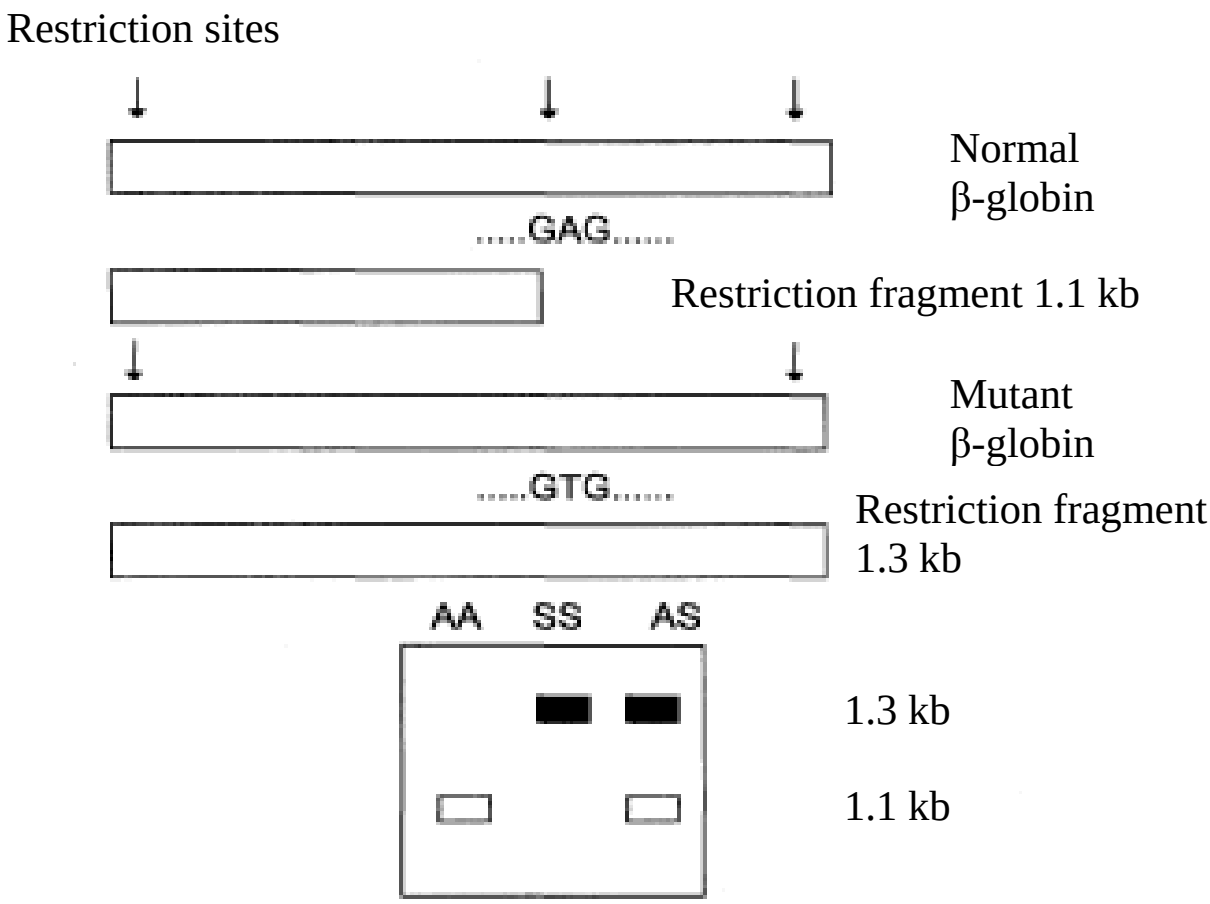


Figure 8.1 – Restriction fragment length polymorphisms

Blot transfer procedure

One of the fundamental steps in the evolution of molecular biology was the discovery that DNA could be transferred from a semisolid gel onto a nitrocellulose membrane in such a way that the membrane could act as a permanent record of the DNA information in the gel and could be used for multiple-probe experiments. The process whereby the DNA is transferred to the membrane was first described by E.Southern, but subsequent techniques based on the transfer of RNA and proteins have adopted the direction theme and are called Northern and Western blots, respectively.

Table 8.1. – **Blots used in molecular biology**

Blot	**Probe**	**Target**
Southern	nucleotide	DNA
Northern	nucleotide	RNA
Western	antibody	protein

Southern blotting. If DNA is digested by a restriction enzyme, the resulting digest can be separated on the basis of size by gel electrophoresis. A solution of digested DNA is placed in a well in an agarose gel, and an electric current applied. The rate of migration of DNA fragments depends on their size, the smallest fragments moving furthest and the largest moving least. Following electrophoresis, the gels are soaked in a strong alkali solution to render the DNA fragments single-stranded. These single-stranded fragments can be then transferred to a nitrocellulose or nylon membrane to which they bind readily and permanently. The process of transfer involves the passage of solute through the gel and into the filter, passively carrying the DNA and producing an image of the gel on the filter (Fig. 8.2).

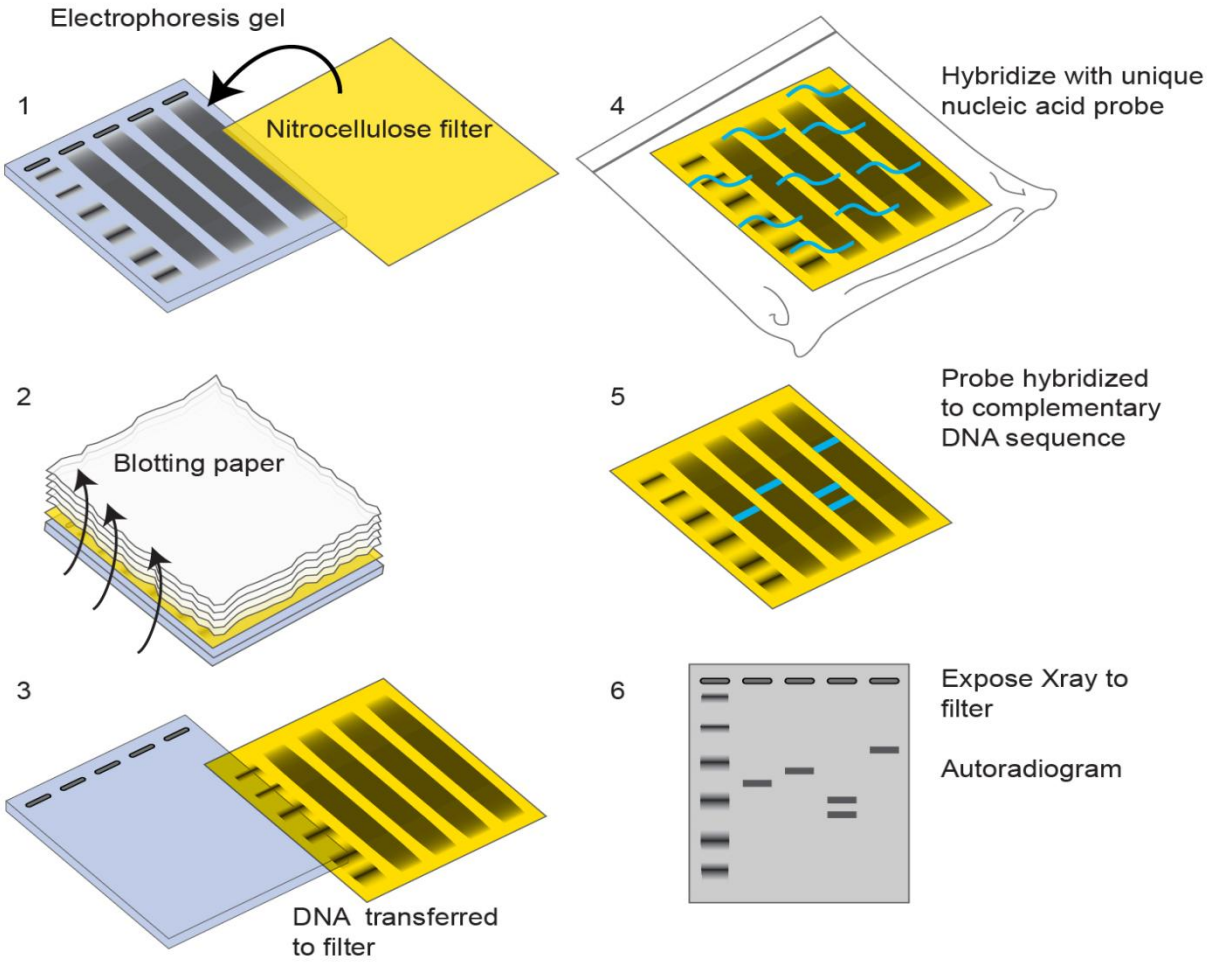


Figure 8.2 – Southern blotting

The filter is then exposed to the labeled DNA probe, which hybridizes to complementary fragments on the filter. After thorough washing, the filter is exposed to X-ray film, which is developed to reveal several specific bands corresponding to the DNA fragment that recognized the sequences in the DNA probe. The RNA, or **Northern blot,** is conceptually similar. In the protein, or **Western, blot**, proteins are electrophoresed and transferred to nitrocellulose and then probed with a specific **antibody**.

DNA amplification and cloning

The amplification of DNA is central to the study of molecular biology and genetics. DNA amplification makes possible an enormous increase in the number of copies of a desired DNA sequence. Two important approaches to the amplification of DNA are:

- **cell-based DNA cloning**: DNA is amplified in vivo by a cellular host so that the number of copies of the desired DNA template increases simply due to the exponential increase in numbe DNA ating host cells;
- **enzyme-based DNA cloning** (cell-free probe hod is represented by the **polymerase chain reaction** (PCR) and involves entirely *in vitro* DNA amplification.

Cell-based cloning is based on the ability of replicating cells, e.g. bacteria, to sustain the presence of recombinant DNA within them. Recombinant DNA refers to any DNA molecule that is artificially constructed from two pieces of DNA not normally found together. One piece of DNA will be the target DNA that is to be amplified and the other will be the replicating vector or replicon, a molecule capable of initiating DNA replication in a suitable host cell.

Bacteria may contain extra-chromosomal double-stranded DNA that can undergo replication. One such example is the bacterial plasmid. Plasmids represent ideal replicons for the amplification of target DNA and methods involving the use of plasmids are widespread throughout molecular biology. Target DNA is introduced into a replicon by using restriction enzymes. DNA ligase then covalently joins the target to the ends of the vector to form a closed circular recombinant plasmid. The next step is to introduce the plasmid into a host cell to allow replication to occur. Only a small fraction of cells may take up plasmid DNA. Following transformation, the cells are allowed to replicate, usually on a standard agar plate containing a suitable antibiotic to kill cells that do not harbor a plasmid. Colonies (clones of single cells) are then 'picked' and transferred to

tubes for growth in liquid culture and a second phase of exponential increase in cell number. Thus, from a single cell and a single molecule of DNA, an extremely large number of cells containing identical recombinant plasmids can be generated in a relatively small time. Cell-based cloning is used to produce clinically important proteins.

Polymerase chain reaction (PCR)

The polymerase chain reaction (PCR), now a central part of biotechnology, was conceived by Kary Mullis in 1983.The use of PCR for the amplification of DNA in vitro has revolutionized molecular biology; it is a method of copying a single template DNA molecule.

A standard PCR reaction requires the following:

- **DNA template**: DNA containing the sequence to be amplified;
- **amplimers**: small oligonucleotide primers that will hybridize with complementary DNA sequences and act as a starting point for the synthesis of the newly amplified DNA strands;
- **polymerase enzyme**: a heat-stable polymerase that will catalyze the formation of the DNA during the amplification reaction;
- **dNTPs**: are essential for the synthesis of the new DNA strands by the polymerase.

PCR consists of a series of programmed temperature changes that serve to bring about a round of DNA synthesis from the primers (Fig. 8.3). The average PCR involves about 30-35 cycles of reactions, which provide sufficient DNA, as much as a billion copies of the original template, so that it can be visualized following electrophoresis on an agarose gel.

The cycle of the PCR comprises three steps that are repeated continuously by varying temperature in a cyclic fashion:

- **denaturation**: heating of the reaction to approximately 95°C to ensure template and primers are single stranded;
- **annealing**: cooling of the mixture to allow heteroduplexes of primer and template to form. The temperature for this is typically in the 50 to 65°C range;
- **elongation**: DNA polymerase elongates the newly synthesized DNA strand from the site of the annealed primer along the template strand (typically about 70°C).

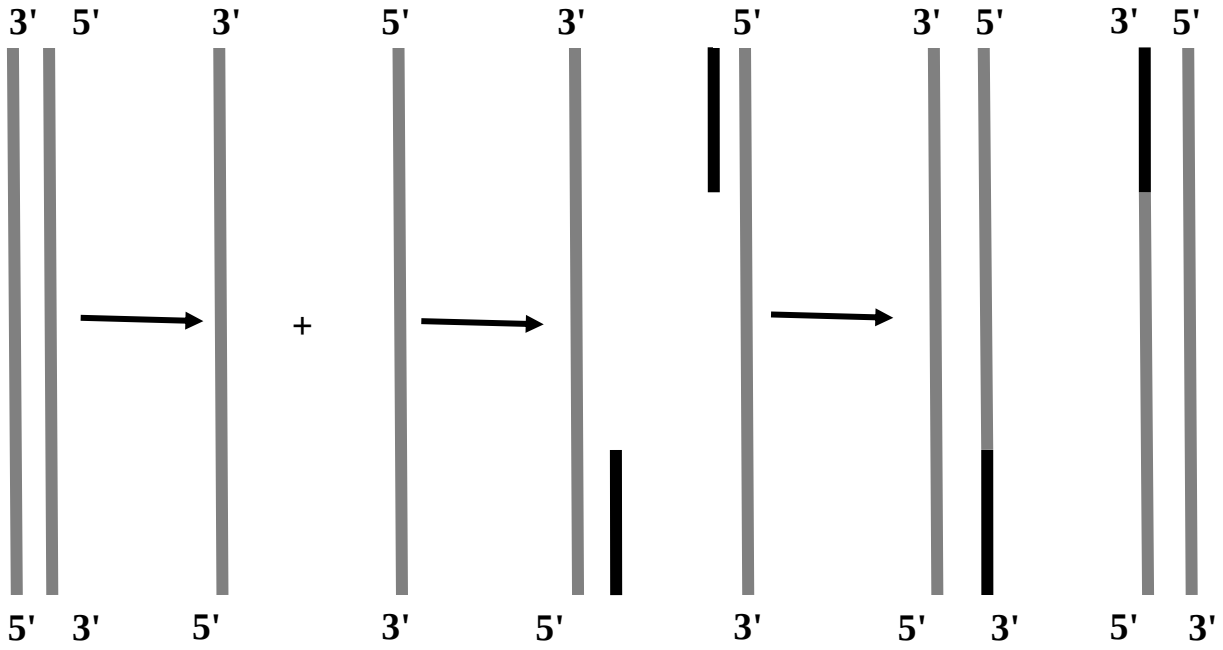


Figure 8.3 – Polymerase chain reaction

Applications of PCR:

- genetic marker typing;
- detection of point mutations;
- amplification of double-stranded DNA;
- DNA sequencing;
- genomic DNA cloning;
- genome walking;
- introduction of mutations *in vitro* to test their effect in biological systems.

DNA sequencing

The technologies now used in the sequencing of DNA have become extremely advanced, and automated sequencing of large amounts of DNA is now the standard practice in many centers around the world.

Chain termination (Sanger) DNA sequencing. In this method, originally developed by Fred Sanger, the sequencing primer is designed to be complementary to the region flanking the sequence of interest and acts as the starting point of chain elongation. DNA polymerization requires dNTPs to allow the strand to be elongated but, in addition, radioactive chain-terminating dideoxynucleotides (ddNTPs) are added. These are analogs of the dNTPs but differ in that they lack the 3'-

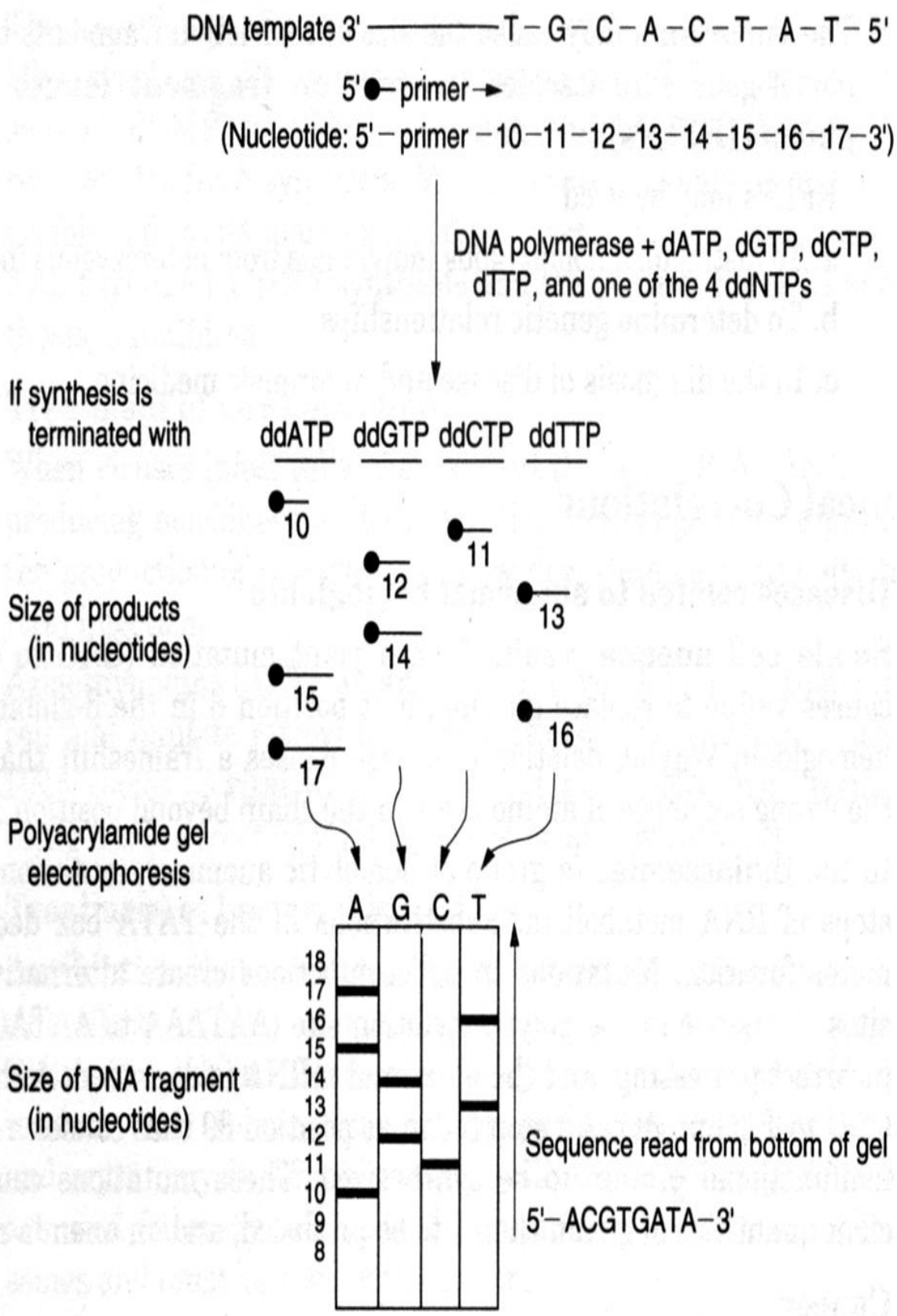


Figure.8.4 – Chain termination DNA sequencing

hydroxyl group required for formation of a covalent bond with the 5'-phosphate group of the incoming dNTP. Therefore, during DNA polymerization, if a growing DNA chain incorporates a ddNTP, growth of the nucleotide chain is halted.

A total of four reactions are carried out in parallel, each containing the primer, template, polymerase, and dNTPs. However, to each of the four reactions, a small amount of one radioactive ddNTP is added (ddATP, ddCTP, ddTTP, ddGTP) so that four separate reactions, the A, T, G, and C reactions, are conducted in parallel. As the chains elongate, ddNTPs, which are present at lower concentration than the natural dNTP, will be incorporated into the chain in place of the corresponding dNTP on a random basis. It means that in any reaction mixture, there will be many chains of varying lengths, which, when pooled together, represent the total collection of fragments that could terminate at that base. The DNA chains of differing lengths can be separated by electrophoresis on polyacrylamide gels. These gels allow DNA fragments that differ by only one nucleotide in length to be separated and, if the reaction involves a labeled group, either a dNTP or the primer, then electrophoresis of the four reactions in parallel, with subsequent autoradiography, will allow the sequence of the DNA to be determined (Fig. 8.4). The chain termination method can be modified by replacing radioactive groups with fluorescence-labeled primers.

CHAPTER 9

INTRODUCTION INTO METABOLISM

Metabolism is the set of life-sustaining chemical and physical transformations within the cells of living organisms. It represents **all enzymatic reactions and physical processes in the body**. In metabolism some substances are broken down to yield energy for vital processes, while other substances are synthesized.

The main purposes of metabolism are:

- Uptake of substances from the outside *(nutrition, respiration).*
- Conversion of food/fuel to energy to run cellular processes.
- Conversion of food/fuel to building blocks for proteins, lipids, nucleic acids, and some carbohydrates.
- Excretion of end products from the body.

Metabolic pathways are linked series of enzymatic reactions that begin with a particular reactant and terminate with an end product. The reactants, products, and intermediate substrates of enzymatic reactions are known as **metabolites** or **intermediates.** The product of one enzymatic reaction acts as the substrate for the next reaction in the pathway.

Four common types of metabolic pathways are described in the table below.

Linear metabolic pathway represents the series of reactions when substrate A is converted by the enzyme E_1 to produce product B. In turn B is a substrate for E_2 to produce C. This process continues until the final product P. (E.g.: *Glycolysis*).

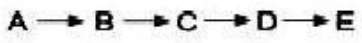
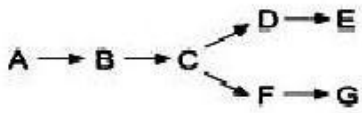
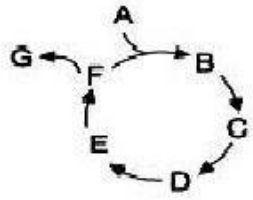
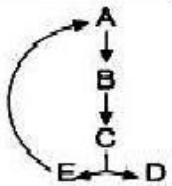
In **cyclic metabolic pathway** the starting molecule is regenerated during one run of the cycle *(E.g.: Tricarboxylic acid cycle, urea cycle).*

In **branched metabolic pathway** the intermediate substrate C can be converted either into the substrate D by the enzyme E3, or the substrate F by the enzyme E4. Each of these intermediates serves as the beginning of new pathway *(E.g.: Synthesis of purine nucleotides).*

In **spiral metabolic pathway** the enzyme E3 cleaves substance C into D and F. After that intermediate F serves as the beginning of new cycle *(E.g.: β-oxidation of fatty acids).*

The reactions of metabolism are divided into two categories, catabolism and anabolism.

Table 9.1. – **Types of metabolic pathways**

Scheme	Types of metabolic pathways	Examples
	Linear	Glycolysis
	Branched	Synthesis of purine nucleotides
	Cyclic	Tricarboxylic acid cycle
		Urea cycle
	Spiral	Beta-oxidation of fatty acids

Catabolism consists of all reactions that lead to the degradation of complex molecules to simpler components. In this process, energy is released. Ex.: *digestive enzymes break down dietary molecules.*

Anabolism consists of all reactions that lead to the synthesis of complex molecules from simpler components. This process requires energy. Ex.: *Biosynthesis of proteins.*

The catabolic and anabolic pathways are closely interrelated. In general, energy is released during anabolism and required during anabolism. During catabolic reactions complex molecules from food or constituents of cells break down to provide heat and utilizable energy in form of the molecules of **adenosine triphosphate (ATP).** Energy produced in catabolic processes is used for anabolic reactions, in which biopolymers are synthesized from intermediate substances formed in catabolic reactions.

Anabolic and catabolic pathways in eukaryotes often occur independently of each other. They are separated either physically by

compartmentalization within organelles or by the requirement of different enzymes and cofactors.

Table 9.2. – **Compartmentalization of some metabolic pathways in the cell organelles**

Cytosol	Glycolysis, most of gluconeogenesis, activation of amino acids.
Plasma membrane	Energy-dependent transport systems
Nucleus	DNA replication, synthesis of some types of RNA
Ribosome	Biosynthesis of proteins
Lysosomes	Hydrolytic breakdown of macromolecules
Golgi apparatus	Formation of plasma membrane and secretory vesicles
Endoplasmic reticulum	Synthesis of lipids
Mitochondria	Tricarboxylic acid cycle, electron transport chain, oxidative phosphorylation, β-oxidation of fatty acids

Specific and common pathways of catabolism

There are three stages in extraction of energy from food, referred as **specific and common pathways of catabolism** *(Fig.9.1)*.

Two of them represent **specific pathways** of catabolism.

Stage I (specific) - digestion. The stage includes the digestion, in which large, complex molecules are digested into relatively small, simple components in the digestive tract or inside the cells. The most common reactions are hydrolytic, in which proteins break down to amino acids, polysaccharides are converted to monosaccharides (primary glucose, galactose, and fructose), and lipids are converted to fatty acids and glycerol.

Stage II (specific) – production of acetyl CoA. In the second stage the smaller molecules from food are taken up by cells and degraded to even simpler units, primarily two-carbon acetyl portion of acetyl coenzyme A **(acetyl CoA)**.

Stage III is referred to as **the common catabolic pathway.** It consists of **the citric acid cycle** followed by the **electron transport chain (ETC)** and **oxidative phosphorylation**. Acetyl CoA undergoes utilization in the citric acid cycle to produce two molecules of CO_2, and reduced cofactors – NADH and $FADH_2$. After that NADH and $FADH_2$ produced in the citric acid cycle are oxidized in the Electron transport chain. The energy liberated

from their oxidation appears in the form of energy-rich molecules of ATP in the process called oxidative phosphorylation.

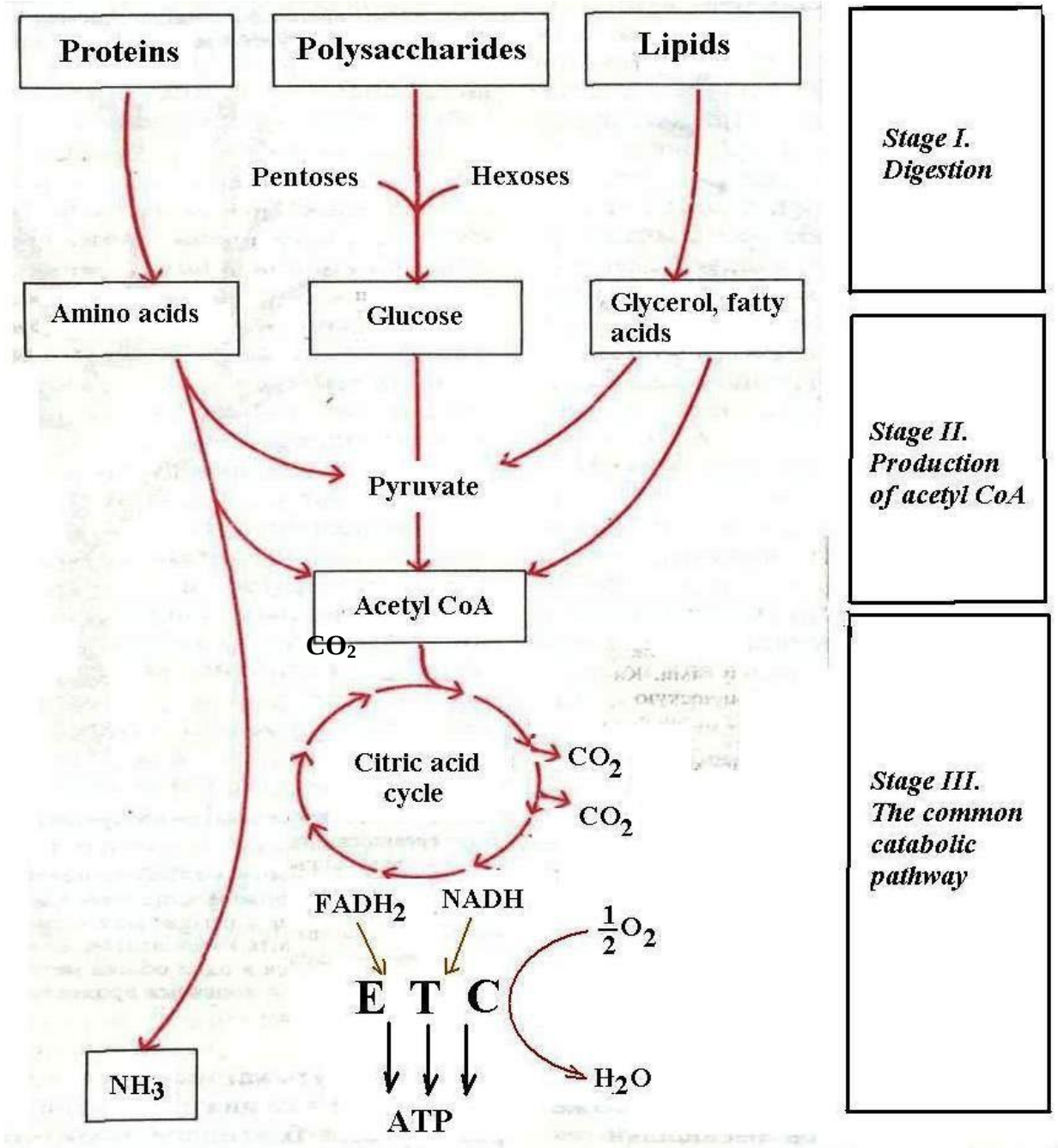


Figure 9.1 – Common and specific pathways for catabolism of carbohydrates, proteins, and lipids

Major end products of catabolism

The major classes of compounds in the food are **carbohydrates, lipids**, and **proteins**. In catabolism the major classes of organic compounds in the body may undergo cleavage to the simplest **end products**, which are

not further metabolized. Lipids and carbohydrates containing C, H, and O break down with formation of CO_2 and H_2O. Catabolism of proteins and nucleic acids results in production of nitrogen-containing end products (*urea*, *ammonia*, *creatinine*, *uric acid*). Their levels are measured in biological liquids (urine, blood, cerebral spinal fluid) for the diagnostics matters.

Table 9.3. – **End products of catabolism**

Dietary molecules	***End products***
Carbohydrates	CO_2, H_2O
Lipids	CO_2, H_2O
Proteins (amino acids)	CO_2, H_2O, NH_3, urea, creatinine, indican
Nucleic acids, nitrogenous bases	CO_2,, H_2O, NH_3, uric acid

EXPERIMENTAL STUDY OF METABOLISM

Metabolic pathways may be studied at different **levels of organization:**

1. Whole organism.
2. Isolated organs.
3. Organ slices.
4. Cell cultures (including microorganisms).
5. Homogenates of tissues.
6. Separated subcellular organelles.
7. Purified molecules of enzymes to study their kinetics and responses to inhibitors, other purified molecules, and their fragments.

Homogenate is a homogenized sample equal in composition. Homogenate usually lacks cell structures and contains mixture of cell organelles and membrane fragments. **Homogenization** is a process whereby a biological sample is brought to a state such that all fractions of the sample are equal in composition. **Homogenizer** is equipment used for homogenization.

Subcellular fractionation is the process used to separate various cellular components *(nuclei, mitochondria, microsomes).*

Methods used for the studies of metabolism

In biochemistry metabolic pathways are studied through estimation of the levels of substrates, determination of the enzymatic activity. For these purposes numerous methods are used.

- Homogenization,
- Dialysis,
- Electrophoresis,
- Chromatography,
- Colorimetric (spectrophotometric) techniques,
- Isotope methods, etc.

Metabolomics methods refer to a field of study in biology ending in -*omics*, such as ***lipidomics***, ***proteomics,*** or ***glycomics***. The aim of omics' studies is the collective characterization and quantification of pools of specific biological molecules in the cell at a system level. The metabolome represents the set of metabolites and their products of a given cell, tissue, organ, or organism.

Use of isotope tracers in medicine and biochemical research

There are radioactive isotopes such as **^{3}H (tritium), ^{32}P, ^{14}C, ^{35}S, ^{131}I (iodine)**, etc. They may be included into some molecule so that **labeled compounds** are formed. These labeled compounds may be injected into the experimental organism. The use of radioisotope tracers helps obtain biochemical information about the tissues of the human body in a non-invasive way. For example **^{32}P and ^{33}P** are used for labeling of nucleotides to study their metabolism. **^{3}H, ^{14}C,** and **^{13}C** are used for labeling of glucose, amino acids to study the rates of their metabolism.

CHAPTER 10

BIOLOGICAL MEMBRANES

Membrane is the thin layer that forms the outer boundary of a living cell or of an internal cell compartment.

The outer boundary of the cell is the **plasma membrane**, and the compartments enclosed by internal membranes are called organelles.

Inside the cell there are:

1. Nucleic membrane,
2. Single-membrane endoplasmic reticulum,
3. Single-membrane Golgi apparatus,
4. Outer and inner mitochondrial membranes,
5. Double lisosomal membranes.

Primary **functions** of biological membranes are:

1. **Separation**. They separate vital but incompatible metabolic processes conducted within organelles. Membranes separate: the cell content – from the external environment, one cell –from another, different parts of the cell.
2. **Barrier function**. They keep toxic substances out of the cell and maintain different concentration of substances on the both sides of the membrane.
3. **Transport of molecules**. They allow the exchange of specific molecules, such as ions, nutrients, wastes, and metabolic products, between organelles and between the cell and the outside environment. Membranes contain channels and pumps thus providing selective transport of ions and substrates.
4. The plasma membrane provides **cell-to-cell interactions,** due to specific carbohydrate molecules on the surface.
5. **Receptor function**. Receptors on the membranes can recognize and bind substances, e.g. hormones, and provide transmembrane signaling.
6. **Catalytic function**. Some membranes contain enzymes on their surfaces.

Properties of biological membranes

1. **Self-assembly** (ability to assembly after destruction).
2. **Asymmetry** (outside surfaces differ from inside surfaces in composition of lipids and proteins).

3. **Viscosity** (as sun-flower oil).

4. **Mobility**. Lipids and proteins can easily move within one layer and this is called lateral diffusion.

5. High electrical resistance.

6. **Fluidity**. It is increased due to the increase of body temperature, or due to the increase of the content of unsaturated fatty acids or cholesterol in the membrane. Phospholipids and proteins are held together **by non-covalent interactions**, and due to these relatively weak bonds, lipids and most proteins are in a constant state of lateral motion. Phospholipids move in flip-flop way across the membrane, membrane proteins can drift within the bilayer. Proteins much larger than lipids move more slowly.

7. **Selective permeability** means that the cell membrane is mostly hydrophobic and serves as a barrier for polar and charged molecules. Therefore:

- polar molecules, such as sugars and ions (Na^+, K^+), do not cross the membrane easily,
- non-polar (hydrophobic) molecules, such as hydrocarbons, can dissolve in the lipid bilayer and pass through the membrane rapidly.

Structure of membranes

The **Fluid Mosaic Model** proposed by Singer and Nicolson in 1972. It states that a biological membrane consists **of phospholipid bilayer** (*double layer*), and each phospholipid has a hydrophilic (*water-soluble*) head and hydrophobic (*water-insoluble*) tails *(Fig. 10.1).*

The membrane is a mosaic (mixture) of different protein molecules floating in the bilayer of phospholipids. The forces holding phospholipids and proteins in the membrane are intermolecular and electric in nature. It means that lipids and proteins do not remain stationary, but are in condition state of lateral (sideways) motion. Some proteins are embedded in the bilayer but can move around or flip over. Other proteins are associated outside the membrane and float with the lipid sea. According to this Model, membrane proteins are often described as ***"…..icebergs that are floating in the sea of predominantly fluid phospholipid molecules".***

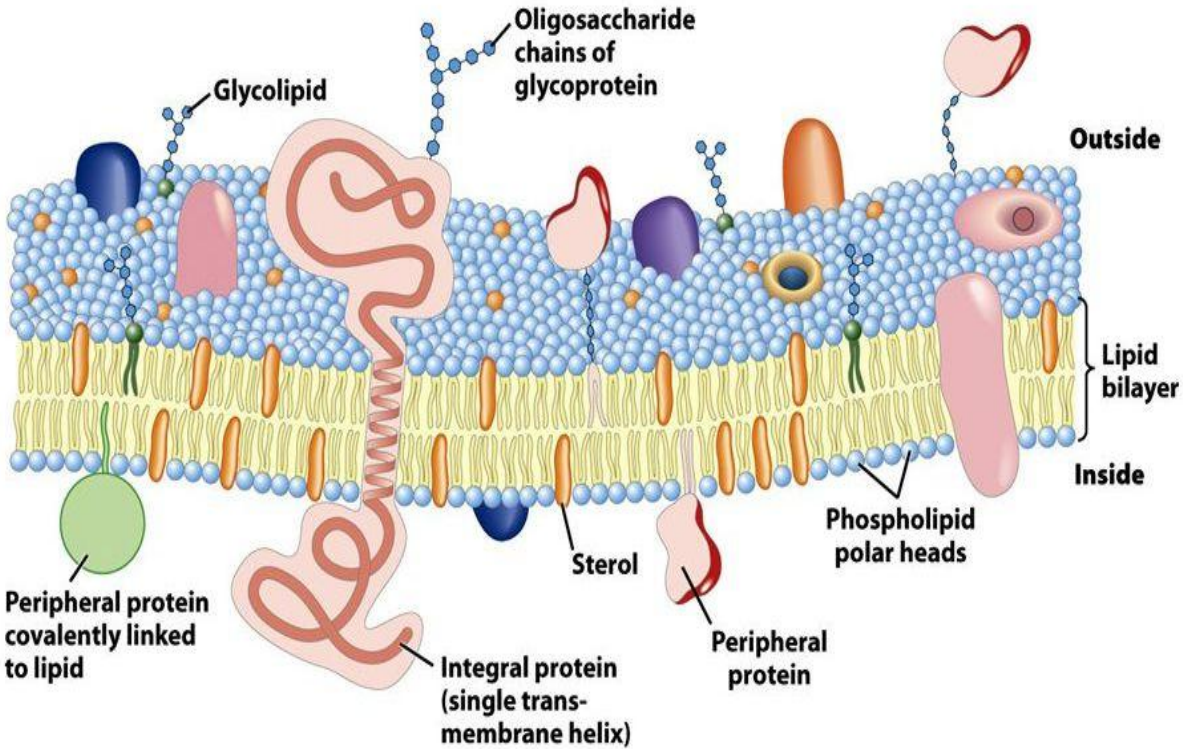


Figure 10.1 – Biological membranes are heterogeneous lipid bilayers with embedded proteins (from Lehninger Principles of Biochemistry, Fifth Edition)

COMPOSITION OF MEMBRANES

Chemically cell membrane is made up of 3 components namely:

1. Lipids (phospholipids, cholesterol),
2. Proteins,
3. Carbohydrates (glycolipids, glycoproteins).

Lipids

Membrane lipids are mostly amphipatic compounds containing both hydrophobic and hydrophilic regions.

The major membrane lipids are:

1. **Phospholipids** (derivatives of glycerol – glycerophospholipids).

This is the major class of the membrane lipids. Representatives of membrane phospholipids are:

- Phosphatidylcholine
- Phosphatidylethanolamine
- Phosphatidylserine
- Phosphatidylinositol
- Acetalphosphatides

- Cardiolipins

Phospholipid molecule generally consists of two hydrophobic fatty acid "tails" and a hydrophilic “head" having a phosphate group. The two components are joined together by a glycerol molecule. The phosphate groups can be modified with simple nitrogen-containing molecules such as choline, ethanolamine, serine, etc. Fatty acids are of both types: saturated and unsaturated.

2. **Sphingolipids** (derivatives of the alcohol sphingosine). One of the sphingolipids is **sphingomyelin** found in nerve cells. They play important roles in signal transduction and cell recognition.
3. **Glycolipids (cerebrosides, gangliosides, sulphatides)** contain carbohydrate chains oriented towards the extracellular side of the membrane. They also play a role in signal transduction and cell recognition in nervous tissue.
4. **Cholesterol** composes about 30% of all animal cell membranes. Cholesterol is steroid molecule that interacts with the phospholipid fatty-acid chains, and **decreases the fluidity of the membrane** because it forces the phospholipids closer together. Cell membranes contain mainly “free” cholesterol with free OH-group attached to the carbon at the 3rd position.

In membranes, all the lipids (except cholesterol) form a bilayer, with the polar heads oriented towards the extracellular side and cytoplasmic side, i.e. towards the aqueous environment. Hydrophobic tails are oriented towards each other. Triacylglycerols (fats) are not constituents of membranes.

The main functions of membrane lipids:

1. To stabilize the native conformation of membrane proteins.
2. To anchor certain membrane proteins.
3. To be precursors of secondary messenger molecules used in signal transduction and lipid signaling in biological cells *(inositol trisphosphate, diacylglycerol)*.
4. To serve as allosteric activators of the membrane-bound enzymes.

Membrane proteins

Membrane proteins may constitute from 30% to 70% of all components in the membrane. They are of three types:

1) **Integral** proteins are firmly and deeply embedded in the lipid bilayers.

2) **Anchored proteins** are connected by covalent bond to the particular membrane lipids.

3) **Peripheral** proteins are loosely bound to the outer or inner surface of the membrane.

Some proteins of a membrane contain oligosaccharide chains oriented towards the extracellular space.

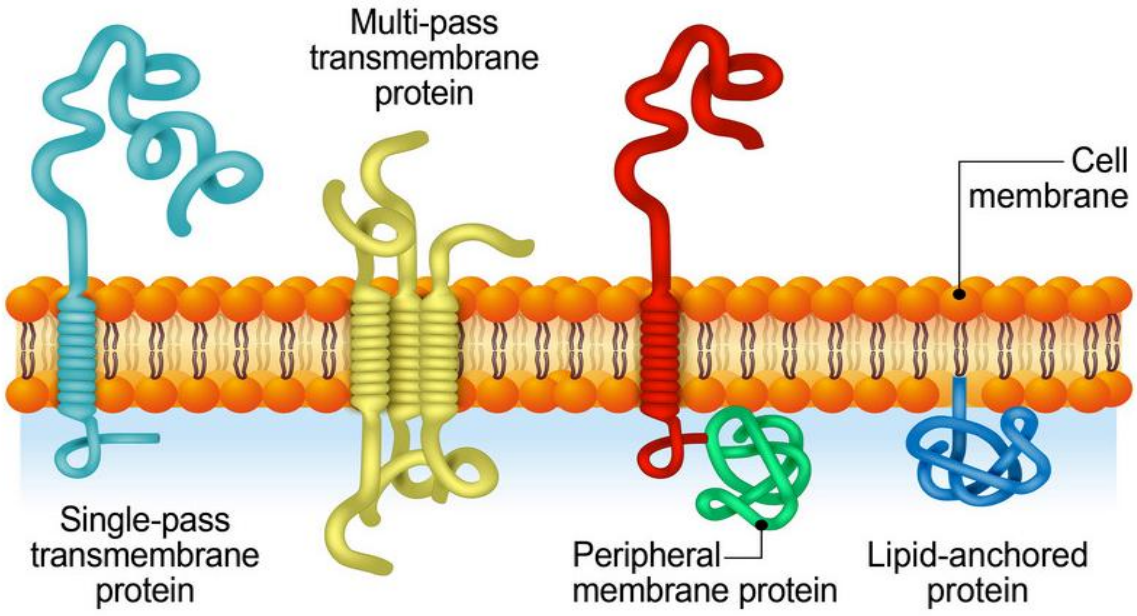


Figure 10.2 – Localization of proteins in the biological membrane

Major functions of membrane proteins

1. Transport (for ions and polar molecules);
2. Enzymatic activity (ATP-ases, etc);
3. Signal transduction;
4. Cell-cell recognition;
5. Intercellular joining;
6. Attachment to the cytoskeleton and extracellular matrix.

Examples of the enzymes associated with several different membranes are shown in the table 10.1. Some of these enzymes are located only in certain membranes and can therefore be used as markers to follow the purification of these membranes.

Table 10.1. – **Enzymatic markers of different membranes**

Membrane	***Enzyme***
Plasma	Adenylyl cyclase Na^+-K^+-ATPase
Endoplasmic reticulum	Glucose-6-phosphatase
Golgi apparatus	Galactosyl transferase
Inner mitochondrial membrane	ATP synthase

Membrane carbohydrates

Membrane carbohydrates (mainly oligosaccharides) constitute about 0.5-10% of all membrane components. In membranes they can be covalently bond to proteins to form **glycoproteins.** Glycoproteins are integral proteins. They play an important role in the immune response and protection.

Membranes contain sugar-containing lipid molecules known as **glycolipids**. In the bilayer, the sugar groups of glycolipids are exposed at the cell surface, where they can form hydrogen bonds. Glycolipids perform a vast number of functions in the biological membrane that are mainly communicative, including cell recognition and cell-cell adhesion. Cells recognize each other by binding to surface molecules, often containing carbohydrates, on the extracellular surface of the plasma membrane.

TRANSPORT MECHANISMS ACROSS MEMBRANES

Transport of substances across the cell membrane is provided by several mechanisms, depending on energy requirements and concentration gradient (*Fig. 10.3*).

1. **Passive transport** (does not require energy).
   - Simple diffusion;
   - Facilitated diffusion;
   - Osmosis.

2. **Active transport (**requires energy).
   - Primary;
   - Secondary;
   - Transport of macromolecules **(**exocytosis and endocytosis).

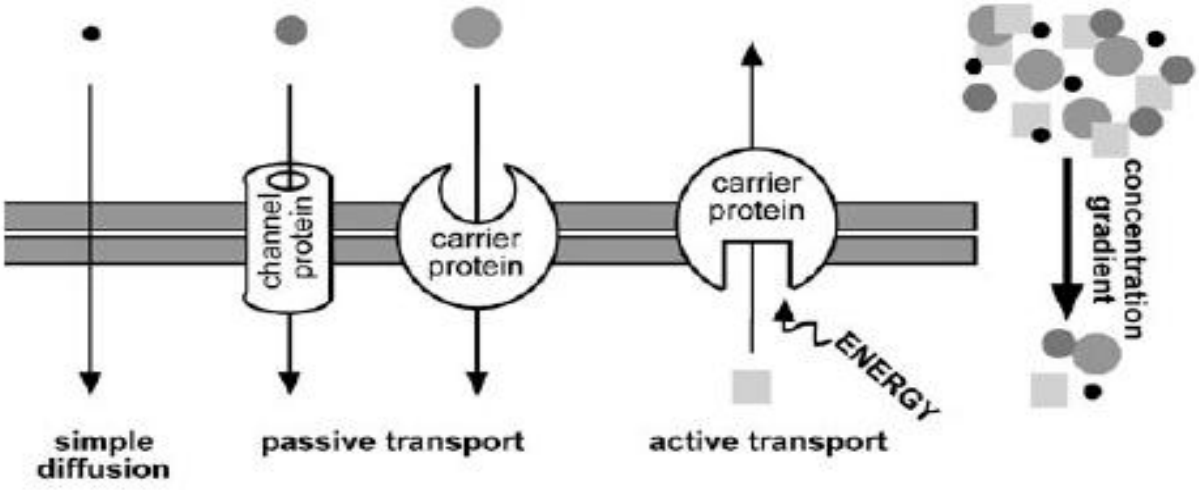


Figure 10.3 – Types of membrane transport

PASSIVE TRANSPORT

In passive transport, molecules are transported across the membrane **down their concentration gradient** and this process does not require energy.

- **Simple diffusion** is the flow of solute from a higher concentration to a lower concentration due to random thermal movement. (e.g. transport of gases CO_2, O_2).
- **Facilitated diffusion** is passive transport of substance from a higher concentration to a lower concentration, mediated by a **specific protein transporter**. Transporter proteins are of different types (permeases, translocases, or carrier proteins). Activity of these transporters is regulated by hormones. (eg, insulin activates the glucose transporter to transfer the carbohydrate into skeletal muscle cells and adipocytes).
- **Osmosis** is the movement of water molecules from high concentration to a low concentration of water trough a partially permeable membrane.

ACTIVE TRANSPORT

In active transport cell energy is used to move ions and molecules across the cell membrane. The substances are moved **against the concentration gradient** i.e. from where there is less to where there is more. The active transport is performed by specific proteins embedded in the membranes.

Primary active transport utilizes the energy liberated by ATP hydrolysis to move ions against their gradients (ATP-driven pumps). These proteins maintain ion gradients across both the plasma membrane and intracellular membranes.

E.g. Na^+,K^+-ATPase (or sodium-potassium pump) is an enzyme embedded in the plasma membrane that hydrolyze ATP so that Na^+ and K^+ ions can be transported against their concentration gradients.

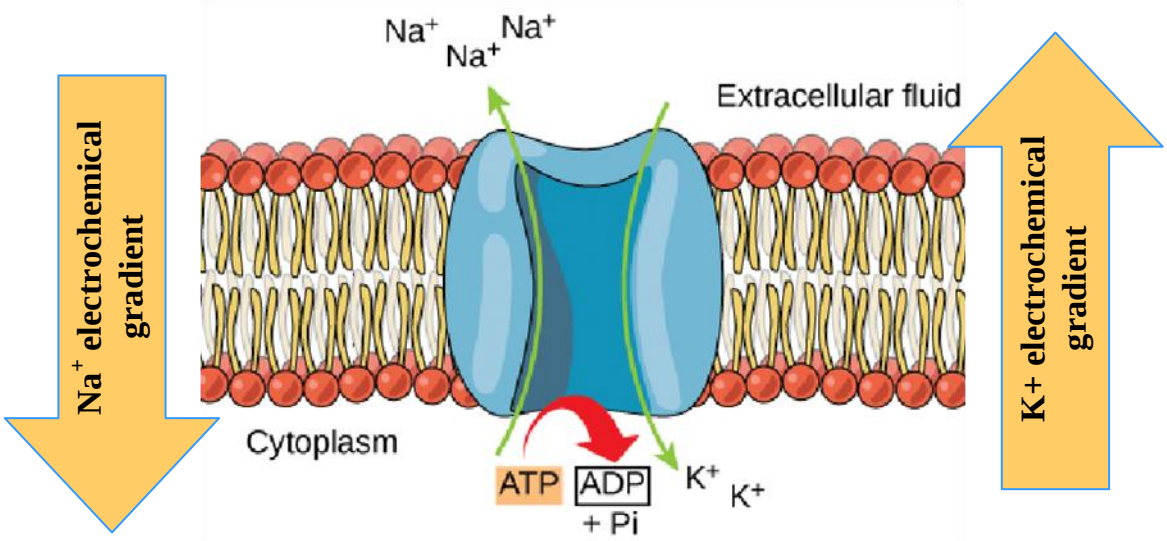


Figure 10.4 – Na^+,K^+-ATPase

Secondary active transport uses the energy stored in ion gradients to actively transport molecules across membranes. Because they rely on the gradients generated by ion pumps, coupled transporters are also known as secondary active transporters. Most often, the source of energy for coupled transporters is the flow of Na^+ down its electrochemical gradient (which is generated by the Na^+/K^+ pump).

E.g.: The *Na^+-driven glucose pump* on the apical side of intestinal epithelial cells.

Classification of transport mechanisms on the number of molecules moved and the direction of movement

Uniport system moves one type of molecules in one direction (Fig. 10.5).

In cotransport system, the transfer of one solute is coupled with subsequent transfer of another solute.

Symport occurs when the carrier protein transports two different solutes in the same direction. (eg, Na^+-sugar transporters for glucose and certain other sugars).

Antiport occurs when one substance traverses the membrane in one direction and another one is transported in the other direction. (eg, Na^+ in and Ca^{2+} out).

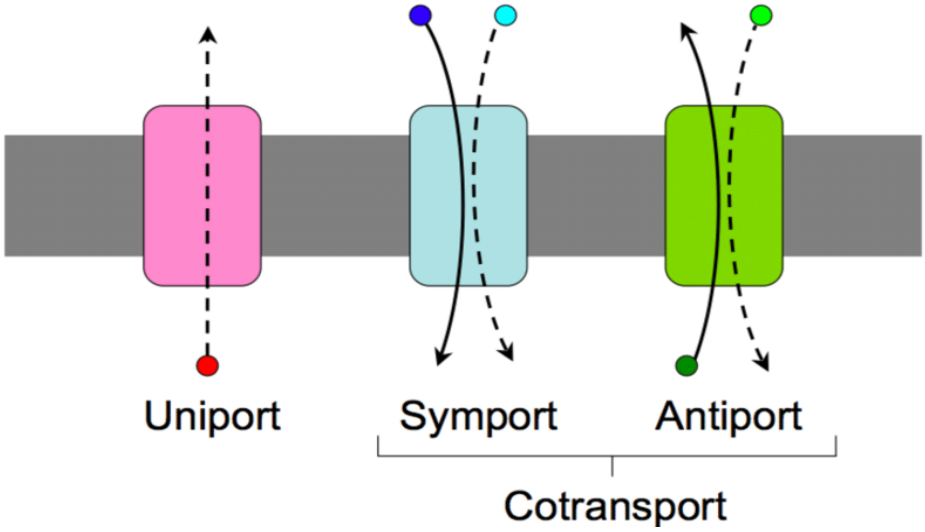


Figure 10.5 Classification of transport mechanisms

Transport of macromolecules

Endocytosis is the process by which cells take up large molecules (eg, proteins, polysaccharides, polynucleotides).

First, invagination of membrane takes place. Then the matter is surrounded by the membrane so that endocytic vesicle is formed and subsequently is engulfed. This process requires energy (ATP), calcium ions, microfilaments, and microtubules.

There are two types of endocytosis:

• **Pinocytosis** ("cell drinking") is the cellular uptake of fluid and dissolved substances.

• **Phagocytosis** ("cell eating") is the process by which a cell engulfs a solid particle to form an internal compartment known as a phagosome. Phagocytosis occurs only in specialized cells, such as macrophages and granulocytes.

In **exocytosis** a cell releases macromolecules to the exterior. In this process the proteins are synthesized in the endoplasmic reticulum, and Golgi are carried in vesicles that fuse with the plasma membrane. The examples are secretory enzymes (trypsinogen), hormones (insulin) and neurotranmitters (acetyl choline) released from the appropriate cells.

CHAPTER 11

ENERGY METABOLISM

Bioenergetics of the cell. Free energy

Living cells of human organism obtain energy needed for the cell functioning by oxidizing substrates (fuels such as carbohydrates or fats). The portion of energy provided by oxidation reactions is conserved in the form of ATP and is then used for performing work (functions of cells, tissues, organs).

Any molecule (or chemical process) contains certain types of energy:

- **enthalpy (ΔH)** is the **total energy** (or heat content) which may be available from any system, or molecule, or chemical reaction.
- **Bound energy (TΔS)** is the portion of energy which c**annot be converted to work**.
- **Free energy** (the Gibbs' energy, ΔG**)** is portion of the energy which can be **used to perform work** at the constant temperature and pressure.

Under the conditions existing in biological systems, changes in free energy, enthalpy, and entropy are interrelated by the equation:

$$\Delta H = \Delta G + T\Delta S;$$
$$\Delta G = \Delta H - T\Delta S,$$

where T is the absolute temperature and ΔS is the change of **entropy**. The entropy is a measure of the disorder of the system. The value of ΔG may be expressed in joules per mole (J/mol), or in calories per mole (cal/mol).

Exergonic reactions. If ΔG is negative (─ΔG), the reaction will proceed spontaneously with the release of energy, and the reaction is called exergonic reaction.

Endergonic reactions. If ΔG is positive (+ΔG), the reaction will not proceed spontaneously and has to be supplied with energy from outside; such a reaction is called endergonic reaction.

Certain portion of free energy (ΔG) may be accumulated in high-energy substrates which are referred to as macroergic compounds.

High-energy compounds (macroergic compounds)

Macroergic compounds contain energy-rich chemical bond, or macroergic bond. **Macroergic bond** is the bond which hydrolysis is

accompanied by the release of free energy (─ΔG) greater than **5 kcal / mol (21 kJ / mol)**. High-energy bond is designated as the sign " ~ " (tilda).

There are two types of macroergic compounds.

1) Phosphate-containing macroergic compounds: phosphoenolpyruvate, 1,3-bisphosphoglycerate, creatine phosphate, carbamoyl phosphate, ATP.

2) Sulfur-containing macroergic compounds (thioesters): acetyl-CoA, acyl-CoA, succinyl-CoA.

Biological role of macroergic compounds

The energy accumulated in high-energy bonds of macroergic compounds may be transferred from one molecule to another or may be used for performing various types of work. E.g. 1,3-bisphosphoglycerate, phosphoenolpyruvate, and creatine phosphate take part in reactions of substrate-level phosphorylation. Besides, **creatine phosphate** is present in muscle cells and serves as a stored source of energy (**depot of energy**) for muscle contraction.

ATP is universal energy currency because only this compound can immediately **give its energy** (accumulated in the macroergic bond) **for performing any type of work in the living cell**. Thus, only ATP can give its energy to drive, push forward, or force numerous energy-requiring processes. Only a few exceptions are represented by GTP (its energy is used for the protein synthesis), CTP (is used in the synthesis of complex lipids), and UTP (is used in the synthesis of polysaccharides).

Thioesters participate in some biochemical reaction, such as those of the TCA cycle or synthesis of heme (succinyl CoA), lipid metabolism (acyl CoA, acetyl CoA), synthesis of urea or pyrimidine nucleotides (carbamoyl phosphate), and aerobic glycolysis (acetyl CoA).

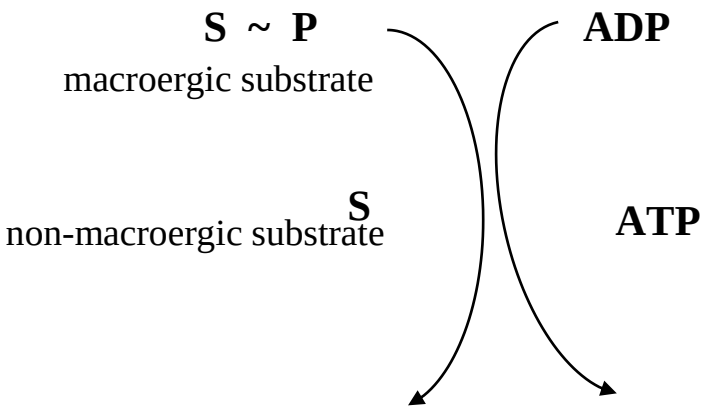
ATP: ways of its formation and use

The molecule of ATP contains 2 macroergic bonds. There are two pathways of the ATP synthesis: oxidative phosphorylation and substrate-level phosphorylation (Fig. 11.1).

Oxidative phosphorylation is synthesis of ATP from ADP and Pi with the use of energy produced in the electron transport chain (the ETC). Approximately 90% of ATP in the cell is formed due to oxidative phosphorylation.

Substrate-level phosphorylation is synthesis ATP from ADP and Pi with the use of energy released by macroergic bond of a high-energy substrate. About 10% of ATP in the cell is formed due to this pathway.

The schematic reaction of the substrate-level phosphorylation may be represented as follows:



In the reaction of substrate-level phosphorylation, ADP accepts the macroergic phosphate group from a high-energy compound (such as 1,3-bisphosphoglycerate, phosphoenolpyruvate, and creatine phosphate) to produce ATP.

ATP plays central role in energy metabolism. ATP is constantly being consumed and synthesized. During a normal day, a human can use and resynthesize 40-60 kg of ATP. The molecule of ATP is universal energy currency in the living cells. The free energy released when ATP is hydrolyzed, is used in many energy-requiring processes (various types of work and processes) (Fig. 11.1).

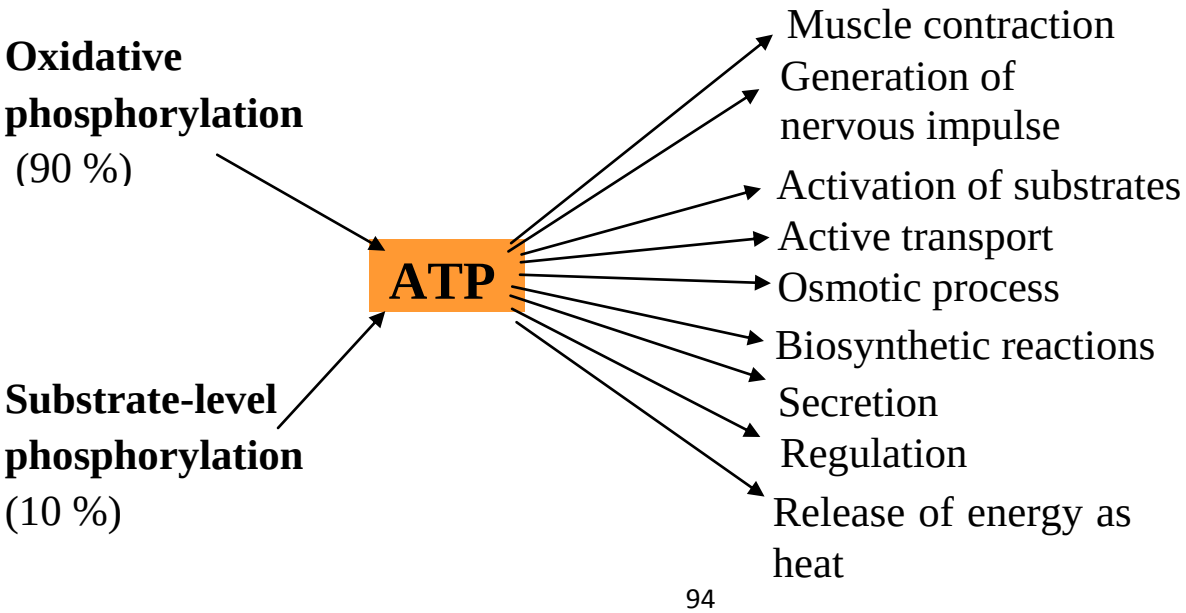


Figure 11.1 – Ways of the ATP formation and use

Electron transport chain

Living cells constantly perform work. They require energy (ATP) to fulfill any type of work. Oxidation of substrates is a source of energy to cells.

- **Oxidation** is the removal of two electrons or two hydrogen atoms from a substrate;
- **reduction** is the gain of them.

Hydrogen atom includes one proton and one electron: $H = H^+ + \bar{e}$; $2H = 2H^+ + 2\bar{e}$.

Oxidation of one substance is always accompanied by reduction of some other substance.

Molecule from which hydrogen atoms or electrons are removed is called **donor** of hydrogen or electrons;

molecule to which hydrogen atoms or electrons are transferred is called **acceptor** of hydrogen or electrons.

Biological oxidation is a process of removal of hydrogen atoms from the reduced substrates with the use of specialized intermediate carriers and participation of oxygen as the final acceptor of protons and electrons. Human organism is absolutely dependent on oxygen. Cells use oxygen to produce energy in the form of ATP. Thus, the major function of biological oxidation is providing energy to the needs of cells. The major way of the ATP synthesis (oxidative phosphorylation) takes place due to functioning of the electron transport chain (the ETC) located in the inner mitochondrial membrane.

The ETC consists of several components (carriers) which follow one another in the definite sequence *(Fig. 11.2)* and transfer hydrogen atoms (protons and electrons) or only electrons from reduced substrates (SH_2) or from reduced coenzymes (such as $NADH+H^+$ and $FADH_2$) to oxygen with the resultant formation of water. Each of the intermediate carriers of the ETC is capable of binding electrons from the previous component and give them to the next one. Thus, **in the ETC the number of oxidation-reduction reactions takes place, due to which free energy is produced**. A portion of this energy (about 50-75%) is accumulated in the phosphate bonds of ATP, and the other portion of the free energy is released as heat.

The components of the ETC are mainly represented by enzymes (proteins), except for ubiquinone (coenzyme Q, CoQ). Each enzyme (protein) of the ETC contains specific non-protein component as integral part.

Enzymes that catalyze reactions of dehydrogenation are called dehydrogenases. Their non-protein parts are considered to be primary acceptors of hydrogen to enter the ETC. There are two types of reduced substrates for subsequent dehydrogenation (oxidation):

1) substrates which undergo dehydrogenation (removal of hydrogen atoms from substrates) by **NAD-dependent dehydrogenases**; NAD^+ is the acceptor of $2\bar{e}$ and H^+ from these substrates to form NADH; one more proton is transferred to the medium; the group of substrates that are oxidized by NAD-dependent dehydrogenases include **pyruvate, isocitrate, α-ketoglutarate, malate, glutamate, β-hydroxybutyrate**;

2) substrates which undergo dehydrogenation by **FAD-dependent dehydrogenases**; FAD is the acceptor of 2 hydrogen atoms ($2H^+$ and $2\bar{e}$) from these substrates to form $FADH_2$; the group of substrates that are oxidized by FAD-dependent dehydrogenases include **succinate, acyl CoA, glycerol 3-phosphate**.

Besides FAD-dependent dehydrogenase, there is also **FMN-dependent dehydrogenase**, which **oxidizes only NADH**.

COMPONENTS OF THE ETC

NAD (NADP)-dependent dehydrogenases

NAD (NADP)-dependent dehydrogenases are conjugated enzymes. Their non-enzymatic part is represented by NAD^+ (nicotinamide adenine dinucleotide) or $NADP^+$ (nicotinamide adenine dinucleotide phosphate) which are derivatives of **vitamin PP** (nicotinamide).

The reaction catalyzed by NAD-dependent dehydrogenases:

$$SH_2 + NAD^+ \rightleftharpoons S + NADH + H^+$$

In this reaction, one molecule of NAD^+ accepts two $\bar{e}$ and one H^+ from the substrates to form NADH; one more proton remains in the medium. The substrates for NAD-dependent dehydrogenases include: pyruvate, isocitrate, α-ketoglutarate, malate, glutamate, β-hydroxybutyrate.

NADP-dependent dehydrogenases located in the **cytoplasm**, participate in **biosynthetic reactions** (e.g. in biosynthesis of fatty acids, cholesterol, etc.), **microsomal oxidation**, and in **ammonia detoxification**.

FAD (FMN)-dependent dehydrogenases

FAD (FMN)-dependent dehydrogenases are conjugated enzymes. Their non-enzymatic part is represented by FAD (flavin adenine dinucleotide) or FMN (flavin mononucleotide), which are derivatives of **vitamin B_2** (riboflavin).

FMN-dependent dehydrogenase, or NADH-dehydrogenase, catalyzes dehydrogenation of NADH only; $2\bar{e}$ and H^+ are transferred from NADH to FMN, and one more proton is taken from the matrix with resultant formation of $FMNH_2$:

$$NADH + H^+ + FMN \rightleftharpoons NAD^+ + FMNH_2.$$

The reaction catalyzed by FAD-dependent dehydrogenases:

$$SH_2 + FAD \rightleftharpoons S + FADH_2.$$

The **substrates** for FAD-dependent dehydrogenases include: **succinate, acyl CoA, glycerol 3-phosphate**.

Both FAD- and FMN-dependent dehydrogenases transfer hydrogen to CoQ.

Coenzyme Q

Coenzyme Q (CoQ), or **ubiquinone**, is not an enzyme, but a lipid-soluble coenzyme, and it is not derivative of vitamin.

Coenzyme Q accepts two hydrogen atoms from $FMNH_2$ or from $FADH_2$ and **divides the hydrogen flow into two parts**: protons and electrons. Protons are released into the medium, and electrons are transferred to cytochrome *b*.

Cytochromes

Cytochromes transfer **electrons** from $CoQH_2$ onto oxygen. The sequence of cytochromes in the ETC is as follows: *b*, c_1, *c*, and aa_3. The cytochromes are enzymes containing heme groups similar to that of hemoglobin. The cytochromes differ from each other by their prosthetic groups, protein parts, absorption spectra, and redox potentials.

When electrons are transferred in the ETC from one cytochrome to another, the heme iron changes its valency between Fe^{2+} and Fe^{3+}:

$$Fe^{2+} \underset{+\,\bar{e}}{\overset{-\,\bar{e}}{\rightleftharpoons}} Fe^{3+}$$

Cytochromes *b*, c_1, and *c* are the intermediate electron carriers, while cytochrome aa_3 (also called **cytochrome oxidase**) is the terminal enzyme which interacts directly with oxygen. The **atom** of oxygen (**½ O_2**) is the final electron acceptor at the end of the ETC. The oxygen atom accepts two electrons from cytochrome aa_3 and then binds with protons to produce water. Besides iron, cytochrome aa_3 contains copper which is reversibly reduced and oxidized between Cu^{2+} and Cu^{+}:

$$Cu^{2+} \underset{-\bar{e}}{\overset{+\bar{e}}{\rightleftharpoons}} Cu^{+}$$

Iron-sulfur proteins

Iron-sulfur proteins (Fe-S proteins, Fe-S centers) contain non-heme iron which participates in one-electron transfer reactions in the ETC. Fe-S proteins are bound to flavoproteins (within FMN- and FAD-dependent dehydrogenases) and cytochrome *b*. Iron-sulfur proteins are present in complexes I, II, and III (see below).

Structural organization of the ETC

There are two types of the ETC: the long chain and the short chain.
In the **long chain**, the standard order of carriers is as follows:

NADH→FMN→Fe-S→CoQ→cyt *b*→ Fe-S →cyt c_1→cyt *c*→aa_3→ O_2.

In the **short chain** the order of components is somewhat different:

FAD → Fe-S →CoQ → cyt *b*→ Fe-S →cyt c_1 → cyt *c* → aa_3 → O_2.

All the components of the ETC are organized into four large protein complexes in the inner mitochondrial membrane (Fig.11.2). Complexes I, III, and IV use energy of the electron transfer in the ETC for pumping out protons from the matrix to the outer surface of the inner mitochondrial membrane.

Complex I is called **NADH: ubiquinone oxidoreductase**, or **NADH-CoQ oxidoreductase.** Its non-protein part contains FMN and FeS. Complex I catalyzes two simultaneous processes:

1) oxidizes NADH by transferring 2ē and one H^+ to coenzyme Q plus one more proton takes up from the matrix with intermediary formation of $FMNH_2$ and resultant formation of $CoQH_2$;

2) transfers four protons ($4H^+$) from the matrix into the intermembrane space of mitochondria.

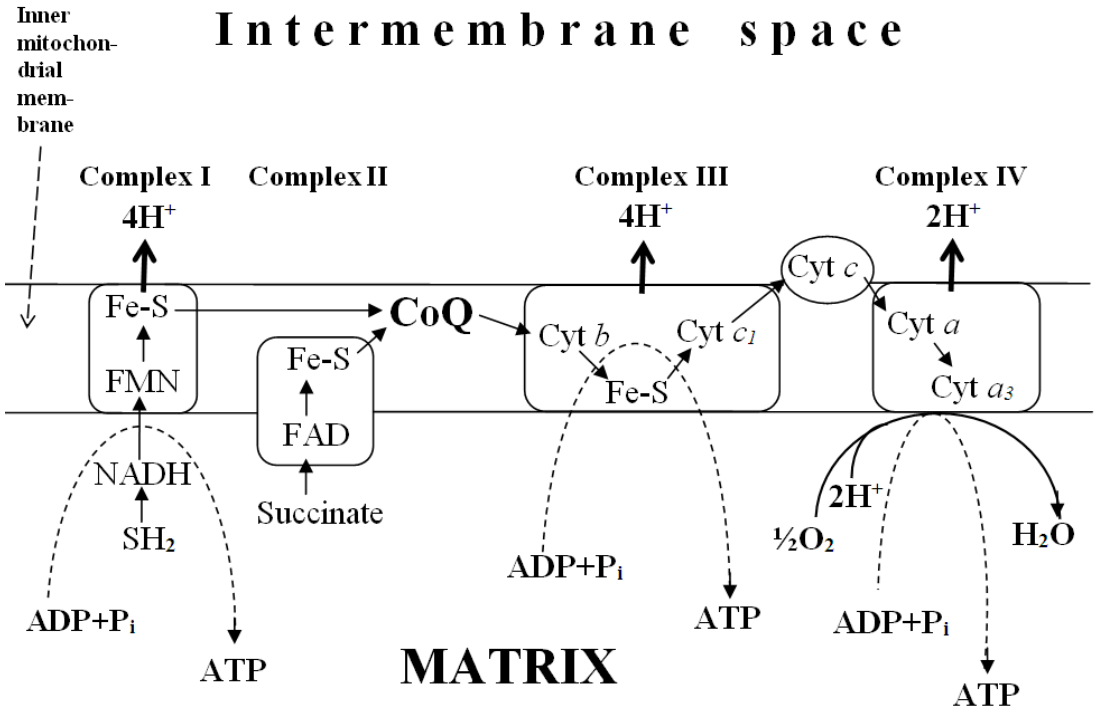


Figure 11.2 – Scheme of the ETC: sequence of components (carriers) in the inner mitochondrial membrane, participation of complexes in the transfer of electrons and protons, sites of coupling the transfer of electrons with oxidative phosphorylation (synthesis of ATP). Symbol "cyt" designates cytochromes; arrows between carriers show the flow of electrons in the ETC.

Complex II – **succinate-CoQ oxidoreductase**, or **succinate dehydrogenase**. Its non-protein part contains FAD and Fe-S. This complex doesn't pump out protons from the matrix into the intermembrane space. The role of this complex is to supply the ETC with additional amount of electrons at the level of ubiquinone, due to oxidation of succinate and transferring electrons and protons (hydrogen atoms) from the substrate to CoQ.

Complex III is called **$CoQH_2$-cytochrome *c* oxidoreductase**, or **QH_2-dehydrogenase**. This complex contains cytochromes *b*, c_1 and iron-sulfur proteins. In the ETC, complex III participates twice per electron pair, therefore complex III couples the transfer of electrons from $coqh_2$ to cytochrome *c* with the simultaneous transport of 4 protons from the matrix into the intermembrane space.

Complex IV is called **cytochrome oxidase**, or **cytochrome *c* oxidase**. This complex includes cytochromes *a* and a_3. The complex transfers 2 electrons from cytochrome *c* to one atom of molecular oxygen

(½ O_2). Each transfer of 2ē through complex IV to the atom oxygen in the ETC is accompanied by taking up two protons from the matrix with the resultant formation of H_2O:

$2\bar{e} + ½\ O_2 \rightarrow O^{--}$

$O^{--} + 2H^+ = H_2O$.

The energy of this oxidative-reduction reaction (within the complex IV) is also used for the simultaneous pumping out 2 protons from the matrix into the intermembrane space.

In the ETC there are sites in which the amount of energy released due to oxidation-reduction reactions is enough to be accumulated in macroergic bonds of ATP. These sites are as follows:

1) between NADH and FMN;

2) between cytochromes *b* and c_1;

3) between cytochromes *a* and a_3.

Thus, in the long chain **three molecules of ATP** are produced (or, in oxidation of NADH in the ETC, 3ATP are produced); in the short chain **two molecules of ATP** are produced (or, in oxidation of $FADH_2$ in the ETC, 2ATP are produced).

Summarizing the characteristic features of structural organization and functioning of the ETC it is very important to underline the following statements.

1) Complexes I and II catalyze transfer of electrons to ubiquinone (coenzyme Q) from two separate donors: from NADH (complex I) and from succinate (complex II). Complex III transfers electrons from reduced ubiquinone ($CoQH_2$) to cytochrome *c*, and complex IV transfers electrons from cytochrome *c* to O_2.

2) CoQ is not included into any complex (CoQ serves as a mobile carrier of electrons). $CoQH_2$ passes electrons to complex III, and the latter passes electrons to another mobile connecting link, cytochrome *c*, which is also not included into any complex.

3) The flow of electrons from the reduced substrates through complexes I, III, and IV to oxygen is accompanied by the simultaneous transfer of protons from the matrix into the intermembrane space. For each pair of electrons transferred to atom oxygen (½ O_2), four protons are pumped out by complex I, four by complex III, and two by complex IV (totally 10 protons per one pair of the electrons transferred to atom oxygen).

Oxidative phosphorylation

In the ETC, two simultaneous processes take place: 1) **oxidation** (i.e. the transfer of electrons from oxidizable substrates, NADH, and $FADH_2$ in the chain of specific carriers to oxygen), and 2) **phosphorylation** of ADP by P_i, or binding P_i to ADP, with the resultant formation of ATP. Oxidation is accompanied by the release of energy, a portion of which is used for synthesis of ATP.

Thus, the transfer of electrons in the ETC is coupled with the formation of ATP (phosphorylation of ADP). The term "oxidative phosphorylation" stands for synthesis of ATP from ADP and P_i due to energy produced in the ETC.

The mechanism of oxidative phosphorylation was described by **chemiosmotic theory** proposed by P.Mitchell (nobel laureate). According to this theory, mitochondrial transfer of electrons in the ETC is coupled to the ATP synthesis through a proton gradient.

The components of the ETC form large protein complexes in the inner mitochondrial membrane. The flow of electrons through complexes I, III, and IV provides energy for pumping out protons from the matrix across the inner mitochondrial membrane into the intermembrane space (fig. 11.3). The inner mitochondrial membrane is impermeable to protons, they cannot return to the matrix, and consequently, the outer surface of the inner mitochondrial membrane acquires positive charge, and the inner surface of the inner membrane becomes negatively charged. As a result, an **electrochemical potential** is generated on the inner mitochondrial membrane. This electrochemical potential is the driving force for the ATP synthesis. The increase of proton concentration (proton gradient) in the intermembrane space activates ATP-synthase – the enzyme which is responsible for the synthesis of ATP.

ATP-synthase, or ATP-synthase complex (often also called **complex V**) contains proteins which form a proton channel in the inner mitochondrial membrane. The electrochemical potential forces protons to move through the channel of ATP-synthase from the zone of higher H^+ concentration (from the intermembrane space) into the zone of lower H^+ concentration (into the matrix). This transfer of protons through the channel of ATP-synthase is accompanied by the release of free energy which is used for the generation of ATP from ADP and P_i. Thus, the mitochondrion is a battery in which the energy for ATP synthesis is stored as a **proton gradient**.

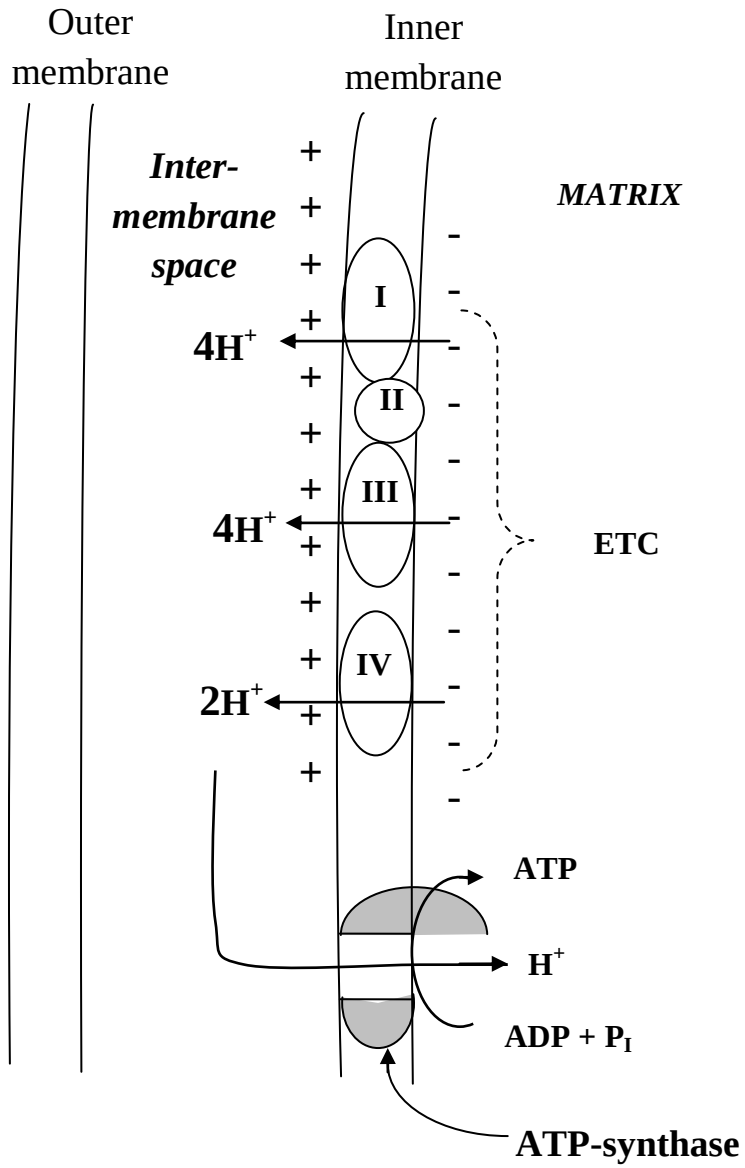


Figure 11.3 – Mechanism of oxidative phosphorylation of ADP (theory of P.Mitchell).

The most active transport of protons from the matrix to the intermembrane space, required for the formation of proton gradient and electrochemical potential, takes place exactly at sites of location of complexes I, III, and IV. These are sites at which the ETC and oxidative phosphorylation are coupled.

The ATP molecules formed by ATP-synthase complex are transported from the matrix into the cytoplasm by translocase in exchange for ADP and P_i.

Normally, during the transfer of electrons through the ETC, about 50-75% of the energy produced is accumulated in macroergic bonds of ATP, and about 25-50% of the energy produced in the ETC is released as heat.

The P/O ratio. The P/O ratio is used for the characteristics of energy production in both types of the ETC (both long and short). In the P/O ratio, "P" means phosphate residue, and "O" means **atom** oxygen. The P/O ratio indicates the number of inorganic phosphate molecules incorporated into ATP per one atom of oxygen consumed, or the number of the ATP molecules formed per one **atom** of oxygen used in the ETC.

At the long ETC, the P/O ratio is equal to 3; at the short ETC the ratio is equal to 2. In other words, if NADH undergoes oxidation in the ETC, 3 ATP is produced, and if $FADH_2$ undergoes oxidation in the ETC, 2 ATP is generated.

Regulation of the ETC: activators and inhibitors of the ETC. Uncoupling agents

Activators activate the ETC and increase synthesis of ATP. The activators of the ETC include: ADP, P_i, oxygen, and reduced substrates (SH_2).

Inhibitors inhibit the ETC, and decrease synthesis of ATP. They block transfer of electrons in the ETC. The inhibitors of the ETC:

- inhibitors of complex I – sleeping-draughts (barbiturates, amytal, chloropromasin), rotenon (insecticide), piericidin (antibiotic) – inhibit the electron transfer from FMN and Fe-S to ubiquinone;
- inhibitors of complex II – malonate (inhibitor of succinate dehydrogenase);
- inhibitors of complex III – antibiotics (antimycin a) – blocks the electron transfer from cytochrome *b* to cytochrome c_1;
- inhibitors of complex IV – such poisons as cyanide and carbon monoxide (CO) inhibit cytochrome oxidase (the transfer of electrons from cytochrome a_3 to oxygen).

Uncouplers (uncoupling agents).

Normally, the ETC and oxidative phosphorylation (i.e. synthesis of ATP) are coupled**;** when the ETC is functioning, synthesis of ATP takes place.

At normal conditions, when the ETC and oxidative phosphorylation are coupled, a portion of energy produced in the ETC (50-75%) is accumulated in ATP, and the other portion of energy is released as heat.

Uncoupler agents uncouple the ETC and oxidative phosphorylation. As a rule, uncouplers are lipophilic substances which can easily cross the membrane. They are capable of binding protons and transferring them from the intermembrane space to the matrix bypassing the proton channels of ATP-synthase. As a result, the ETC keeps functioning (substrates are oxidizing, oxygen is consuming, and the energy is generating) but the ATP is not synthesized, and all the energy formed in the ETC is released as heat.

Symptoms of the uncoupler action are **hyperthermia** (rise of the body temperature because of the intensive heat generation) and **muscle weakness** (because of the decreased ATP synthesis).

The examples of uncoupler agents: 2,4-dinitrophenol, hormones thyroxine and progesterone, some antibiotics (valinomycin), calcium ions, dicoumarol (anticoagulant agent), fatty acids, components of the brown adipose tissue (protein thermogenin), constituents of microbial cells. In a new-born organism, protein thermogenin is present in large amounts in mitochondria of the brown adipose tissue. This protein, functioning as an uncoupler agent, contribute to the maintenance of body temperature of new-born children.

DISORDERS OF ENERGY METABOLISM

Hypoxia

The living cell has constant need of ATP, because various energy-requiring processes keep proceeding every moment. There are no ATP stores in the cell. Hence, the cell must be continuously supplied with nutrients (reduced substrates) and oxygen for the maintenance of ATP synthesis. In starvation, endogenous substances of tissues serve as sources for energy production.

Hypoxia (lack of oxygen or oxygen deficiency) is the most common reason of hypoenergetic states.

There are two types of hypoxia: exogenous and endogenous.

Exogenous hypoxia is due to the reduced PO_2 (partial oxygen pressure) in the inspired air. This may occur at high altitudes or if oxygen-supply systems are out of order in the cabin of an aircraft.

Endogenous hypoxia is due to pathologic processes affecting the supply of oxygen to tissues or due to impaired utilization of oxygen by tissues.

Several subtypes of endogenous hypoxia are distinguished:

a) the **respiratory hypoxia** is caused by the decreased alveolar **ventilation** (obstruction of airways by inhaled object, inflammation of respiratory tract, pulmonary edema, pneumonia, constriction of bronchi occurred in asthma).

b) the **cardiovascular** (**circulatory**) **hypoxia** may occur due to impaired circulation leading to the deficient supply of organs and tissues with blood (e.g. heart failure, profuse bleeding and blood loss, thrombosis, arterial spasm).

c) the **hematic hypoxia** may be observed in anemia and is associated with the decreased amount of red blood cells or with the sharp drop of hemoglobin content in erythrocytes.

The hematic hypoxia is also observed in:

i) hemoglobinopathies (genetic defects of hemoglobin such as sickle cell anemia and thalassemia);

ii) poisoning with carbon monoxide (due to this, carboxyhemoglobin is formed which is unable to transport oxygen);

iii) poisoning with methemoglobin-forming agents, such as nitrates, aniline, aniline dyes, some drugs (sulfanylamides, amyl nitrite, etc.).

d) the **histotoxic hypoxia** is due to impaired tissue uptake of oxygen from the blood. This type of hypoxia is caused by inhibitors of the ETC, e.g. cyanide poisoning. After CN^- ions enter the cell they interact with Fe^{3+} and thus inhibit the end enzyme of the ETC (cytochrome oxidase), which in turn inhibits consumption of oxygen by the cells.

Disorders of energy metabolism in vitamin deficiency

Besides hypoxia, deficiency of vitamins, which participate in energy production (in the ETC), may lead to disorders of energy metabolism. E.g. NAD is a coenzymatic form of vitamin PP; FAD and FMN are derivatives of vitamin B_2. The deficiency of these vitamins (hypovitaminoses) causes hypoenergetic state.

The tricarboxylic acid cycle (the TCA cycle, or Krebs cycle)

The TCA cycle is also called the citric acid cycle or the Krebs cycle, after its discoverer, Hans Krebs. Normal functioning of the TCA cycle is absolutely essential to sustain life. The TCA cycle is located in the mitochondrial matrix.

Acetyl CoA is the starting metabolite for the TCA cycle. Acetyl CoA is a common end-product of catabolism of carbohydrates, fatty acids, and

amino acids, and is oxidized to produce coenzymes ($NADH+H^+$ and $FADH_2$) which are oxidized through the ETC (electron transport chain).

The TCA cycle begins with reaction between acetyl CoA and oxaloacetate to form citrate. The oxaloacetate is regenerated on completion of the cycle.

The scheme of the TCA cycle is represented in fig. 11.4.

The functions of the TCA cycle

1. **Catabolism of acetyl CoA**. Acetyl CoA is a product of catabolic reactions (specific pathways) in the carbohydrate, lipid, and amino acid metabolism. The TCA cycle is a **common pathway** for catabolism of acetyl CoA. Since acetyl residue in the molecule of acetyl, CoA contains two carbon atoms, therefore two molecules of CO_2 are formed due to catabolism of acetyl CoA in the TCA cycle. Decarboxylation reactions in the TCA cycle produce most of the body's carbon dioxide.

2. **Energy production**. The TCA cycle is the major energy-producing pathway in the cell. There are five energy-producing reactions in the TCA cycle:

a) three of them produce $NADH+H^+$. These are the reactions catalyzed by isocitrate dehydrogenase, α-ketoglutarate dehydrogenase, and malate dehydrogenase. Each of these reactions produces $NADH+H^+$. Then $NADH+H^+$ undergoes oxidation in the ETC and forms 3 ATP. Totally, three $NADH+H^+$ produce9 ATP.

b) one reaction produces $FADH_2$. This reaction is catalyzed by succinate dehydrogenase. $FADH_2$ undergoes oxidation in the ETC and generates 2 ATP.

c) one high-energy compound, GTP (guanosine triphosphate), is produced in the TCA cycle in the reaction called substrate-level phosphorylation. This GTP is then transphosphorylated with ADP to form ATP.

Thus, totally, 12 ATP molecules are produced due to catabolism of one molecule of acetyl CoA in the TCA cycle.

3. **Anabolic function** of the TCA cycle. Metabolites of the TCA cycle may serve as substrates in a variety of biosynthetic reactions. E.g.: oxaloacetate is a precursor for synthesis of glucose; α-ketoglutarate is used for synthesis of glutamate, and oxaloacetate – for synthesis of aspartate (subsequently these amino acids are used for synthesis of protein); acetyl

CoA may be used for synthesis of fatty acids, cholesterol, and ketone bodies; succinyl CoA – for synthesis of heme.

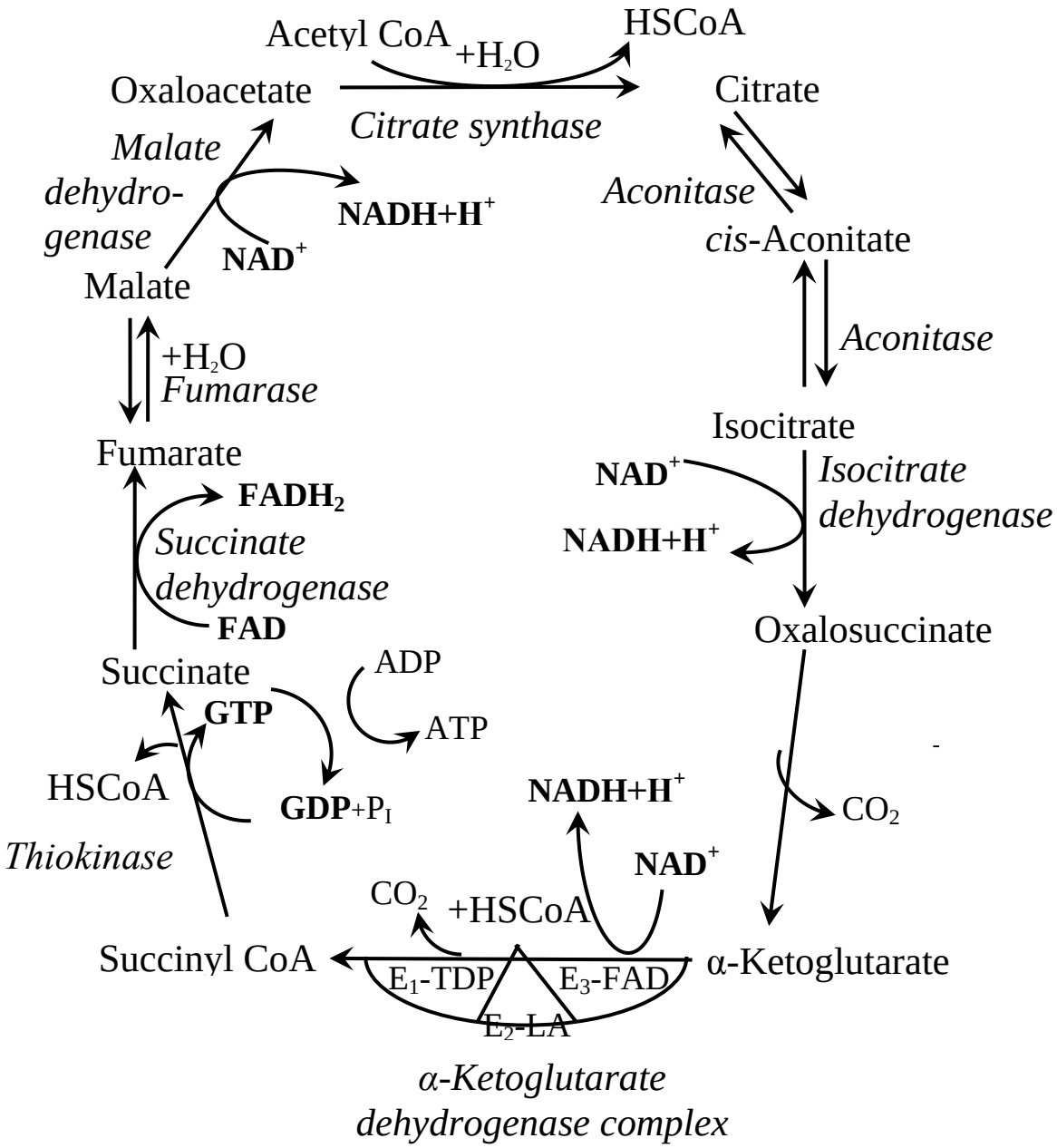


Figure 11.4 – The scheme of the TCA cycle

4. **Integrating function**. All types of metabolism (carbohydrate, lipid, and amino acid metabolism) can be interrelated through the TCA cycle by conversion of substrates of one type into others.

Regulation of the TCA cycle

The reactions involved into regulation of the TCA cycle are as follows: **citrate synthase, isocitrate dehydrogenase, and α-ketoglutarate dehydrogenase**. Citrate synthase is allosterically inhibited by ATP. Isocitrate dehydrogenase and α-ketoglutarate dehydrogenase are inhibited by $NADH+H^+$ (i.e. by the product of the TCA cycle), and ATP. Succinyl CoA inhibits α-ketoglutarate dehydrogenase and citrate synthase. ATP, citrate are also inhibitors of citrate synthase. ADP is allosteric activator of citrate synthase.

Thus, the TCA cycle is inhibited when energy is abundant. When energy stores (ATP and $NADH+H^+$) are low, the TCA cycle is activated, i.e. ADP and NAD^+ activate the TCA cycle.

CHAPTER 12

TYPES OF OXIDATION. ANTIOXIDANT SYSTEMS

Oxygen performs dual role in the body, i.e. oxygen may cause both positive and negative effects on the organism.

Positive role of oxygen. Oxygen takes part in oxidation reactions due to which **energy** is generated, many useful compounds are produced, detoxification of xenobiotics takes place.

Negative role of oxygen. Oxygen can produce oxygen radicals (reactive oxygen species) which are toxic and can kill a cell.

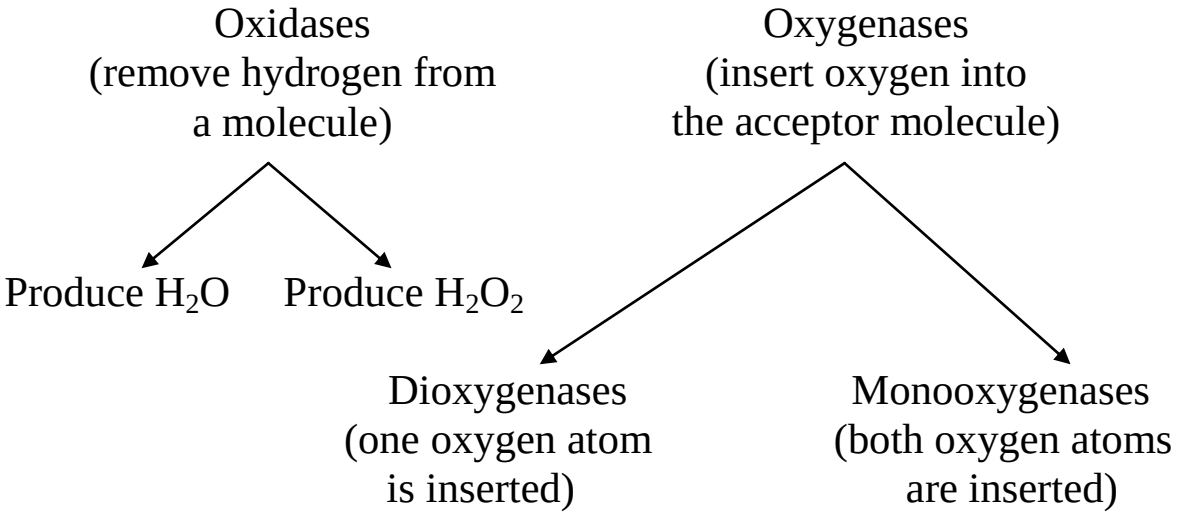
Oxidation of chemical substances in the body is performd in two ways:

1) by removal of 2H atoms (i.e. 2 protons, H^+, and 2ē) or only 2ē from a substrate; the reactions are catalyzed by **oxidases**;

2) by incorporation of oxygen into a substrate; the reactions are catalyzed by **oxygenases.**

Oxydases and oxygenases are not synonyms.

Both oxidases and oxygenases are divided into two subclasses:



Types of oxidation

1) **Oxidase** type (it takes place in the ETC):

$$AH_2 + ½O_2 \rightarrow A + H_2\mathbf{O}.$$

Most oxygen in the cell (80-90 %) is used precisely by this way of oxidation. The process takes place in the inner mitochondrial membrane, in the ETC (electron transport chain), where, due to oxidative phosphorylation, energy is produced and accumulated in the form of ATP.

2) **Peroxidation** type (it takes place in oxidation of purines, biogenic amines, aldehydes, amino acids):

$AH_2 + O_2 + FAD$ (or FMN) → $A + FADH_2$ (or $FMNH_2$) + H_2O_2.

H_2O_2 is toxic and is destroyed by **catalase.**

3) **Dioxygenase** type (incorporation of both atoms of molecular oxygen into a substrate):

$$A + O_2 \rightarrow A\mathbf{O_2}.$$

The reaction is catalyzed by **dioxygenase**, e.g. oxidation of homogentisic acid (in the metabolic pathway called oxidative degradation of phenylalanine and tyrosine).

4) **Monooxygenase** type (incorporation of one atom of molecular oxygen into a substrate with the formation of hydroxyl group). The other oxygen atom is reduced to water, and for this purpose, a donor of hydrogen (usually $NADPH+H^+$) is required:

$$A\text{-}H + O_2 + NADPH+H^+ \longrightarrow A\text{-}\mathbf{O}H + NADP^+ + H_2O.$$

Monooxygenases are divided into two subgroups:

a) soluble enzymes located **in the cytoplasm**, and
b) membrane bound enzymes located **in microsomes** (endoplasmic reticulum).

- Monooxygenases located in the cytoplasm also called hydroxylases catalyze the reaction of hydroxylation:

i) in the synthesis of adrenaline (in adrenal medulla)

Tyrosine —(O_2, H_2O)→ DOPA —(CO_2)→ Dopamine —(O_2, H_2O)→ Noradrenaline → Adrenaline;

ii) in the synthesis of melanin (in melanocytes)

Tyrosine —(O_2, H_2O)→ DOPA → → → Melanin;

iii) in the conversion of phenylalanine to tyrosine

Phenylalanine —(O_2, H_2O)→ Tyrosine.

- Monooxygenases located in microsomes participate in microsomal oxidation.

Microsomal oxidation

Microsomal oxidation occurs in microsomes (fragments of EPR). In microsomal oxidation, **one atom** of molecular oxygen is getting **incorporated into a substrate** liable to oxidation. The **other atom** of molecular oxygen accepts two hydrogen atoms to form **water**.

The microsomal chain (Fig. 12.1) involved in microsomal oxidation contains several components:

1) $NADPH+H^+$ – donor of $2\bar{e}$ and $2H^+$;
2) Flavoprotein (FAD-containing enzyme) which is reffered to as **NADPH-cytochrome P_{450}-reductase**;
3) Cytochrome P_{450} (cyt P_{450}).

Flavoprotein takes up 2 hydrogen atoms (i.e. $2H^+$ and $2\bar{e}$) from $NADPH+H^+$ and divides them into two parts: two protons are released to form subsequently water, and $2\bar{e}$ are transferred to cyt P_{450}.

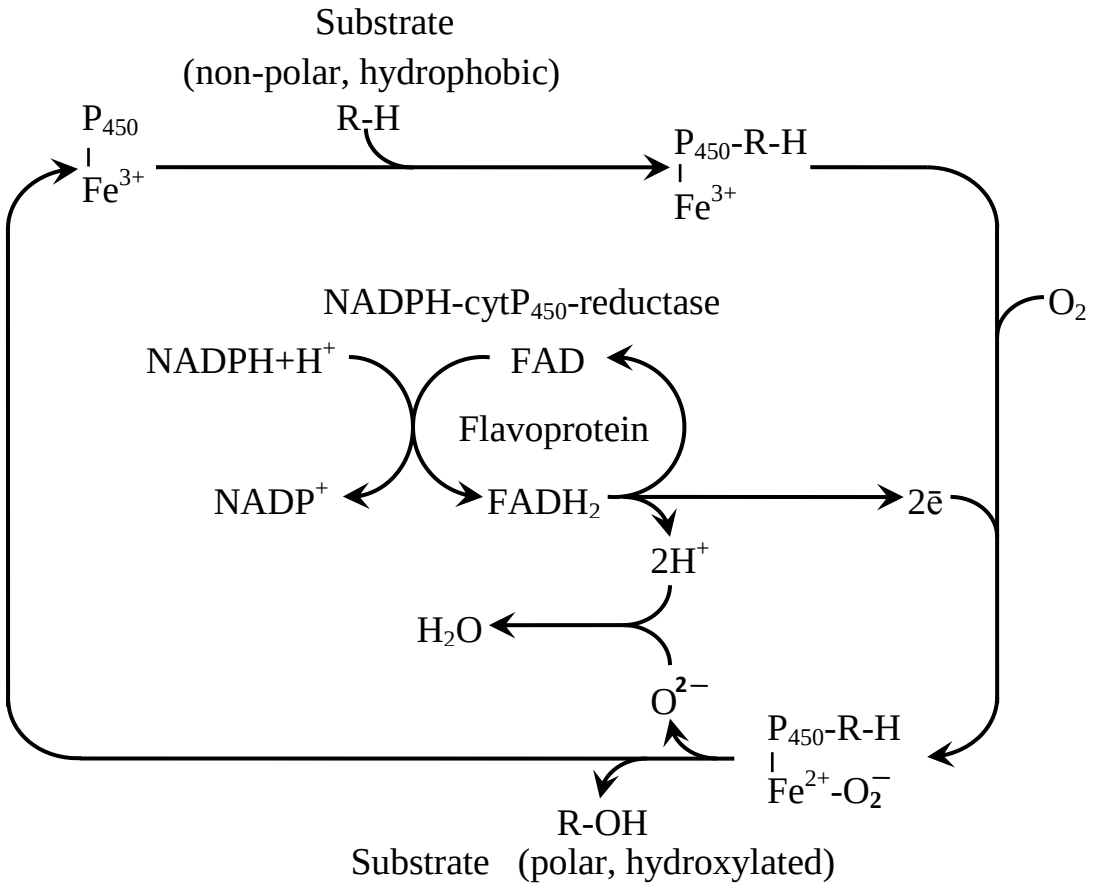


Figure 12.1 – Scheme of microsomal oxidation

Functions of cyt P_{450}

1) It binds with any non-polar (hydrophobic) substrate;

2) It binds O_2 and transfers to it 2ē which were previously taken from $NADPH+H^+$ and then transferred to cyt P_{450}.

After accepting 2ē, O_2 is divided by cyt P_{450} into two unequal parts:

- one oxygen atom with 2ē is released to meet with $2H^+$ to form H_2O;
- the other oxygen atom is inserted into the substrate to form hydroxyl group.

Biological role of microsomal oxidation

1. **Detoxification of xenobiotics.** Xenobiotics (from Greek *xenos*, strange, and *bios*, life) are compounds which may be ingested as drugs (medicines) taken for therapeutic purposes, or may be ingested accidentally (poisons, toxins, etc.) or with food products, like preservatives, food additives, adulterants, etc. Due to microsomal oxidation, xenobiotics become more polar, water soluble and readily excreted from the body.

2. **Synthesis of many** useful **compounds**. In steroidogenic tissues such as adrenal cortex, testis, ovary, and placenta, microsomal oxidation participates in biosynthesis of steroid hormones from cholesterol. In the liver, microsomal oxidation is involved in synthesis of bile acids, in kidney enzymes of microsomal oxidation chain participate in synthesis of the active form of vitamin D). In all tissues microsomal oxidation takes part in synthesis of unsaturated fatty acids, prostaglandins and other eicosanoids.

3. Microsomal oxidation may **increase toxicity** of some substances (e.g., the nontoxic benzopyrene contained in tobacco smoke is converted to hydroxybenzopyrene, a potent carcinogen).

Oxygen radicals (reactive oxygen species, ROS)

Free radical is a molecule or its fragment containing unpaired electron in its outer orbital. Free radical is denoted by superscript dot ($^{\bullet}R$).

Molecular oxygen can undergo one-electron reduction. Reduction of molecular oxygen in a series of one-electron steps (Fig. 12.2) produces superoxide anion, hydrogen peroxide, and hydroxyl radical. The complete reduction of oxygen produces water.

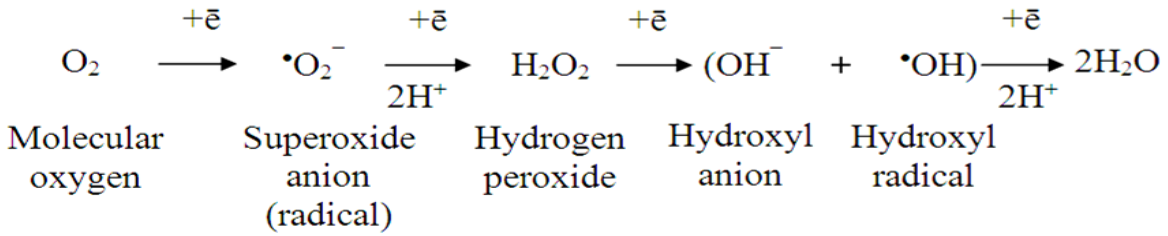


Figure 12.2 – Reduction of molecular oxygen and formation of reactive oxygen species

The products of partial reduction of oxygen are highly reactive (superoxide anion, $^{\bullet}O_2^-$; hydroxyl radical, $^{\bullet}OH$), and are named **oxygen free radicals**, or **reactive oxygen species** (**ROS**), or activated forms of oxygen.

Superoxide anion (O_2^-) can accept proton to form peroxide radical ($H^{\bullet}O_2$): $^{\bullet}O_2^- + H^+ \longrightarrow H^{\bullet}O_2$

Hydrogen peroxide is not a free radical (H_2O_2 does not have superscript dot). But because of its extreme reactivity, it is included in the group of reactive oxygen species. Hydrogen peroxide may be reduced by superoxide anion to form hydroxyl radical ($^{\bullet}OH$), **Haber-Weiss** reaction: $H_2O_2 + {}^{\bullet}O_2^- \longrightarrow {}^{\bullet}OH + OH^- + O_2$.

About 2-3 % of oxygen entering the body is converted to oxygen free radicals and H_2O_2. This creates an "oxidative stress", with which the body must deal.

The free radicals are constantly produced in the body during the normal oxidation of substrates, e.g. due to leaks in the electron transport chain (ETC) in mitochondria. Ionizing radiation damages tissues by producing hydroxyl radicals, hydrogen peroxide, and superoxide anion. Ultraviolet radiation in sunlight also produces oxygen radicals leading to suntans or burns.

$$H_2O \xrightarrow[\text{(radiolysis of water)}]{\gamma,\ \text{UV radiation}} H + {}^{\bullet}OH.$$

The capacity of producing tissue damage by H_2O_2 is minimal as compared to other free radicals. But in the presence of iron, H_2O_2 can generate $^{\bullet}OH$ (hydroxyl radical) which is highly reactive (**Fenton reaction**):

$$H_2O_2 + Fe^{2+} \longrightarrow {}^{\bullet}OH + OH^- + Fe^{3+}.$$

Properties of ROS

1) Extreme reactivity (when ROS react with some molecule other ROS are generated, i.e. chain reaction takes place);
2) Short life-span;
3) Damage to various tissues.

Harmful effects of ROS

Almost all biological macromolecules are damaged by ROS:

Proteins – oxidation of HS-groups of proteins leads to the loss of protein functions and inactivation of enzymes.

Heteropolysaccharides – ROS cause their degradation.

DNA – ROS lead to degradation of DNA strands, mutations which cause carcinogenesis, cell death; and contribute to the cell ageing.

Lipids – ROS may react with membrane lipids and initiate the lipid peroxidation which, in turn, leads to destruction of cell membranes and loss of membrane function.

Hemoglobin – ROS may react with hemoglobin to convet it to met-hemoglobin (the latter cannot transport oxygen).

Lipid peroxidation

Lipid peroxidation is the result of damaging effect of ROS on polyunsaturated fatty acids (PUFAs), which are constituents of membrane phospholipids. Due to lipid peroxidation, hydroperoxides of PUFAs are formed (Fig. 12.3). Hydroperoxides are unstable and undergo degradation to form free radicals which involve other molecules of PUFAs into the peroxidation process (the chain reaction mechanism).

Oxygen free radicals are capable of taking up hydrogen from methylene group ($-CH_2-$) adjacent to double bond in the molecule of PUFA. As a result, methylene group is converted to the **carbon centered radical** of fatty acid ($-^{\bullet}CH-$). Such carbon centered radical of fatty acid rapidly reacts with **molecular oxygen** to form a **peroxyl radical** of fatty acid, which can attack another polyunsaturated lipid molecule. This, another molecule of PUFA is converted to another carbon centered radical, and the former peroxyl radical is simultaneously converted to **organic hydroperoxide**. Organic hydroperoxides are very unstable molecules, and split to form two free radicals.

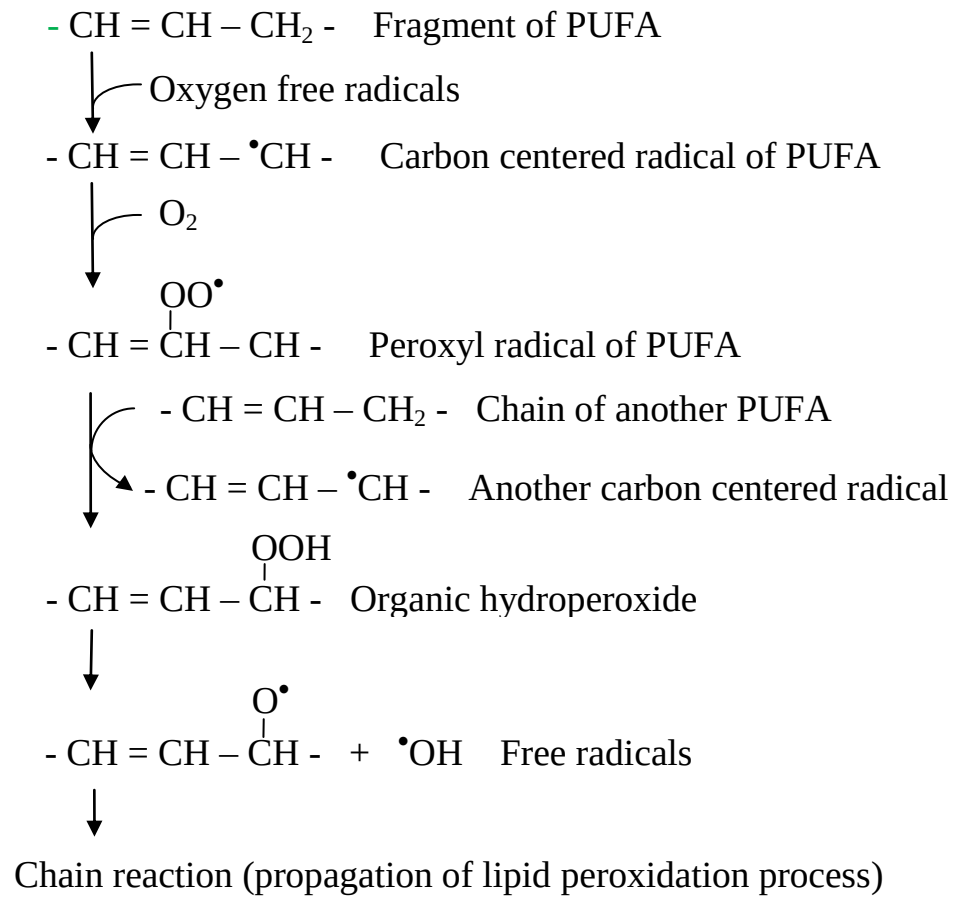
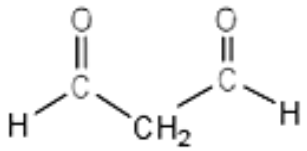


Figure 12.3 – Scheme of lipid peroxidation

Hence, the process would lead to continuous production of free radicals, their quantity is increased. These free radicals can initiate further peroxidation: they take up hydrogen from other neighbouring molecules of PUFA, thus resulting in a **chain reaction** or **propagation** of lipid peroxidation process.

The progression of this chain of events will destroy PUFA present in the membrane lipids and **damage the integrity of cell membrane**. Impairment of cell membrane will cause increased permeability to sodium ions, rapid influx of calcium, osmotic entrance of water into the cell, leading to **cell destruction and its death**. Process of lipid peroxidation may contribute to the development of **cancer**, **inflammatory diseases, atherosclerosis, and ageing**.

One of the consequences of lipid peroxidation is the rupture of carbon-carbon bond adjacent to hydroperoxyde group. As a result of this rupture, aldehyde groups are formed, and a consequent degradation product **malon dialdehyde**



is generated. Malon dialdehyde binds with the amino groups of proteins with the resultant formation of insoluble lipid-protein complexes which are called “pigments of ageing”or lipofuscins. Their accumulation in a cell accelerates cell ageing.

Lipid hydroperoxides impede cell division and thus decrease healing of damaged tissues. Destruction of lysosomal membranes due to lipid peroxidation results in the release of lysosomal emzymes into the cytoplasm and cell destruction (autolysis).

Antioxidant systems

Antioxidants protect the organism against harmful effects of ROS. Antioxidant systems include: enzyme antioxidant systems and non-enzymatic antioxidants.

Enzyme antioxidant systems:

a) superoxide dismutase (SOD) – catalyzes the reaction:

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

H_2O_2 is then destroyed by catalase and peroxidases.

b) catalase – catalyzes the reaction: $2H_2O_2 \rightarrow 2H_2O + O_2$.

c) glutathione peroxidase. The enzyme contains selenium as prosthetic group and catalyzes destruction of H_2O_2 and lipid hydroperoxides by reduced glutathione. As a result of this reaction, oxidized glutathione is formed which, in turn, undergoes reduction by **glutathione reductase** in the presence of **NADPH+H$^+$**.

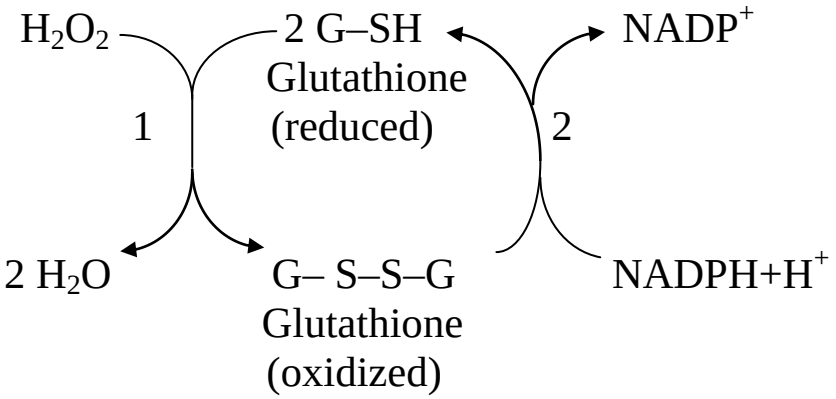


Figure 12.4 – Glutathione peroxidase system.
1 – glutathione peroxidase; 2 – glutathione reductase

Glutathione peroxidase is the major cell mechanism preventing accumulation of organic hydroperoxides and H_2O_2, and protecting membrane lipids and hemoglobin against oxidation by peroxides. Destruction of H_2O_2 with participation of the glutathione peroxidase system is shown in Fig. 12.4.

Non-enzymatic antioxidants:

a) **water-soluble antioxidants**: vitamin C, glutathione, uric acid, ceruloplasmin, ferritin, transferrin, caffeine, ferritin, bilirubin, taurin, carnosin.
b) **fat-soluble antioxidants**: vitamin A, β-carotene, vitamin E, ubiquinone, vitamin K.

Among all the antioxidants, there are three of them of the greatest importance: vitamin C, vitamin E, and β-carotene. They are especially wide-spread in the nature.

Vitamin C inhibits lipid peroxidation by two mechanisms. 1) It reduces the oxidized form of vitamin E and maintains concentration of vitamin E in the cell membranes. 2) Vitamin C interacts with reactive oxygen species and inactivates them.

Vitamin E inactivates free radicals in the hydrophobic layer of membranes and thus prevents propagation of chain reaction in lipid peroxidation.

The molecules of β-carotene (precursor of vitamin A) also inhibits lipid peroxidation.

Diet enriched with vitamins E, C, carotenes lowers the risk of developing atherosclerosis, cardiovascular diseases; protects against cancer. Their action is related to inhibition of lipid peroxidation and inactivation of reactive oxygen species and hence to maintenance of normal structure of cell components.

CHAPTER 13

GENERAL CHARACTERISTICS OF HORMONES. MECHANISMS OF ACTION OF HORMONES

Hormones are organic substances produced by endocrine glands. Hormones are secreted directly into the bloodstream, and transported via the blood to target tissues where they exert their biological action, i.e. **hormones regulate metabolism, functions, growth, division, development, and differentiation of cells during ontogenesis, support homeostasis of the organism.**

Classification of hormones

I. On the chemical structure:

1) polypeptide hormones (hormones of pituitary gland, hypothalamus, insulin, glucagon, hormones of parathyroid glands);

2) derivatives of amino acids (thyroxine, epinephrine, or adrenaline);

3) steroid hormones (hormones of adrenal cortex, male and female sex hormones);

4) eicosanoids – tissue hormones, or hormone-like substances, which are derivatives of arachidonic acid (polyunsaturated fatty acid) that cause local effect on tissues.

II. On the place of their synthesis:

hormones of hypothalamus, pituitary gland, thyroid gland, parathyroid glands, pancreas, adrenal glands (medulla and cortex), hormones of male and female sex glands, local or tissue hormones.

III. On their effects on biochemical processes and functions:

1) hormones regulating metabolism of proteins, lipids and carbohydrates (insulin, glucagon, epinephrine, cortisol);

2) hormones regulating the salt and water balance (aldosterone, vasopressin);

3) hormones regulating metabolism of calcium and phosphate (parathyroid hormone, calcitonin, calcitriol);

4) hormones stimulating growth (growth hormone, sex hormones, thyroid hormones, cortisol, insulin);

5) hormones controlling reproductive function (male and female sex hormones);

6) hormones regulating functions of other endocrine glands (adrenocorticotropic hormone, thyroid stimulating hormone, follicle-stimulating hormone, prolactin, or lactotropic hormone, luteinizing hormone).

7) hormones mediating response to stress (epinephrine, glucocorticoids);

8) hormones effecting the highest nervous activity, i.e. memory, attention, mentation, behaviour, mood (glucocorticoids, parathyroid hormone, thyroxine, adrenocorticotropic hormone).

Properties of hormones

1. **High biological activity**. Concentration of hormones in the blood is extremely low (10^{-8} M), but their action is very noticeable; therefore the slightest increase or decrease of hormone content in the blood can immensely change metabolism and functions.

2. **Short "life span"** (ranging from a few minutes to a half an hour). After hormones exert their physiological effects, they undergo degradation or inactivation, but their action may last for hours and up to a day (24 hours) or days.

3. **Distance of action**. Hormones are synthesized in organs of one type (endocrine glands) and act in distant organs of the other type (target tissues).

4. **High specificity of the action**. Hormones exert their action only after binding with a receptor of a hormone. **Receptor** is a conjugated protein (glycoprotein) consisting of two parts – the carbohydrate and the protein ones. Hormone binds with the carbohydrate part of the receptor. The structure of the carbohydrate part is unique, specific and, on its three-dimensional conformation, corresponds to the structure of the hormone. Therefore the hormone always binds with its receptor unerringly, precisely, specifically, notwithstanding the small concentration of the hormone in the blood.

Receptors to some hormones (such as thyroxine, glucocorticoids, or insulin) are present in many tissues. Receptors to some other hormones are present in a few tissues (e.g. oxitocin). Tissues which contain receptors to the definite hormone are called **target tissues** for this hormone.

MECHANISM OF ACTION OF POLYPEPTIDE HORMONES AND ADRENALINE (EPINEPHRINE)

Receptors to these hormones are located on the external surface of the cell membrane, and the hormone doesn't enter the cell. But the action of the hormone is transferred into the cell due to so called **second messengers**: cyclic AMP **(**cAMP), cyclic GMP **(**cGMP), inositol triphosphate, diacylglicerol, and calcium ions (Table 13.1). They are named the second messengers because in the system of transmitting the regulatory signal the hormone is the first messenger.

Each of the second messenger stimulates specific **protein kinase** which phosphorylates the cell proteins (enzymes), thus changing the activity of proteins (enzymes).

Table 13. 1. – **Second messengers and protein kinases**

Second messenger	***Specific protein kinase stimulated by the second messenger***
cAMP	cAMP-dependent protein kinase (protein kinase A)
cGMP	cGMP-dependent protein kinase (protein kinase G)
Ca^{2+}	Ca^{2+}-calmodulin-dependent protein kinase
Inositol triphosphate	Ca^{2+}-calmodulin-dependent protein kinase
Diacylglicerol	Protein kinase C

The major intracellular second messenger is cAMP. Most hormones act via this compound. But other hormones acting via their specific protein kinases are able to alter the cAMP concentration in the cell due to the increase or decrease of the activity of enzymes which generate or degrade cAMP.

Cyclic AMP

Cyclic AMP is synthesized from ATP by the **adenylate cyclase system** which consists of 3 components: 1) specific receptor, 2) G-protein, and 3) adenylate cyclase (Fig. 13.1).

The hormone binds with the receptor to form the hormone-receptor complex. G-protein is called so because it binds with guanylic nucleotides (either GDP or GTP). The G-protein is active when it is bound to GTP, and vice versa, being bound to GDP it is non-active.

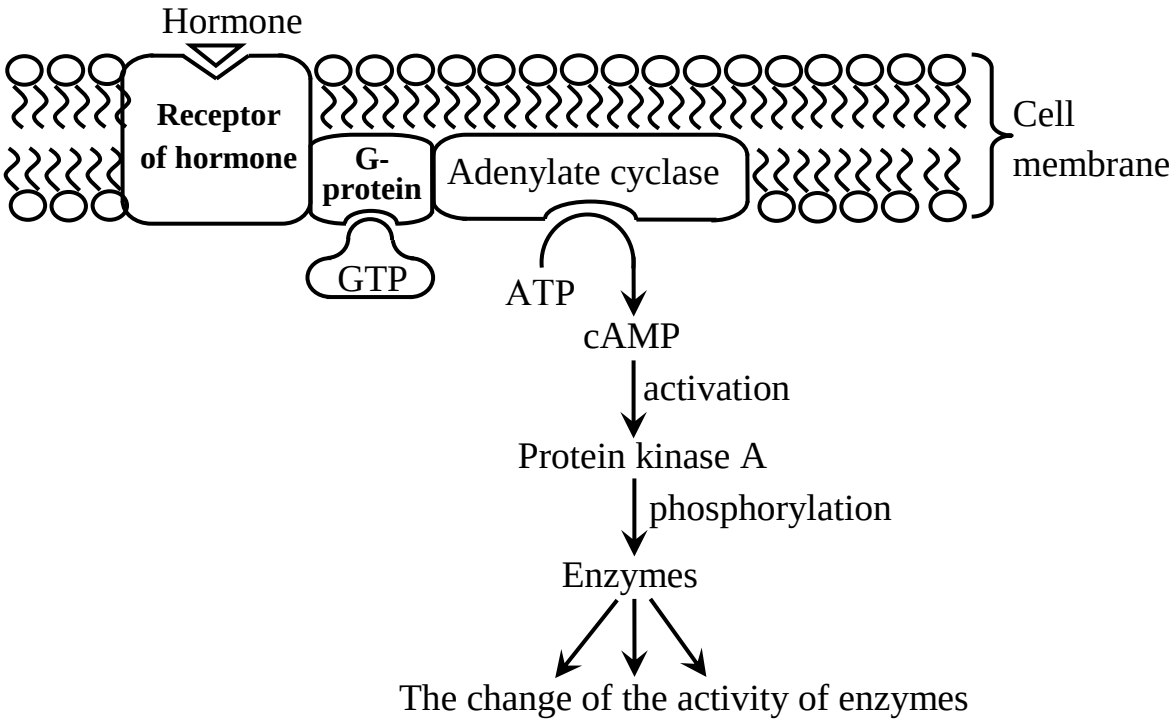


Figure 13.1 – Adenylate cyclase system

Binding of the hormone with its receptor causes consequent changes in the three-dimensional structure of all the components of the adenylate cyclase system. As a result, G-protein exchanges its GDP for GTP, thus becoming active, and stimulates adenylate cyclase which converts ATP to cAMP. cAMP stimulates protein kinase A.

Protein kinase consists of four subunits: two of them are regulatory subunits, and the other two are catalytic ones (Fig. 13.2). Four-subunit protein kinase is not active. After binding four molecules of cAMP to regulatory subunits, the whole enzyme disintegrates, releasing active catalytic subunits which phosphorylate proteins (enzymes). Phosphirylation of proteins (enzymes) leads to the change of their activity or functions.

The enzyme **phosphodiesterase** cleaves cAMP to form AMP, thereby decreasing intracellular level of cAMP.

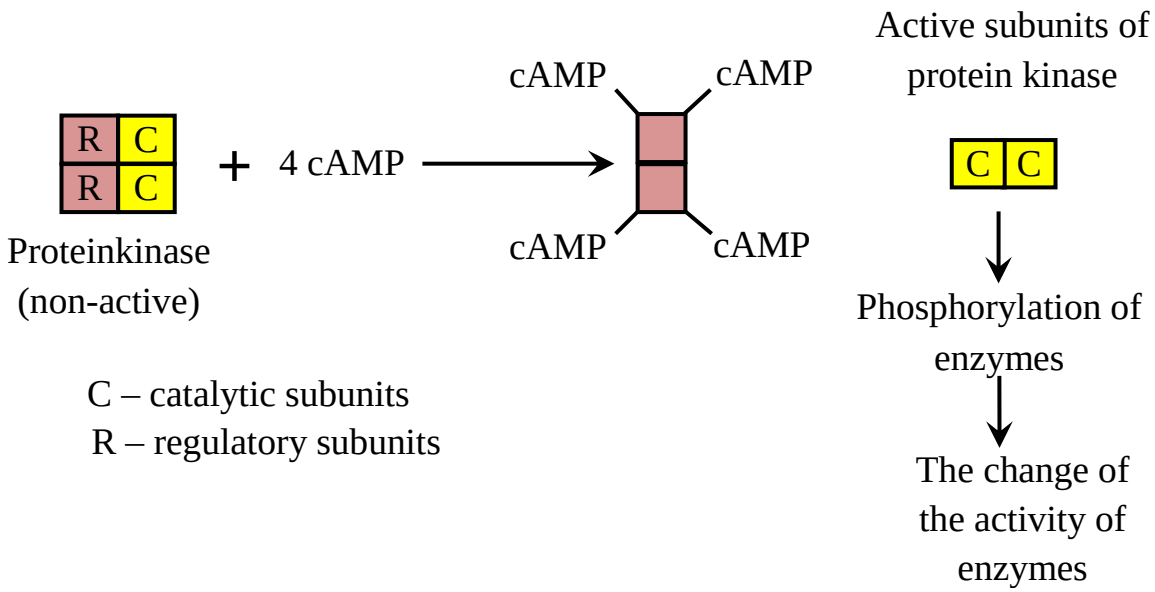


Figure 13.2 – Activation of protein kinase A by cAMP

Cyclic GMP

Cyclic GMP is formed from GTP due to the activity of guanylate cyclase similar to the formation of cAMP. cGMP activates **protein kinase G** which phosphorylates proteins providing specific effects. As well as cAMP, cGMP is cleaved by **phosphodiestherase**.

Calcium ions

Concentration of Ca^{2+} in the extracellular fluid is about 10,000 as much as that in cytoplasm. Such content of Ca^{2+} inside the cell would be lethal for it. The cell is pumping out the excess of Ca^{2+} from cytoplasm due to the functioning of Ca^{2+}-ATPase located in the cell membrane.

Some hormones can increase the cytoplasmic calcium concentration. They open calcium channels within the cell membrane, allowing extracellular calcium to move into the cell. Besides these hormones cause mobilization of intracellular stores of Ca^{2+}, i.e. its release from endoplasmic reticulum, cell membrane, and mitochondria. The increase of calcium concentration inside the cell results in binding of Ca^{2+} to the protein **calmodulin**. Each molecule of this protein is capable of binding 4 calcium ions so that **calcium-calmodulin complex** is formed. This complex activates specific **Ca^{2+}-calmodulin-dependent protein kinase** which, in turn, catalyzes phosphorylation of numerous intracellular enzymes (proteins), thereby changing their activity.

Inositol triphosphate (IP_3) and diacylglycerol (DAG)

IP_3 and DAG are derivatives of the membrane phospholipid called phosphatidylinositol (Fig. 13.3.).

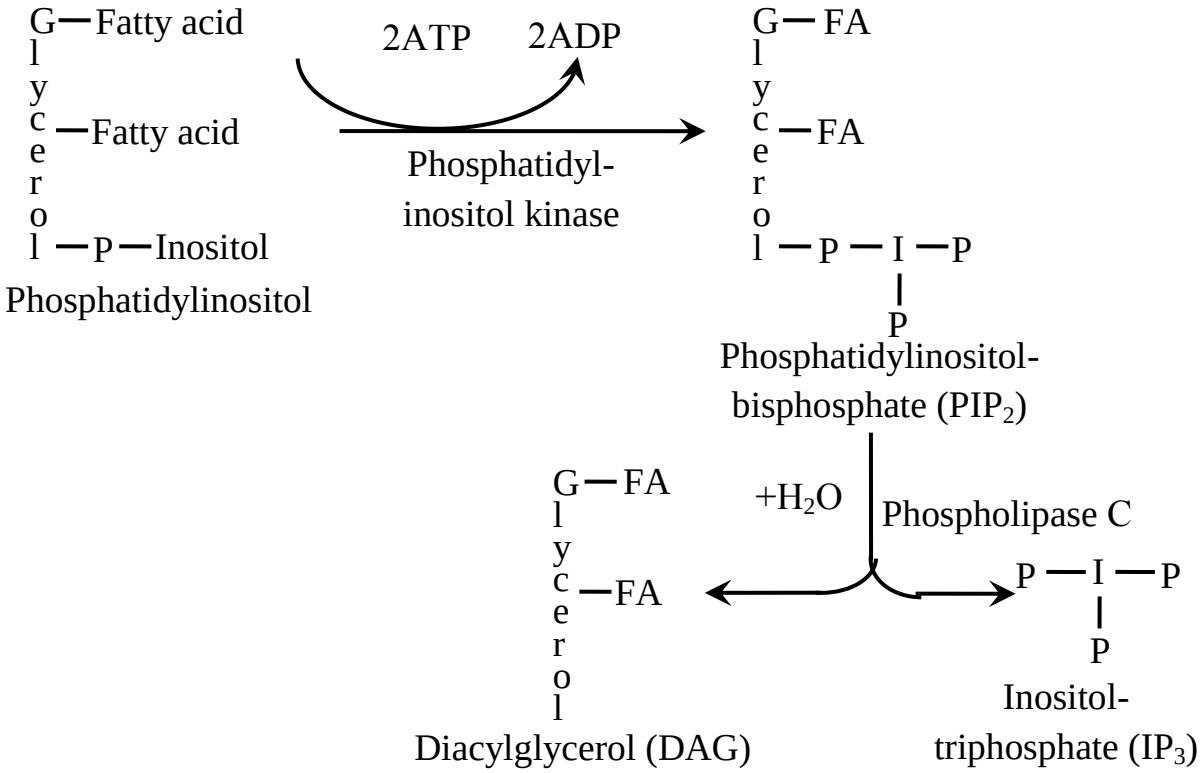


Figure 13.3 – Scheme of formation of diacylglycerol and inositoltriphosphate from phosphatidylinositol. FA – fatty acid; I – inositol; P – phosphate inorganic.

This phospholipid is phosphorylated in the membrane by the enzyme phosphatidylinositol kinase to form phosphatidylinositol bisphosphate (PIP_2). Some hormones can activate the membrane-bound enzyme phospholipase C which cleaves PIP_2 in the cell membrane to produce two second messengers – IP_3 and DAG.

Biological effects of these second messengers are realized differently. DAG activates Ca^{2+}-dependent protein kinase C which phosphorylates certain proteins, altering their activity. IP_3 binds to the specific receptor on the endoplasmic reticulum causing Ca^{2+} to be released from intracellular stores (EPR) to the cytoplasm. Ca^{2+} binds then with calmodulin and activates Ca^{2+}-calmodulin-dependent protein kinase which phosphorylates enzymes, altering their activity. Besides, Ca^{2+} can directly interact with proteins and cause the change of their activity.

Mechanism of action of steroid and thiroid hormones

Steroid and thyroid hormones enter the cell and bind with intracellular receptors (Fig.13.4). The hormone-receptor complex is transferred into the nucleus, where it binds with DNA. This leads to stimulation of the mRNA synthesis. Translation of the mRNA produces proteins which are responsible for certain biological effects.

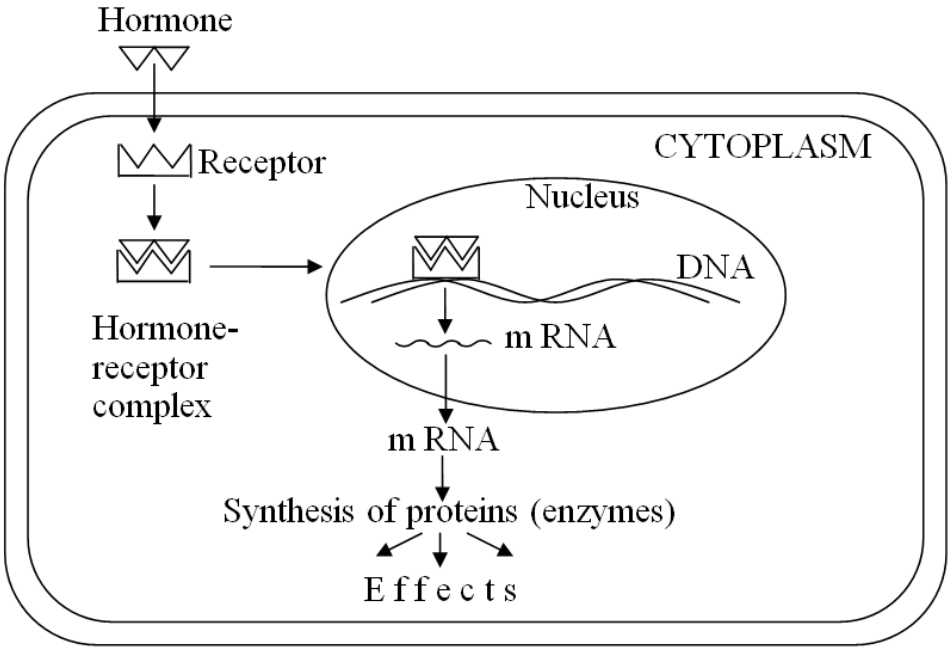


Figure 13.4 – Mechanism of action of steroid hormones and thyroxine

Thus, peptide hormones mainly change the **activity** of enzymes, and steroid hormones change the **amount** of enzymes.

CHAPTER 14

HORMONES OF ENDOCRINE GLANDS. PROSTAGLANDINS

THE SYSTEM OF HYPOTHALAMUS-HYPOPHYSIS IN REGULATION OF ENDOCRINE GLANDS

Synthesis of hormones and their secretion into the blood are regulated by requirements of the organism. Hormones are released into the blood in response to the appropriate stimulation. The impulses from receptors reach (via afferent nerves) the CNS, there the impulses are analyzed and then (via efferent nerves) sent to the periphery. But the nervous regulation doesn't cover all functions of all organs; therefore it is supplemented by hormonal regulation. The site of joining the nervous and hormonal regulation is the hypothalamus. Under the influence of nervous impulses from the CNS, **liberins** and **statins** are formed in hypothalamus. **Liberins** stimulate, and **statins** inhibit synthesis of **tropic hormones** of the **hypophysis (pituitary gland),** i.e. the anterior lobe of hypophysis known as **adenohypophysi**s. Hormones generated here enter the blood, are transported to the peripheral endocrine glands and stimulate production of definite hormones.

The hormones of **adenohypophysis** include:

1. **Growth hormone** (**GH**; **somatotropin)** acts on the bone tissue to accelerate its growth.

2. **Thyroid stimulating hormone (TSH**) stimulates growth of the thyroid gland and secretion of thyroxine.

3. **Adrenocorticotropic hormone (ACTH)** stimulates growth of the adrenal cortex and increases mainly secretion of cortisol.

4. **Gonadotropic hormones: follicle-stimulating hormone (FSH), luteinizing hormone (LH),** and **prolactin** (or **lactotropic hormone, LTH**)**.** They influence development and the hormone secretion of ovaries in females and testes in males. Prolactin stimulates lactation.

The posterior lobe of hypophysis called **neurohypophysis** contains hormones **oxytocin** and **vasopressin,** which are synthesized in supraopthical and paraventricular nuclei of hypothalamus but are stored in the posterior lobe of the hypophysis.

1. **Oxytocin** stimulates the uterus to contract during the childbirth and causes production of milk from the mammary glands.

2. **Vasopressin**, or **antidiuretic hormone (ADH)** stimulates

reabsorption of water by the kidney tubules and causes vasoconstriction resulting in the increase of the blood pressure. In the posterior lobe atrophy, diabetes insipidus is developed (urinary excretion is extremely large, 10-20 liters per day).

Of all hormones produced in the adenohypophysis, growth hormone and ACTH exert the most expanded biochemical and physiological effects on the organism.

Growth hormone

GH is a protein with absolute species specificity: GH of animals does not influence humans.

The GH secretion is stimulated under definite conditions such as:

- sleep, especially during the first hours of getting asleep (if children d sleep little, they grow up poorly);
- coldness (cold outer temperature) – on average, people of some northern populations are taller than some southern ones;
- physical exercise – as a rule, sportsmen are taller than "not-sportsmen";
- stress – a child deprived of ordinary everyday stress situations reveals relatively slow growth.

Effects of GH on the organism.

1) **Anabolic effect.** GH increases synthesis of nucleic acids and proteins in bones (osteogenesis), cartilages (chondrogenesis), and soft tissues. Cartilages of epiphyses are mostly sensitive to GH; due to this effect the bone can grow longways.

2) **Diabetogenic effect.** In the liver, GH increases gluconeogenesis by way of stimulation of glucagon secretion. In the muscle and adipose tissue, GH decreases membrane permeability for glucose to enter the cell. Excess of GH in the organism leads to the **insulinoresistancy of peripheral tissues**, and may result in the **somatotropic diabetes**.

3) **Lipolytic effect.** In children, during the period of intensive growth, the adipose stores are absent because in the adipose tissue GH increases cleavage of triacylglycerols (lipolysis). The fatty acids formed due to lipolysis enter the blood and are utilized by tissues as energy sources to meet energy requirements (for synthesis of construction materials during growth of the body). Also, fatty acids are taken up by the liver and utilized in β-oxidation to form acetyl CoA. The latter compound is used for synthesis of ketone bodies; therefore in excess of GH, the enhanced amount

of ketone bodies is produced in the liver, and their concentration in the blood is increased.

Hypersecretion of GH. Excessive secretion of GH may occur as a result of the benign tumor of the adenohypophysis. If the hypersecretion begins prior to closure of growth centers in the long bones, **gigantism** occurs. The main manifestations of gigantism are as follows: excessive height, the extremities are disproportionally long. If hypersecretion begins after the growth centers had been closed, this results in **acromegaly** (from the Greek *acron*, extremity, and *megale*, great). The disease is characterized by the intensive enlargement of individual parts of the skeleton bones (the prominent parts of facial skeleton, superciliary archs, cheekbones, jaw, and chin) as well as the enlargement of the soft tissues of the face (lips, nose, tongue). Hands and feet are also abnormally large in size. Overgrowth of endocrine glands is also occurred, which may be accompanied by their hyperfunction. Chronic GH excess may often lead to glucose intolerance because of its diabetogenic action.

Hyposecretion of GH. Hyposecretion of GH, or hypophyseal **dwarfism**, may be due to the inborn underdevelopment of pituitary gland and the decreased GH secretion. As a result, the proportional underdevelopment of the skeleton and the whole body is observed. Unlike in cretinism, there is no psychic abnormalities in such children: the patients are normal mentally and no skeletal deformations take place.

Adrenocorticotropic hormone

This hormone stimulates growth of the adrenal cortex and increases secretion of cortisol (primarily) and aldosterone (at less extent).

The major target tissues for ACTH are:

1) adrenal cortex, where ACTH increases synthesis and secretion of glucocorticoids and (to less extents) mineralocorticoids;
2) adipose tissue, where ACTH stimulates cleavage of triacylglycerols;
3) liver, where ACTH activates cleavage of glycogen.

THE THYROID GLAND

The thyroid gland produces thyroid hormones: **thyroxine** (tetraiodothyronine, T_4) and **triiodothyronine** (T_3).

Biochemical features of the thyroid gland:

1) The thyroid gland (follicular cells) takes up iodine from the blood.

2) The follicular cells contain specific protein – thyroglobulin which contains many residues of amino acid tyrosine. Iodination of tyrosine residues within the molecule of thyroglobulin results in the formation of monoiodotyrosine and diiodotyrosine which are then condensed to form T_3 and T_4.

Such an iodinated molecule of thyroglobulin is secreted into the lumen of the follicle. Thyroid stimulating hormone (TSH) stimulates pinocytosis of thyroglobulin by the follicular cells. Then lysosomal proteases cleave peptide bonds of iodinated thyroglobulin, and the free T_3 and T_4 are released into the blood. So, TSH stimulates the release of T_3 and T_4 from the thyroid gland.

In the blood thyroid hormones bind to transporting proteins and reach target tissues. The concentration of T_4 in the blood is 10 times as much as that of T_3; therefore **T_4** is considered to be the **major form** of thyroid hormones **in the blood**. But T_3 is 10 times as active as T_4.

Target tissues for the thyroid hormones are almost all tissues of the body except for the spleen and testes.

In the target tissues, thyroid hormones are separated from the transporting proteins and enter the cell. In the cell, 90% of T_4 loses one atom of iodine and converts to T_3. Thus, **the major intracellular form** of thyroid hormones **is T_3**.

The action of thyroid hormones depends on their concentration in the blood: in small (normal, physiological) concentrations, thyroid hormones exert anabolic effect; in high (excessive) concentrations, they cause catabolic effect.

Action of physiological concentrations of thyroid hormones

The major effects of thyroid hormones at physiological concentrations include:

1) **stimulation of both nucleic acid and protein synthesis, and**
2) **stimulation of energy metabolism.**

1) The increase of the nucleic acid and protein synthesis stimulates growth, development, cell division and differentiation of all organs and tissues. This effect is especially important for the growing organism.

Thyroid hormones are absolutely required for the structural, biochemical, and functional maturation of the brain. In the CNS, cells keep

dividing during 1-1.5 years after birth. Therefore thyroid-hormone deficiency occurred at fetal life or at early ages leads to the decrease of protein synthesis in the whole organism, and in the brain tissue in particular. As a result, the differentiation of large hemispheres and cerebellum is impaired which is accompanied by mental and physical retardation. Hypothyroidism in children is called **cretinism**. The earlier the age at which the thyroid hormone deficiency appeared is the more it impairs the CNS development.

2) Thyroid hormones stimulate energy metabolism, i.e. both the use and synthesis of ATP. As these two processes are activated simultaneously, the equilibrium is preserved between them. The outer sign of the existing energy equilibrium is the maintenance of the normal body temperature. Thus, **thyroid hormones maintain the energy equilibrium in the organism.**

The preserving of the energy equilibrium in the cell is of great importance for the normal functioning of the nervous tissue, i.e. for coordination of excitement and inhibition processes in the brain. Under normal conditions, due to participation of thyroid hormones, processes of excitation and inhibition in the brain are coordinated. Both excess and deficiency of thyroid hormones cause impairment of energy equilibrium and electrochemical processes in the CNS, and this is accompanied by certain symptoms of brain dysfunctions.

The action of high concentrations of thyroid hormones

The action of excessive concentrations of thyroid hormones is observed in hyperthyroidism (Graves' disease).

In **hyperthyroidism**, the energy balance (the balance between production and wasting of ATP) is impaired.

High concentrations of thyroid hormones act on mitochondria where in the inner mitochondrial membrane the ETC is located. Normally, major portion of energy produced in the ETC is stored in the phosphate bonds of ATP (oxidative phosphorylation), and the other portion of energy is dissipated as heat for the maintenance of the normal body temperature. **High concentrations of thyroid hormones uncouple the ETC and oxidative phosphorylation.** As a result, the ETC keeps functioning, substrates are oxidized, oxygen is consumed, and energy is generated, but because of uncoupling the ETC and oxidative phosphorylation, the ATP is not formed, and all the energy produced is dissipated as heat. That is why

the symptoms of hyperthyroidism include the **enhanced body temperature** (**hyperthermia**, due to intensive heat generation) and **muscle weakness** (because ATP is required for both muscle contraction and maintenance of the muscular tonus). As excessive quantities of thyroid hormones exert catabolic action, the degradation of body fuels (carbohydrate, lipid and protein stores) is increased which results in severe **body weight loss**.

The other symptoms of hyperthyroidism include enlarged thyroid gland (goitre), protruding eyes (exophthalmos), palpitations, the increased heart rate (tachycardia), enhanced systolic blood pressure and different psychic abnormalities, such as nervousness, tremors, excitement, anxiety (a sense of restlessness), emotional lability, sleeplessness (insomnia). Due to the increased heat production, patients with hyperthyroidism have sensation of heat intolerance, as well as excessive perspiration which occurs because of the need to dissipate heat through increased sweating. Therefore the skin of patient with hyperthyroidism is wet, reddened (hyperemic), and hot with palpation.

Hypothyroidism (deficiency of thyroid hormones) in adults is called **myxedema** (from the Greek *myxa*, mucus, and *oidema*, swelling). Mucus-like substances (glycosaminoglycans) are accumulated in subcutaneous tissues and on the vocal cords; therefore the common symptoms in hypothyroidism are mucoid-like swelling of tissues, weight gain, and husky (hoarse) voice. In adult patients with hypothyroidism, the generation of ATP is decreased, which leads to general brain disorder and psychic aberrations, such as a sense of weakness, fatigue, lethargy (somnolence), apathy, decreased memory, slowed mentation, psychical inertness, hypokinesis (slow in action), the speech becomes slow and indistinct (unclear), mimics is unexpressive.

The heat production is diminished which causes the sense of cold intolerance and decreased sweating. The skin is dry, pale, and cool in palpation, the body temperature is decreased, and patients severely suffer from coldness. The heart rate is slowed, and the blood pressure may be decreased. Obesity, hyperlipemia, hypercholesterolemia, loss of hair and teeth are also observed.

In children, hypothyroidism is called **cretinism** (see above).

The special form of hypothyroidism is called **endemic goitre**. It occurs as a result of insufficient dietary iodine supply. Most commonly, this disease is observed in the mountain regions, where the iodine content in water and plant (and, consequently, in the diet) is low. The iodine

deficiency leads to the comp**e**nsatory enlargement (hypertrophy) of the thyroidal tissue at the expense of prevalent growth of the connective tissue; however, due to the lack of iodine the enlargement of the thyroid gland is not accompanied by the increased secretion of thyroid hormones.

PARATHYROID GLANDS

Parathyroid glands produce parathyroid hormone (PTH) in response to low calcium blood levels. **PTH increases Ca^{2+} and decreases phosphate concentration in the blood**. The major target tissues of PTH are bones, kidney, and intestine.

- In **bones** PTH**:**
  1) inhibits collagen synthesis in osteoblasts;
  2) increases Ca^{2+} and phosphate mobilization from bone and their release into the blood. As a result, Ca^{2+} and phosphate concentrations are increased in the blood.
- In the **kidney** PTH acts on renal tubules to increase reabsorption of Ca^{2+} and stimulates excretion of phosphate into the urine.
- In the **intestinal epithelial cells** PTH increases absorption of both Ca^{2+} and phosphate from the lumen into the blood.

Hyperparathyroidism. Increased secretion of parathyroid hormone is mainly observed in tumor of parathyroid glands.

The increased release of Ca^{2+} from bones leads to demineralization of the skeleton (osteoporosis) and "spontaneous" fractures.

Calcium ions released from the bones enter the blood and result in **hypercalcemia** (increased blood calcium concentration). Chronic hypercalcemia leads to:

a) calcification of internal organs;
b) calcification of the hurts and bruises;
c) the decrease of neuromuscular excitability which in turn results in:
- muscle atrophy and weakness,
- psychoses, slowed mentation, impairments of memory and attention, change of personality.

Chronic renal filtration of blood, rich in calcium, leads to saturation of the tubular fluid with calcium salts; as a consequence, renal calculi (kidney and urinary tract stones) may occur.

Due to the excess of PTH, secretion of gastrin in the stomach is increased. Enhanced gastrin secretion stimulates production of HCl and pepsin, and may lead to stomach ulcers.

Hypoparathyroidism. It is observed after operations on the thyroid gland when parathyroid glands were accidentally removed. In child organism, hypoparathyroidism may occur due to infections of respiratory tracts.

Deficiency of PTH leads to the lowered levels of calcium in the blood (hypocalcemia) which causes the increase of neuromuscular excitability. The latter may be manifested as:

1) **tetany** (condition of continuous muscle contraction, convulsions, cramps, involuntary twitching of muscles);

2) **spasmophilia** (cyanosis and apnoe of a crying child because of spasms of respiratory muscles; i.e. a crying child stops breathing and gets cyanotic, or has dark-blue colour of skin).

Calcitonin

Calcitonin is synthesized in the thyroid gland. **Calcitonin decreases Ca^{2+} and phosphate concentration in the blood.**

The major target tissues of calcitonin, as well as those of PTH, are bones, kidney, and intestine, but the action is mainly opposite, and only in kidney calcitonin (as well as PTH) increases phosphate excretion into the urine.

Thus, calcitonin:

- decreases release of Ca^{2+} and phosphate from the bone into the blood and increases their deposition in bones;
- decreases Ca^{2+} and phosphate reabsorption by renal tubular cells;
- decreases Ca^{2+} and phosphate absorption by intestinal epithelial cells.

No kinds of pathology involving calcitonin have been described.

THE PANCREAS

Insulin

Insulin is polypeptide hormone synthesized by β-cells of the pancreas and deposited in secretory granules bound with zinc. The pancreas releases insulin into the blood in response to the increased blood glucose levels.

On their sensitivity to insulin, tissues may be divided into three

groups:

1) The **absolutely dependent** on insulin (major target-tissues): adipose tissue and muscle tissue. These tissues exhibit the maximal sensitivity to insulin. Glucose may enter these cells and metabolize in them only in the presence of insulin.

2) **Absolutely independent** on insulin (or absolutely insensitive to insulin) tissues. Glucose may enter cells of these tissues even in the absence of insulin, and glucose is the only energy substrate for these cells. The most important (vital, or essential to life) functions of the organism are fulfilled by these tissues:

- the brain – central regulation;
- medulla of kidney – secretion;
- erythrocytes – O_2 and CO_2 exchange in the tissues;
- intestinal epithelial cells – nutrition (absorption of products of digestion);
- testicles – breeding.

The brain consumes 50% of free glucose of the blood, erythrocytes and kidneys – 20%, total 70%; thus, it is extremely important to the organism that major metabolic glucose pool and life-providing functions of the organism are independent on insulin.

3) **Relatively dependent** on insulin tissues – these are all other tissues.

The action of insulin

1. Insulin is the only hormone which **decreases the blood glucose levels**. Mechanism:

- Insulin increases transport of glucose from the blood into the cell.
- Insulin activates utilization of glucose (activates metabolic pathways: glycolysis and glycogen synthesis).
- Insulin decreases production of glucose in the body, i.e. inhibits both gluconeogenesis (synthesis of glucose from non-carbohydrate precursors) and cleavage of glycogen.

2. Insulin is a **universal anabolic hormone**: insulin activates syntheses of DNA, RNA, proteins, triacylglycerols, fatty acids, glycogen, and decreases their breakdown.

Hyperinsulinemia. The excess of insulin in the organism may occur in **insulinoma** (insulin-secreting tumor) and in **overdose of insulin** which may take place in the course of the diabetes mellitus treatment. The major symptoms of hyperinsulinemia include: hypoglycemia, cramps, convulsions, loss of consciousness. Severe hypoglycemia may lead to death.

Hypoinsulinemia. Insulin deficiency results in **type I** (insulin-dependent) **diabetes mellitus**. In this condition, the plasma contains antibodies to the β cells which produce insulin. **In type II** (insulin-independent) diabetes mellitus, the adipose and muscle tissues have resistance to insulin action (tissues have insensitivity to insulin and are unable to take up glucose despite the presence of insulin). The major **symptoms** in diabetes mellitus are as follows: **hyperglycemia** (increased levels of glucose in the blood), **glucosuria** (excretion of glucose into the urine); the **increased catabolism** (degradation) and decreased synthesis of glycogen, proteins, triacylglycerols; glycolysis is decreased and gluconeogenesis is increased; concentrations of **ketone bodies** in the blood and urine are risen.

Glucagon

Glucagon is generated in α_2-cells of Langerhans islets. The liver is the major target tissue for the glucagon action. The other target tissues include adipose tissue and kidney cortex.

The **liver**. The maximal amounts of glucagon are released from the islets during **starvation**. This is the main hormone which maintains the blood glucose levels in starvation. During the first day of starvation, glucagon increases cleavage of glycogen (glycogenolysis) in the liver. However the glycogen storages appear to be completely depleted after 24 hours of starvation. Therefore beginning from the 2^{nd} day of starvation, glucagon stimulates gluconeogenesis, i.e. synthesis of glucose form amino acids which are produced due to the protein degradation. Unlike epinephrine, glucagon doesn't affect muscle glycogen. Thus, in starvation, the blood glucose is entirely of the liver origin.

Other effects of glucagon in the liver are: the decrease of the glycogen synthesis, inhibition of glycolysis, the increase of the ketone bodies production.

In **adipose tissue**, glucagon increases lipolysis (triacylglycerol degradation) and decreases lipogenesis (triacylglycerol synthesis).

In **all target tissues** glucagon stimulates proteolysis (cleavage of protein) and inhibits its synthesis.

In the **kidney cortex**, the hormone stimulates gluconeogenesis.

The excess of glucagon in the organism may occur in **glucagonoma** (glucagon-secreting tumor).

ADRENAL MEDULLA

The adrenal medulla produces catecholamines – dopamine, norepinephrine (noradrenaline) and epinephrine (adrenaline) which are synthesized from phenylalanine. Epinephrine makes up 80% of catecholamines in the adrenal medulla.

The essential amino acid phenylalanine is hydroxylated to form tyrosine which in turn is converted by hydroxylation to dihydroxyphenylalanine (DOPA). Subsequent decarboxylation of DOPA forms neurotransmitter dopamine. Hydroxylation of dopamine on its aliphatic chain yields the neurotransmitter norepinephrine. Methylation of norepinephrine forms hormone epinephrine.

The adrenal medulla secretes both adrenaline and noradrenaline into the blood. Adrenaline is not produced elsewhere in the organism beyond the adrenal medulla. Noradrenaline is also synthesized in terminals of sympathetic nervous system, functioning as a neurotransmitter.

Biochemical characteristics of adrenaline and noradrenaline

1) The maximal amount of both epinephrine and norepinephrine is secreted into the blood in response to **stress** and **physical exertion**.

2) The organism responds to epinephrine very fast.

3) Adrenaline and noradrenaline help the organism resist to crisis situations and fulfill (perform) **quick and intensive work**.

Normally only 1-4% of adrenaline is execreted into the urine. This amount is too little to be detected by the routine methods; therefore normally **adrenaline is** considered **not** to be **present in the urine**.

Degradation of adrenaline and noradrenaline takes place in the liver. The major degradation product excreted into the urine is **vanillylmandelic acid** which is used **for diagnostics**.

The target tissues for epinephrine and norepinephrine are the liver, muscles, adipose tissue, and cardiovascular system.

The liver. The hormone stimulates glycogenolysis (degradation of the liver glycogen **to form glucose**), and increases the blood glucose concentration.

Muscles. The hormone stimulates cleavage of glycogen stores **to form lactic acid** and increases its concentration in the blood.

Adipose tissue. The hormone stimulates lipolysis (the breakdown of triacylglycerol stores **to form fatty acids**) in adipose tissue, and increases the fatty acid concentration in the blood.

Cardiovascular system. Adrenaline raises blood pressure, increases heartbeat and respiration, causes tachycardia, bronchodilatation, and hypertension. The hormone acts as **vasoconstrictor** (narrows blood vessels) on the **skin**, mucous membranes and ***vas afferentis*** **of the kidney**; therefore, during times of stress, paleness and **anuria** are observed. Nevertheless the hormone causes vasodilatation in the heart, skeletal muscles and inner organs. Via the cardiovascular system, adrenaline affects almost all functions of practically all organs resulting in efficient mobilization of the organism for resisting to the stressful situations. The hormone relaxes the smooth muscles of both bronchi, gastrointestinal tract and bladder, but contracts sphincters of digestive tract and bladder, **dilate pupil** of the eye, and contracts muscles rising skin hair.

Pathology. Hyperproduction of adrenaline and noradrenaline takes place in **pheochromocytoma** (tumor). The concentration of epinephrine and norepinephrine in the blood increases in 500 and more times. Concentrations of both **glucose** and **fatty acids** are increased **in the blood**. **In the urine, epinephrine and glucose** are observed (they are normally absent in the urine), and the excessive amount of vanillylmandelic acid is also present.

ADRENAL CORTEX

This is the outer section of the adrenal gland. The region produces steroid hormones: **corticosteroids** (glucocorticoids and mineralocorticoids) and **sex hormones** (male and female). All of them are synthesized from cholesterol. Inactivation of corticosteroids takes place in the liver. The products of their inactivation called 17-ketosteroids (17-KS) are excreted into the urine and used in diagnostics.

Glucocorticoids

Representatives of glucocorticoids (GCs): **cortisol,** or hydrocortisone, (the major glucocorticoid in humans), **cortisone,** and **corticosterone**.

Target-tissues for GCs include the liver, as well as the muscle, adipose, connective, and lymphoid tissues. In the liver, GCs stimulate anabolic processes and increase transport of substrates into the cell, and in other target-tissues they, vice versa, activate catabolism and decrease transport of substrates into the cell.

The influence of GCs on metabolism

Carbohydrate metabolism. GCs inhibit glycolysis in all the target-tissues. In the **liver**, GCs increase gluconeogenesis and synthesis of glycogen. In **other target-tissues**, GCs decrease transport of glucose into the cell. In muscles GCs increase degradation of glycogen.

The **excess** of GCs in the organism (this may take place in the use of GCs for the **treatment** in high doses or for a long period, or this may be due to the **increased production** of GCs in pathology) leads to the increase of the blood glucose level. The long-term increase of glucose levels in the blood may result in hyperstimulation of insulin production by β-cells of the islets Langerhans with the subsequent islets depletion, which may cause **steroid diabetes**.

Lipid metabolism. In the **liver**, GCs increase synthesis of triacylglycerols, VLDL (very low density lipoproteins), and ketone bodies. In the **adipose tissue** GCs stimulate degradation of triacylglycerols on the extremities but increase deposition of triacylglycerols on the trunk and the face. Therefore, the excess of GCs in the organism leads to the **spider-like obesity**; the increased levels of **ketone bodies** in the blood are also observed.

Protein and amino acid metabolism. In the **liver**, GCs increase synthesis of proteins and decrease their degradation. In the **other target-tissues**, GCs decrease synthesis of proteins, increase their degradation, therefore the following symptoms are observed:

1) muscle atrophy and weakness;
2) the decrease of collagen synthesis which results in:
   a) retardation of wounds' healing;
   b) osteoporosis (which is manifested by bone fragility and fractures inminimal trauma);
3) in the lymphoid tissue GCs decrease synthesis of antibodies, lymphocyte formation and cause destruction of these cells, therefore:
   a) GCs can be used for treatment of some allergic reactions;
   b) GCs are used in transplantation of organs because they suppress the immune response;
   c) in stressful situations accompanied by the enhanced production of GCs, these hormones may result in the increase of susceptibility to infections.

The systemic effects of GCs

1) **GCs increase the HCl secretion in the stomach** due to the decrease of synthesis of prostaglandins which in turn inhibit secretion of

HCl. Therefore the excess of GCs in the organism may result in the developing of **stomach ulcers**.

2) GCs have **anti-inflammatory effects** and may be used for the treatment of inflammation. They inhibit all the stages of inflammatory process, mainly decrease the membrane permeability and synthesis of prostaglandins (the latter are known to be the tissue inflammatory factors).

3) GCs **decrease the enhanced reactivity** of the organism, i.e. **hypersensitivity**; therefore they may be used for the treatment of allergy (e.g. anaphylactic shock).

4) GCs exert **permissive effects**, i.e. they increase the action of other hormones. For example, the excess of GCs in the organism is accompanied by hypertension (high blood pressure) mediated by the cortisol-stimulated overproduction of adrenaline.

Mineralocorticoids

Representatives of mineralocorticoids are **aldosterone** and **dehydroxycorticosterone**. These hormones regulate metabolism of mineral salts (**sodium, potassium**) and **water** balance in the organism.

Aldosterone is the major mineralocorticoid hormone. The **epithelial cells of the distal renal tubules** are its major target-tissue. In the kidney, aldosterone increases reabsorption of sodium, thus enhancing its concentration in the blood serum; therefore aldosterone is called **sodium-retaining hormone**.

As sodium attracts water (water follows the flow of sodium) the increase of the circulating blood volume is observed. Therefore, the **excess of aldosterone** in the organism results in the increased blood pressure and swelling of tissues (edema), and intensification of inflammatory processes. The sodium reabsorption from the urine stimulated by aldosterone is accompanied by excretion of potassium into the urine. In the **aldosterone excess**, the concentration of potassium is lowered in the blood, which leads to the increased excitement of myocardium and the heartbeat impairments, characteristic changes in an electrocardiogram, heart failure, and heavy weakness.

Sweat glands are another target-tissue for aldosterone. The high outer temperature stimulates aldosterone production due to which, at the hot weather, the excessive sodium loss via the sweat is prevented.

Deficiency of aldosterone in the organism results in the loss of sodium and water with the urine and dehydration of the body.

Glucocorticoids, especially corticosterone, exert partial mineralocorticoid effects on the organism; therefore in the use of glucocorticoids as therapeutic agents, the potassium-containing medicines should be prescribed to the patient.

Hyperfunction of adrenal cortex (hypercorticism) may be represented by either hypersecretion of all the steroid hormones in the adrenal cortex, or hyperproduction of only one group of the hormones. In the latter case, three types of hypercorticism are observed:

1) **Glucocorticoid excess**. It appears due to hyperfunction of *zona fasciculata* of adrenal cortex, where glucocorticoids are mostly synthesized. Glucocorticoid excess takes place both in **Cushing's** syndrome (malignant adrenal cortex tumor) and Cushing's disease (non-tumor hyperplasia, or benign abnormal growth of the adrenal glands).

2) **Mineralocorticoid excess** is observed in **Konn's** disease (hyperfunction of *zona arcuata* in which mineralocorticoids are mainly synthesized).

3) **Adrenal virilism or adrenogenital syndrome.** It occurs due to hyperproduction of male sex hormones (androgens) in *zona reticulata* of adrenal cortex. Excess of adrenal androgen secretion in females leads to virilism (appearance of male signs); in males, the increase of male signs is observed; in children – premature sex developing (maturation before puberty) takes place. Excess of adrenal estrogens (female sex hormones) in males leads to the appearance of abnormal feminine characteristics.

Hypocorticoidism (hypocorticism). Hypofunction of the adrenal cortex is called **Addison's (or bronze) disease**. Hypocorticism may be caused by **tuberculosis or atrophy of adrenal cortex** and is accompanied by lack of both mineralocorticoids and glucocorticoids which in turn leads to miscellaneous changes of metabolism and functions.

The major symptoms of Addison's disease include: fatigue, weakness, weight loss, bronze pigmentation of the skin, enhanced sensitivity to stress situations, hypoglycemia (hunger intolerance caused by lack of glucocorticoids), the lowered blood pressure (caused by lack of mineralocorticoids), enhanced water and sodium excretion and, related to the latter, subconscious preference of salt meals.

Both lack and excess of corticosteroids influence deeply the mood and person's adaptability to the real life conditions. Patients with Addison's disease may often experience depression or anxiety (apprehensions), and

these symptoms may be relieved by adequate replacement therapy. The long-term use of glucocorticoids for the treatment of diseases may cause euphoria, hyperirritability, and even psychoses in persons liable to such conditions.

FEMALE SEX HORMONES

Female sex hormones are divided into 2 groups: estrogens and progestins.

Representatives of estrogens: **estradiol** (the major hormone of ovaries), **estriol** (is generated by placenta), and **estrone** (is produced in adrenal cortex). The main progestin is progesterone which is secreted by *corpus luteum* of ovaries. Small amounts of estrogens are also produced in the testicles.

Estrogens are inactivated in the liver by way of conjugation with glucuronic or sulfuric acids, and then are excreted in the urine. In a conjugated form, estrogens are deprived of biological activity, therefore *per os* administration of estrogens is of a small effectiveness.

The target-tissues for estrogens are divided into sex organs and non-sex organs.

The effects of estrogens on the sex organs

Estrogens are responsible for the development and functioning of sex organs, and formation of secondary female sex characteristics during the period of sexual maturation (at puberty). Progesterone is responsible for the preparation and maintenance of the uterus in pregnancy.

The effects of estrogens on the non-sex organs

CNS, hypothalamus, hypophysis. Estrogens are responsible for the formation of sexual behaviour, instinct, and psychical status of a female.

Skeleton and larynx. Estrogens are responsible for the formation of the female type of the skeleton, larynx, and voice. These hormones increase ossification of epiphyses where the growth zone of the bone is located. Therefore, **lack of estrogens** in a girl’s organism may be the possible reason of tall height. In women, **excess** of estrogens increases deposition of calcium in the bone cavities where the red bone marrow is located; therefore, in such patients, **anemia** may take place.

Skin. Estrogens promote growth of hair on the so called female type, hamper hair growth on the trunk and the face, inhibit secretory activity of the sebaceous glands thus decreasing sebaceousness of the skin.

Liver. Estrogens stimulate synthesis of specific liver proteins such as blood **clotting factors** (II, VII, IX, X), and **angiotensinogen**; therefore **excess** of estrogens may cause **thromboses and hypertension**.

Estrogens increase synthesis of both very low density lipoproteins (VLDL) and high density lipoproteins (HDL).

VLDL contain a lot of triacylglycerols. These lipoproteins are released from the liver into the blood and transport triacylglycerols to adipose tissue in which the fat is deposited. Therefore, in female, muscles are always covered by the layer of subcutaneous adipose tissue.

HDL remove cholesterol off the organism; therefore atherosclerosis and myocardial infarction, as consequences of increased cholesterol levels in the blood, are mostly observed in men than in women.

Adipose tissue. Estrogens increase synthesis of triacylglycerols in adipose tissue and decrease their degradation, promote formation of the typically female fat depositions.

Kidney. Estrogens increase the sodium retaining in the organism. Progesterone, vice versa, increases excretion of sodium into the urine. Especially large amounts of progesterone are produced in pregnancy; the loss of sodium with the urine may explain the subconscious preference of the salt at pregnancy.

MALE SEX HORMONES

Male sex hormones (androgens) are represented by **testosterone** and **androsterone**. They are secreted by testes, adrenal cortex, prostate gland. Small amounts of androgens are also produced in the ovaries.

Androgens are inactivated in the liver with the resultant formation of 17-ketosteroids which are excreted into the urine.

Androgens exert **generalized anabolic effect** on the organism: they stimulate synthesis of nucleic acids and proteins, retain nitrogen and calcium in the organism, and increase synthesis of the membrane phospholipids.

The target-tissues for androgens are divided into sex organs and non-sex organs.

The effects of androgens on the sex organs

Androgens exert so called **androgenic effect** on the sex organs. These hormones are responsible for the development and functioning of sex organs, and formation of secondary male sex characteristics during the period of sexual maturation (at puberty). Testosterone is responsible for

masculinization during early development (at adolescence) and plays a role in spermatogenesis in the adult male.

The effects of androgens on the non-sex organs

In the non-sex organs androgens cause **anabolic action**.

CNS, hypothalamus, hypophysis. Androgens are responsible for the formation of sexual behaviour, instinct, and psychical status of a male. Androgens promote the brain development on the male type. **Lack** of androgens during fetal period in certain critical spells of the brain development may cause appearance of variants of sexual behaviour (sexual preferences) in adolescents and adult men. **Excess** of androgens may be a reason of aggressiveness.

Skeleton and larynx. Androgens are responsible for the formation of the male (masculine) type of skeleton, larynx, and voice (hoarseness). These hormones increase ossification of epiphyses where the growth zone of the bone is located. Therefore, **excessive** production of androgens in adolescents may lead to premature ossification of epiphyses and height shortening.

Muscles. Androgens stimulate protein synthesis in the skeletal muscle, resulting in the increase of prominence (relief), mass, and strength of muscles.

Adipose tissue. Androgens decrease synthesis of triacylglycerols in adipose tissue and increase their degradation; therefore in men the subcutaneous fat layer is thinner than in women.

Skin. Androgens promote growth of hair on the male type, stimulate hair growth on the trunk and face, pigmentation of the skin (promotes the tanning), secretory activity of the sebaceous glands, thus increasing sebaceousness of the skin. **Excess** of androgens may be a reason of baldness.

Other effects. Androgens increase protein synthesis in the liver and kidney, promote **hemopoiesis**.

Anabolic steroids. Marked anabolic effects of male sex hormones prompted the investigation of compounds which would exhibit maximal anabolic action and minimal androgenic effect on the organism. Chemical analogues of androgens with such properties were finally synthesized. They are named anabolic steroids (anabolics).

Initially anabolic steroids were used for the quick and effective increase of cattle’s body weight (muscle mass). But subsequently, anabolics were

revealed to be harmful for the human organism: they didn't metabolize in human tissues and didn't degrade during cooking (thermal processing of meals); therefore if the meat contained anabolic steroids, which had been consumed with meals this often, caused the heavy damage of the liver (the liver cancer). Lately most countries refused the use of anabolic steroids in the herd breeding. However some sportsmen administer these substances for improvement of their sports results. Apart from the above mentioned adverse effects on the human's health, the use of anabolics during at least 2 years, at the age spell of 18-26, causes sexual impotence and impairment of spermatogenesis (oligospermia due to decreased production of androgens) in men as well as cancer of the breast in women.

Small doses of anabolic steroids are used as therapeutic agents, in patients over 35, for the increase of body weight, stimulation of appetite, improvement of wounds healing during recovery period after heavy trauma, operations, myocardial infarction.

PROSTAGLANDINS AND OTHER EICOSANOIDS

This is a group of so called local, or tissue hormones, or hormone-like substances, because unlike "real" hormones that are synthesized in one type of organs but act in the other one, eicosanoids are both formed and act in the same tissues. These compounds are called eicosanoids because they are produced from eicosatetraenoic, or arachidonic, acid.

Eicosanoids (prostaglandins, prostacyclins, thromboxanes, and leukotrienes) are synthesized from arachidonic acid (Fig. 14.1.). This polyunsaturated fatty acid is released from membrane phospholipids by phospholipase A_2. The enzyme is inhibited by glucocorticoids (anti-inflammatory agents). Arachidonic acid is oxidized by cyclooxygenase to form prostaglandins, prostacyclins, and thromboxanes. Cyclooxygenase is inhibited by aspirin, indomethacin, and other nonsteroidal anti-inflammatory agents. Leukotrienes can be produced from arachidonic acid by a pathway in which lipoxygenase participates; its activity is inhibited by vitamin E and vitamin P.

Prostacyclins are produced by vascular endothelial cells. Prostacyclins dilate coronary arteries, decrease blood pressure, and inhibit platelet aggregation.

Thromboxanes are formed in platelets. Thromboxanes promote vasoconstriction and platelet aggregation. The ratio prostacyclins/ thromboxanes in the vessel wall is very important in the development of

thromboses or their prevention.

Leukotrienes take part in inflammation, allergic reactions, and immune response, attract leucocytes to the place of inflammation, constrict bronchi, and increase secretion of bronchial mucus.

Prostaglandins are synthesized in all cells excepting erythrocytes and are degraded very quickly – in 20 minutes. Prostaglandins have multiple effects which differ from one tissue to another and from one type of these compounds (prostaglandins of E group) to another (prostaglandins of F group).

Prostaglandins increase functional activity of endocrine glands (hypophysis, thyroid gland, pancreas, adrenal gland), thus promoting effects of other hormones.

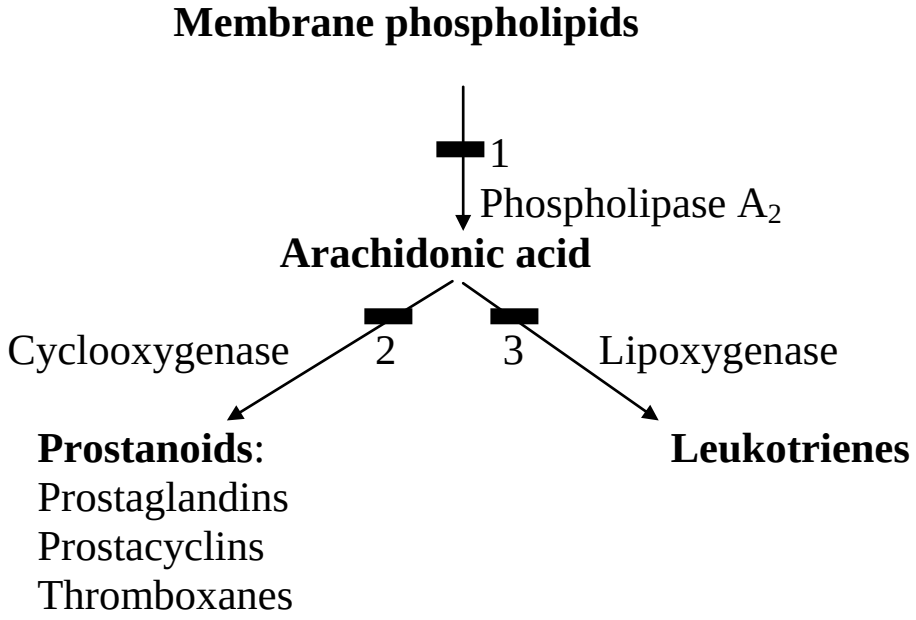


Figure 14.1 – Synthesis of eicosanoids. Inhibition of enzyme activity by: 1 – glucocorticoids; 2 – aspirin, indomethacin and other non-steroidal anti-inflammatory agents; 3 – vitamin E, vitamin P

Prostaglandins E:

1) inhibit cleavage of triacylglycerols and glycogen;
2) are the tissue inflammatory factors; prostaglandins E are generated in the focus of inflammation in large amounts, increase permeability of vessels and cell membranes, dilate capillaries; besides they are powerful pyrogenic agents, i.e. they enhance the body temperature: therefore aspirin (as an inhibitor of prostaglandin synthesis) is used to decrease body temperature;

3) cause pulsating headache, which may be revealed in 20 minutes by the administration of aspirin;
4) decrease blood pressure because of vasodilatation effect; therefore they are used in hypertension;
5) dilate bronchi, therefore may be used in bronchial asthma;
6) decrease secretion of hydrochloric acid in the stomach, thus preventing it from ulceration; therefore prostaglandins E are used in the therapy of ulcers (aspirin and glucocorticoids decrease synthesis of prostaglandins which inhibit HCl secretion; therefore the improper use of aspirin or the prolonged or uncontrolled therapy with glucocorticoids may lead to ulcer formation in the stomach);

Prostaglandins F:

1) stimulate peristalsis of the bowel;
2) constrict bronchi;
3) stimulate the smooth muscle of the uterus, therefore they are used for stimulation of the infant delivery.

THE USE OF HORMONES IN MEDICAL PRACTICE

The use of hormones in medical practice is usually directed towards the following aims:

1) Replacement therapy. Hormones as medicines (drugs) are used in hypofunction of endocrine glands:

- vasopressin is used in diabetes insipidus;
- insulin – in diabetes mellitus;
- thyroxine – in hypofunction of thyroid gland (hypothyroidism);
- growth hormone – in hypophyseal dwarfism;
- glucocorticoids (deoxycorticosteron) are used in hypocorticoidism;
- mineralocorticoids (aldosteron) – in Addison's disease (hypocorticoidism);
- estrogens – in hypofunction of ovaries;
- androgens – in hypofunction of testicles.

2) The use of mechanisms of hormonal action on biological processes and functions:

- oxitocin is used for the stimulation of labour;
- adrenalin – for the increase of the decreased blood pressure;
- glucocorticoids – are used as anti-inflammatory drugs, antiallergic drugs, and immunosuppressors (tratment of autoimmune diseases,

anaphylactic shock, allergy, in transplantation of organs to suppress the immune response);

- sex hormones – for the treatment of some hormone-dependent tumors (estrogens – for the treatment of prostate cancer, androgens – for the treatment of mammary gland cancer);
- prostaglandins E are used in arterial hypertension, bronchial asthma, gastric ulcer, prostaglandins F – for stimulation of the infant delivery.

3) The use of analogues of hormones:

- oral contraceptive pills are derivatives of female sex hormones;
- anabolic steroids (derivatives of male sex hormones) are used as therapeutic agents at small doses, in patients over 35, for the increase of body weight, stimulation of appetite, improvement of wounds healing during recovery period after heavy trauma, operations, myocardial infarction.

CHAPTER 15

BIOCHEMISTRY OF NUTRITION

Nutritiology (from the greek *"nutricia*" – food) is a science about foods, nutrients, and other components in food, their interactions and role in maintaining health or disease, the processes of consumption, uptake, transport, utilization (spending), and excretion.

From the external environment, the body receives the organic and inorganic substances which are exposed to various chemical reactions. Nutrients are used to update the components of the cells of tissues and organs, for growth of the organism, as well as for energy purposes. All nutrients are divided into six main groups – carbohydrates, proteins, fats, vitamins, minerals, and water.

In the oxidative decomposition of organic food substances, releases chemical energy, which is used for life. The need for food is determined by the physiological state of the organism.

The main issues faced by the biochemistry of nutrition include:

1) What is the substance and the quantity required by the body for life?
2) What is biological role of the nutrients?
3) What are the symptoms of excessive or insufficient nutrient intake?

Food provides the following functions:

- plastic role (provides growth, development, and renewal of body tissues);
- the energy supply of the cell;
- dietary intake of essential substances.

To meet all these functions, the diet must be complete and maintain the **principles of nutrition**, namely:

1. Caloric intake should provide the body energy costs, which depend on age, gender, type of physical or mental activity (for students of 2200-3000 kcal/day).

2. Rational ratio of proteins, fats and carbohydrates, which for the average person is 1:1,5:4. Most food carbohydrates are of plant origin. Usual daily ration comprises 400-500 g carbohydrates, of which 60-80% are polysaccharides (mainly starch, a lesser amount – glycogen and fiber – cellulose) 20-30% of oligosaccharides (sucrose, lactose, maltose), the rest

quantity – monosaccharides (glucose, fructose, and pentoses). Approximately equal ratios of dietary fat (100 g/day) should be present saturated, monounsaturated, and polyunsaturated fatty acids. Normal nutritional amount of protein from 80 to 100 g/day, and it should be ensured as proteins of plant origin and animal (in equal parts).

3. Presence of essential components in food, many of which are present in minimal quantities (minor substances) essential amino acids, essential fatty acids (linoleic, linolenic, arachidonic), vitamins, minerals, fiber, flavoring components, essential oils, as well as water.

4. Mode of eating, which includes the multiplicity of reception and distribution of the daily diet, morning – dinner – evening.

5. Correspondence of a diet to physiological (or pathological) status of the organism (restriction of carbohydrates in diabetes, proteins - with renal disease, lipids – in atherosclerosis).

6. Food must be subjected to cooking to increase the organoleptic properties and safety to the organism.

Major disturbances of diets are as follows:

- excessive consumption of animal fats;
- lack of polyunsaturated fatty acids;
- full deficit of proteins;
- deficiency of most vitamins;
- deficiency of mineral elements - calcium, iron;
- lack of micronutrients - iodine, fluorine, selenium;
- pronounced deficiency of dietary fiber.

Currently, for the correction of diets extensive use of biologically active additives (BAA) to food is offered. BAA are concentrated natural or identical to natural biologically active substances, intended for direct reception by the administration or in foods.

The use of dietary supplements is allowed to eliminate shortages of essential nutrients, individualize specific healthy or sick person, depending on the needs and condition, increase nonspecific resistance, accelerate binding and excretion of xenobiotics from the body, as well as directional change of toxic substances exchange.

General characteristics of the main components of food

Proteins

Nutritional value of protein is provided by the presence of essential amino acids, carbon skeletons which can not be synthesized in the human body, and they accordingly must come from food. They are also the main sources of nitrogen. Daily protein requirement is 80-100 g, half of which should be of animal origin.

Protein requirement is the amount of protein which provides all of the metabolic needs of the body. We should consider the physiological condition of the organism and the properties of dietary protein themselves. From the properties of the components of the diet depends on the digestion, absorption, and metabolic utilization of amino acids.

The need for protein is composed of two components. The first is to satisfy the need in total nitrogen biosynthesis, providing essential amino acids and other nitrogen containing endogenous bioactive substances. The second component is determined by the need of the human body in essential amino acids that are not synthesized in the body.

Animal proteins contain a complete set of essential amino acids. However, proteins have advantages and disadvantages, the main of which are quite toxic products of catabolism (ammonia, food rotting proteins in the large intestine) and rather complex metabolic pathways.

Carbohydrates

The main carbohydrate foods are monosaccharides, oligosaccharides, and polysaccharides, which should arrive in an amount of 400-500 g per day. Carbohydrates are the main energy food material for the cell; they provide 60-70% of daily energy consumption. Carbohydrate metabolism is characterized by simple metabolic pathways, and for their oxidation small amount of oxygen is necessary. The end products of catabolism are harmless substances.

However, there are several disadvantages of carbohydrates. They contain a small amount of essential components and are quite common in violation of their metabolism with the development of the disease.

Fiber from food (cellulose) in the digestive tract is not digested, but it stimulates the bowel peristalsis and removes toxic end products. Therefore, it should also be present in the diet.

Lipids

The main food lipids are triacylglycerols (neutral fats), phospholipids, cholesterol, and higher fatty acids. The daily requirement of lipids is 70 - 145 g.

Lipids are sources of energy (when 1 g of lipid destroys 9,3 kcal/g is released, whereas in destruction of protein and carbohydrates – 4,1 kcal/g is released).

Higher fatty acids are components of membrane phospholipids and triacylglycerols. Among the higher fatty acids so called essential higher fatty acids are present, which include linoleic, linolenic, and arachidonic fatty acids. They are collectively named as “vitamin F”.

Food phospholipids are sources of: choline, inositol, used for the synthesis of neurotransmitters, complex lipids of the cell membrane. Cholesterol (1,5 g/day) is also included in the membrane, is a precursor of steroid hormones, bile acids, and vitamin D.

The main disadvantage of lipid in food is conditioned by a large amount of oxygen necessary for its oxidation. With overeating and obesity, fatty infiltration of internal organs (adipose degeneration) often develops.

CHAPTER 16

BASICS OF VITAMINOLOGY

Vitamins are indispensable components of food that are present in small amounts in the diet and ensure the normal course of biochemical and physiological processes by participating in the regulation of metabolism in the body.

Vitamins have a high biological activity and are required in very small amounts – from a few micrograms to a few tens of milligrams per day. Unlike other essential nutritional factors (amino acids, fatty acids, etc.), vitamins are not plastic material or energy source.

Biological functions of vitamins

Most vitamins are precursors of coenzymes and prosthetic groups of enzymes which catalyze biochemical reactions in the body. Some vitamins function as inducers of protein synthesis (vitamin A); exhibit hormonal activity (vitamin D); exert an antioxidant action (vitamins A, E, C). In addition, each vitamin is characterized by a specific function fulfilled in the body.

Classification of vitamins

On the physico-chemical properties (in particular, solubility), vitamins are classified into two groups: water-soluble and fat-soluble. To identify each vitamin, there is a literal character, the chemical name and the name of the subject to be treated with vitamin disease with the prefix "anti".

Water-soluble vitamins:

1. Vitamin B_1, thiamine (antinevritic).
2. Vitamin B_2, riboflavin (vitamin of growth).
3. Vitamin B_5, pantothenic acid (antidermatic).
4. Vitamin B_6, pyridoxine (antidermatic).
5. Vitamin B_{12}, cyanocobalamin (antianemic; B_9).
6. Vitamin PP, nicotinamide, nicotinic acid, niacin (antipellagric).
7. Vitamin B_c, folic acid (antianemic).
8. Vitamin H, biotin (anti-seborrhoeic).
9. Vitamin C, ascorbic acid (antiskorbic).
10. Vitamin P, rutin.

Fat-soluble vitamins:

1. Vitamin A, retinol (antikseroftalmic).
2. Vitamin D, calciferol (antirahitic).
3. Vitamin E, tocopherols (antisteril, vitamin of reproduction).
4. Vitamin K, naphthoquinones (antihemorrhagic).

Vitamin-like compounds are a group of chemicals, some of which are synthesized in the body, but possess vitamin properties.

1. B_4, choline (lipotropic factor).
2. B_8, inositol (lipotropic factor).
3. B_{13}, orotic acid (growth factor).
4. B_{15}, pangamic acid.
5. B_t, carnitine.
6. N, lipoic acid (lipotropic factor).
7. U (anti-ulcer).
8. PABA, para-aminobenzoic acid.
9. F (linoleic, linolenic and arachidonic acid).
10. Coenzyme Q.

Table 16.1. – **Main characteristics of water-soluble vitamins**

Name	***Daily requirement, mg***	***Coenzyme form***	***Biological functions***	***Characteristic signs of avitaminosis***
B_1 (thiamine)	2-3	TDP	Decarboxylationα-keto acids, transferactivealdehyde (trans-ketolase)	Polyneuritis
B_2 (riboflavin)	1,8-2,6	FAD, FMN	As part of the respiratory enzymes, hydrogen transfer	Ocular (keratitis, cataracts)
B_5 (panto-thenic acid)	10-12	CoA -SH	Transport of acyl group	Degenerative changes in the adrenal glands and nervous tissue
B6 (pyridoxin)	2-3	Pyridoxine phosphate	Exchange of amino acids (transamination, decarboxylation)	Increased excitability of the nervous system, dermatitis
PP (niacin)	15-25	NAD, NADP	Acceptors and hydrogen-carriers	Symmetrical dermatitis on exposed skin, dementia and diarrhea
H (biotin)	0,01-0,02	Biotin	Activation of CO_2 reaction carboxylation(e.g.,pyruvate and acetyl-CoA)	Dermatitis accompanied by enhanced activity of the sebaceous glands
B_C	0,05-0,4	THFA	Transport of one-carbon	Anemia, leukopenia

Name	*Daily requirement, mg*	*Coenzyme form*	*Biological functions*	*Characteristic signs of avitaminosis*
(folic acid)			groups	
B_{12}(Cyanocobalaminun)	0,001-0,002	Desoxyadenosilandmethyl-cobalamin	Transport of methyl groups	Megaloblastic anemia
C (ascorbic acid)	50-75	-	Hydroxylation of proline, lysine(collagen), an antioxidant	Bleeding gums, loosening of teeth, bruising, swelling
P (rutin)	not installed	-	Together with vitamin C is involved in redox processes, inhibits the action of hyaluronidase	Bleeding gums and petechiae

Table 16.2. – **Main characteristics of the fat-soluble vitamins**

Name	*Daily requirement, mg*	*Biological functions*	*Characteristic signs of avitaminosis*
A (retinol)	1-2,5	Participates in the act of vision, regulates cell growth and differentiation	Night blindness (hemeralopia), xerophthalmia, keratomaliaton, hyperkeratosis of epithelial cells
D (calciferol)	0,012-0,025	Regulation of metabolism of phosphorus and calcium in the body	Rickets
E (tokoferol)	5	antioxidant	Not studied
K (naphthoquinone)	1-2	engaged in the activation of coagulation factors: II, VII, IX, XI	Clotting disorder

In accordance with vitamin participation in biological processes, all vitamins are divided into three groups:

• vitamins-coenzymes (B_1, B_2, B_6, B_{12}, PP, K, C, folic acid, biotin, etc.);

• vitamins-prohormones, active forms which have hormonal activity (D, A, hormonal form of which is retinoic acid, which plays an important role in the growth and differentiation of epithelial tissues);

•vitamins-antioxidants (C, E, beta-carotene, and other carotenoids, bioflavonoids).

However we should consider that some vitamins have polyfunctional nature. For example, vitamin C, along with the antioxidant effect, as a

cofactor is involved in the enzymatic hydroxylation processes.

Metabolism of vitamins

Vitamins do not perform their functions in metabolism in the form in which they are supplied with food. Stages of vitamins metabolism:

• absorption in the intestine with the help of special transport systems;

• transport to disposal sites via transport proteins;

• conversion of vitamins in coenzyme form using special enzyme systems;

• co-operation with relevant apoenzyme coenzymes.

Supply of body with vitamins

The source of vitamins for humans is food. An important role in the formation of vitamins belongs to intestinal bacteria that synthesize a number of vitamins. Water-soluble vitamins are not accumulated in tissues (except vitamin B_{12}), and therefore must be ingested daily. Fat-soluble vitamins can accumulate in tissues. Their deficiency is less common. Imbalance of vitamins in the body can be caused by their shortage as well as excess.

Shortage in vitamins intake with food causes a disease called **hypovitaminosis**. In the complete absence of food **avitaminosis** (complete vitamine deficiency disease) develops. Excess dose or excessive accumulation of vitamins in the tissues, accompanied by clinical and biochemical signs of disturbance, is called **hypervitaminosis**. It is characteristic of fat-soluble vitamins. Some vitamins enter the body with food in the form of inactive precursors – provitamins which tissues are converted into biologically active forms of vitamins.

Hypovitaminosis

The human need for vitamins depends on gender, age, physiological condition, and the intensity of labor. The climatic conditions and a nature of food have significant impact on an individual's need for vitamins (the predominance of carbohydrate or protein in the diet, the quantity and quality of fat).

In clinical practice, the most frequent is hypovitaminosis. Vitamin deficiencies can be hidden or pronounced, appearing in relevant diseases. Inadequate intake of vitamins negatively affects the growth and development of children, reduces endurance, physical, and mental

performance, increases the impact of unfavorable environmental factors. Vitamin deficiency reduces the activity of the immune system, accelerates the aging process.

The main causes of hypovitaminosis are:

- lack of vitamins in food;
- malabsorption in the digestive tract;
- decay of vitamins in the gut microflora due to its development;
- increased need for vitamins (stress, physical activity, smoking, alcohol consumption);
- congenital defects in the enzymes involved in the conversion into vitamin coenzymes;
- effects of structural analogues of vitamins (antivitamins).

Hypervitaminosis

Diseases arising from excessive intake of soluble vitamins have not been described. Physiologically essential part of vitamins entering the body is used, and their excess is excreted in the urine.

The cause of hypervitaminosis of fat-soluble vitamins (A and D) is the excessive consumption of these vitamins in preparations, or with exotic food (shark liver and white bear). Hypervitaminosis is manifested in common symptoms: loss of appetite, disorder of motor function of the gastrointestinal tract, headaches, hair loss, peeling skin, increased excitability of the nervous system, and some specific features inherent in this vitamin. Hypervitaminosis can be fatal.

Methods of the estimation of the human body with vitamins

Nowadays, almost each vitamin can be assessed in the organism using different methods. For example, vitamin content or products of their metabolism can be found in the blood or urine (direct method), activity of enzymatic processes in which vitamin is directly involved like coenzyme (functional methods). For this purpose, HPLC X-ray assays analyses are widely used. This method is based on detection of the activation of enzymes by adding vitamin-depending coenzymes. Biochemical tests can pick up early preclinical stage of insufficient supply of vitamins, characterized by the occurrence of primary metabolic disorders.

Application of vitamins in medicine

Use of vitamins in prophylactic and therapeutic purposes can be

organized as follows:

As a preventive measure:

1. Primary prevention of hypovitaminosis caused by:

- insufficient intake of vitamins from food;
- increased need for vitamins (stress, physical and mental stress, exposure to harmful environmental factors, pregnancy).

2. Increase of host defenses, reduction of the risk of respirotary, cardiovascular diseases, cancer, etc.

As a therapeutic measure:

1. Treatment of primary deficiency diseases;
2. Prevention and (or) treatment of secondary metabolic disorders and disfunctions of vitamins due to:
   - pathological processes;
   - surgical interventions;
   - drugs and physiotherapy;
   - dietary restrictions.
3. Correction of congenital disorders of metabolism and functions of vitamins;
4. The use of high doses of vitamins in the treatment of various diseases.

Insufficient intake of vitamins weakens the host defenses, reducing its resistance to various diseases, adverse environmental effects, contributes to the development of chronic diseases, and accelerates the aging process. Insufficient supply of the body with vitamins leads to diseases of the gastrointestinal tract, liver and kidneys, in which the absorption and utilization of vitamins destroys. Drug therapy (antibiotics, etc.), diet, surgery, stress exacerbate vitamin deficiency.

Vitamin deficiency in turn, disrupts metabolism and prevents successful treatment of any disease. Therefore, it is reasonable to use multivitamin supplements in complex therapy of various diseases.

The use of vitamins at doses exceeding physiological need, in the treatment of various medical conditions:

1. Vitamin A – increases fertility, tissue regeneration, stimulates the growth and development of children.
2. Vitamin D – prevents from rickets and is used for treatment of skin diseases.
3. Vitamin K – cures bleeding associated with a decrease in blood clotting.

4. Vitamin E – prevents miscarriage, liver disease, muscle atrophy, congenital disorders of erythrocyte membranes in the newborn.

5. Vitamin B_1– is used in diabetes mellitus (to improve digestion of carbohydrates), inflammation of the peripheral nerves, and lesions of the nervous system, heart, and dystrophy of skeletal muscles.

6. Vitamin B_2 – is used in dermatitis, poorly healing wounds and ulcers, keratitis, conjunctivitis, liver damage.

7. Pantothenic acid – is used in skin diseases, liver disease, heart muscle dystrophy.

8. Vitamin PP – is used in dermatitis, lesions of peripheral nerves, heart muscle dystrophy.

9. Vitamin B_6 – is used in polyneuritis, dermatitis, toxemia of pregnancy, hepatic dysfunction.

Antivitamins

Antivitamins are substances that cause a reduction or complete loss of the biological activity of vitamins.

Antivitamins can be divided into two main groups:

1) antivitamins which inactivate the vitamins by their destruction or binding molecules to inactive forms;

2) antivitamins replacing coenzymes (vitamin derivatives) in the active sites of enzymes.

Examples of action of antivitamins of the first group:

a) egg protein **avidin** binds to biotin and avidin-biotin formed complex, in which biotin is void of activity, is insoluble in water, and is not absorbed from the intestine and can not be used as a coenzyme;

b) the enzyme **ascorbate oxidase** destroys ascorbic acid;

c) the enzyme **thiaminase** destroys thiamine (B_1) ;

d) enzyme **lipooxydase** destroys provitamin A – carotene by oxidation.

The second group of substances includes structural analogues of vitamins. They interact with the apoenzyme to form an inactive enzyme complex type of competitive inhibition. Structural analogues of vitamins can have a significant impact on metabolic processes in the body, most of them are used:

a) as therapeutic agents, specifically acting on certain biochemical and physiological processes;

b) for creation of experimental animals avitaminosis.

Table 16.3. – **Antivitamins**

Vitamin	***Antivitamin***	***Mechanism of action***	***Applicationof antivitamin***
Para-amino benzoic acid (PABA)	Sulfanilamide	Sulfonamides - structural analogs of PABA. They inhibit the enzyme by replacement of PABA complex, enzyme synthesizes folic acid, which leads to the inhibition of bacterial growth.	For the treatment of infectious diseases.
Folic acid	Pteridine	Incorporated into the active site of enzymes folatzavisimyhand blocks the synthesis of nucleicacids (cytostatic effect), inhibitedcell division.	For the treatment of acute leukemia, some forms of cancer.
Vitamin K	Coumarins (Varfarin, etc)	Coumarins block the formation of prothrombin prokonvertina and other clotting factors in the liver (have anticoagulant effect).	For prevention and treatment of thrombosis (angina, thrombophlebitis, etc.).
Vitamin PP	Isonicotinic acid hydrazide(INH) and its derivatives (tubazid, ftivazid, metozid)	Antivitamins included in the structure of NAD and NADP, forming false coenzymes, which are notable to participate in redox reactions and other biochemical systems of Mycobacterium tuberculosis, the most sensitive to these Antivitamins.	For the treatment of tuberculosis.
Thiamine (B_1)	Oxy-thiamin, pyri-thiamine	Replace thiamine coenzymes in enzymatic reactions.	To create experimental deficiency of vitamin B_1.

Antivitamins are widely used in clinical practice as antitumor agents and antibiotics, inhibiting synthesis of nucleic acids and proteins in bacteria and tumor cells.

CHAPTER 17

GENERAL CARACTERISTICS OF CARBOHYDRATES

Carbohydrates are aldehyde or ketone derivatives of the higher polyhydric alcohols, or compounds which yield these derivatives on hydrolysis. Many, but not all, carbohydrates have the general formula $Cx(H_2O)y$; some also contain nitrogen, phosphorus or sulfur. Carbohydrates are widely distributed in plants and animals. In plants, glucose is synthesized from CO_2 and H_2O by photosynthesis and stored as starch or used to synthesize the cellulose of the plant cell walls. Animals can synthesize carbohydrates from amino acids, but most are derived ultimately from plants. Carbohydrates are major constituents of animal food.

There are three major classes of carbohydrates: monosaccharides, oligosaccharides, and polysaccharides (Fig. 17.1).

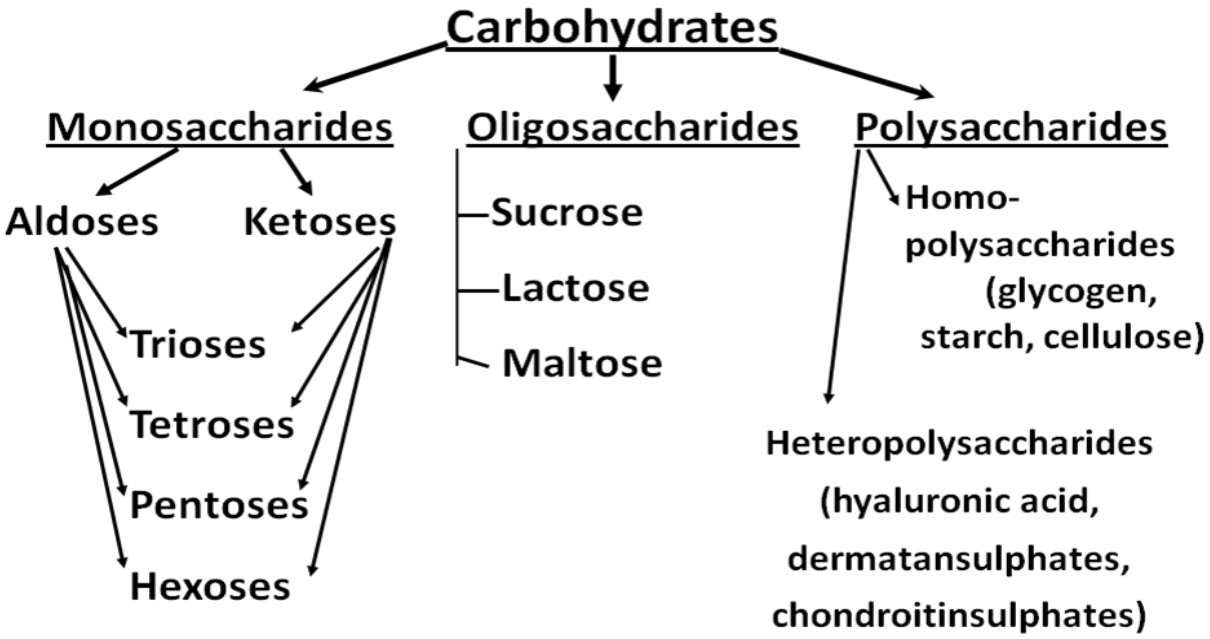


Figure 17.1 – Classification of carbohydrates

Functions of carbohydrates

- **Energetic** (major metabolic fuel);
- **Structural** (glycosaminoglycans of connective tissues, glycolipids in membranes);
- **Metabolic** (lipids and some aminoacids can synthesized from carbohydrates);

- **Protective** (components of immunoglobulins);
- **Receptative** (glycoproteins of membranes);
- **Antigenic** (glycoproteins of erythrocytes define the group of blood);
- **Plastic** (elements in the structure of DNA, RNA, FAD, NAD(P), etc);
- **Antitoxic**.

Table 17.1. – **Dietary carbohydrates** (300-500 g/day)

Carbohydrates	***Representatives***	***Sources***	***Daily consumption***
Polysaccharides	Starch	Bread Potatoes cereals	250-400 g/day
Disaccharides	Sucrose Lactose Maltose	Sugar Milk Sweets Cakes, etc	50-100 g/day
Monosaccharides	Glucose Fructose galactose	Fruits berryes	0-50 g/day

In addition, some complex carbohydrates of plant origin (cellulose, pectins, lignins) are indigestible by the human gut and constitute what is termed “fiber”. Fiber is present in unprocessed cereals, legumes, vegetables, and fruits.

Biological role of dietary fibers:

- provider of substrates for bacterial fermentation in the large intestine;
- regulation of gut transit and motility;
- the main component of feces;
- sorbent for different toxins.

Digestion of carbohydrates

Dietary carbohydrates enter the gut as mono-, di-, and polysaccharides. Disaccharides and polysaccharides require hydrolytic cleavage prior to absorption. During the eating process and homogenization that occurs with mastication in the mouth and the action of gastric folds, dietary polysaccharides become hydrated. Hydration of polysaccharides is essential for the appropriate action of amylase. This enzyme is specific for

internal α1→4-glycosidic linkages. The cleaved units thus formed are the trisaccharide maltotriose, the disaccharide maltose, and an oligosaccharide with one or more α1→6 branches, and containing on average eight glycosyl units termed the "α-limit dextrin". These compounds are then further cleaved to glucose units by oligosaccharidase and α-glucosidase, the latter removing single glucose residues from α1→4-linked oligosaccharides (including maltose) from the nonreducing end of the oligomer. A sucrase-isomaltase complex activated to two separate active polypeptide enzymes, one of which (isomaltase) is responsible for the hydrolytic cleavage of α1→6 glycosidic linkages. Disaccharides are acted upon by membrane-bound disaccharidases on the intestinal mucosal surface (Table 17.2).

Table 17.2. – **Digestion of carbohydrates**

Section of GIT	***Enzymes***	***Localisation of enzymes***	***Products of hydrolysis***
Oral cavity	Amylase	Saliva	Dextrins
Duodenum	Amylase	Pancreatic juice	Maltose, maltotriose, glucose, small branched dextrins
Small intesine	maltase sucrase-isomaltase complex lactase	Brush border of the intestinal mucosal cells	Mono-saccharides

Dietary disaccharides such as lactose, sucrose, and trehalose are hydrolyzed to their constituent monomeric sugars by a series of specific disaccharidases, which are attached to the small intestinal brush-border membrane.

Absorption of monosaccharides in the small intestine

There are active and passive transport systems which transport carbohydrates across the brush-border membrane. Glucose, fructose, and galactose are the primary monosaccharides produced by the digestion of dietary carbohydrates. The absorption of these sugars occurs via specific

carrier-mediated mechanisms. In addition, all monosaccharides can cross the brush-border membrane by a simple diffusion process, although it is extremely slow.

At least two carrier-mediated transport systems for monosaccharides exist – an Na^+-dependent co-transporter and an Na^+-independent transporter.

At the brush-border membrane both **glucose** and **galactose** are transported by the **Na^+-dependent glucose transporter**. This membrane-linked protein binds with glucose (galactose) and Na^+ at separate sites and transports both into cytosol. The Na^+ is thus transported down its concentration gradient, carrying glucose against its concentration gradient. This transport mechanism is linked to Na^+-dependent ATPase, which then removes Na^+ from the cell in exchange for K^+, with the concomitant hydrolysis of ATP. The transport of glucose (galactose) is thus an **indirect active process**.

Fructose is transported across the brush-border membrane by an Na^+-independent facilitated diffusion process involving a specific membrane-associated protein, possibly glucose transporter (GLUT 5), which is present on the luminal side of the enterocyte, and GLUT 2 present on the antiluminal side.

Transport of glucose from the bloodstream to the tissues

Glucose transporters are essential for facilitated diffusion of glucose into cells. According to the latest data, the glucose transporter family comprises 15 major species, named GLUT. Cells in insulin-sensitive tissue such as muscle and adipose have GLUT 4. Insulin stimulates translocation of GLUT 4 from intracellular vesicles to the plasma membrane, facilitating glucose uptake during meals.

Fructose metabolism

Fructose is a component of the disaccharide sucrose, table sugar. Fructose is metabolized by two pathways in cells. It may be phosphorylated by hexokinase, an enzyme that is present in all cells; however, hexokinase has a strong preference for glucose. In liver, fructose is phosphorylated to fructose-1-phosphate (Fru-1-P) by fructokinase, and the liver aldolase, called aldolase B, can cleave Fru-1-P, as well as fructose-1,6-bisphosphate (Fru-1,6-BP). In contrast, muscle aldolase, called aldolase A, is specific for Fru-1,6-BP. The products of aldolase B are dihydroxyacetone phosphate

and glyceraldehyde (not glyceraldehyde phosphate). The glyceraldehyde must then be phosphorylated by triokinase in order to be metabolized in the glycolytic pathway.

Lack of hepatic **fructokinase** causes a relatively asymptomatic condition, **essential fructosuria.**

Absence of hepatic **aldolase B** leads to **hereditary fructose intolerance**. Consequence of hereditary fructose intolerance is fructose-induced hypoglycemia, despite the presence of high glycogen reserves. The accumulation of fructose1-phosphate and fructose 1,6-bisphosphate allosterically inhibits the activity of liver phosphorylase. The sequestration of inorganic phosphate also leads to depletion of ATP and hyperuricemia. Hereditary fructose intolerance can be managed by removing fructose from the diet.

Galactose metabolism

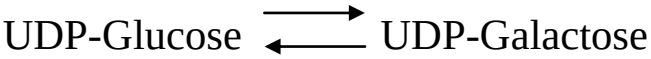
Galactose is an important component of our diet, because it is one of the sugars in the milk disaccharide, lactose. Galactose is first phosphorylated by a specific hepatic enzyme, galactokinase, to form galactose-1-phosphate (Gal-1-P). The conversion of Gal-1-P to Glc-1-P involves the nucleoside diphosphate sugar intermediate, UDP-Glc. The enzyme galactose-1-phosphate uridyl transferase catalyzes an exchange between UDP-Glc and Gal-1-P to form UDP-Gal and Glc-1-P. The Glc-1-P arising from galactose metabolism can be converted to Glc-6-P by phosphoglucomutase, and thus enter glycolysis.

Galactosemia may be caused by inherited defects in galactokinase, galactose-1-phosphate uridyl transferase, or uridine diphosphogalactose 4-epimerase. A deficiency in **galactose-1-phosphate uridyl transferase** is the best known cause.

The galactose concentration in the blood is reduced by aldose reductase to galactitol, which accumulates in lens of the eye, causing cataract. In galactose-1-phosphate uridyl transferase deficiency, galactose 1-phosphate accumulates and depletes the liver of inorganic phosphate. Ultimately, liver failure and mental deterioration occurs. As the uridine diphosphogalactose 4-epimerase is present in adequate amounts, the galactosemic individual can still form UDP-Gal from glucose, and normal growth and development can occur regardless of the galactose-free diets used to control such symptoms as: cataract, liver failure, and mental deterioration.

Lactose metabolism

Lactose is synthesized from UDP-Gal and glucose in mammary glands during lactation. The reversible convertion of UDP-Glucose to UDP-Galactose is catalyzed by uridine diphosphogalactose 4-epimerase.



Then UDP-Gal condenses with glucose to yield lactose, catalyzed by lactose synthase.

Lactose synthase is formed by the binding of α-lactalbumin to the galactosyl transferase. α-Lactalbumin, which is expressed only in the mammary glands during lactation, converts galactosyltransferase to lactose synthase by lowering the enzyme's Km for glucose, leading to preferential synthesis of lactose. α-Lactalbumin is the only known example of a "specifier" protein that alters the substrate specificity of an enzyme.

Lactose intolerance is a physiologic change resulting from acquired lactase deficiency, leading to diarrhea and intestinal discomfort. Lactase activity decreases with increasing age in children but the extent of the decline in activity is genetically determined and demonstrates ethnic variation. Lactase deficiency in the adult black population varies from 45-95%. If symptoms of malabsorption occur after the introduction of milk to adult diets, the diagnosis of acquired lactase deficiency should be considered.

CHAPTER 18

PATHWAYS OF GLUCOSE METABOLISM

Glucose is the major carbohydrate on Earth, the backbone and monomer unit of cellulose and starch. Glucose is the most important carbohydrate. Most dietary carbohydrates are absorbed into the bloodstream as glucose, and other sugars are converted to glucose in the liver. Glucose is the major metabolic fuel of mammals. It is the precursor for synthesis of all other carbohydrates in the body, including glycogen for storage; ribose and deoxyribose for nucleotides; galactose in lactose of milk, in glycolipids, glycoproteins and proteoglicans. The major pathways of glucose metabolism are presented in the Fig. 18.1.

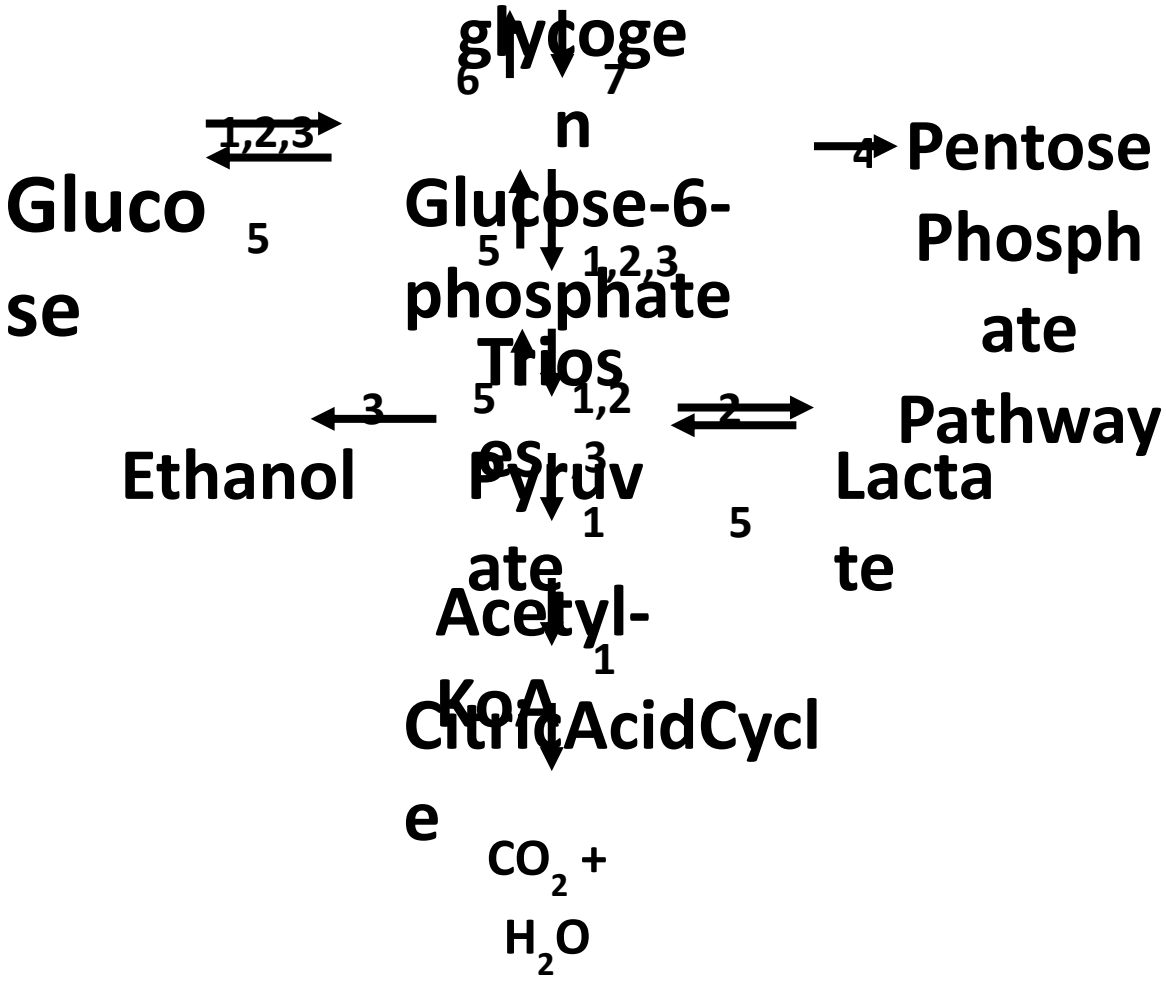


Figure 18.1 – The general scheme of pathways of glucose metabolism
1-aerobic glycolysis; 2-anaerobic glycolysis; 3-alcohol fermentation;

4-pentose phosphate pathway; 5-gluconeogenesis; 6-glycogen synthesis; 7-glycogen degradation

Glucose enters metabolism by phosphorylation to glucose 6-phosphate, catalyzed by **hexokinase**. Hexokinase has a high affinity (low Km) for glucose, and is saturated under all normal conditions and so acts at a constant rate to provide glucose 6-phosphate to meet the cell's need. Hexokinase is inhibited allosterically by its product, glucose 6-phosphate. Liver and pancreatic β islet cells also contain an isoenzyme of hexokinase, **glucokinase**. The glucokinase differs from hexokinase:

- the glucose concentration at which glucokinase is half-saturated (about 10 mM) is higher than the usual concentration of glucose in the blood. When blood glucose is high, as it is after a meal rich in carbohydrates, excess glucose is transported into hepatocytes, where glucokinase converts it to glucose 6-phosphate, which will be used for glycogen synthesis;
- glucokinase is not inhibited by glucose 6-phosphate, and it can therefore continue to operate when the accumulation of glucose 6-phosphate completely inhibits hexokinase;
- glucokinase is subject to inhibition by the reversible binding of a regulatory protein specific to liver. Glucokinase is also regulated at the level of protein synthesis. Circumstances that call for greater energy production or a high-carbohydrate diet cause increased transcription of the glucokinase gene.

Glycolysis

All cells begin the metabolism of glucose by a pathway termed glycolysis, i.e. carbohydrate (*glyco*) splitting (*lysis*). Glycolysis, the major pathway for glucose metabolism, occurs in the cytosol of all cells. It can function either aerobically or anaerobically, depending on the availability of oxygen and the ETC. Glycolysis is also the main pathway for the metabolism of fructose, galactose, and other carbohydrates.

The roles of glycolysis:

- to produce energy;
- to produce intermediates for metabolic pathways.

Anaerobic glycolysis is the pathway of glucose degradation under anaerobic conditions to lactate as end product (Fig. 18.2)

During glycolysis, two molecules of ATP are expended to build up a glucose-6-phosphate and a fructose-1,6-bisphosphate, which is then cleaved to two three-carbon triose phosphates. These are converted into pyruvate. Two moles of ATP are formed from each mole of triose phosphate.

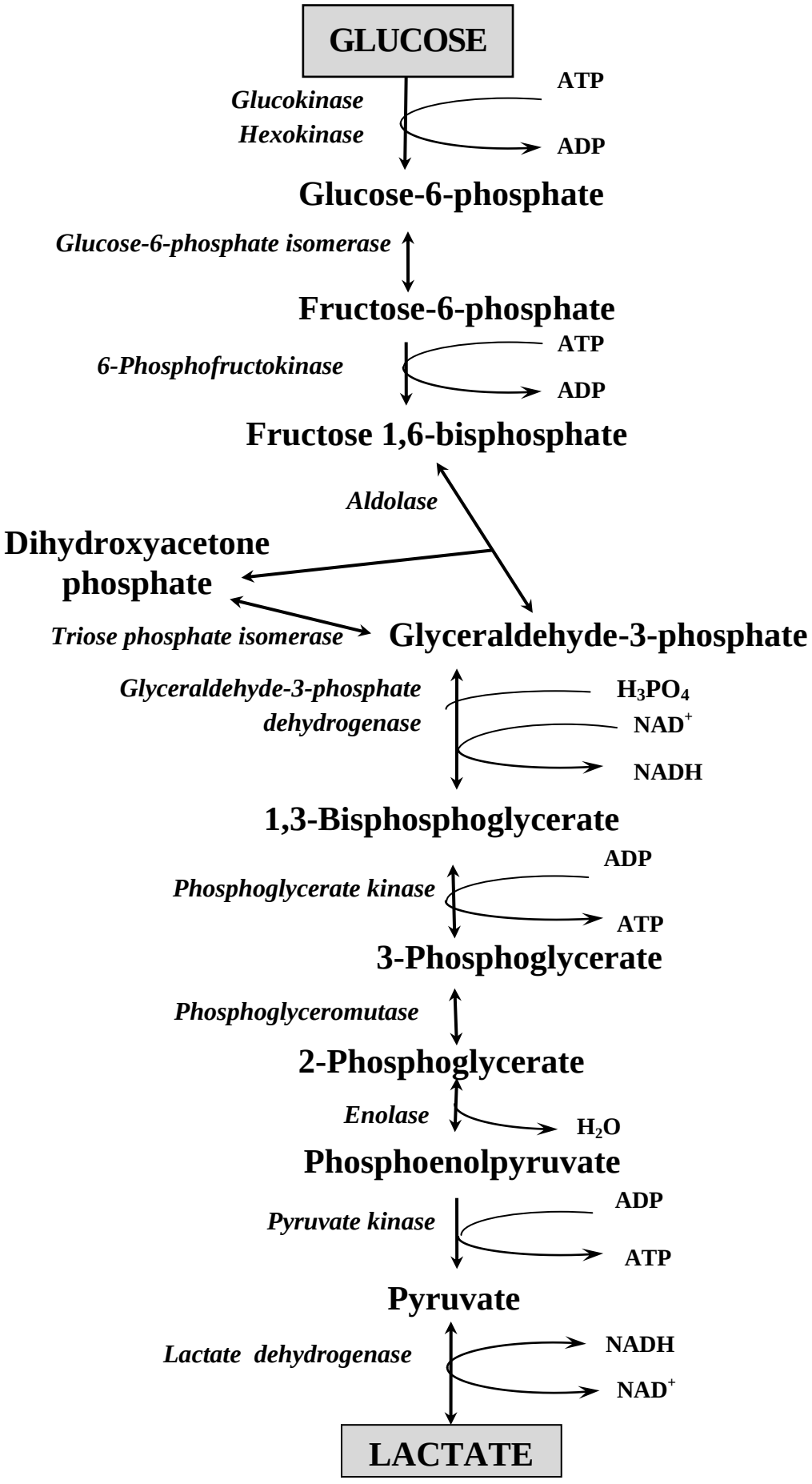


Figure 18.2 – The scheme of anaerobic glycolysis

The synthesis of ATP is accomplished by kinases that catalyze **substrate-level phosphorylation**, a process in which a high-energy phosphate compound transfers its phosphate to ATP. Phosphoglycerate kinase and pyruvate kinase catalyze the ATP-generating reactions of glycolysis, yielding 2 moles of ATP per mole of triose phosphate or a total of 4 moles of ATP per mole of glucose. After adjustment for the ATP invested in the hexokinase and phosphofructokinase reactions, the net energy yield is 2 moles of ATP per mole of glucose converted into lactate. The oxidation of NADH is accomplished under anaerobic conditions by lactate dehydrogenase which catalyzes reduction of pyruvate to lactate by $NADH+H^+$, regenerating NAD^+. In mammals, all cells have lactate dehydrogenase, and lactate is the end product of glycolysis under anaerobic conditions.

Despite their capacity for oxidative metabolism, however, some cells may at times form lactate, e.g. in muscle during oxygen debt and in phagocytes in pus or in poorly perfused tissues. Under anaerobic conditions or in red cells, lactate is excreted into blood, where it is retrieved by liver for use as a substrate for gluconeogenesis.

Regulation of glycolysis

Glycolysis appears to be regulated simply by the energy needs of the cell, i.e. the requirement for ATP. The balance between ATP consumption and production is controlled allosterically at three sites, the **hexokinase, phosphofructokinase**, and **pyruvate kinase** reactions. Hexokinase is subject to feedback inhibition by its product glucose 6-phosphate. Phosphofructokinase is the primary site of regulation of glycolysis, controlling the flux of fructose 6-phosphate to fructose 1,6-bisphosphate. Phosphofructokinase activity is sensitive to the energy status of the cell. ATP is both a substrate and an allosteric inhibitor of phosphofructokinase. The increasing ADP and AMP concentrations relieve the inhibition of phosphofructokinase by ATP, activating glycolysis.

Under normal conditions, the activity of phosphofructokinase is suppressed by ATP. AMP, which is present at much lower concentration, relieves this inhibition. Pyruvate kinase is allosterically activated by fructose 1,6-bisphosphate.

Aerobic glycolysis (aerobic degradation of glucose) comprises next stages:

1. oxidation of glucose with formation of two molecules of pyruvate;
2. oxidative decarboxylation of pyruvate to acetyl-CoA and its oxidation in the common pathway of catabolism (citric acid cycle);
3. electron transport chain coupled with dehydration reactions in the pathway of glucose degradation.

Under aerobic conditions, mitochondria oxidize NADH to NAD^+ and convert pyruvate to CO_2 and H_2O. 38 moles of ATP are available by complete oxidation of 1 mole of glucose to CO_2 and H_2O.

Oxidative decarboxylation of pyruvate

Inside the mitochondrion, pyruvate oxidatively decarboxylated to acetyl-CoA by multienzyme pyruvate dehydrogenase complex. The complex contains multiple copies of each of the three enzyme subunits: pyruvate dehydrogenase (E_1), dihydrolipoyl transacetylase (E_2), dihydrolipoyl dehydrogenase (E_3). The complex requires the following coenzymes: TDP, lipoic acid, CoA-SH, FAD, NAD^+.

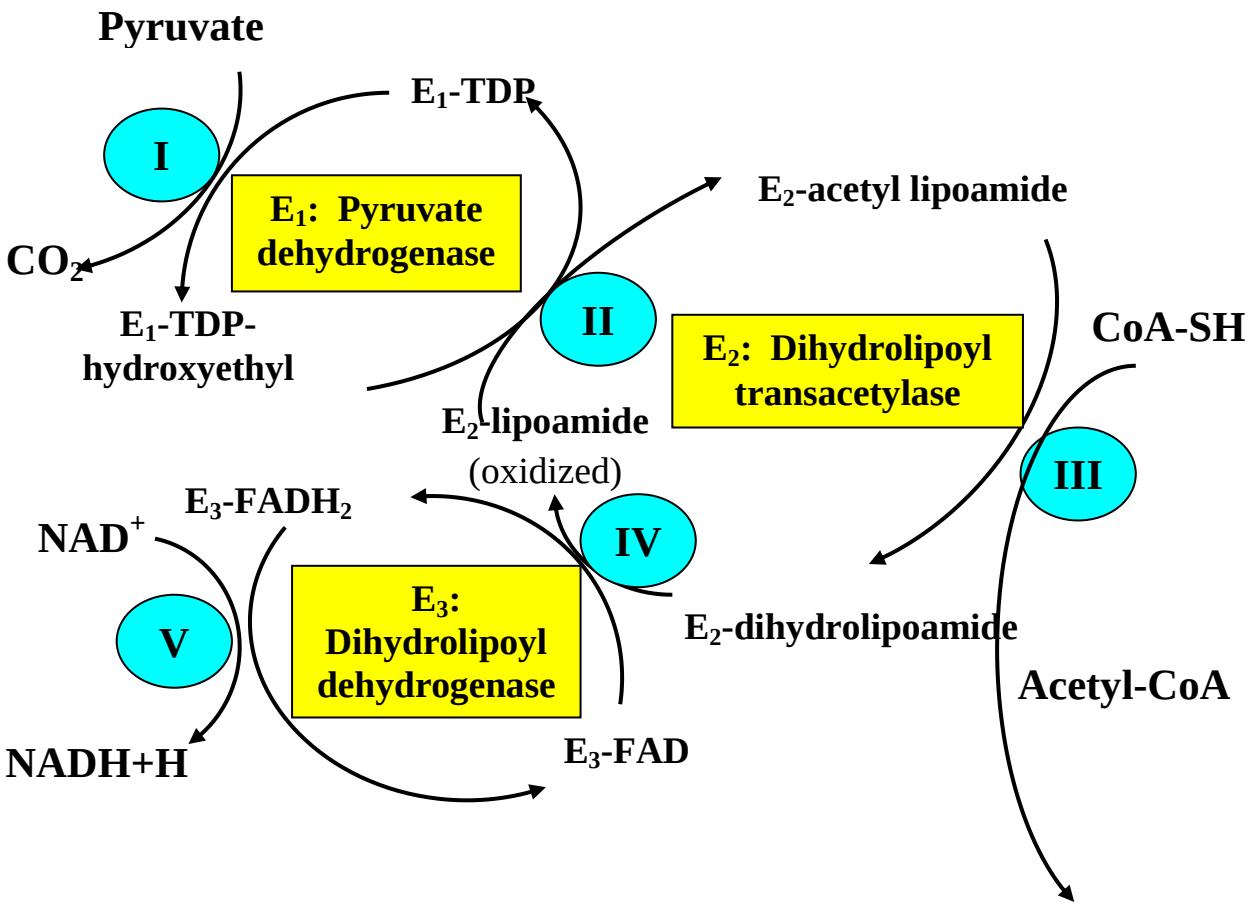


Figure 18.3 – Oxidative decarboxylation of pyruvate by pyruvate

dehydrogenase complex

The raction proceeds in five stages (Fig.18.3):
I – pyruvate decarboxylated by the pyruvate dehydrogenase to the hydroxyethyl derivative of the TDP;
II – this derivative reacts with lipoamide (procthetic group of dihydrolipoyl transacetylase) to form acetyl lipoamide;
III – acetyl lipoamide reacts with coenzyme A to form acetyl-CoA and reduced lipoamide;
IV – the reduced lipoamide oxidized by dihydrolipoyl dehydrogenase, containing FAD;
V – the reduced flavoprotein is oxidized by NAD^+.

Regulation of pyruvate dehydrogenase complex

Pyruvate dehydrogenase complex is regulated by phosphorylation (catalyzed by PDH kinase), resulting in decreased activity and by dephosphorylation (catalyzed by PDH phosphatase) that causes an increase in activity. Activators and inhibitors of PDH are listed below.

Activators of pyruvate dehydrogenase:	**Inhibitors of pyruvate dehydrogenase:**
AMP	acetyl-CoA
NAD^+	ATP
Ca^{2+}	$NADH+H^+$
insulin (in adipose tissue)	fatty acids

Gluconeogenesis

Gluconeogenesis is a process of synthesizing glucose from noncarbohydrate precursors in the **liver, kidney,** and **small intestine**.

During fasting and starvation, when hepatic glycogen is depleted, gluconeogenesis is essential for maintenance of blood glucose homeostasis. Unlike glycogenolysis, which can be turned on rapidly in response to hormonal stimulation, gluconeogenesis is a slower response, reaching maximal activity over a period of hours; it becomes the primary source of our blood glucose concentration about 8 hours into the post-absorbtive state.

Gluconeogenesis requires both a source of energy and a source of carbons for formation of the backbone of the glucosemolecule. The energy

is provided by metabolism of fatty acids released from adipose tissue.

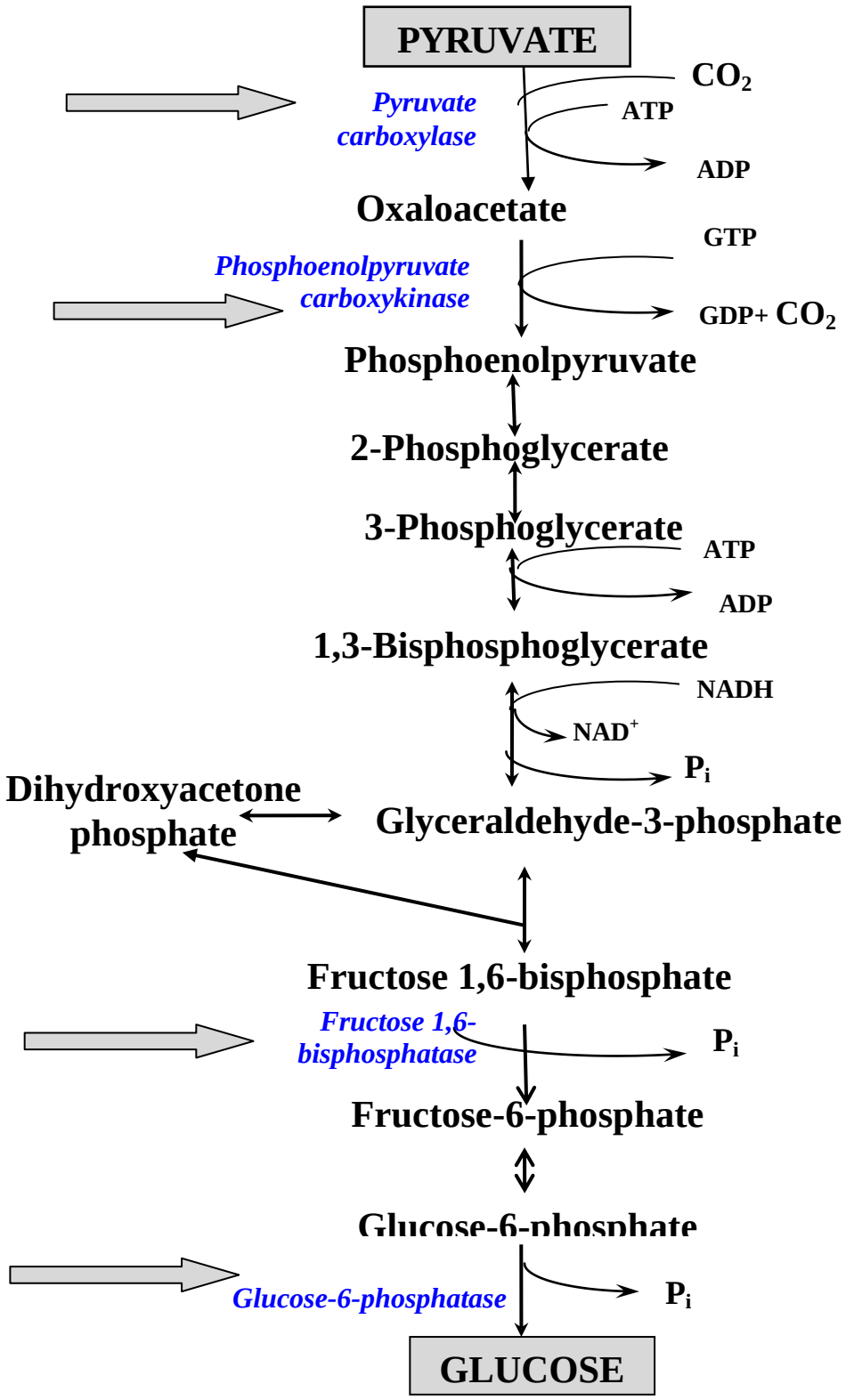


Figure 18.4 Glyconeogenesis
(→ - key reactions)

The carbon skeletons are provided from three primary sources:

- **lactate** produced in tissues such as the red cell by anaerobic glycolysis;
- **amino acids** derived from muscle proteins;
- **glycerol** released from triglycerides during lipolysis in adipose tissue.

Gluconeogenesis is conceptually the opposite of anaerobic glycolysis (Fig.18.4), but proceeds by a different pathway, involving both mitochondrial and cytosolic enzymes. Lactate is the end product of anaerobic glycolysis - blood lactate is derived primarily from anaerobic glycolysis in red cells and exercising muscle. During hepatic gluconeogenesis lactate is converted back into glucose, using, in part, the same glycolytic enzymes involved in conversion of glucose into lactate. The lactate cycle involving the liver, red cells, and muscle, is known as the Cori cycle.

To circumvent the three irreversible reactions of glycolysis, the liver uses four unique enzymes: pyruvate carboxylase in the mitochondrion and phosphoenolpyruvate carboxykinase in the cytoplasm to bypass pyruvatekinase, fructose-1,6-bisphosphatase to bypass phosphofructokinase, and glucose-6-phosphatase to bypass hexokinase.

Regulation of gluconeogenesis

Gluconeogenesis is regulated primarily by hormonal mechanisms. The regulatory process involves regulation of glycolysis and gluconeogenesis, largely by phosphorylation/dephosphorylation of enzymes, under control of glucagon and insulin. The primary control point is at the regulatory enzymes **phosphofructokinase** and **fructose-1,6-bisphosphatase**, which are sensitive to the allosteric effector fructose 2,6-bisphosphate. Fructose 2,6-bisphosphate is an activator of phosphofructokinase and an inhibitor of fructose-1,6-bisphosphatase.

Gluconeogenesis is also regulated in the mitochondrion by acetyl CoA. The influx of fatty acids from adipose tissue, stimulated by glucagon to support gluconeogenesis, leads to an increase in hepatic acetyl CoA, which is both an inhibitor of pyruvate dehydrogenase and an activator of pyruvate carboxylase. In this way, fat metabolism inhibits the oxidation of pyruvate and favors gluconeogenesis in liver. In muscle, the utilization of glucose is limited both by the low level of GLUT-4 in the plasma membranes (because of the low plasma insulin concentration) and by inhibition of pyruvate dehydrogenase by acetyl CoA. Active fat metabolism and high levels of acetyl CoA in muscle promote the

excretion of a significant fraction of pyruvate as lactate, even in the resting state.

Pentose phosphate pathway

The pentose phosphate pathway is a cytosolic pathway present in all cells, named so because it is the primary pathway for formation of pentose phosphates for synthesis of nucleotides. The pentose phosphate pathway is divided into an irreversible redox stage, which yields both NADPH and pentose phosphates, and a reversible interconversion stage, in which excess pentose phosphates are converted into glycolytic intermediates (Fig.18.5).

Biological role of Pentose phosphate pathway:

- formation of NADPH for synthesis of fatty acids and steroids;
- the synthesis of ribose for nucleotide biosynthesis.

NADPH is a major product of the pentose phosphate pathway in all cells. In tissues with active lipid biosynthesis, e.g. liver, adrenal cortex or lactating mammary glands, the NADPH is used in redox reactions required for biosynthesis of fatty acids, cholesterol, steroid hormones, and bile salts. The liver also uses NADPH for hydroxylation reactions involved in the detoxification and excretion of drugs. In RBC the NADPH is used primarily for the reduction of glutathione (GSH), an essential cofactor for antioxidant protection.

Alcohol fermentation

Fermentation is a general term for anaerobic metabolism of glucose. During fermentation in yeast, the pathway is identical with glycolysis, except that pyruvate is converted into ethanol. The pyruvate is first decarboxylated by pyruvate decarboxylase to acetaldehyde, releasing CO_2. The NADH produced in the glyceraldehyde-3-phosphatedehydrogenase reaction is then re-oxidized by alcohol dehydrogenase, regenerating NAD^+ and producing ethanol. Ethanol is a toxic compound, and yeast die when the ethanol concentration in their medium reaches about 12%, which is the approximate concentration of alcohol in natural wines.

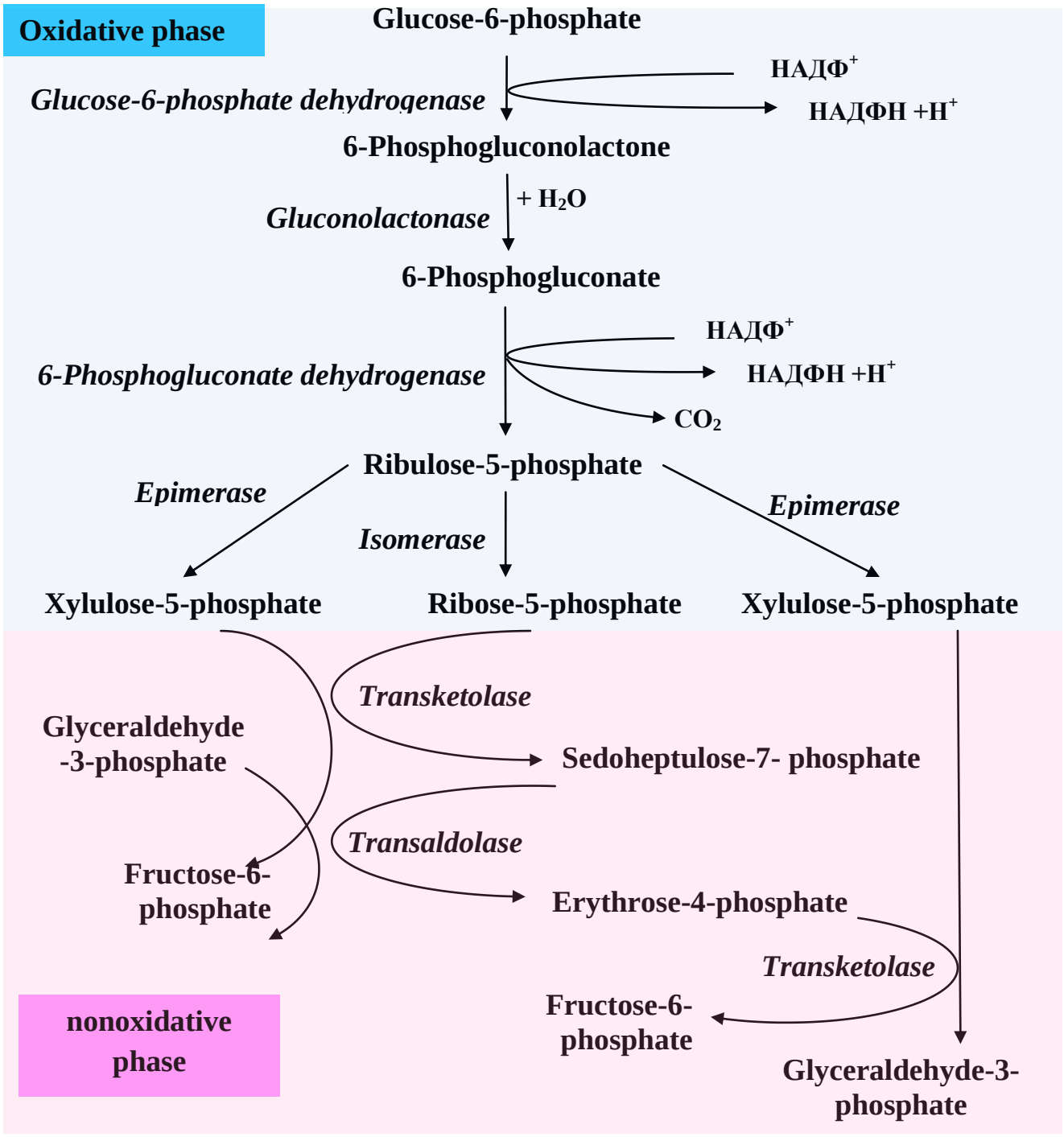
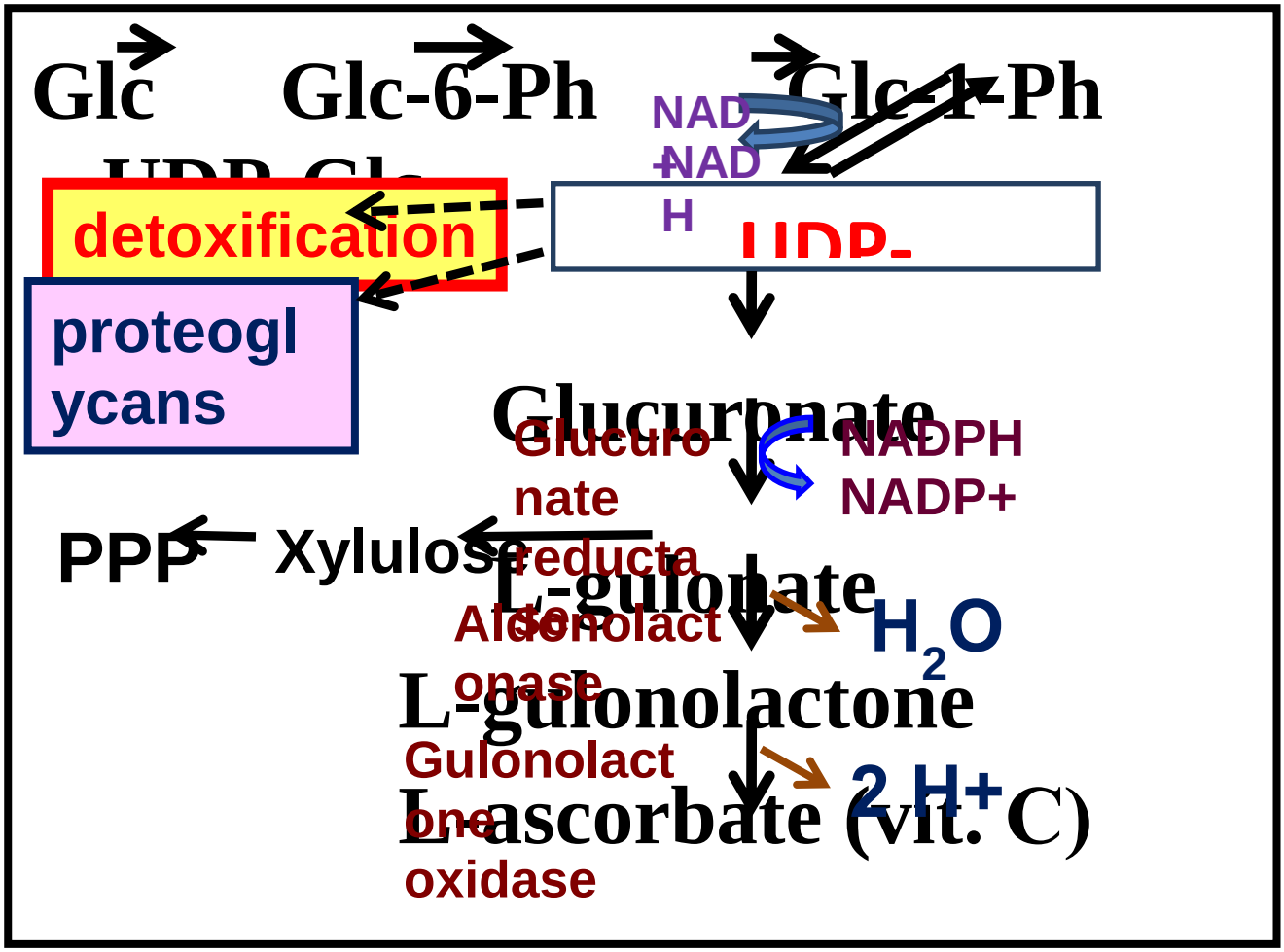


Figure 18.5 – The scheme of pentose phosphate pathway

Glucuronic acid pathway

UDP-glucose is the precursor of other essential sugars, such as glucuronic acid and xylose, which are required for proteoglycan biosynthesis. Oxidation of UDP-glucose by UDP-glucose dehydrogenase leads to the activated form of glucuronic acid, UDP-glucuronic acid. This nucleotide is the donor of glucuronic acid both for the formation of proteoglycans and for conjugation and detoxification reactions that occur in the liver. In that organ, glucuronic acid is conjugated to steroid hormones, bilirubin, and many drugs. UDP-glucuronic acid undergoes a decarboxylation reaction to form UDP-xylose, the activated form of xylose, the pentose sugar that serves as the link between protein and glycan in proteoglycans. UDP-glucuronic acid is also a precursor of ascorbate (vitamin C) in most mammals, except primates and guinea pigs.



CHAPTER 19

METABOLISM OF GLYCOGEN

Glycogen, a polysaccharide storage form of glucose, is our first line of defense against declining blood glucose concentration. During and immediately following a meal, glucose is converted in the liver into glycogen (a process known as **glycogenesis**). Hepatic glycogen is gradually degraded between meals, by the pathway of **glycogenolysis**, releasing glucose to maintain blood glucose concentration. However, total hepatic glycogen stores are sufficient for maintenance of blood glucose concentration during a 12-h fast. During sleep, when we are not eating, there is a gradual shift from glycogenolysis to gluconeogenesis.

Glycogen is also stored in muscle, but this glycogen is not available for maintenance of blood glucose. Glucose, derived from glycogen, is essential for muscle energy metabolism. The tissue concentration of glycogen is higher in the liver than in muscles, but because of the relative masses of muscles and the liver, the majority of glycogen in the body is stored in muscles.

Glycogen contains only two types of glycosidic bonds, chains of α1→4-linked glucose residues with α1→6 branches spaced about every 4-6 residues along the α1→4 chain.

Glycogen synthesis (glycogenesis)

Glucose is channeled into glycogen, providing a carbohydrate reserve for maintenance of blood glucose during the post-absorptive state.

The pathway of glycogenesis from glucose involves next steps (Fig. 19.1):

- phosphorylation of glucose;
- conversion of glucose 6-phosphate into glucose1-phosphate by phosphoglucomutase;
- activation of glucose 1-phosphate to the sugar nucleotide uridine diphosphate-glucose (UDP-glucose) by the enzyme UDP-glucose pyrophosphorylase;
- transfer of glucose to glycogen in α1→4 linkage by glycogen synthase;
- when the α1→4 chain exceeds eight residues in length, glycogen branching enzyme, a transglycosylase, transfers some of the α1→4-

linked sugars to an α1→6 branch, setting the stage for continued elongation of both α1→4 chains, until they become long enough for transfer by branching enzyme.

Glycogen synthase is the regulatory enzyme for glycogenesis.

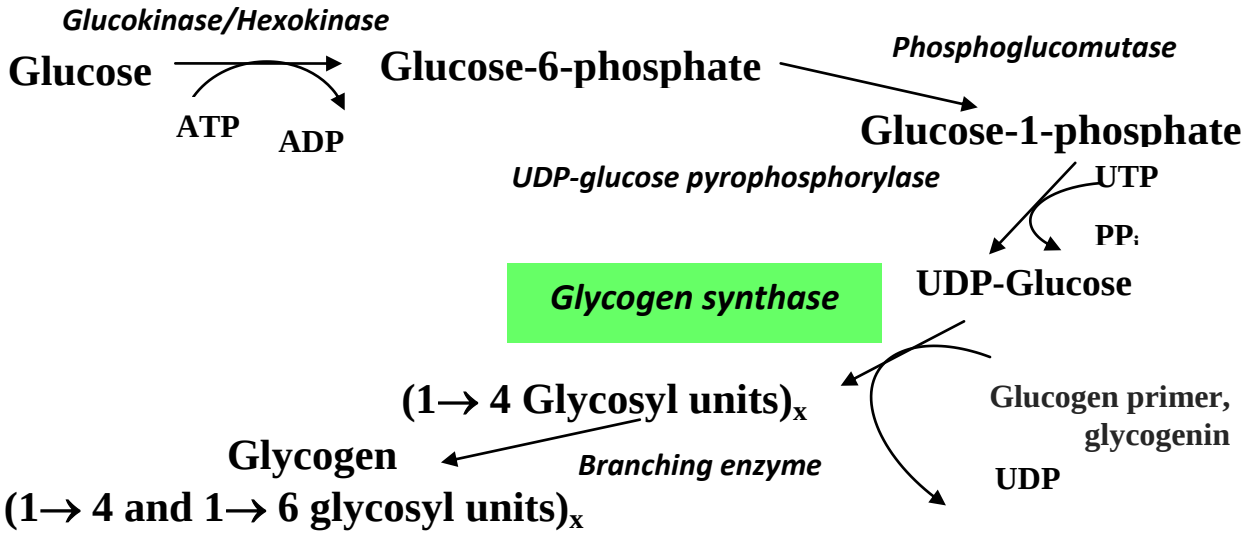


Figure 19.1 – The scheme of glycogen synthesis

Glycogen degradation (glycogenolysis)

The pathway of glycogenolysis begins with removal of the external α1→4-linked glucose residues in glycogen. This is accomplished by **glycogen phosphorylase**, an enzyme that uses cytosolic phosphate and releases glucose from glycogen in the form of glucose 1-phosphate, which is converted into glucose 6-phosphate by phosphoglucomutase. In the liver the glucose is released from glucose 6-phosphate by glucose-6-phosphatase, and exits into blood. The regulatory step in glycogenolysis is catalyzed by phosphorylase, the first enzyme in the pathway (Fig. 19.2).

Phosphorylase is specific for α1→4 glycosidic linkages; it cannot cleave α1→6 linkages. Thus phosphorylase cleaves the external glucose residues, until the branches are three or four residues long, then debranching enzyme, which has both transglycosylase and glucosidase activity, moves a short segment of glucose residues bound to the α1→6 branch to the end of an adjacent α1→4 chain, leaving a single glucose residue at the branch point. This glucose is then removed by the exo-1,6-glucosidase activity of branching enzyme, allowing glycogen phosphorylase to proceed with degradation of the α1→4 chain, until another branch point is approached, setting the stage for a repeat of the transglycosylase and

glucosidase reactions. About 90% of the glucose is released from glycogen as glucose1-phosphate, and the remainder, derived from the α1→6 branching residues, as free glucose.

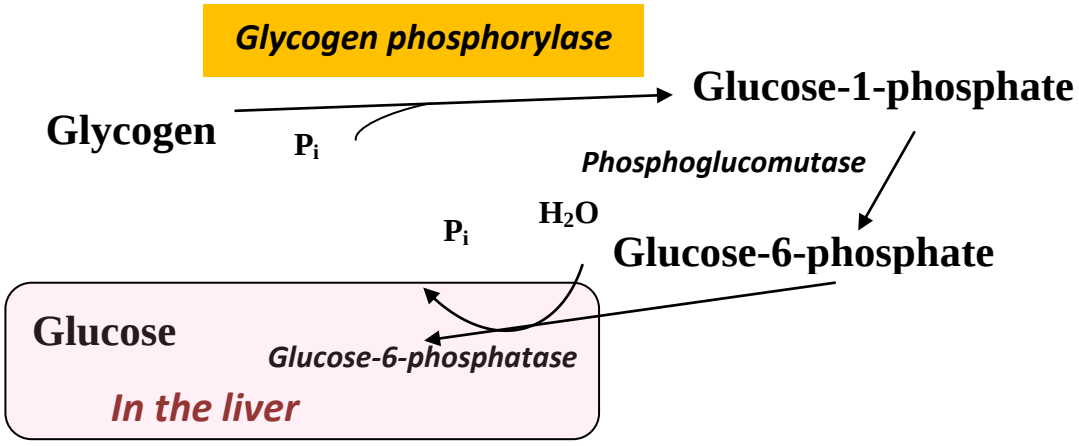


Figure 19.2 – The scheme of glycogen degradation

Regulation of glycogen metabolism

Glycogenolysis is activated in the liver in response to a demand for blood glucose, either because of its utilization during the post-absorptive state or in preparation for increased glucose utilization in response to stress. There are three major hormonal activators of glycogenolysis: **glucagon, adrenaline,** and **cortisol.**

The primary function of glucagon is to activate hepatic glycogenolysis for maintenance of normoglycemia. Blood glucagon increases between meals, decreases during a meal, and is chronically increased during fasting or on a low-carbohydrate diet.

Glycogenolysis is also activated in response to both acute and chronic stress. Stress causes an activation of glycogenolysis through the action of the adrenaline released from the adrenal medulla. During prolonged exercise, both glucagon and adrenaline contribute to the stimulation of glycogenolysis.

Increased blood concentrations of cortisol also induce glycogenolysis.

Glucagon binds to a hepatic plasma-membrane receptor and initiates a cascade of reactions that lead to mobilization of hepatic glycogen. On the inside of the plasma membrane there is a class of proteins, known as G-proteins, that bind GTP and GDP. GDP is bound in the resting state. Binding of glucagon to the plasma membrane receptor stimulates exchange

of GDP for GTP on the G-protein, and the G-protein then undergoes a conformational change that leads to dissociation of one of its subunits, which then binds to and activates the plasma membrane enzyme, adenylyl cyclase. This enzyme converts cytoplasmic ATP into cAMP, a second messenger for action of glucagon (and other hormones). cAMP binds to the cytoplasmic enzyme protein kinase A, which then initiates a series of protein-phosphorylation reactions. Phosphorylation of phosphorylase initiates glycogenolysis, leading to production of glucose 6-phosphate in liver, which is then hydrolyzed to glucose and exported into blood.

Glycogenolysis and glycogenesis are opposing pathways. Theoretically, glucose 1-phosphate produced by phosphorylase could be rapidly activated to UDP-glucose and reincorporated into glycogen. To prevent this wasteful cycle, protein kinase also acts directly on glycogen synthase, in this case inactivating the enzyme. Thus, the **activation of glycogenolysis is coordinated with inactivation of glycogenesis**.

Adrenaline action on glycogenolysis proceeds by two pathways. One of these, through the β-adrenergic receptor, is similar to that for glucagon, involving a plasma-membrane adrenaline-specific receptor, G-proteins, and cAMP. Adrenaline also works simultaneously through an α-receptor, but by a different mechanism. Binding to α-receptors also involves G-proteins, but in this case, the G-protein is specific for activation of a membrane isozyme of phospholipase C, which is specific for cleavage of a membrane phospholipid, phosphatidylinositol bisphosphate. Both products of phospholipase C action, diacylglycerol and inositol trisphosphate, act as second messengers of adrenaline action. Diacylglycerol activates protein kinase C, which initiates a series of protein-phosphorylation reactions. Inositol trisphosphate promotes the transport of Ca^{2+} into the cytosol. Ca^{2+} then binds to the cytoplasmic protein calmodulin, which binds to and activates **phosphorylase kinase**, leading to phosphorylation and activation of phosphorylase, providing glucose for blood.

The tissue localization of hormone receptors provides tissue specificity to hormone action. Only those tissues with glucagon receptors respond to glucagon. Muscle may be rich in glycogen, even during hypoglycemia, but it lacks both the glucagon receptor and glucose-6-phosphatase. Therefore muscle glycogen cannot be mobilized to replenish blood glucose. Muscle glycogenolysis is activated in response to adrenaline through the β-adrenergic receptor, providing a supply of carbohydrate for the energy needs of muscle. This occurs not only during “fight or flight”

situations, but also during prolonged exercise.

There are also two important **hormone-independent mechanisms** for activation of glycogenolysis in muscle. First, the influx of Ca^{2+} into the muscle cytoplasm in response to nerve stimulation activates the basal, unphosphorylated form of phosphorylase kinase by action of the Ca^{2+}-calmodulin complex. This hormone-independent activation of phosphorylase provides for rapid activation of glycogenolysis during short bursts of exercise, even in the absence of adrenaline action. A second mechanism for activation of muscle glycogenolysis involves direct allosteric activation of phosphorylase by AMP. Increased usage of ATP during a rapid burst of muscle activity leads to rapid accumulation of ADP, which is converted in part into AMP by action of the enzyme myokinase (adenylate kinase), which catalyzes the reaction. AMP activates both the basal and phosphorylated forms of phosphorylase, enhancing glycogenolysis either in the absence or presence of hormonal stimulation. AMP also relieves inhibition of phosphofructokinase by ATP, stimulating the utilization of glucose through glycolysis for energy production. The stimulatory effects of Ca^{2+} and AMP assure that the muscle can respond to its energy needs, even in the absence of hormonal input.

Glycogenesis is under the control of the **insulin**. Insulin is secreted into blood following a meal, tracking blood glucose concentration. It has two primary functions in carbohydrate metabolism: firstly, insulin reverses the actions of glucagon in phosphorylation of proteins, turning off glycogen phosphorylase and activating glycogen synthase, promoting glucose storage; secondly, it stimulates the uptake of glucose into muscle and adipose tissue, facilitating synthesis and storage of glycogen and triglycerides.

The liver also appears to be directly responsive to blood glucose concentration, increasing glycogen synthesis following a meal, even in the absence of hormonal input. Thus, the increase in hepatic glycogenesis begins more rapidly than the increase in insulin concentration in blood, and perfusion of liver with glucose solutions *in vitro*, in the absence of insulin, also leads to inhibition of glycogenolysis and activation of glycogenesis. This appears to occur by direct allosteric inhibition of phosphorylase by glucose and secondary stimulation of protein phosphatase activity.

Glycogen storage diseases

There are a number of autosomal recessive genetic diseases affecting glycogen metabolism. Glycogen is degraded in response to a falling blood glucose concentration. A defect in the glycogenolytic pathway can lead to insufficient glucose supply and may cause hypoglycemia. This happens in patients with inherited deficiencies of enzymes controling glycogen metabolism. These diseases are known as glycogen storage diseases. Over ten types of glycogen storage disease are known. They are very rare. The symptoms of glycogen storage diseases vary and depend on the site of the enzyme defect. For instance, type 1 glycogen storage disease (von Gierke's disease) is a deficiency of glucose-6-phosphatase, which leads to a fasting hypoglycemia unresponsive to adrenaline and glucagon. On the other hand, patients with type V disease (McArdle's disease), which is caused by muscle phosphorylase deficiency, do not experience hypoglycemia, but have a limited ability to perform strenuous exercise.

Predictably, glycogen storage diseases affecting hepatic glycogen metabolism are commonly characterized by fasting hypoglycemia and may be life-threatening. Defects in muscle glycogen metabolism are characterized by muscle fatigue during exercise.

CHAPTER 20

TISSUE LIPIDS, DIGESTION AND TRANSPORT

Lipids are chemically heterogeneous group of substances of biological origin, common property of which is hydrophobicity and the ability to dissolve in nonpolar organic solvents. There are several classifications of lipids: physico-chemical, biological or physiological, and structural. The most challenging one is the structural classification based on the structural features of these compounds. According to this classification, all lipids are divided into saponifiable and unsaponifiable. Saponifiable ones include those compounds which are formed by alkaline hydrolysis of fatty acid salts (soap); unsaponifiable lipids are not subjected to alkaline hydrolysis.

Classification of lipids:

I. Saponifable:
   1) Simple:
      a. Waxes;
      b. Neutral fats (triacylglycerols, TAG).
   2) Complex:
      a. Proteolipids;
      b. Glycolipids;
         - Sulfatides*;
         - Gangliosides*;
         - Cerebrosides*.
      c. Phospholipids:
         - Glycerophosholipids;
            - Phosphatidylcholine;
            - Phosphatidylethanolamine;
            - Phosphatidylserine;
            - Phosphatidyinositol;
            - Plasmalogens;
         - Sphingomielines*.

II. Non-saponifable:
   1. Steroids;
   2. Carotenoids;
   3. Terpenoids.

Note! In some classifications sphingomyelins, sulfatides, gangliosides, and cerebrosides are combined into a group of **sphingolipids, since they all contain the amino alcohol sphingosine.*

According to physic-chemical properties of lipids (degree of polarity), they are classified into neutral lipids or nonpolar (non-charging), and polar (charge carriers), such as phospholipids and fatty acids. According to physiological value, lipids are divided into structural and reserve. Reserve lipids are deposited in large quantities and then consumed for energy needs of the body. Reserve lipids are triacylglycerols (TAG). All other lipids can be attributed to structural. They have no particular energy value, but are involved in the construction of biological membranes and protective covers.

Characteristic structural component of most lipids is fatty acid. These long-chain organic acids consist of 4-24 carbon atoms and contain a carboxylic group and a long non-polar hydrocarbon "tail". As part of TAG fatty acids function as the energy deposition. Phospholipids, sphingolipids, and fatty acid form an inner hydrophobic layer of membranes. Free fatty acids are present in the body in small amounts, for example in blood, where they are transported in a complex with a protein albumin.

Some fatty acids in the human body (e.g. l**inoleic** and **linolenic**) are not synthesized, so must come with food. These acids are called **essential**. These also include **arachidonic** acid, which can be synthesized in the body from linoleic acid.

Functions of lipids are important and varied:

- **substrate-energy**: fat in the body is a very efficient source of energy (in the form of reserves of adipose tissue);
- **structural** (plastic): lipids bound with proteins are the structural elements of cell membranes and the cell organelles;
- **transport**: lipids determine the transport of substances into cells;
- **mechanical protection**: body fat protects the body and organs from mechanical damage;
- **thermal isolation**: with pronounced low thermal conductivity, lipids retain heat in the body;
- **electrically isolation**: lipids are electrically isolating material, thus participating in the transmission of nerve impulses and thus the functioning of the nervous system;
- **emulsifier**: phosphoglycerols and bile acids stabilise the emulsion at the interface of oil-water phase;
- **hormonal** (regulatory): steroid hormones are synthesised from cholesterol, are involved in the regulation of water-salt metabolism, sexual function, eicosanoids (derived polyene fatty acids) and cause a variety of

biological effects;

- **vitamins**: some vitamins are fat-soluble (A, D, E, K) and essential fatty acids (F);

- **solvent**: some lipids are solvents of other lipid substances.

Lipids of human tissues. Lipids comprise 10-12% of body weight. The average adult body contains approximately 10-12 kg of lipids: 2-3 kg are structural lipids, and the remainder are reserve lipids. The reserve lipids are mainly (about 98%) localized in adipose tissue and are represented by TAG. These lipids are a potential source of chemical energy available during periods of fasting.

The lipid content in human tissues differs significantly. In adipose tissue, they constitute up to 75% of dry weight. In nervous tissue lipid contains up to 50% of dry weight: phospholipids and sphingomyelins (30%), cholesterol (10%), gangliosides and cerebrosides (7%). In the liver, the total amount of lipids normally does not exceed 10-14%.

Fatty acids are typical for the human body, containing an even number of carbon atoms, usually – 16 to 20. The main saturated fatty acid in the human lipids is palmitic acid (30-35%). Unsaturated fatty acids are represented by monoenoic and polyene ones. Double bonds in fatty acids in the human body have a *cis* configuration. Fats and phospholipids at normal body temperature have a liquid consistency due to a certain content of unsaturated fatty acids. In phospholipids of membrane unsaturated acids can reach 80-85%.

Food lipids, their absorption and digestion. An adult requires from 70 to 145 g of lipids per day depending on work, sex, age, and climatic conditions. With a balanced diet fats should provide no more than 30% of total calories. Daily meals should contain at least one-third of liquid fats (oils).

In the mouth and stomach of an adult there are no conditions for enzyme digestion of lipids. The main cleavage site of lipids is the small intestine. To increase the contact surface with hydrophilic enzymes fats should be emulsified (break into small drops). Emulsification takes place under the action of bile salts. Emulsification also promotes peristalsis and bubbling CO_2 which neutralizes acidic stomach contents by bicarbonate, secreted in the pancreas.

The main dietary lipids are represented mostly by TAG, and to a lesser extent by phospholipids and steroids. Stepwise hydrolysis of TAG is carried out by pancreatic lipase. It is secreted in an inactive form in the intestine and

activates colipase and bile acids. Pancreatic lipase hydrolyzes fats mainly in positions 1 and 3, so the main hydrolysis products are glycerol, free fatty acids, monoacylglycerols (Fig.20.1).

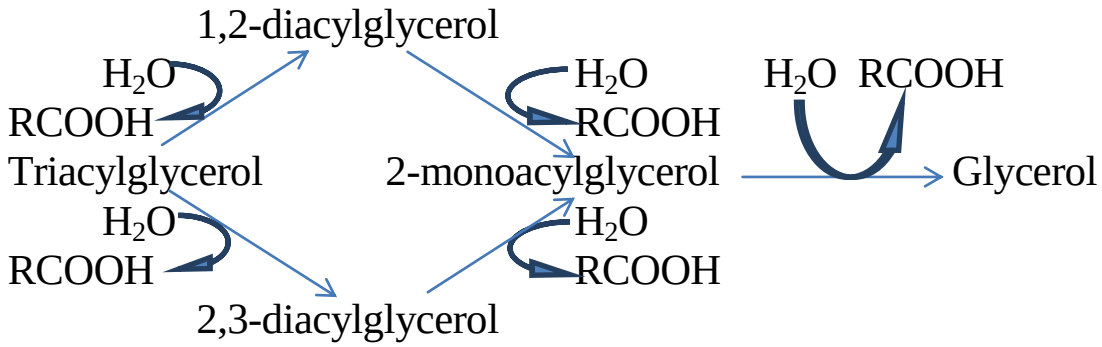


Figure 20.1 – Hydrolysis of TAG in small intestine

Phospholipids are hydrolyzed by pancreatic phospholipases A_1, A_2, C, and D (Fig. 20.2). Digestion products are glycerol, fatty acids, phosphoric acid, and the nitrogenous alcohols (choline, ethanolamine, serine, inositol). Esters of Cholesterol digested pancreatic cholesterol esterase on cholesteroland fatty acids. Enzyme activity is manifested in the presence of bile acids.

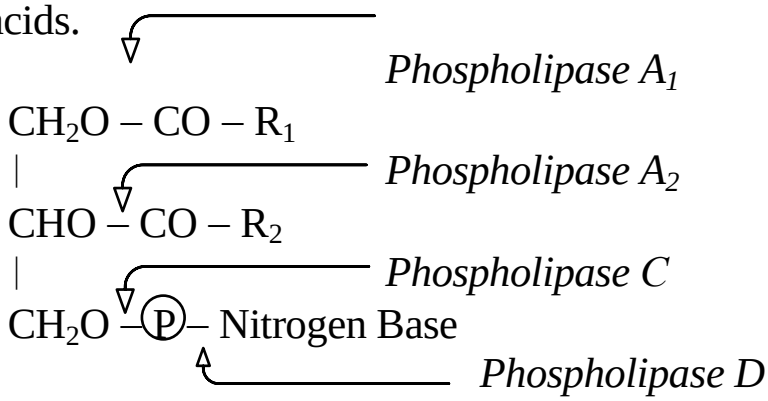


Figure 20.2 –Hydrolysis of phospholipids in small intestine

Lipid absorption occurs in the proximal part of the small intestine. 3-10% of food fats are absorbed without hydrolysis in the form of triacylglycerols. The main part of lipids is absorbed only in the form of degradation products. The hydrophilic digestion products (glycerol fatty acid with carbon number less than 12, phosphoric acid, choline, serine, ethanolamine, etc.) diffuse into the epithelial cells of the intestine. The hydrophobic products (cholesterol, long chain fatty acid di-and monoglitserols) form micelles. Micelles are absorbed by the small intestine

epithelil cells. Major role in the formation of micelles is played by bile acids.

Micelle is a spherical complex, in the center of which hydrophobic products of digestion, surrounded by bile acids, are transported. Micelles are coming closer to the brush border cells of the intestinal mucosa, and lipid components of the micelles diffuse through the membrane into cells. Together with the products of the hydrolysis of lipids and fat-soluble vitamins bile salts are absorbed.

Bile acids are further returned through the portal vein to the liver, and their lipid component is included in the resynthesis process. Resynthesis of TAG involves not only fatty acids, absorbed from the bowel, but also fatty acids synthesized in the body, therefore, the composition resynthesis differs from fats derived from food.

Monoacylglycerol + R – CO – S-CoA →Diacylglycerol + HS-CoA
Diacylglycerol + R – CO – S-CoA → Triacylglycerol + HS-CoA

Figure 20.3 –Resynthesis of TAG in the intestinal wall.

In intestinal mucosal cells, the synthesis of phospholipids and cholesterol ester formation is catalysed by acetylcholesterolacetyltransferase.

Transport of lipids. Lipids are insoluble in aqueous medium; therefore their transport in the body is performed by lipoprotein (LP, complex of lipids with proteins). There are exogenous and endogenous types of lipid transport: exogenous – transport of lipid, received from food, and endogenous – transport of lipids synthesized by the body.

There are several types of LP, but they all have a similar structure – a hydrophobic core and a hydrophilic layer on the surface. The hydrophilic layer is formed by proteins, called ***apolipoproteins (Apo)***, amphiphilic molecules and lipids (phospholipids and cholesterol). The hydrophilic groups of the molecules are directed towards the aqueous phase, and the hydrophobic groups are a central part of LP, in which the lipids are transported. Apoproteins perform several functions:

• form the structure of lipoproteins (e.g. B-48 – basic protein of chylomicrons (ChM), B-100 – the main protein of VLDL, IDL, LDL);

• interact with receptors on the cell surface, determining what tissue is captured by this type of lipoproteins (apoprotein B-100, E);

• are enzymes or enzyme activators acting on lipoproteins (C-II – activator of lipoproteinlipase, A-I – activator lecithin: cholesterol

acyltransferase).

Table 20.1. – **Characteristics of lipoproteins**

Characteristic (feature)	***ChM***	***VLDL***	***IDL***	***LDL***	***HDL***
Composition,%					
Protein	2	10	11	22	50
Phospholipids	3	18	23	21	27
Cholesterol (C)	2	7	8	8	4
Cholesterol Ethers (CE)	3	10	30	42	16
TAG	85	55	26	7	3
Functions	Transport of exogenous lipids	Transport of endogenous lipids	Precursor of LDL	Transport of C into tissues	Transport of C from tissues, donor of apo A and C-II
Place of synthesis	Intestine	Liver	Blood	Blood	Liver
Diameter, nm	> 120	30-100		21-100	7-15
Main apolipoproteins	B-48 C-II E	B-100 C-II	B-100 E	B-100	A-I C-II E

When exogenous TAG resynthesized in enterocytes, they form ChM together with phospholipids, cholesterol and proteins, and are secreted into the lymph first, and then enter the blood. In the lymph and blood HDL transfer apoproteins E (apoE) and C-II (apoC-II) into ChM, thus converting into "mature" ChM. ChM is quite large, so after a fatty meal they give blood plasma opalescent, like milk. Getting into the circulatory system, ChM quickly undergo catabolism, and disappear within a few hours. Decay time depends on hydrolysis of TAG in ChM by action of lipoprotein lipase (LPL).

This enzyme is synthesized and secreted by adipose and muscle tissues, cells of the mammary glands. Secreted LPL binds to the surface of endothelial cells of the capillaries of the tissues where it is synthesized. Regulation of secretion has tissue specificity. In adipose tissue LPL synthesis is stimulated by insulin, allowing the entry of fatty acids synthesis and storage in the form of TAG. In diabetes, when insulin deficiency is noted, the level of LPL is reduced. As a result, blood accumulates a large amount of LP. In muscle, where LPL is involved in the delivery of fatty acids to oxidation between meals, insulin inhibits the formation of the enzyme.

On the ChM surface there are two factors necessary for LPL activity – apo C-II and PL. ApoC-II activates the enzyme, and PL is involved in binding the enzyme to the surface of ChM. As a result of LPL action on ChM, TAG breaks down into fatty acids and glycerol.

Thereafter, fatty acids are transported into the tissue, where they can be deposited as TAG (in adipose tissue) or used as an energy source (in muscles). Glycerol is transported to the liver where in postabsorptive period may be used for the synthesis of fats.

As a result of LPL activity, the amount of neutral fats in ChM decreases by 90%, particle sizes decrease as well; apoC-II is transferred back to HDL. The formed particles are called residual ChM (remnant). They contain PL, C, fat-soluble vitamins, apo B-48, and apo E.

Residual ChM enter hepatocytes that have receptors that interact with these apoproteins. Under theaction of lysosomal enzymes, proteins and lipids are hydrolyzed, and then recycled. The fat-soluble vitamins and exogenous ChL are used in the liver or transported to other organs.

At endogenous transport, sinthesized in the liver, TAG and PL form VLDL, which includes apo B-100 and apo C. VLDL is the main form of transport for endogenous TAG. Once in the blood, VLDL gets apo C-II and apo E from HDL and converts under LPL action. In this process, VLDL is first converted to IDL, and then to LDL. Cholesterol becomes the main lipid of LDL and is transferred to the cells of all tissues. Formed during hydrolysis, fatty acids enter the tissue and glycerol is transported to the liver, which can be used again for the synthesis of TAG.

All changes in LP content in plasma (increase, decrease or complete absence) is called dyslipoproteinemia. Dislipoproteinemia can be either a specific primary manifestation of disturbances in lipid and lipoprotein metabolism, a concomitant syndrome in some diseases of the internal organs (secondary dislipoproteinemia). With successful treatment of the underlying disease they disappear.

Hypolipoproteinemias include the following conditions:

1**.** ***Abetalipoproteinemia*** occurs as a rare inherited disorder – a defect of apoprotein B gene, when protein synthesis of apo B-100 in the liver and apo B-48 in the intestine is disrupted. As a result, in the cells of the intestinal mucosa ChM is not formed, while VLDL is not synthesized in the liver, and the cells of these organs accumulate fat droplets.

2. ***Familial hypobetalipoproteinemia***: concentrationof LP, containing apo B, is only 10-15% of normal levels, but the body is capable of forming

ChM.

3. ***Familial alfa-lipoprotein deficiency*** (Tangier disease): in plasma HDL is almost not detectable, and large amounts of cholesterol esters are accumulated in the tissues. The absence of apo C-II (activator of LPL) in patients leads to a characteristic increasein TAG concentration in plasma.

There are following types of **hyperlipoproteinemias**:

Type I –familial lipoprotein lipase deficiency (Hyperchylomicronemia). Removal rate of ChM from the bloodstream depends on LPL activity, the presence of HDL, supply of apoproteins C-II and E to ChM, activity of apoC-II and apoE transport on ChM. Genetic defects in any of the proteins involved in the metabolism of ChM, lead to the development of family hyperchylomicronemia– ChM accumulation in the blood. The disease manifests itself in early childhood, characterized by hepatosplenomegaly, pancreatitis, abdominal pains. Treatment: diet with a low lipid content (up to 30 g/ day) and high carbohydrate content.

Type II – familial hypercholesterolemia (hyper-beta-lipoproteinemia). This type is divided into *two subtypes*: IIa characterized by high blood levels of LDL and IIb – with elevated levels of both LDL and VLDL. The disease is associated with impaired reception and catabolism of LDL (defect of cellular receptors for LDL or change in the structure of LDL), accompanied by increased biosynthesis of cholesterol, apo-B and LDL. This is the most serious pathology in LP metabolism: the risk of coronary heart disease in patients with this type of violation increases 10-20 times compared with healthy individuals. As a secondary phenomenon, hyperlipoproteinemia type II may develop hypothyroidism, nephrotic syndrome. Treatment: a diet low in cholesterol and saturated fat.

Type III – familial dysbetalipoproteinemiya (broad beta desease) occurs due to an anomalous composition of VLDL. They are rich in free cholesterol, but have a deficit of apo-E, inhibiting the activity of hepatic TAG lipase. This leads to violations of the catabolism of VLDL and XM. The disease manifests itself in the age of 30-50 years. The condition is characterized by high VLDL remnants, and hypercholesterolemia is revealed by xanthoma and atherosclerotic lesions of peripheral and coronary vessels. Treatment: diet therapy aimed at weight reduction.

*Type IV – familial hypertriacylglycerolemia.*The primary form occurs due to a decrease in LPL activity, increased TAG in plasma. ChM accumulation is not observed. The state occurs only in adults and is characterized first by the development of atherosclerosis of coronary

arteries, and then peripheral arteries. The disease is often accompanied by a decrease in glucose tolerance. Secondary form occurs in pancreatitis, alcoholism. Treatment: diet therapy aimed at reducing weight.

Type V – mixed hyperlipoproteinemia familial. In this type of pathology changes in blood lipoprotein fractions are complex: content of ChM and VLDL fractions increases, LDL and HDL decreases severely. Patients often are overweight, may develop hepatosplenomegaly, pancreatitis. Atherosclerosis does not develop in all cases. As a secondary phenomenon, hyperlipoproteinemia type V may occur with insulin-dependent diabetes mellitus, hypothyroidism, pancreatitis, alcoholism, type I glycogen storage disease. Treatment: diet therapy aimed at reducing weight (a diet low in carbohydrates and fat).

Disturbances of digestion and absorption of lipids. Received dietary fats if their intake is moderate (no more than 100-150 g), are almost completely absorbed, and normal digestion feces contain no more than 5% fat. Remains of fatty food are allocated mainly in the form of soaps. In case of disturbances of the digestion and absorption of lipids the excess of lipids in the stool, steatorrhea (fatty stools) is observed. There are 3 types of steatorrhea.

Pancreatogenous steatorrhea occurs in case of pancreatic lipase deficiency. The reasons for such a state can be chronic pancreatitis, pancreatic inborn hypoplasia, congenital or acquired deficiency of pancreatic lipase, as well as cystic fibrosis, where the pancreas is damaged along with other glands. In this case, the feces contain bile pigments, reduced levels of free fatty acids and increased TAG.

Hepatogenous steatorrhea is caused by blockage of the bile ducts. This occurs in congenital biliary atresia, resulting in narrowing of the bile duct by gallstones, or pinched tumor growing in the surrounding tissues. Decreasing the secretion of bile emulsification leads to disruption of dietary fat, and, consequently, blockage of their digestion. In the feces of patients there are no bile pigments, but high content of TAG, fatty acids, and soaps can be found.

Enterogenous steatorrhea is observed in intestinal lipodistrophy, amyloidosis, extensive resection of the small intestine, in processes accompanied by a decrease in metabolic activity of the intestinal mucosa. This pathology is characterized by fecal pH shift to the acid medium, the growth of fatty acids content in feces.

CHAPTER 21

METABOLISM OF TRIACYLGLYCEROLS AND FATTY ACIDS

TAG (neutral fats) is the main form of energy deposition. Deposited fat can provide the body with energy during fasting for a long time (up to 7-8 weeks). TAG synthesis occurs in absorptive period in the liver and adipose tissue. However, if the adipose tissue participates only in fat deposition, the liver plays an important role in converting carbohydrates originating from food in fats, which are then secreted into the blood as part of VLDL and delivered to other tissues. The immediate substrates for the synthesis of fats are the acyl-CoA and glycerol-3-phosphate. The metabolic pathway of synthesis of fats in the liver and adipose tissue is the same, except for the different pathways of glycerol-3-phosphate synthesis (Fig.21.1).

The liver is the main organ where synthesis of fatty acids from the products of glycolysis takes place. In the smooth endoplasmic reticulum of hepatocytes fatty acids, interacting with glycerol-3-phosphate, are activated and immediately used for the synthesis of TAG. Synthesised fats are packaged in VLDL and secreted into the blood.

In adipose tissue for TAG synthesis mainly fatty acids, released by the hydrolysis of ChM and VLDL fats, are used. Fatty acids come into adipocytes, where they are transformed into derivatives of CoA and react with glycerol-3-phosphate. Furthermore in these cells synthesis of fatty acids from products of glycolysis occurs. TAG molecules in adipocytes are combined into larger oil droplets, containing no water, which is the most compact form of fuel storage molecules.

Mobilization of fat (hydrolysis to glycerol and fatty acids) occurs in the post absorptive period of fasting or active physical exercise. The process is carried out under the influence of hormonsensitive TAG- lipase and includes 3 stages. At first this enzyme cleaves the external fatty acid, which is attached to the first carbon atom of the glycerol with diacylglycerol formation. Then another external fatty acid is cleaved and monocylglycerol is formed, which is hydrolyzed to glycerol and one fatty acid, entering the bloodstream. Glycerol, as the water-soluble substance, is freely transported in the blood, while fatty acids are transported in the complex of plasma with albumin.

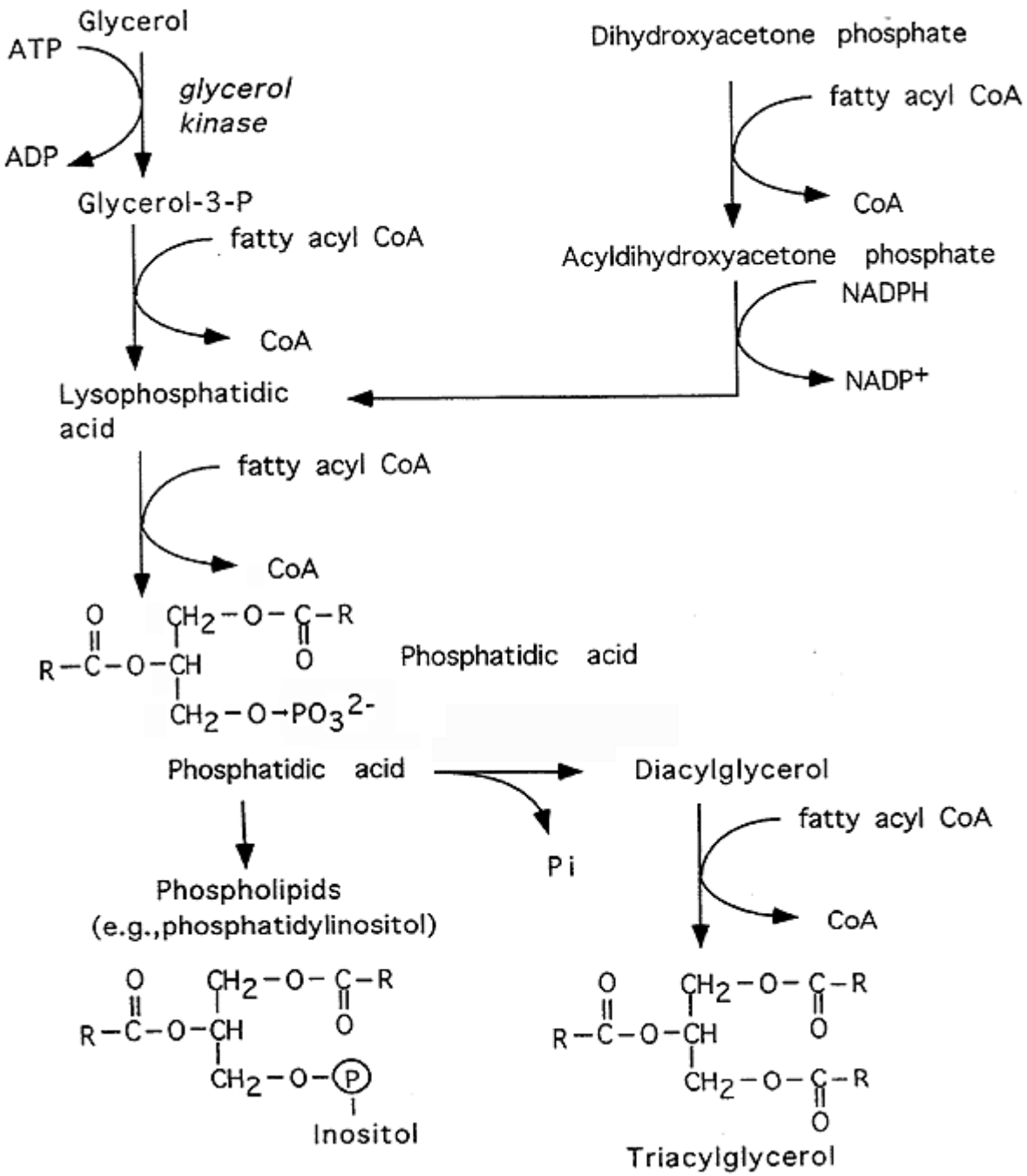


Figure 21.1 –TAG synthesis

Regulation of triacylglycerols synthesis

In absorptive period the ratio of insulin/glucagon increases and activates the synthesis of TAG in the liver. In adipose tissue synthesis lipoproteinlipase (LPL) is induced, and in this period the entry of fatty acids into adipocytes increases. Simultaneously, insulin activates protein,

participating in glucose transport – GLUT-4, which leads to an increased entrance of glucose in adipocytes and activation of glycolysis there. As a result, the required substances for the synthesis of fats are produced: glycerol-3-phosphate and activated fatty acid. In the liver, as a result of increased amount of insulin, activity of regulatory enzymes of glycolysis and the enzymes involved in the synthesis of fatty acids from acetyl- CoA increase. The result of these changes is the increase of the synthesis and secretion of TAG in their blood as part of VLDL. VLDL delivers fat in adipose tissue capillaries, where the action of LPL provides rapid entry of fatty acids into adipocytes, where they are deposited as part of the TAG.

Regulation of mobilization of triacylglycerols

Mobilization of deposited TAG is stimulated mostly by glucagon and epinephrine, and to a lesser extent, by somatotropin and cortisol. In the post absorptive period and fasting, glucagon, acting on adipocytes through adenylatecyclase system, activates hormone sensitive lipase that

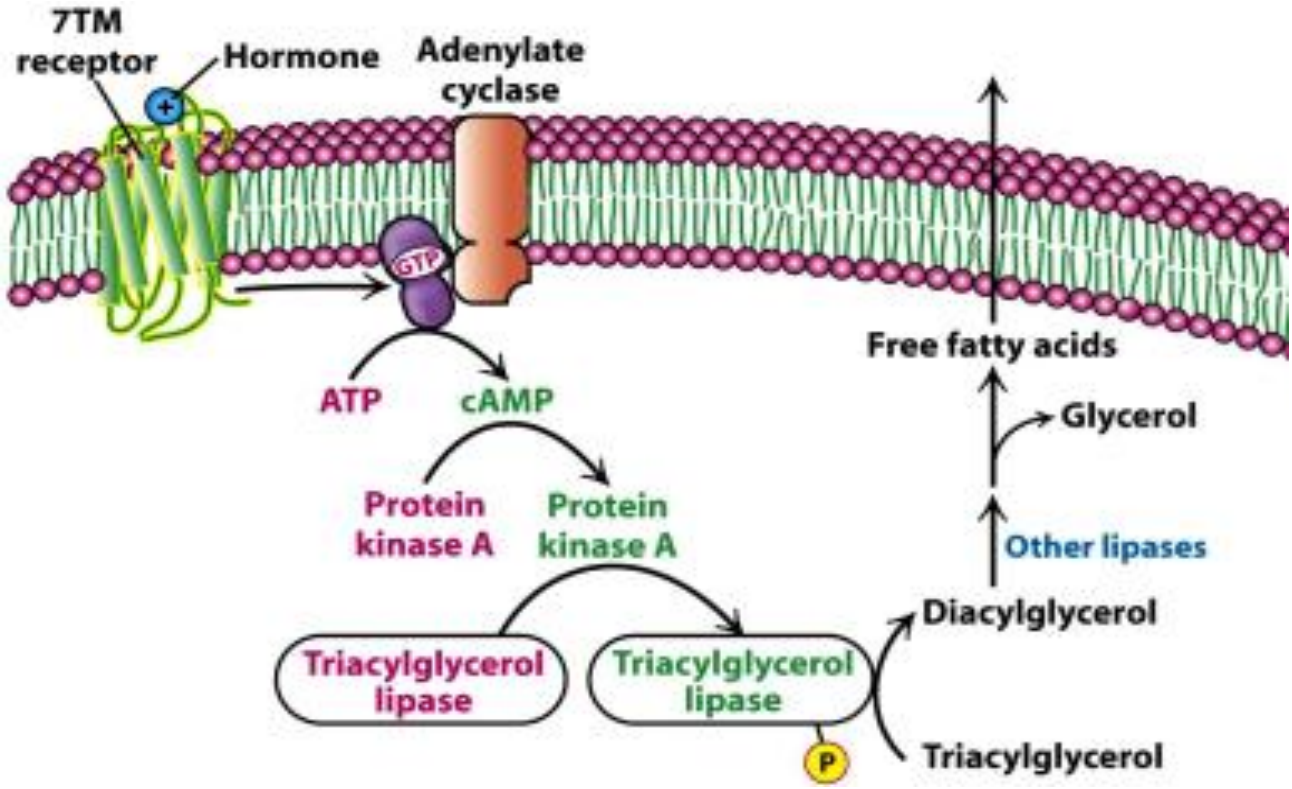


Figure 21.2 – Regulation of mobilization of TAG

activates lipolysis and releases fatty acids and glycerol in the blood. In physical activity, the secretion of adrenaline increases, which activates lipolysis through the adenylatecyclase system. It is known that the action

of adrenaline is realized in two ways: at low concentrations in the blood; its antilipolytic action through α2-receptors dominates, while high – dominate lipolytic action occurs through β-receptors (Fig. *21.2*).

As a result of TAG mobilization, concentration of fatty acids in the blood increases by about 2 times, but they are disposed quickly enough. For muscle, heart, kidney, liver, during fasting or physical work, fatty acids are an important source of energy. The liver processes some of the fatty acids into ketone bodies used by the brain, nervous and some other tissues as sources of energy. When the post absorptive period is replaced by absorptive, insulin, through intermediate mechanisms, inhibits the activity of hormonsensitive lipase and stops the breakdown of fats.

Obesity

Primary obesity. A condition where the body weight by 20% is more than ideal for a given individual is considered obese. It develops when the adipose tissue processes of lipogenesis predominate. Adipocyte formation occurs in utero from the last trimester of pregnancy, and ends in the prepubertal period. After that fat cells can increase in size with obesity or decrease in weight loss, but their number does not change throughout the life. One classification of obesity is based on the size and number of adipocytes. The increase of total number of these cells indicates ***hyperplastic obesity*** (developing in infancy, hereditary). The enlarged size of adipocytes leads to ***hypertrophic obesity***. According to another classification, primary and secondary obesity is observed.

Primary obesity develops as a result of nutritional imbalance, i.e. excessive caloric intake compared with the cost of energy. As a result, 80% of cases are genetic disorders. Metabolic differences between obese and normal people can not be uniquely determined up to day. One of the supposed reasons for these differences is the fact that people who are prone to obesity have different ratio of aerobic and anaerobic glycolysis, the differences in the activity of Na^+/K^+-ATPase.

Moreover, the scientist found out that humans and animals have the gene of obesity. The expression product of this gene is the protein leptin, which is synthesized and secreted by adipocytes and interacts with receptors in the hypothalamus. As a result, it decreases the secretion actions of neuropeptide Y, stimulating food intake. Most of the patients with obesity have a genetic defect of leptin receptors in the hypothalamus. But as a result the secretion of the neuropeptide Y

extends, which leads to an increase in appetite and consequently weight gain.

Secondary obesity is the form of obesity that develops as a result of an illness, often endocrine one. For example, hypothyroidism, Cushing's syndrome and hypogonadism lead to the development of obesity.

Methabolism of fatty acids

Released upon lipolysis, fatty acids enter the bloodstream and are transported together with serum albumin. Their admission is accompanied by the appearance of free fatty acids in the plasma and glycerol as well. Glycerol may be involved in gluconeogenesis, or included in the glycolytic pathway to form a glycerol-3-phosphate.

After the fatty acids enter the cell, they are activated by the formation of Coenzyme A derivatives:

$$RCOOH + HSCoA + ATP \rightarrow RCO \sim CoA + AMP + PPi$$

The reaction is catalyzed by the enzyme acyl-CoA synthetase. It can be found in cytosol and mitochondria matrix, differing in their specificity for fatty acids, having different carbon chain length. Fatty acids with a chain length of 2 to 4 carbon atoms can penetrate the mitochondrial matrix through diffusion. Activation of such acids occurs in the mitochondrial matrix. Fatty acids having long chain, which are predominant in human body, are activated by acyl-CoA synthetase, located in the outer membrane of mitochondria.

β-oxidation of fatty acids takes place in the mitochondrial matrix, so after activation of these substrates, they must be transported to mitochondria. This process is performed by using carnitine, which comes from the diet or is synthesized from the essential amino acids lysine and methionine.

In the outer membrane of mitochondria (Fig. 21.3) there is an enzyme carnitinacyltransferase-I, catalysing the reaction with the formation of acylcarnitine. The resulting acylcarnitine goes through the intermembrane space to the outer side of the inner membrane, and is transported through carnitin-acyltransferase translocase on the inner surface of the inner mitochondrial membrane, wherein the enzyme carnitin-acyltransferase-II catalyses acyl transfer on intramitochondrial CoA. Thereafter, acyl-CoA becomes included in β-oxidation reaction. Free carnitine returns to the intermembrane space of the same translocase.

β-oxidation of fatty acids is a specific way of fatty acids catabolism into the mitochondrial matrix flowing only under aerobic conditions and ending with the formation of acetyl-CoA. Hydrogen proton of β-oxidation enters the ETC, and acetyl-CoA is oxidized in the citric acid cycle, also supplying hydrogen proton for ETC. Therefore, β-oxidation of fatty acids is the most important metabolic way of providing ATP synthesis in the respiratory chain.

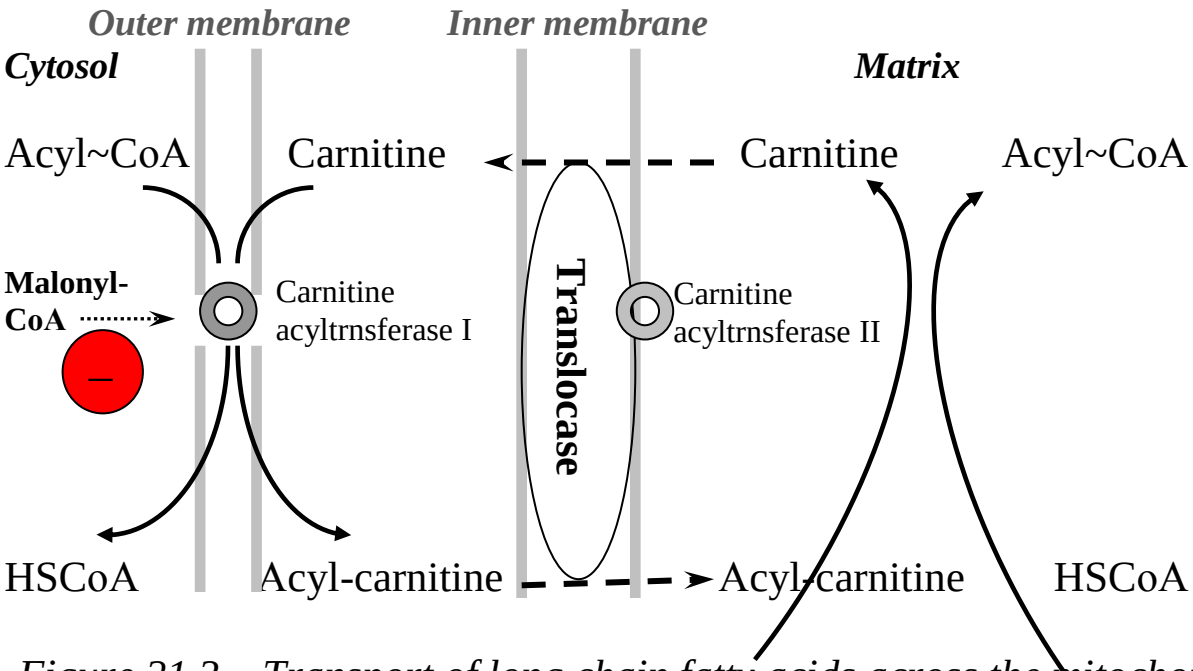


Figure 21.3 – Transport of long chain fatty acids across the mitochondrial membrane.

Products of each cycle of β-oxidation are $FADH_2$, NADH, and acetyl-CoA. Acid residue, which is involved in each subsequent cycle, is shorter by two carbon atoms. In the last cycle, when 4 carbon atoms fatty acid breaks down, two acetyl-CoA molecules are immediately formed (Fig 21.4).

The overall equation of β-oxidation of palmitoyl-CoA can be represented as follows:

$$C_{15}H_{31}CO\text{-}CoA + 7\,FAD + 7\,NAD^+ + 7\,HSKoA \rightarrow$$
$$\rightarrow 8CH_3CO\text{-}CoA + 7FADH_2 + 7(NADH + H^+)$$

Energy input in this case is 131 molecules ATP (21 ATP is formed by oxidation of each of the 7 molecules of NADH at ETC, 14 – by the oxidation of each of the 7 molecules of $FADH_2$ in the ETC, 96 molecules of

ATP synthesis is provided by oxidation of 8 molecules of acetyl-CoA to the TCA cycle). One molecule of ATP is used for activation of a fatty acid. And total energy yield of oxidation of palmitate is 130 ATP.

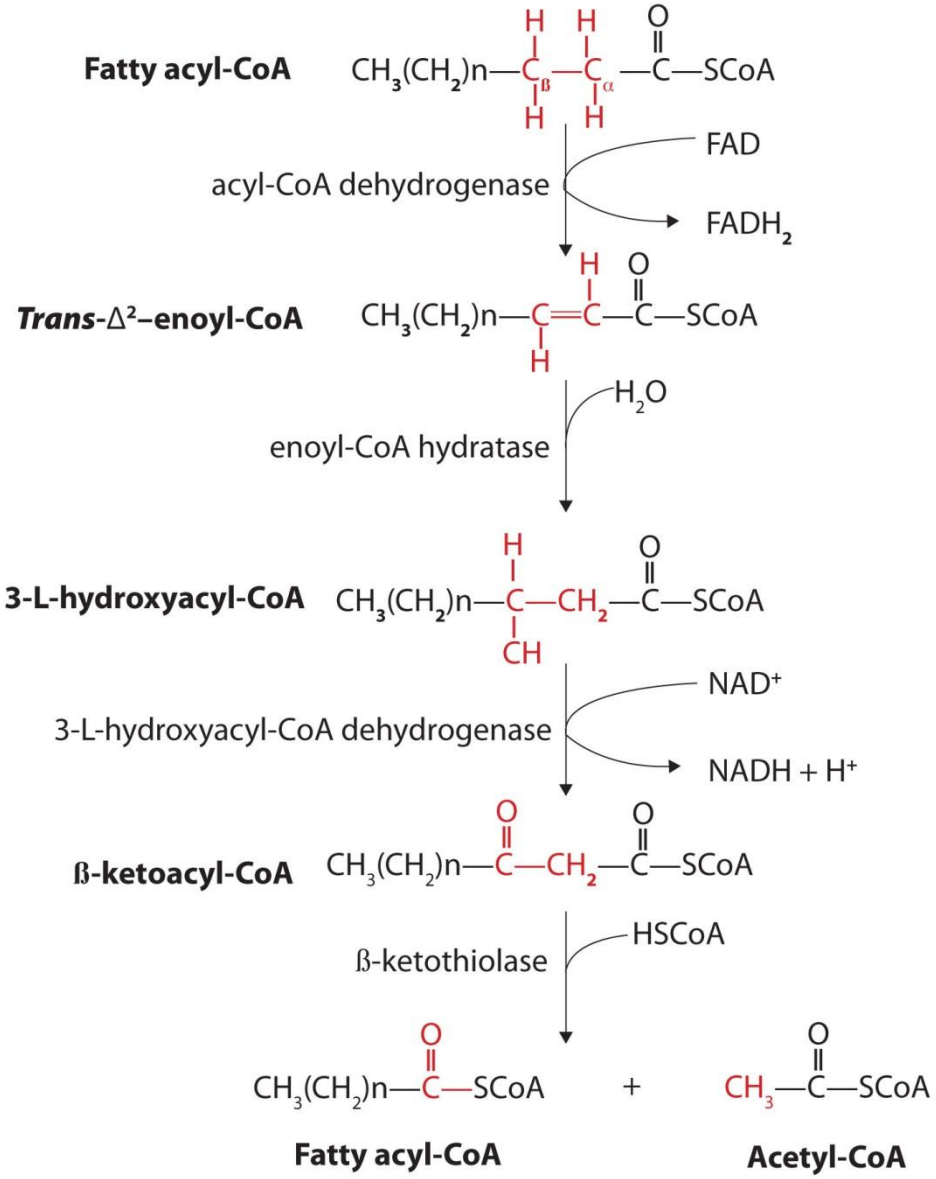


Figure 21.4 – β-Oxidation of fatty acids

Oxidation of fatty acids is an important source of energy in the tissues with high activity of the TCA cycle and respiratory chain (skeletal and cardiac muscle, kidney). The erythrocytes, which lack mitochondria, can't oxidize fatty acids. These compounds do not serve as a source of energy for the brain, as the fatty acids do not pass through the blood-brain barrier.

Regulation of β-oxidation. Speed of the process is depended by the need for energy cells (ratios ATP/ADP, $NADH/NAD^+$). Rate of β-

oxidation also depends on the availability of substrate: the amount of fatty acids entering the mitochondria. Free fatty acids concentration in the blood rises upon activation of lipolysis. Under these conditions, the fatty acid becomes the primary source of energy for muscle and liver, as a result of β-oxidation, formed NADH and acetyl-CoA – inhibitors of pyruvate dehydrogenase complex. Thus, the use of fatty acids as a primary energy source in muscle tissue and liver, saves glucose for nervous tissue and erythrocytes.

Rate of β-oxidation depends on the activity of liver carnitin-acyl transferase-I. In the liver, this enzyme is inhibited by malonyl-CoA formed in the biosynthesis of fatty acids. That is, the malonyl-CoA inhibits the degradation of fatty acids, thus contributing to their use in synthesis of fat.

Other types of oxidation of fatty acids. β-oxidation is the major route of catabolism of fatty acids, but besides it there are α-oxidation and ω-oxidation. α-Oxidation is a sequential cleavage of one-carbon fragments emitted as CO_2 from the carboxyl terminus of the molecule. Such a type of fatty acid oxidation is subjected to chain more than 20 carbon atoms (characteristic for lipid of nervous tissue), and branched fatty acids having a carbon chain (dietary lipids).

ω-Oxidation of fatty acids is normally quite insignificant, it occurs in the liver microsome. The initial step is catalyzed by monooxygenase which requires the presence of NADPH, O_2, and cytochrome P-450. Methyl group transforms into -CH_2OH, and then is oxidized to -COOH. The resulting dicarboxylic acid may be used in β- oxidation reactions.

Oxidation of unsaturated fatty acids occurs in the usual way, until the double bond appears between the third and fourth carbon atoms. There after enzyme enoyl-CoA isomerase moves double bond from position 3-4 to 2-3 and changes the conformation of double bond from cis- to trans- which is required for β-oxidation. In this cycle, β-oxidation of the first dehydrogenation reaction does not occur, since the double bond in the fatty acid radical is already available (Fig. 21.5). Further cycles of β-oxidation continue, not differing from the usual way.

Fatty acids with an odd number of carbon atoms in the final stage of β-oxidation form acetyl-CoA and propionyl-CoA. Three-carbon fragment in the three reactions is converted into succinyl-CoA – metabolite TCA cycle (Fig.21.6).

The acetyl-CoA formed by β-oxidation of fatty acids, cleavage of ketogenic amino acids and the oxidative decarboxylation of pyruvate, is the

starting substrate for a number of important metabolic pathways:

1) oxidation in the TCA cycle;
2) formation of ketone bodies;
3) biosynthesis of cholesterol;
4) biosynthesis of fatty acid.

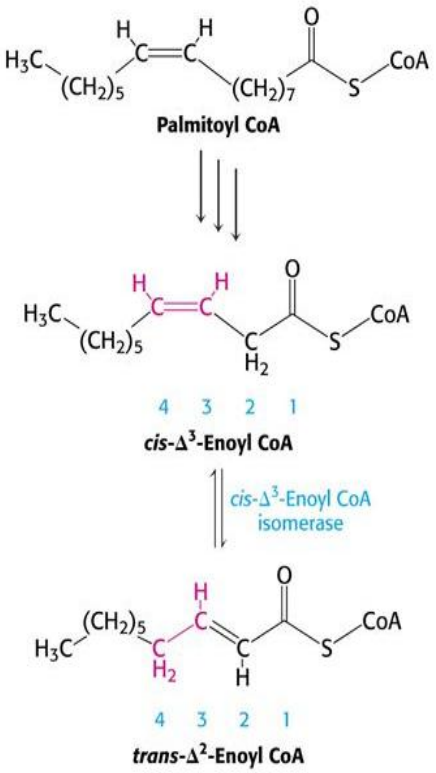


Figure 21.5 – Oxidation of unsaturated fatty acids

Metabolism of ketone bodies

Fasting, prolonged physical exertion, and cases when the cells do not get enough glucose (a diet low in carbohydrates, gastrointestinal disorders, glucosuria, and diabetes melitus) activate the break down of fat in adipose tissue. Fatty acids are transported in the liver in a larger amount than usually which increases speed of β-oxidation. TCA cycle activity is reduced in these conditions, because oxaloacetate is used in gluconeogenesis. As a result, the rate of acetyl CoA formation exceeds the ability of TCA cycle to oxidize it.

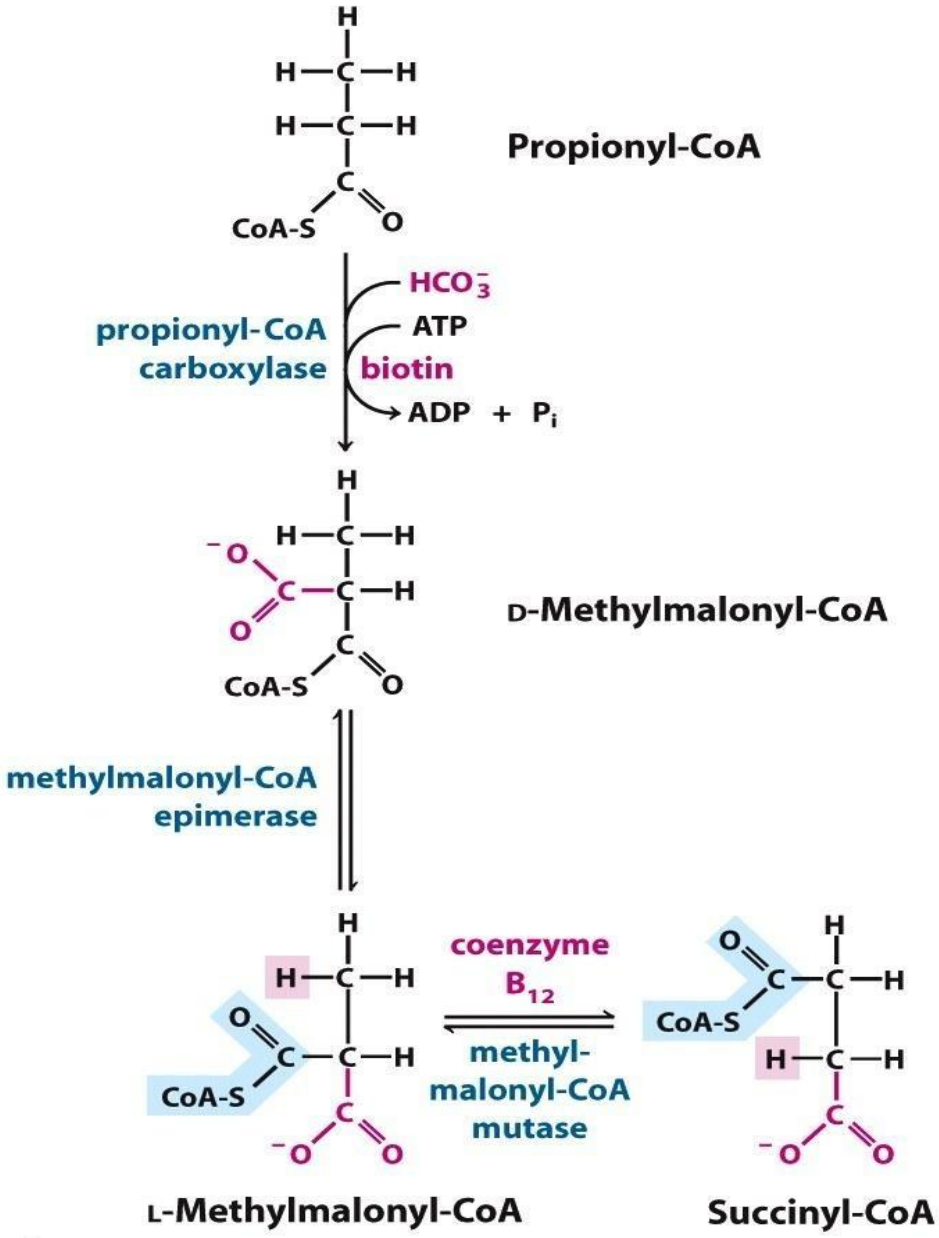


Figure 21.6 – Oxidation of fatty acids with an odd number of carbon atoms

Acetyl-CoA accumulates in the mitochondria of the liver, and is used for the synthesis of ***acetoacetate*** (Fig. 21.7). This substance may be released into the blood by the liver or converted to other ketone body – ***β-hydroxybutyrate*** by reduction. In hepatocite with active β-oxidation, a high concentration of NADH occurs. It helps to transform mostly acetoacetate to β-hydroxybutyrate, so the main blood ketone body is β-hydroxybutyrate. At high concentrations of acetoacetate, its part is decarboxylated non-enzymatically, and turns into ***acetone***. Acetone is not utilized by tissue, but is excreted in the urine and exhaled air. In this way the body removes excess amount of ketone bodies, which do not have time

to oxidize and cause acidosis (Fig. 21.8).

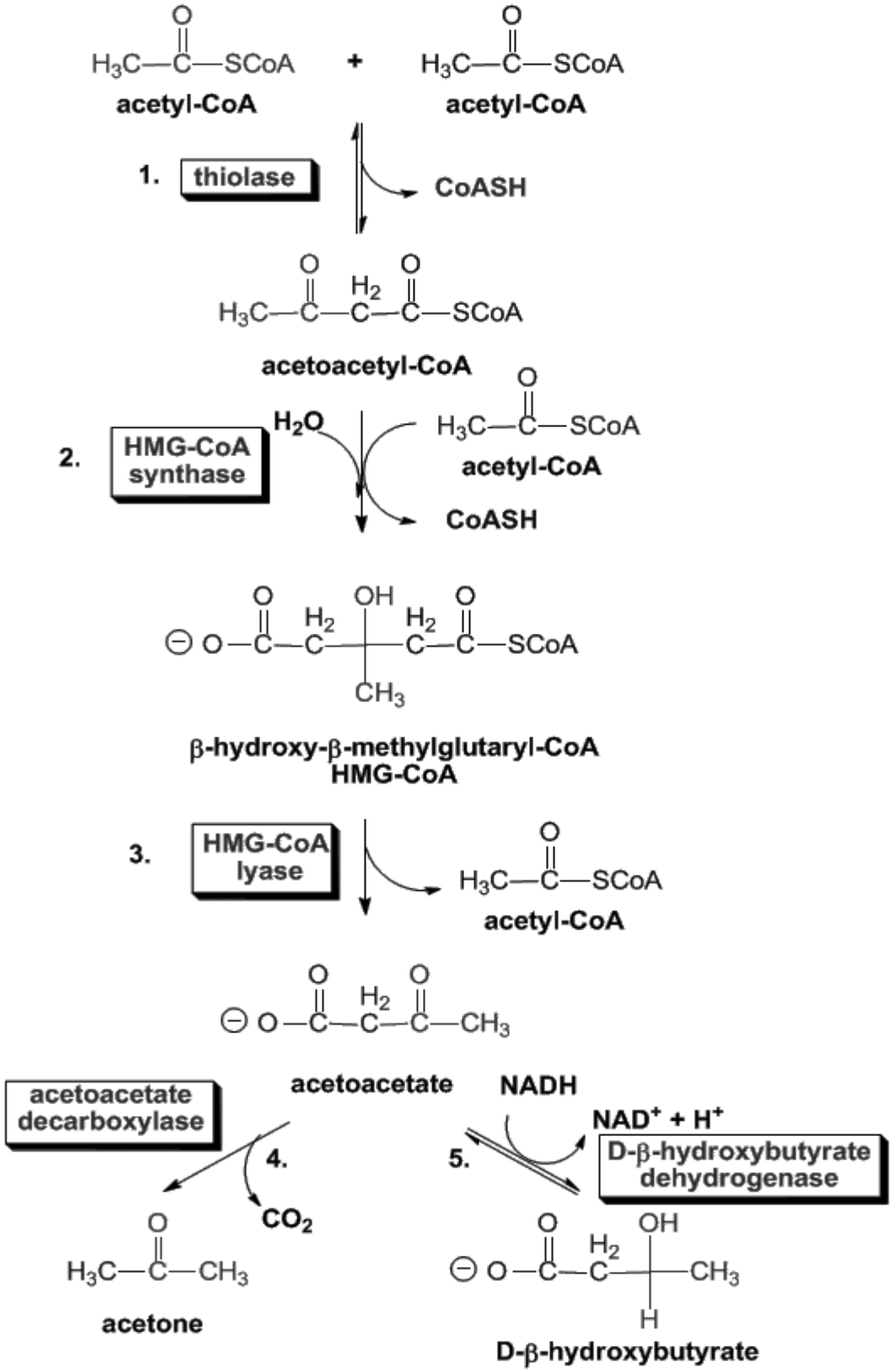


Figure 21.7 – Synthesis of ketone bodies

The rate of synthesis of ketone bodies depends on the activity of 3-

hydroxy-3-methylglutaryl-CoA synthase (HMG-CoA synthase). This enzyme is inducible, its synthesis increases with increasing the concentration of fatty acids in the blood. HMG-CoA synthase is inhibited by high concentrations of free CoA. A small amount of ketone bodies (their concentration in the blood of 10-30 mg/L or up to 0.2 mmol/L) is the norm. In the liver acetoacetate can not be oxidized, so it flows with the blood into skeletal muscle, heart, brain, which is capable of converting acetoacetic acid again to acetyl-CoA.

Content of ketone bodies in the blood increases, when fatty acids become the main source of energy for the body (in the prolonged muscular work, starvation, diabetes mellitus).

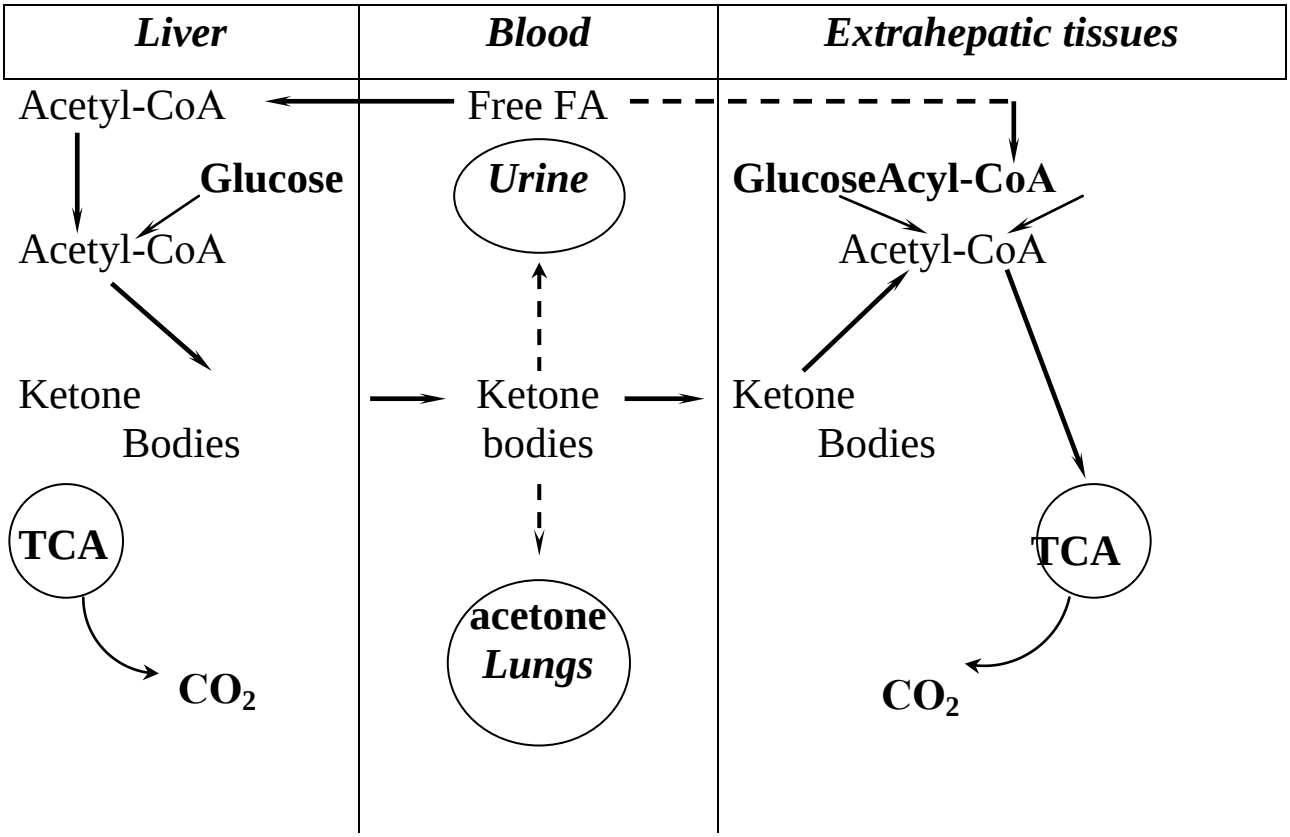


Figure 21.8 – Formation, utilization, and excretion of ketone bodies (the main path shown by continuous arrows)

Increase of ketone bodies concentration in the blood is called **ketonemia**, the allocation of ketone bodies in the urine is called **ketonuria**. Accumulation of ketone bodies in the body leads to **ketoacidosis**: alkali reserve reduces, and in severe cases – a shift of pH occurs, as β-hydroxybutyrate and acetoacetate are water-soluble organic acids capable of dissociation. Acidosis reaches dangerous quantities in case of diabetes

melitus. The content of ketone bodies in the blood in this disease increases 100 and more times, achieving a concentration of 4-5 g/L. Severe form of acidosis is one of the main causes of death in diabetes mellitus.

Synthesis of fatty acids

Synthesis of fatty acid occurs mainly in the liver, to a lesser extent, in adipose tissue, and lactating mammary gland. Glycolysis and subsequent oxidative decarboxylation of pyruvate promote increased concentrations of acetyl-CoA in the mitochondrial matrix. Synthesis of fatty acids occurs in the cytosol and where the substrate is to be transported. For this purpose, in mitochondrial matrix acetyl-CoA is condensed with oxaloacetate and form citrate. Then translocase carries citrate into the cytoplasm. This only occurs when the concentration of citrate in the mitochondria is high, where isocitrate dehydrogenase and alfa-ketoglutarate dehydrogenase are inhibited by high concentrations of ATP and NADH. Such a situation is created in the absorptive period when a liver cell gets enough energy. In the cytoplasm, citrate is split to oxaloacetate and acetyl-CoA. Last is the starting substrate for the synthesis of fatty acids and oxaloacetate by the action of malate dehidrogenase converted to malate, which by the action of malic enzym, tansforms to pyruvate. Pyruvate is transported back to the mitochondrial matrix.

The first reaction of fatty acid synthesis is the conversion of acetyl-CoA to malonyl-CoA. This reaction is carried out by acetyl-CoA carboxylase, determining the rate of subsequent reactions of fatty acid synthesis.

Next, the synthesis of fatty acids proceeds by multienzyme complexes – the fatty acid synthase. This enzyme consists of two identical protomers, each of which has a domain structure, and accordingly, 7 enzymatic centers, having different catalytic activities (atsetyltransferase, malonyltransferase, ketoacylsynthase, ketoacylreductase, hydratase, enoyl reductase, and thioesterase) and **acyl carrier protein (ACP)**.

ACP is not an enzyme, its function is only associated with the transfer of acyl radicals. In the synthesis, the important role is played by SH-group. One of them belongs to the 4-phosphopantetheine, part of the ACP, the second belongs to cysteine of ketoacyl synthase.

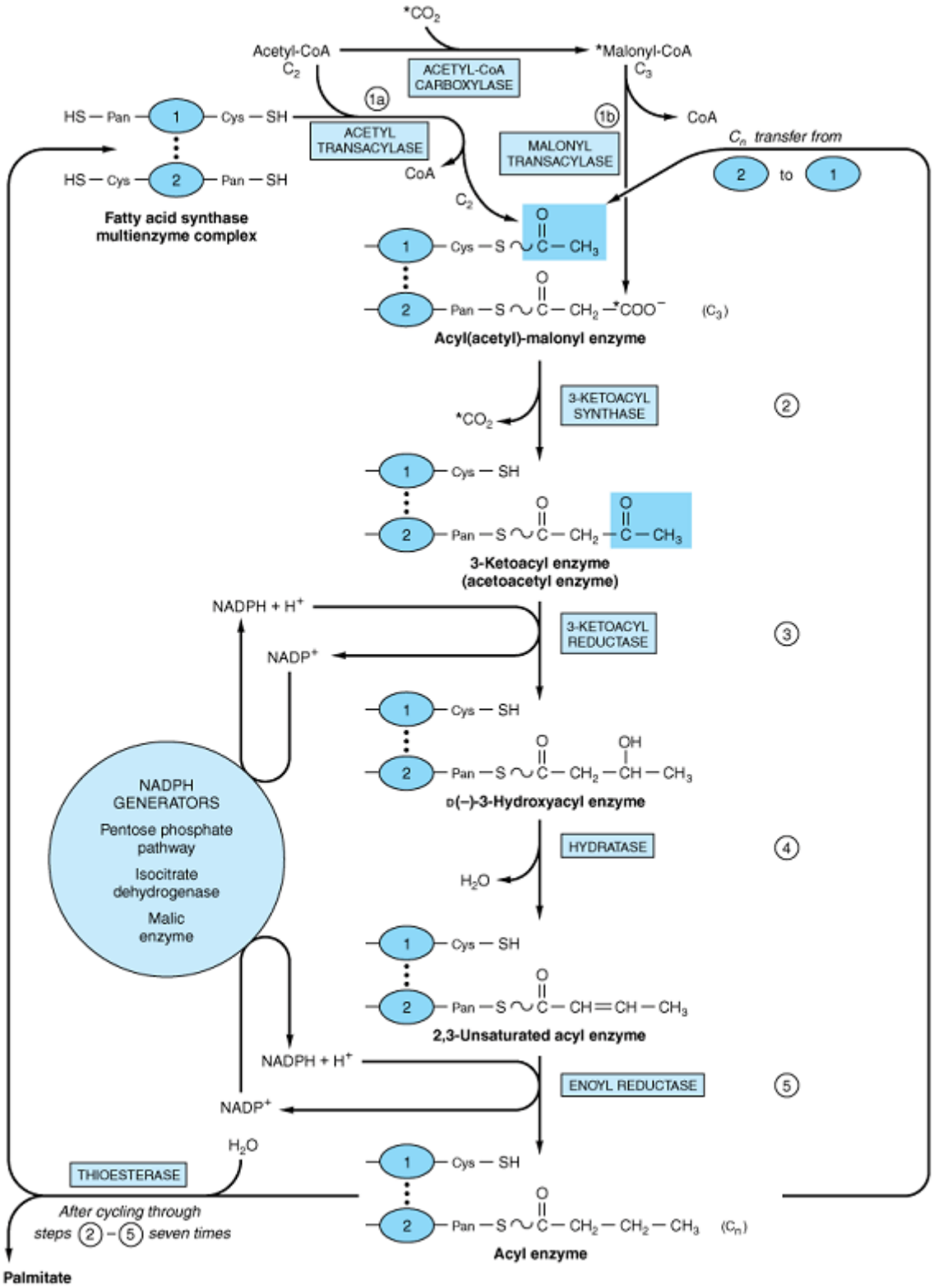


Figure. 21.9 – Synthesis of palmitic acid

This complex series extends fatty acid radical at 2 carbon atoms, which serves as a donor of malonyl-CoA. Reaction cycles are repeated until a radical of palmitic acid is formed, which under the hydrolytic action of tioesterase, is separated from the enzyme complex, turning the free palmitic acid. In each

cycle, in biosynthesis of palmitic acid, there are two reduction reactions in which the hydrogen donor is NADPH (Fig. 21.9).

Regulation of fatty acid synthesis. Regulatory enzyme in fatty acid synthesis is **acetyl-CoA carboxylase**. Its activity is regulated by two ways:

1. Association/dissociation of subunits. In an inactive form of acetyl-CoA carboxylase is an individual complex, consisting of four subunits. Enzyme **activator – citrate** – stimulates association, complexes **inhibitor** – **palmitoyl-CoA** – causes them to dissociate.

2. The phosphorylation/dephosphorylation of acetyl-CoA carboxylase. In the post absorptive state or physical work, **glucagon or adrenaline** through adenylatecyclase system activates protein kinase A, stimulating phosphorylation of subunits of acetyl-CoA carboxylase. In phosphorylated enzyme activity, fatty acid synthesis is stopped.

Another way to enhance the synthesis of fatty acids is the induction of synthesis of the enzymes of the metabolic pathway. This happens in the long-term consumption of food rich in carbohydrates and fats, poor food when insulin stimulates the induction of synthesis of acetyl-CoA carboxylase, fatty acid synthase, isocitrate dehydrogenase, and citrate lyase.

From palmitic acid fatty acids with long carbon chain (more then 16), as well as unsaturated fatty acids, can be synthesized.

Palmitic acid elongation may occur:

a) in mitochondria by the addition of acetyl-CoA by way of reverse reaction of β-oxidation using NADPH instead $FADN_2$;

b) in microsomes through malonyl-CoA and NADPH. The process is the same that in synthase complex in the cytosol, but intermediate products of the process are not bound to ACP.

Formation of double bonds in fatty acid structure also occurs in microsomes using oxidases, wherein NADPH and O_2 are used.

CHAPTER 22

METABOLISM OF COMPLEX LIPIDS

Complex lipids include such compounds which contain both the lipid and non-lipid component (protein, carbohydrate or phosphate). Accordingly there are a proteolipids, glycolipids and phospholipids. Unlike the simple lipid used as the energetic material, the complex lipids perform plastic functions and are used mainly as structural components of biological membranes. Proteolipids are structural components in the myelin sheath of nerve cells, in the synaptic membranes and inner membranes of mitochondria. Glycolipids are involved in the functioning of membranes: the processes involved in the reception, participate in the control and regulation of cell-cell interactions, have the high tissue specificity and act as cell surface antigens. Phospholipids (PhL) play an important role in the structure and function of cell membranes and activation of lysosomal membrane enzymes, nerve impulse conduction, blood coagulation, immunologic reactions, the processes of cell proliferation and tissue regeneration, in the transfer of electrons in the ETC.

Metabolism of phospholipids

Formation of PhL occurs most rapidly in the liver, the intestinal wall, testes, ovaries, and breast. Synthesis of PhL containing choline and ethanolamine begins with activation of nitrogenous bases, with the participation of ATP and related kinases. In the synthesis of phosphatidylinositol at the first stage phosphatidic acid enters into reaction with CTP, resulting in the formation of citidyn diphosphate diacylglicerol, which reacts with inositol to form phosphatidylinositol.

Besides the synthetic routes of individual PhL, there are routs of their interconversion, the importance of which, obviously, is conditioned by the necessity to supply the tissues with the required PhL at the right moment.

For the synthesis of phosphatidylcholine, and to a lesser extent – sphingomyelin choline or methionine is necessary, the demand for which is mainly covered by food sources. Prolonged insufficiency of dietary choline and methionine is observed in the development of **fatty infiltration of the liver**, in which the lipid content, mainly TAG can increase to 45% of the organ dry weight, compared to 7-14% in a healthy person. Mechanism of development of fatty liver disease is associated with a deficiency of phosphatidylcholine and sphingomyelin necessary for the formation of LP.

In the formation of LP, along with PhL significant amounts of TAG and cholesterol are used. LP rich in triacylglycerol (VLDL) are formed in the liver and delivered into the bloodstream. Therefore, the formation of LP can be viewed as an important way to dispose hepatic lipids. Therefore, the lack of synthesis of choline containing PhL in the liver impaires formation of LP and leads to the accumulation of cholesterol and TAG in the body/liver. For this reason, **choline, methionine**, and also **phosphatidylcholine** belong to the group of **lipotropic agents**, received with food, and preventing the development of fatty liver.

Breakdown of phospholipids may occur with the participation of several enzymes, each of which catalyzes the hydrolytic breakdown of srictly localized bond. Hydrolysis of some PhL under the action of phospholipases is important not only as a way of catabolism, but also as a pathway for the **formation of eicosanoids**. Furthermore, phospholipase A_1 and A_2 are involved in changing the fatty acid composition of the PhL, for example in the synthesis of dipalmitoylphosphatidylcholine – surfactant component in the embryonic period.

Metabolism of sphingolipids

At the first stage of glycolipids and sphingomyelin (sphingolipids) synthesis sphingosine is formed. This takes place by condensation with palmitoyl-CoA involving serine, pyridoxal phosphate (PALP), and manganese ions (Figure 22.1).

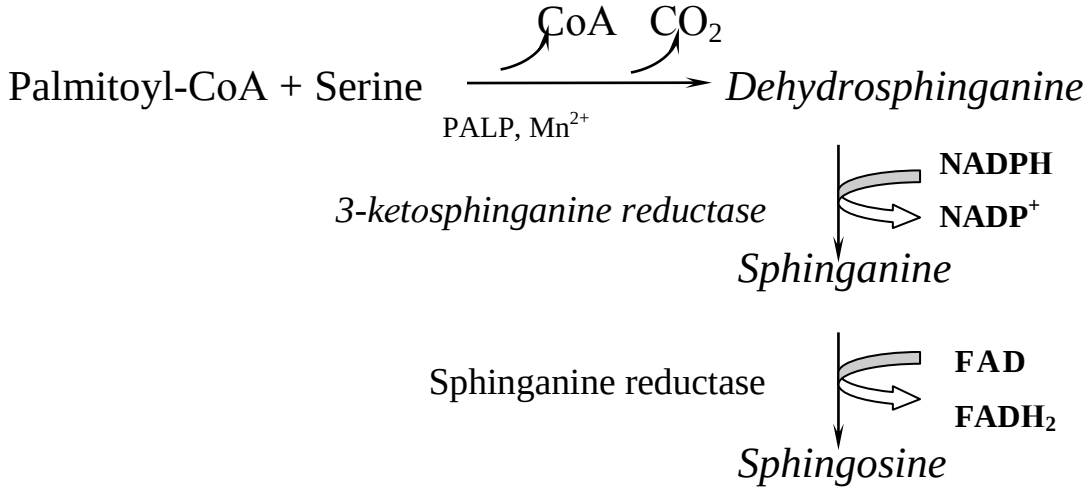


Figure 22.1 – Scheme of the sphingosine synthesis

Sphingosine is subjected to acylation (accession of fatty acid residue). In result ceramide is synthesized which is the precursor of cerebrosides, gangliosides, sulfatides, and sphingomyelin (Figure 22.2).

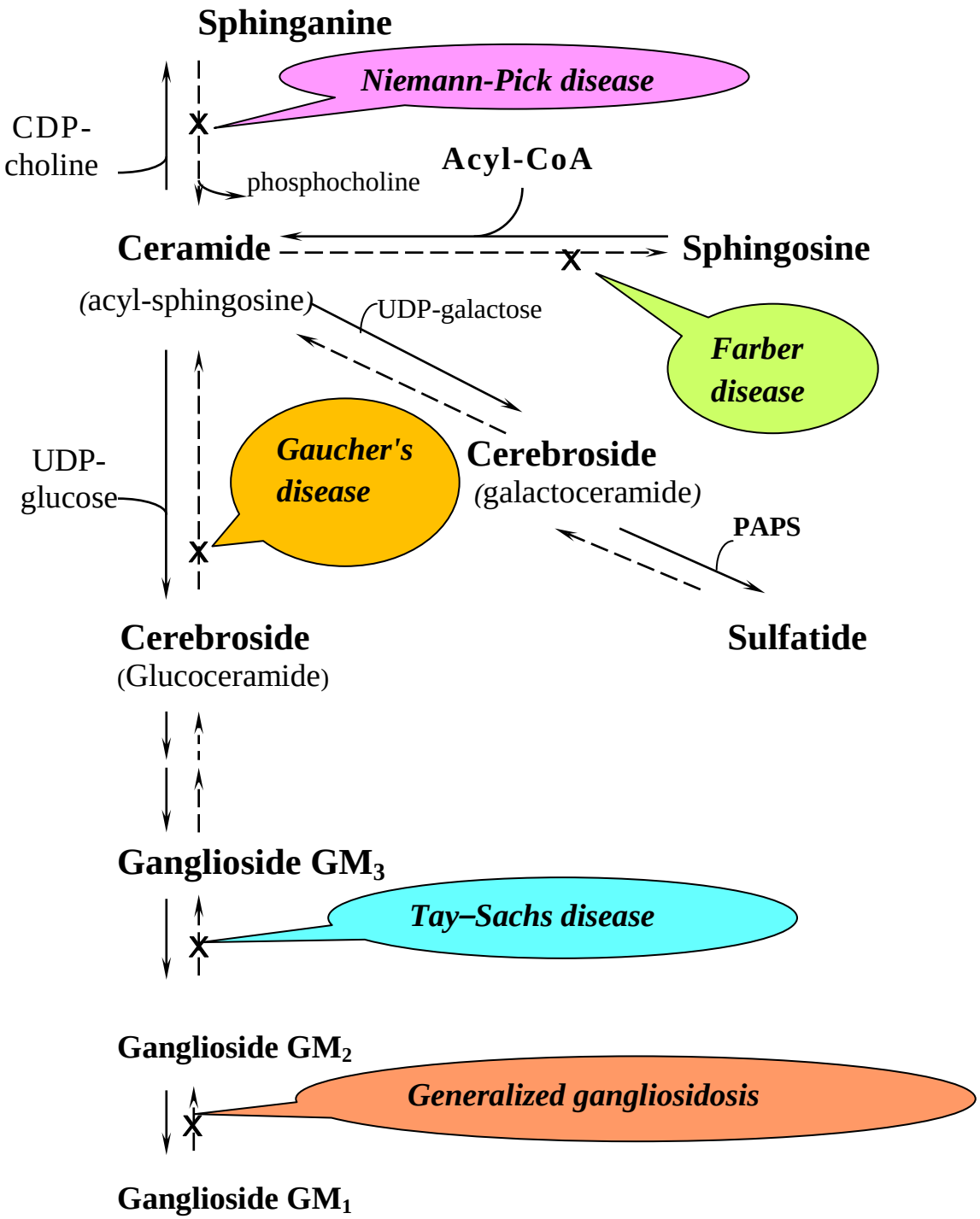


Figure 22.2 – Metabolism of sphingolipids.
Biosynthesis (⟶) and degradation (⇢).

Catabolism of sphingomyelins and glycolipids occurs in lysosomes. A crucial aspect of this process is the existence of more than ten specific lysosomal storage diseases – **sphingolipidoses**. They usually cause mental retardation and lead to death at an early age as a result of nervous tissue cells destruction due to glycolipids accumulation.

In the breakdown of sphingomyelins (Figure 22.2) sphingomyelinase, which cleaves phosphocholine, participates. **Sphingomyelinase** genetic defect causes **Niemann-Pick disease**. Children with this defect die at an early age. Symptoms of the disease are: accumulation of sphingomyelin in lysosomes, mental retardation, hepatosplenomegaly.

Complex glycolipid molecules are cleaved by a sequence of reactions of hydrolysis to glucose, galactose, and other metabolites of ceramide. Genetic defects in any of the enzymes of this class provide lipid catabolism, lead to development of diseases, among which can be mentioned:

• **Gaucher disease** – a consequence of a defect **β-glucosidase**, in which hepatosplenomegaly and mental retardation are observed;

•**Tay-Sachs disease** – a consequence of a defect **β- hexosaminidase**, which is characterized by mental retardation and blindness;

• **Generalized gangliosidosis** caused by decreased activity of **β-galactosidase**, also leading to mental retardation.

Degradation of ceramide to sphingosine and fatty acids is carried out by **ceraminidase**. Genetic block of this enzyme leads to the development of the **Farber disease**, fatal at an early age. In this pathology, ceramide accumulates in lysosomes, there is also hepatosplenomegaly, mental retardation, and joints damage.

CHAPTER 23

CHOLESTEROL METABOLISM. BIOCHEMISTRY OF ATHEROSCLEROSIS

Cholesterol is a steroid, characteristic only of animal organisms. The main place of its synthesis in the human body is the liver, where 50% of cholesterol is synthesized, in the small intestine only 15-20% of cholesterol is formed, the remainder is synthesised in the skin, adrenal cortex and gonads. Sources of formation of cholesterol and the ways of its use are illustrated in the figure 22.1.

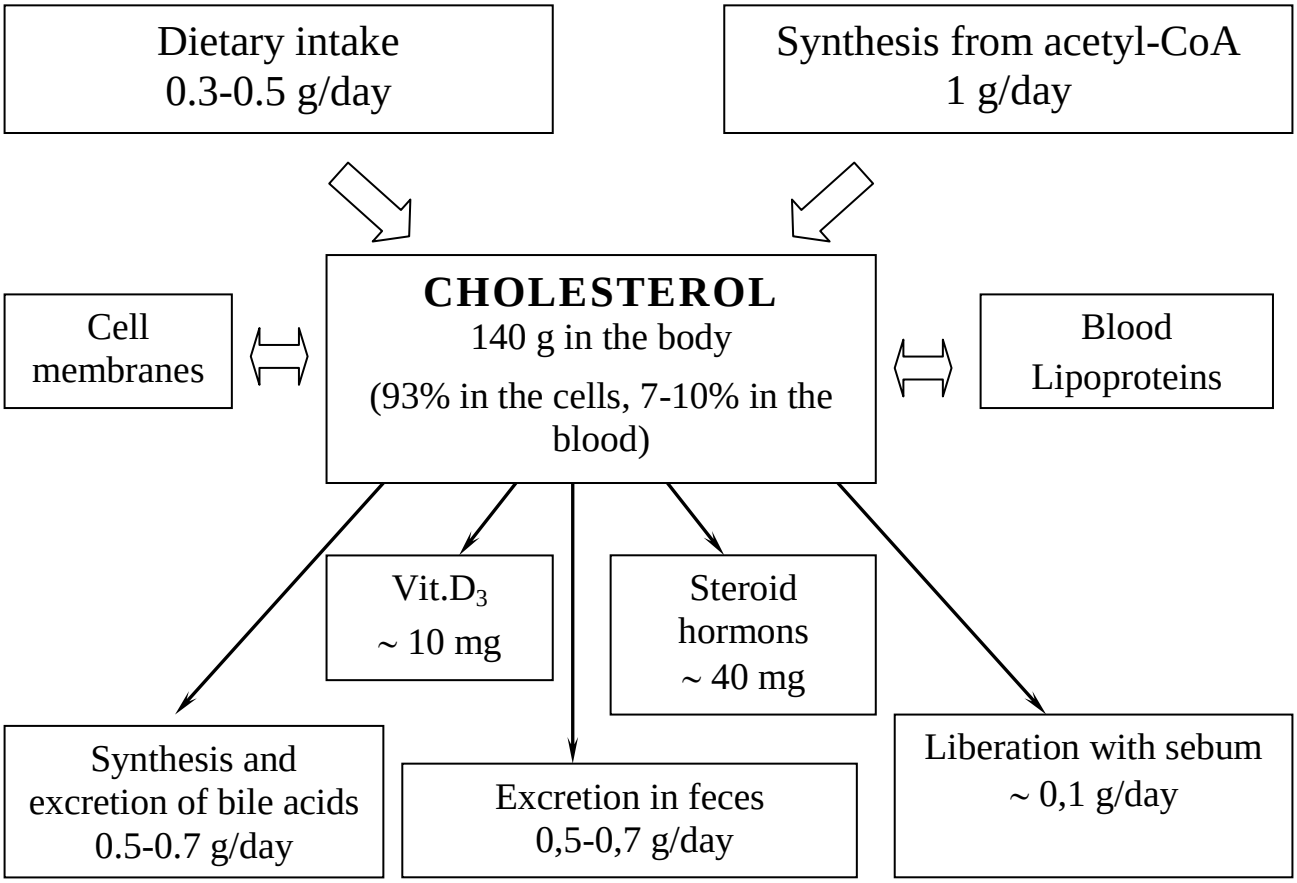


Figure 23.1 – Formation and distribution of cholesterol in the body.

Cholesterol in human organism (total amount about 140 g) can be divided into three pools:

- **pool A** (30 g) quickly metabolized – includes the intestinal wall cholesterol, blood plasma, liver or other organs, renewal occurs after 30 days (1 g/day);

- **pool B** (50 g), slowly methabolised cholesterol of other organs and

tissues;

- **pool C** (60 g), very slowly methabolised cholesterol of spinal cord and brain, connective tissue, its renewal takes years.

Cholesterol synthesis occurs in the cytosol of cells. It is one of the longest of the metabolic pathways in the body. It takes place in three stages: the **first stage** is the **formation of mevalonic acid,** the **second** is the formation of **squalene** (hydrocarbon sceleton consisting of 30 carbon atoms). The **third** phase is convertion of squalene molecule into lanosterol, then there go 20 consecutive reactions converting lanosterol to **cholesterol** (Fig.23.2).

In some tissues, the hydroxyl group is esterified to form cholesterol esters. Reaction is catalyzed by intracellular enzyme acylCoA:cholesterol acyltransferase. The esterification reaction also occurs in blood HDL, where the enzyme lecithin:cholesterol acyltransferase, acts. Cholesterol esters are the form in which it is transported or deposited in the blood cells. In the blood about 75 % of cholesterol is in the form of esters.

Regulation of cholesterol synthesis is carried out by affecting the activity of the enzyme **3-hydroxy-3-methylglutaryl-CoA reductase** (HMG-CoA reductase) and its amount. This is achieved in two ways:

1. **Phosphorylation/dephosphorylation** of HMG-CoA reductase. Insulin stimulates the dephosphorylation of HMG-CoA reductase, thereby converting it into an active state. Therefore, in the absorptive period cholesterol synthesis increases. In this period availability of the starting substrate (acetyl-CoA) increases. Glucagon has the opposite effect: through the protein kinase A stimulates phosphorylation of HMG-CoA reductase, converting it to an inactive state. As a result, the synthesis of cholesterol in the post absorptive period and fasting becomes inhibited.

2**. Inhibition of HMG-CoA reductase**. Cholesterol (final product of the metabolic pathway) reduces transcription of gene of HMG – CoA reductase, thereby inhibiting its own synthesis. Bile acids have the same effect.

Cholesterol transport in the blood is carried out as part of the LP. LP provide a flow of exogenous cholesterol in the tissue, define its flows between the organs and excretion from the body. Exogenous cholesterol is delivered to the liver comprising residual ChM. Along with the synthesized there endogenous cholesterol, it forms the common fund. In hepatocytes TAG and cholesterol are packed in VLDL, and in this form are secreted into the blood. In the blood, under the influence of LP lipase, VLDL hydrolyzes

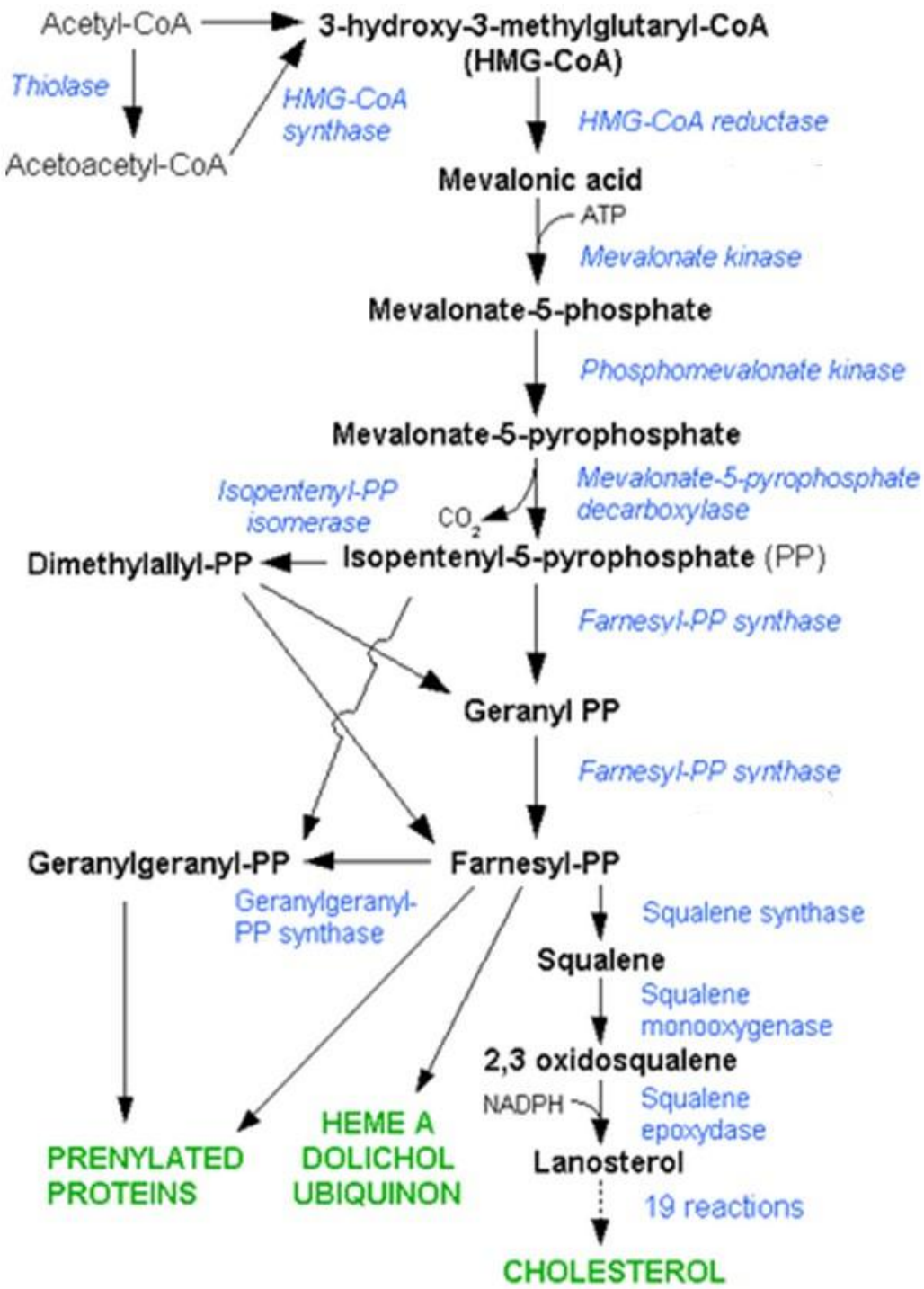


Figure 23.2 – Synthesis of cholesterol

TAG till glycerol and fatty acids in the first transform into LID, and then to LDL containing up to 55% of cholesterol and its esters. LDL is the basic transport form of cholesterol in which it is delivered to the tissue (70% of cholesterol and its esters in blood is composed of LDL). LDL from the blood goes to the liver (75%) and other tissues which have on their surface LDL receptors.

If the amount of cholesterol entering the cell exceeds the demand, the

synthesis of LDL receptors is suppressed, which reduces the flow of blood cholesterol. In reduced concentration of free cholesterol in the cell, on the contrary, the synthesis of activated receptors occurs. In regulation of the synthesis of LDL receptors the following hormones are involved: insulin, triiodothyronine, and sex hormones, which increase the formation of receptors, and glucocorticoids, give the opposit effect.

In the ways ensuring the return of cholesterol to the liver (so-called "reverse cholesterol transport"), the major role is played by HDL. They are synthesised in the liver as immature precursors that are essentially free from cholesterol and TAG. In blood, precursor of HDL is saturated by cholesterol, getting it from other LP and cell membranes. In the transference of cholesterol in HDL enzyme **lecithin:cholesterol acyltransferase** is involved, located on their surface. This enzyme attaches the fatty acid residue of phosphatidylcholine (lecithin) to the cholesterol. In result, the hydrophobic molecule of cholesterol ester is formed, which is moved into HDL. Thus, unmature HDL, enriched by cholesterol, transform into HDL_3 – mature and larger size particles. HDL_3 exchange cholesterol esters on TAG, contained in VLDL and LID with the participation of a specific protein, cholesterol ester transfers between lipoproteins.

The synthesis of bile acids

In the liver, from cholesterol 500-700 mg of the bile acid per day is synthesised. Their formation includes reaction of hydroxyl groups incorporation involving hydroxylases and partial oxidation reaction of cholesterol side chain (Figure 22.3).

The first reaction of synthesis is regulatory (formation of 7-α-hydroxycholesterol). The activity of the enzyme catalyzing this reaction is inhibited by the end product of the way – bile acid. Another mechanism is the regulation of phosphorylation/dephosphorylation of the enzyme (active phosphorylated form of 7-α- hydroxylase). The possible mechanism of regulation is changing the amount of the enzyme: cholesterol induces transcription of the gene 7-α-hydroxylase and bile acids repress. Thyroid hormones induce the synthesis of 7-α-hydroxylase and estrogen inhibits it. This effect of estrogen on bile acid synthesis explains why gallstone disease is common in women 3-4 times more often than in men.

Formed from cholesterol cholic and chenodeoxycholic acids are called "primary bile acids". These acids mostly undergo conjugation, namely, joining molecules of glycine or taurine to the carboxyl group of bile acids.

Conjugation begins with the formation of active forms of bile acid-CoA derivatives and then taurine or glycine becomes attached, and the result is 4 types of conjugates: taurocholic and taurochenodeoxycholic, glycocholic and glycochenodeoxycholic acid.

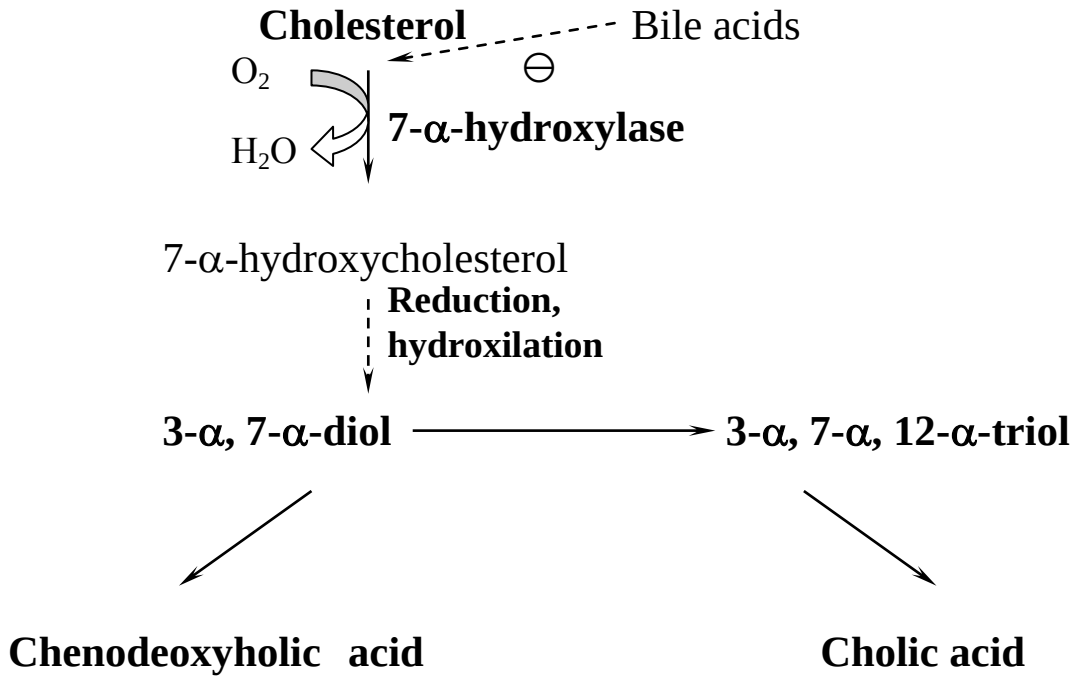


Figure 23.3 – The scheme of the bile acid synthesis

They are much stronger emulsifiers than the primary bile acid. There are 3 times more glycine conjugates than taurine ones, as the amount of taurine in the body is limited. In the intestine, a small amount of primary bile acid conjugates under the action of bacterial enzymes converted into secondary bile acids. Deoxycholic acid, formed from cholic acid, and lithocholic one, formed from deoxycholic acid, have has poor solubility and are slower absorbed in the intestine.

About 95% of bile acids secreted in the intestine return back to the liver via the portal vein, and then again secreted into the bile, and re- used in the emulsification of fats. This path is called bile acid enterohepatic circulation. With feces mainly secondary bile acids are removed.

Gallstone disease is a pathological process in which the gallbladder stones are formed.

Secretion of cholesterol in the bile must be accompanied by a proportional allocation of bile acids and phospholipids, cholesterol retaining

hydrophobic molecules in a micellar state. Causes leading to the change in the ratio of bile acids and cholesterol in bile are foods rich in cholesterol, high-calorie food, stagnation of bile in the gallbladder, violation of the enterohepatic circulation of bile acid synthesis disorders, gall bladder infection.

Most patients with gallstone disease have increased synthesis of cholesterol, and synthesis of bile acids is slowed, which leads to a disruption of the amount of cholesterol, and bile acids are secreted into the bile. As a result, cholesterol begins to precipitate in the gallbladder, forming a viscous precipitate which slowly solidifies. Sometimes it is impregnated by bilirubin, proteins, and calcium salts. Stones can consist only of cholesterol (cholesterol stones) or a mixture of cholesterol, bilirubin, protein, and calcium. Cholesterol stones are usually white, and mixed (different shades of brown).

In the initial stage of stones formation chenodeoxycholic acid can be used as a medicine. Getting into the gallbladder, it gradually dissolves cholesterol stones, but it is a very slow process, lasting several months.

Biochemistry of atherosclerosis

Atherosclerosis is a pathology characterized by the appearance of atherogenic plaques on the inner surface of the vascular wall. One of the main causes of this disease is an imbalance between the supply of cholesterol from food, its synthesis, and excretion from the body. In patients suffering from atherosclerosis, elevated concentrations of LDL and VLDL is observed. There is an inverse relationship between HDL cholesterol concentration and the likelihood of atherosclerosis.

The basic metabolic reason of atherosclerosis development is hypercholesterolemia (high level of cholesterol in the blood).

Hypercholesterolemia develops:

-due to excess income of cholesterol, carbohydrates and fats;

-due to genetic predisposition, genetic defects consisting in LDL receptors or structures of apoB-100 as well as the increased synthesis or secretion of apoB-100 (in case of familial combined hyperlipidaemia, in which HDL and TAG concentration in the blood is increased).

Important role in the mechanisms of atherosclerosis is played by modification of LP. Changes in the normal structure of the lipid and protein composition in LDL make them foreign to the body, and therefore more accessible for gripping by phagocytes. **Modification of LP** may occur

through several mechanisms:

- **glycosylation** of proteins occurs with increasing concentration of glucose in blood;

- **peroxide modification**, leading to changes in lipid and lipoprotein structure of apoB-100;

- **formation of autoimmune complexes** (LP-antibody). Modified LP may cause the formation of autoantibodies.

Modified LDL are ingested by macrophages. This process is not regulated by the amount of cholesterol absorbed as if it enters cells via specific receptors, so macrophages become overloaded with cholesterol and turn into “foam cells” penetrating into the subendothelial space. This leads to the formation of lipid spots or strips in the wall of blood vessels. At this stage, the vascular endothelium can maintain its structure. The increase in the amount of foam cells leads to endothelium damage. This damage facilitates platelets activation. As a result, they secrete thromboxane, which stimulates platelet aggregation and platelet starts to produce a growth factor that stimulates the proliferation of smooth muscle cells. These cells migrate from the medial layer to the inner surface of the arterial wall, thus contributing to plaque growth. Further, there is a germination of the fibrous tissue into a plaque, the cells necrotize under the fibrous sheath and cholesterol is deposited in the intercellular space of the arterial wall. In the late stages of development, the plaque is impregnated with calcium salts and becomes very dense. In the plaques thrombi are often formed overlying the vessel lumen, leading to acute circulatory problems in the relevant section and myocardial tissue (Fig.23.4).

Biochemical basis of atherosclerosis treatment. Important therapeutic factor that reduces the risk of hypercholesterolemia and atherosclerosis is hypocaloric and hypolipidemic diet. Daily cholesterol intake will not exceed 300 mg/day. Curative and preventive factors include food rich in polyene fatty acids, which reduce the risk of thrombosis and promote the excretion of cholesterol from the body. Vitamins C, E, A, antioxidant properties inhibit lipid peroxidation, thereby maintaining normal structure and metabolism of LDL.

In this case, treatment of atherosclerosis is usually integrated. One of the principles of treatment is breaking the cycle of the enterohepatic circulation of bile acids. For this purpose cholisteramin – a polymer which adsorbs bile acid in the intestine, and is excreted with the faeces – reduces the bile acids returning to the liver. In the liver, it increases the capture of

blood cholesterol for the synthesis of bile acids.

The most effective drugs used in the treatment of atherosclerosis are inhibitors of HMG-CoA reductase. Such drugs can almost completely suppress the synthesis of cholesterol in the body. Under these circumstances the LDL flow from blood to the liver increases.

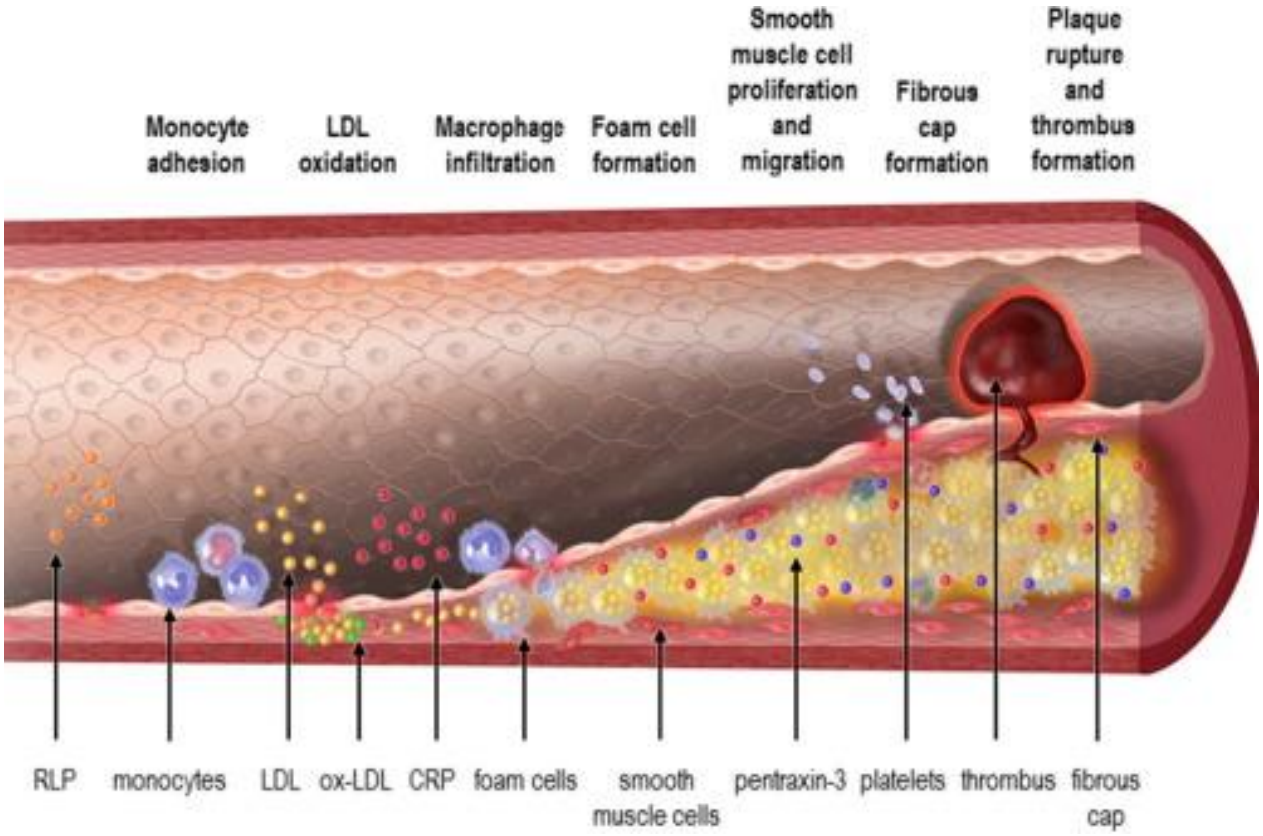


Figure 23.4 – Pathogenesis of atherosclerosis (RLP - remnant-like particle, LDL - low-density lipoprotein, ox-LDL - oxidized low-density lipoprotein, CRP - C-reactive protein).

Medications – fibrates – accelerate the catabolism of VLDL, activating LP lipase. These drugs also enhance the oxidation of fatty acids in the liver, thereby reducing the synthesis of cholesterol esters and TAG and, consequently, secretion of VLDL by the liver. Clofibrate induces the synthesis of enzymes of peroxisomes capable of oxidizing fatty acids. Fibrates are usually used in simultaneous gipertriglitserolemii and hypercholesterolemia. For effective treatment of atherosclerosis, usually, the combined effect of several drugs is used.

CHAPTER 24

DYNAMIC STATE OF BODY PROTEINS. METABOLISM OF AMINO ACIDS

Dynamic state of body proteins

In the body, there is dynamic equilibrium between synthesis and breakdown of substances. Almost all the body proteins are subjected to incessant breakdown and synthesis. The active re-synthesis of proteins occurs even under prolonged starvation, although an intensive degradation of protein is predominant in this state. Usually, **the protein breakdown in one tissue is accompanied by an increased biosynthesis of proteins in another tissue**. E.g., in starvation the liver, muscles, blood plasma, and intestinal mucosa are the first to lose mass, while the mass of the brain and heart remains unaffected. Amino acids released due to protein degradation and are used for the synthesis of absolutely essential proteins, enzymes, hormones. Thus, **body proteins are constantly being renewed.**

The rate of the body protein renewal is characterized by the **half-life** of the protein. E.g., most proteins of the liver, blood plasma, and intestinal mucosa are renewed within 10 days. The proteins of muscle, skin, and brain are renewed at a slower rate. The half-life for antibodies and a number of other blood plasma proteins is about two weeks. For a number of hormones, the half-life is several hours or even several minutes.

Excess amino acids are not stored. Amino acids not immediately incorporated into new proteins, are rapidly degraded: first the α-amino group is removed from an amino acid, and then its carbon skeleton is utilized in other metabolic pathways. The α-amino group is removed from amino acids in the form of ammonia. Free ammonia is very toxic; therefore humans convert it into urea which is excreted in the urine.

Urea is the major form of excreted nitrogen in humans. On an average, more than 80% of the excreted nitrogen is in the form of **urea**. Small amounts of nitrogen (~15% of total nitrogen excretion) are also excreted in the form of **uric acid**, **creatinine**, and **ammonium salts**.

Nitrogen balance

Nitrogen balance is defined as the difference between intake and output of nitrogen. There are three variants of nitrogen balance: nitrogen equilibrium, positive nitrogen balance, and negative nitrogen balance.

In the state of **nitrogen equilibrium**, the total nitrogen loss as end products excreted in the urine each day by the organism is equal to the amount of the total dietary intake of nitrogen supplied in the diet. In the state of nitrogen equilibrium, synthesis of body protein equals degradation. This state takes place in a healthy adult on a balanced diet with the normal daily supply of proteins.

The **positive nitrogen balance** occurs when the amount of nitrogen excreted from the organism is less than the amount of nitrogen supplied in food, and synthesis of body proteins exceeds degradation. Such a state takes place in a growing organism, pregnancy, and lactation.

The **negative nitrogen balance** occurs when the amount of nitrogen excreted by the organism is greater than the daily dietary intake of nitrogen, and degradation of body protein exceeds synthesis. This takes place in starvation, protein deficiency, aged persons, grave infectious, and chronic diseases, when the intensive breakdown of body proteins is not compensated for protein diet.

The intensity of the protein metabolism is regulated by definite **hormones**. Glucocorticoids, glucagon, and high concentrations of thyroid hormones cause active degradation of tissue proteins and lead to the negative nitrogen balance.

Growth hormone, androgens (male sex hormones), and insulin stimulate protein synthesis.

Dietary proteins

Proteins are not deposited in the body like reserves of carbohydrates (glycogen deposited in the liver and muscle) and lipids (triglycerides stored in fat depots); therefore the human organism is in permanent need of alimentary protein.

Dietary protein norms depend on professional occupations, energy expenditures, age. The daily requirement in proteins increases in pregnancy, lactation, and in certain pathologic states (e.g. in acute infectious diseases, burns).

According to the WHO/FAO recommendation in 1985, the level of protein intake for an adult is 0.75 g/kg/day (80-100 g/day).

Dietary proteins. Nutritional value of different proteins is not the same. The closer is the amino acid composition of alimentary protein to the amino acid composition of body proteins the higher is its biological value. Generally, proteins of animal origin are of higher biological value (meat,

milk, fish, cheese, and egg white).

These proteins of animal origin are called full-valued proteins (or **full-fledged proteins**) because they contain essential amino acids which are not synthesized in the human body (Table 24.1).

Thus, **dietary protein is the only source for essential amino acids in the organism**.

Protein synthesis is accomplished only if all the 20 amino acids are available in the cell. The dietary deficiency or lack of only one essential amino acid leads to incomplete assimilation of other amino acids, and may be a limiting factor in the synthesis of all proteins in the body.

Table 24.1. – **Essential and non-essential amino acids**

Essential amino acids	***Non-essential amino acids***
Arginine*	Alanine
Valine	Asparagine
Histidine*	Aspartate
Isoleucine	Glycine
Leucine	Glutamine
Lysine	Glutamate
Methionine	Proline
Threonine	Serine
Tryptophan	Tyrosine
Phenylalanine	Cysteine

* - Semi-essential amino acids

Sources of amino acids in the body

There are two sources of amino acids in the body: exogenous and endogenous. Exogenous source of amino acids is derived from dietary proteins, and it accounts for about ⅓ of the total amino acids presented in the body. Endogenous source of amino acids includes:

a) amino acids which are the product of hydrolysis of endogenous proteins (tissue proteins), and

b) amino acids which were synthesized in the organism *de novo* (non-essential amino acids).

The mixture of exogenous (⅓) and endogenous (⅔) amino acids is called **metabolic pool of amino acids**. It can be used as a source for anabolic and catabolic reactions of nitrogen metabolism (Fig. 24.1).

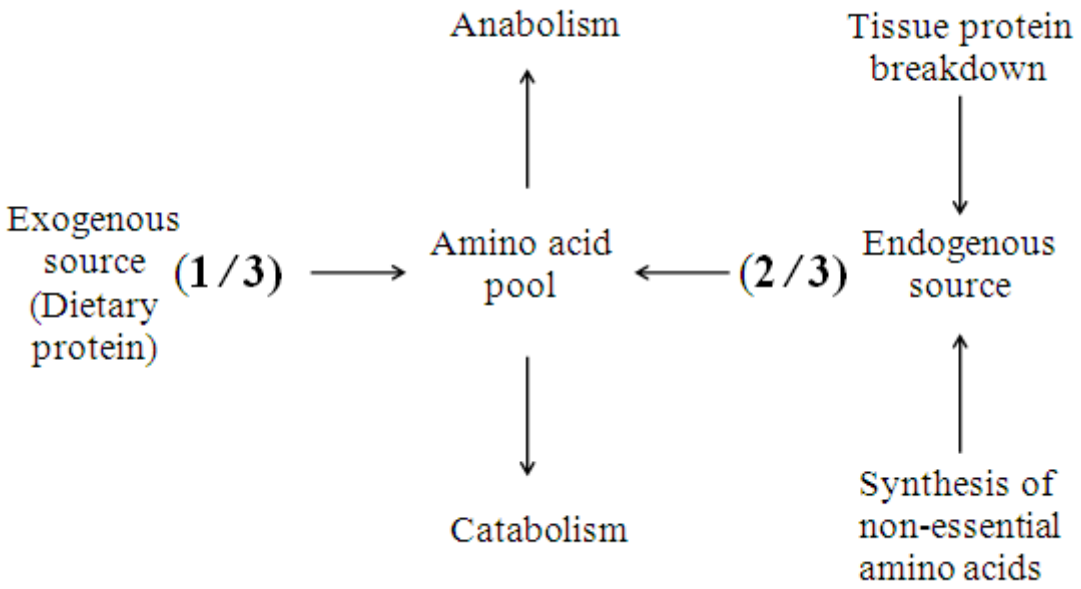


Figure 24.1 – Sources of amino acids in the body

Ways of the amino acids use

Amino acids are used as building blocks for synthesis of proteins and peptides. Some amino acids may be converted to other amino acids (synthesis of non-essential amino acids). The carbon skeletons of some amino acids can be used to produce glucose through gluconeogenesis in the liver; such amino acids are designated as glucogenic amino acids. The carbon skeletons of certain amino acids can produce acetyl CoA or acetoacetate; they are named ketogenic amino acids, and this indicates that they can be precursors of lipids (fat) and ketone bodies.

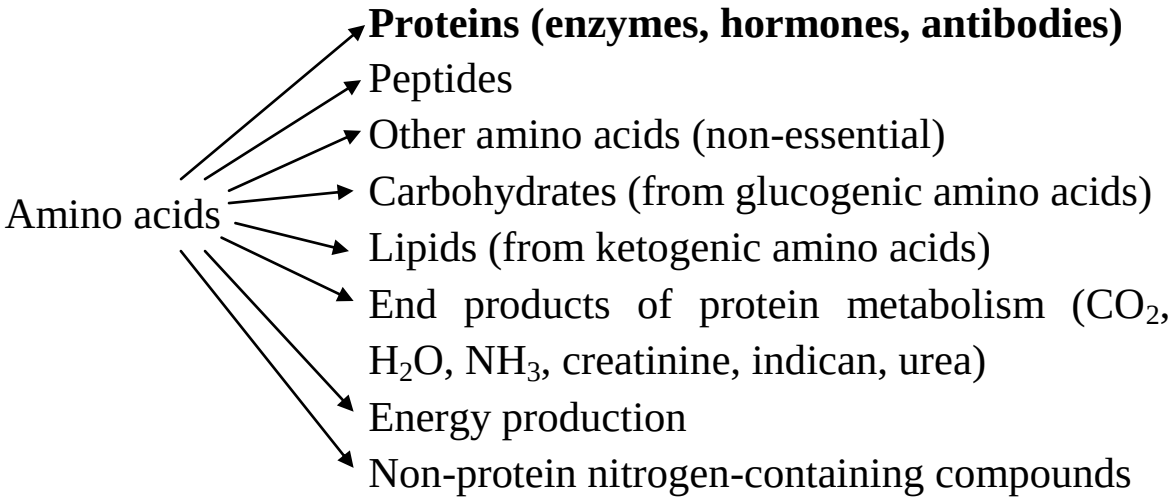


Figure 24.2 – Variety of routes to the use of amino acids in the body

Relatively little portion of amino acids is utilized for energy production, but in starvation, amino acids may act as energy sources.

Due to catabolism of amino acids, the end products are formed, such as CO_2, H_2O, NH_3, creatinine, indican, and urea. A portion of amino acids is utilized for the synthesis of specific non-protein nitrogen-containing compounds.

The variety of routes to the use of amino acids in the body is shown in the figure 24.2 and table 24.2.

Table 24.2. – **Non-protein nitrogen-containing compounds derived from amino acids**

Non-protein nitrogen-containing compounds	***Amino acids used for their synthesis***
Nitrogenous bases (purines, pyrimidines)	Glycine, glutamine, aspartate
Pigments (melanin)	Tyrosine
Porphyrins (heme)	Glycine
Hormones (thyroxine, adrenaline)	Tyrosine
Neurotransmitters: Norepinephrine, dopamine Serotonin Histamine γ-Aminobutyric acid	Tyrosine Tryptophan Histidine Glutamate
Vitamins (vitamin PP)	Tryptophan
Creatine	Arginine, glycine, methionine
Phospholipids: Phosphatidylserine Phosphatidylcholine	Serine Methionine

Digestion of proteins in the gastrointestinal tract

Dietary proteins can be used by the human organism only after their digestion (hydrolysis into free amino acids in the gastrointestinal tract). In the course of digestion, proteins undergo stepwise degradation by proteolytic enzymes (proteases) which cleave peptide bonds between amino acids. The major enzymes that catalyze the hydrolytic breakdown of dietary proteins and the steps of protein digestion are shown in figure 24.3.

Proteolytic enzymes are secreted as inactive zymogens which are converted to their active forms in the intestinal lumen.

Gastric digestion of proteins. In the stomach, hydrochloric acid is secreted. It makes the pH optimum for the action of pepsin and also activates pepsin. The acid also denatures proteins making them more easily digested.

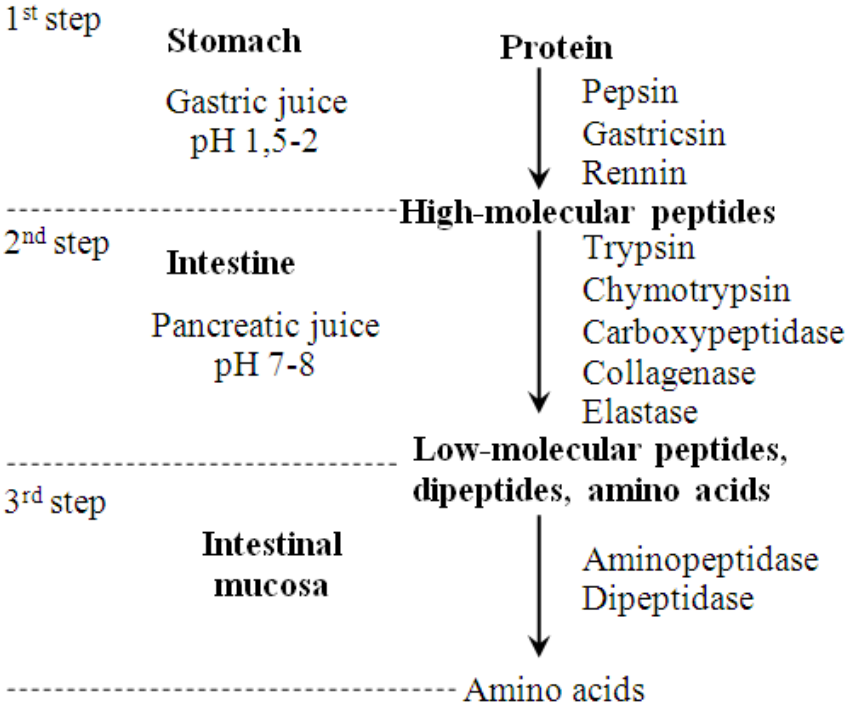


Figure 24.3. – Digestion of proteins in the gastrointestinal tract

The gastric juice contains three enzymes to digest proteins: **pepsin, gastricsin, and rennin.**

Pepsin is secreted by the chief cells of stomach as inactive pepsinogen, which is converted to pepsin. The conversion of pepsinogen to pepsin is brought about by removal of 44 amino acids from the N-terminal end, spontaneously by the hydrochloric acid. The optimum pH for activity of pepsin is around 2.0.

Gastricsin is a pepsin-like enzyme, which optimum pH is 3.0.

Rennin is active in infants and is involved in the curdling of milk. Its optimum pH is 4.5.

Pancreatic digestion of proteins. The optimum pH for the activity of pancreatic enzymes is 8.0. Pancreatic juice contains trypsin, chymotrypsin, carboxypeptidase, collagenase, and elastase. They are also secreted as zymogens (trypsinogen, chymotrypsinogen, procarboxypeptidase, procollagenase, proelastase). Trypsinogen is converted to the active trypsin by enterokinase by the removal of a hexapeptide from N-terminal end. Once activated, the trypsin activates other trypsin molecules.

All other enzymes present in the intestine are also activated by trypsin. These enzymes degrade the proteins into small peptides, dipeptides, tripeptides, and little amount of amino acids.

Intestinal digestion of proteins. Complete digestion of low-molecular peptides to amino acids is brought about by enzymes present in the intestinal juice.

Absorption of amino acids

The absorption of amino acids occurs mainly in the small intestine. There are several major mechanisms for absorption of amino acids.

Active transport of amino acids into intestinal epithelial cells. This is the energy requiring process, and the mechanism is very similar to that described for active transport of glucose. At the brush border membrane, there are Na^+-dependent symporters for amino acids. The transporter uptakes amino acids together with Na^+ and then pumps out Na^+ at the contraluminal membrane, using ATP energy (Fig. 24.4).

On the contraluminal surface, Na^+-independent transporters are present; they allow amino acids to enter the hepatic portal system (**facilitated diffusion**), Fig. 24.4.

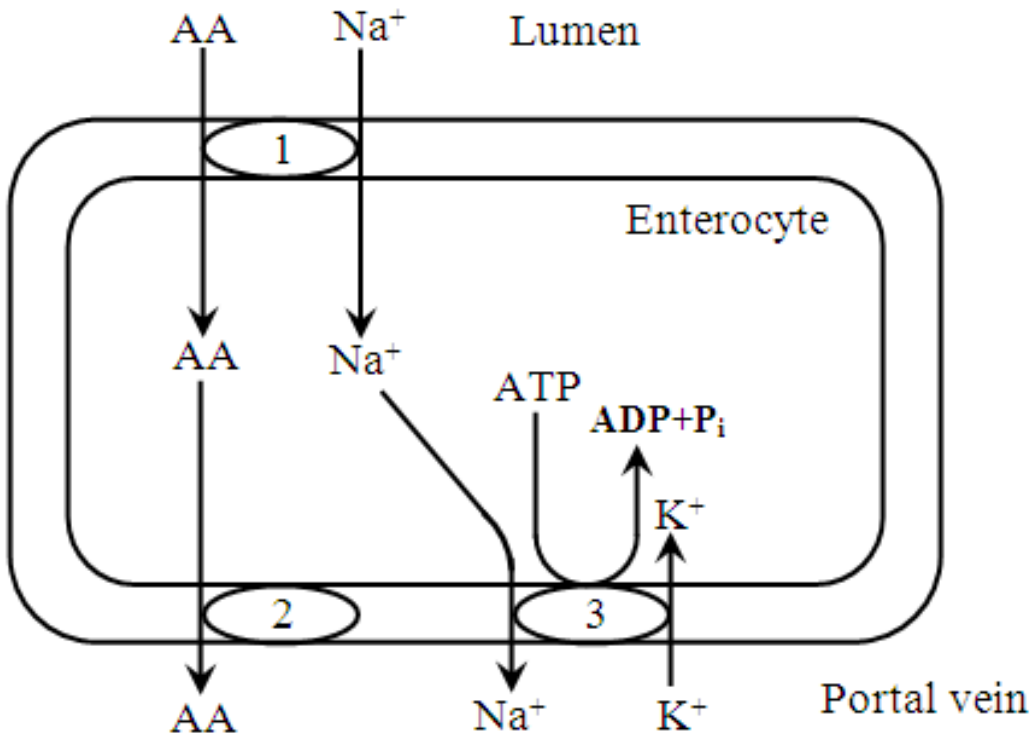


Figure 24.4 – Active transport of amino acids and facilitated diffusion. AA – amino acid; 1 – Na^+-dependent amino acid transporter (symporter); 2 – Na^+-independent amino acid transporter;

3 – Na^+,K^+-ATPase (sodium pump)

Gamma-glutamyl cycle

In the intestine, kidney tubules, and brain, the absorption of neutral amino acids is performed by the gamma-glutamyl cycle. The tripeptide **glutathione** (γ-glutamyl-cysteyl-glycine) consisting of γ-glutamic acid, cysteine, and glycine is involved into this process. Glutathione reacts with an amino acid to form gamma-glutamyl amino acid. The reaction is catalyzed by **γ-glutamyltranspeptidase** (or γ-glutamyltransferase). The glutamyl amino acid is then cleaved to give the free amino acid (inside the cell). Subsequently glutathione is resynthesized (Fig. 24.5).

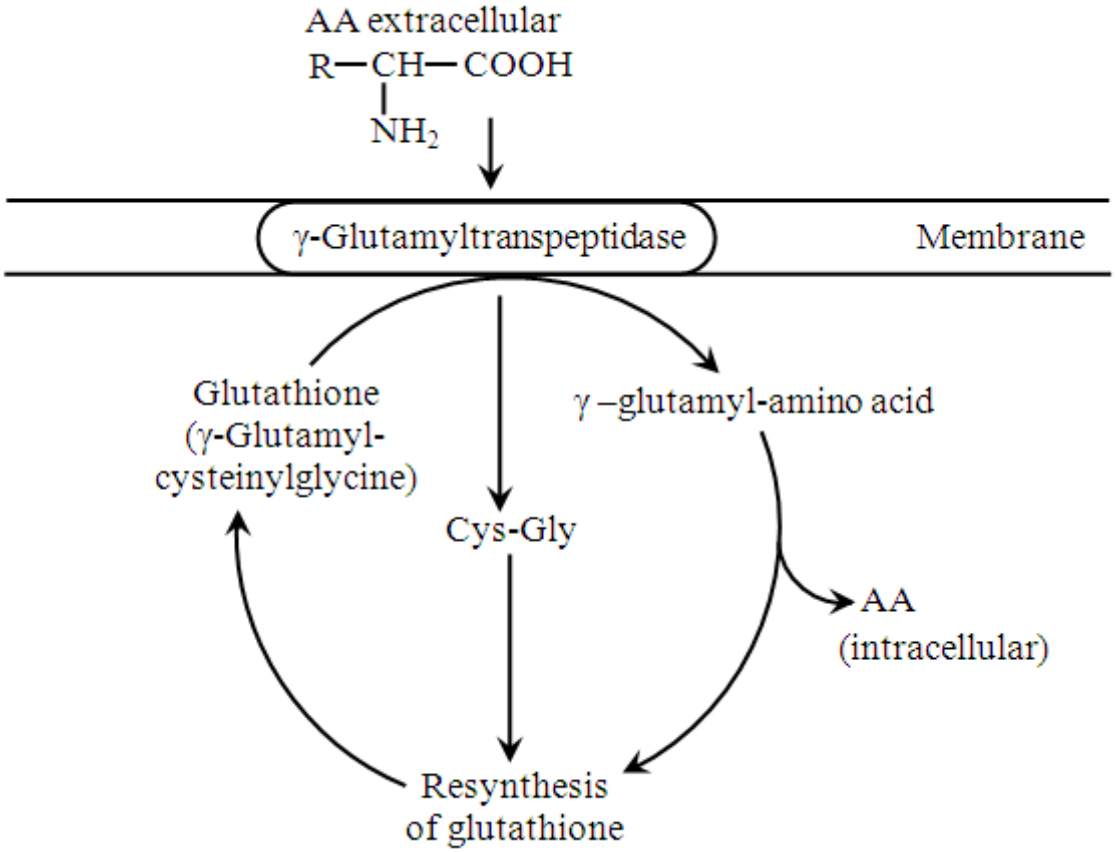


Figure 24.5 – Gamma-glutamyl cycle

Intestinal putrefaction of proteins (conversion of amino acids by intestinal bacteria)

Intestinal bacteria have enzymes which catalyze conversion of amino acids to definite toxic products. This process is called intestinal putrefaction of proteins.

1) Putrefaction of sulfur-containing amino acids (cysteine, cystine,

methionine) produces **hydrogen sulfide** (H_2S) and **methylmercaptan** (CH_3–SH), the products which are removed from the intestine with intestinal gas.

2) Putrefaction of diaminomonocarboxylic acids. Ornithine will produce putrescine, lysine – cadaverine. The toxic products (**putrescine** and **cadaverine**) are detoxified in enterocytes by diaminoxidases.

3) Putrefaction of aromatic amino acids:

Tyrosine → **Cresol** → **Phenol**

Tryptophan → **Scatole** → **Indole**

Skatole is excreted in the feces. Some portion of skatole is converted to indole. All these toxic products are delivered through the portal vein to the liver where they are detoxified. The liver contains enzymes which catalyze transferring the sulfuric acid residue from PAPS (phosphoadenosine phosphosulfate) or the glucuronic acid residue from UDP-glucuronate to any of the toxic products.

The example of indole detoxification:

Indole → Indoxyl → Indoxyl sulphate → **Indican**

Potassium salt of indoxyl sulfate (indican) is excreted in the urine. Determination of indican concentration in the urine indicates the rate of protein putrefaction in the intestine as well as the functional state of the liver. Normal concentration of indican in the blood serum is 0.87-3.13 μmol/L. Normal excretion of indican in the urine is 4-16 μmol/day. The indican levels in the blood and urine are increased when protein putrefaction in the intestine is intensified, and are lowered if detoxification function of the liver is impaired.

General pathways of amino acid metabolism

The reactions involving amino acid conversion include:

1) deamination (removal of the amino group from an amino acid with the release of ammonia);

2) transamination (the transfer of amino group from an amino acid to an α-keto acid, without intermediary formation of NH_3;

3) decarboxylation (removal of the carboxyl group from an amino acid with the release of CO_2);

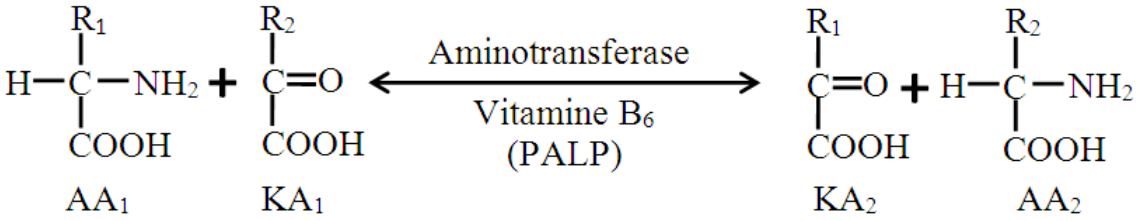
4) polymerization (synthesis of protein);

5) racemization (isomerization of D- and L-amino acids; reactions are typical of microorganisms only);

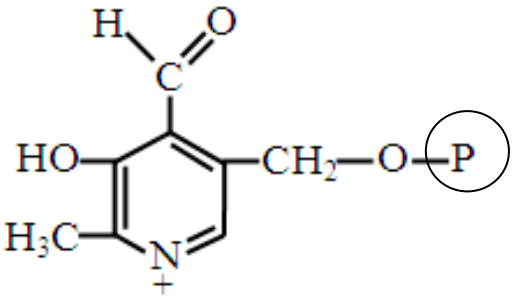
6) modification of a side chain radical.

Transamination of amino acids

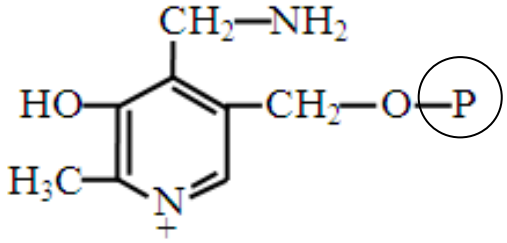
Transamination is the transfer of α-amino group from an amino acid (AA_1) to a keto acid (KA_1) with the resultant formation of another amino acid (AA_2) and another keto acid (KA_2), **without intermediary release of ammonia**. The general scheme of the reaction:



The enzymes catalyzing the reaction are called amino transferases (or transaminases). Pyridoxal phosphate (derivative of vitamin B_6) is the coenzyme of transaminases. In the course of transamination, pyridoxal phosphate may reversibly bind with amino group to form another coenzymatic derivative of vitamin B_6 – pyridoxamine phosphate:



Pyridoxal phosphate
(PALP)



Pyridoxamine phosphate
(PAMP)

Biological role of transamination is synthesis of non-essential amino acids.

Of all, two transaminases are of clinical importance:

1) **alanine aminotransferase** (AlAT or SGPT, GPT – serum glutamate pyruvate transaminase); in the reaction, alanine is transaminated with α-ketoglutarate to form pyruvate and glutamate;

2) **aspartate aminotransferase (**AsAT or SGOT, GOT – serum glutamate oxaloacetate transaminase); in the reaction aspartate is

transaminated with α-ketoglutarate to form oxaloacetate and glutamate.

Measurement of the alanine aminotransferase and aspartate aminotransferase levels in the blood serum is important in some medical diagnoses. Reactions catalyzed by alanine aminotransferase and aspartate aminotransferase are shown in (Fig. 24.6). The activity of transaminases is high in tissues, and is low in the blood serum. In cell destruction (**necrosis**) or increased cell membrane permeability (**inflammation**), transaminases are released from the tissue into the blood plasma. Clinical determination of alanine aminotransferase and aspartate aminotransferase activity in the blood serum is used for diagnostics of certain diseases.

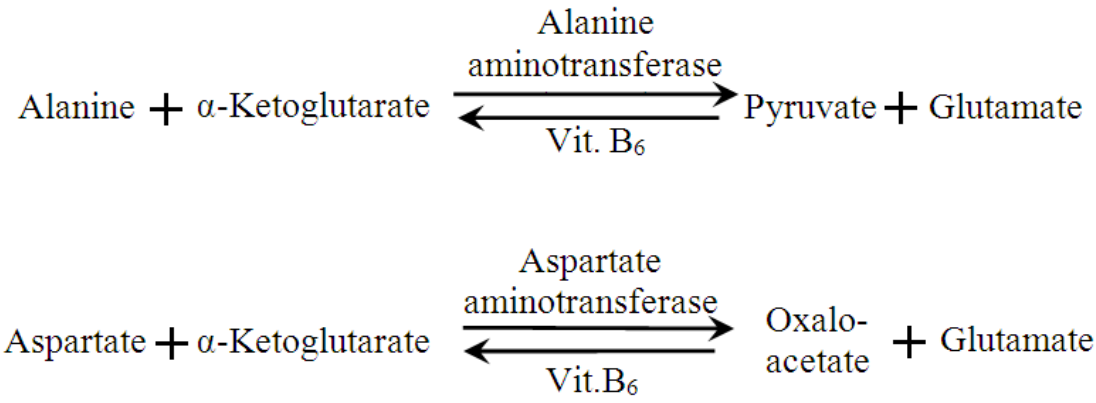


Figure 24.6 – Reactions catalyzed by alanine aminotransferase and aspartate aminotransferase

Normal serum level of alanine aminotransferase is 0.1-0.68 mmol/L/h. It is increased in hepatitis.

Normal serum value of aspartate aminoransferase is 0.1-0.45 mmol/L/h. It is increased in myocardial infarction.

Mechanism of transamination

An amino acid (AA_1) donates its amino group to pyridoxal phosphate to form pyridoxamine phosphate, and thereby the amino acid is converted to an α-keto acid (KA_1). The incoming α-keto acid (KA_2) then accepts the amino group from pyridoxamine phosphate, and departs in the form of the other amino acid (AA_2). Mechanism of transamination is shown in Fig. 24.7.

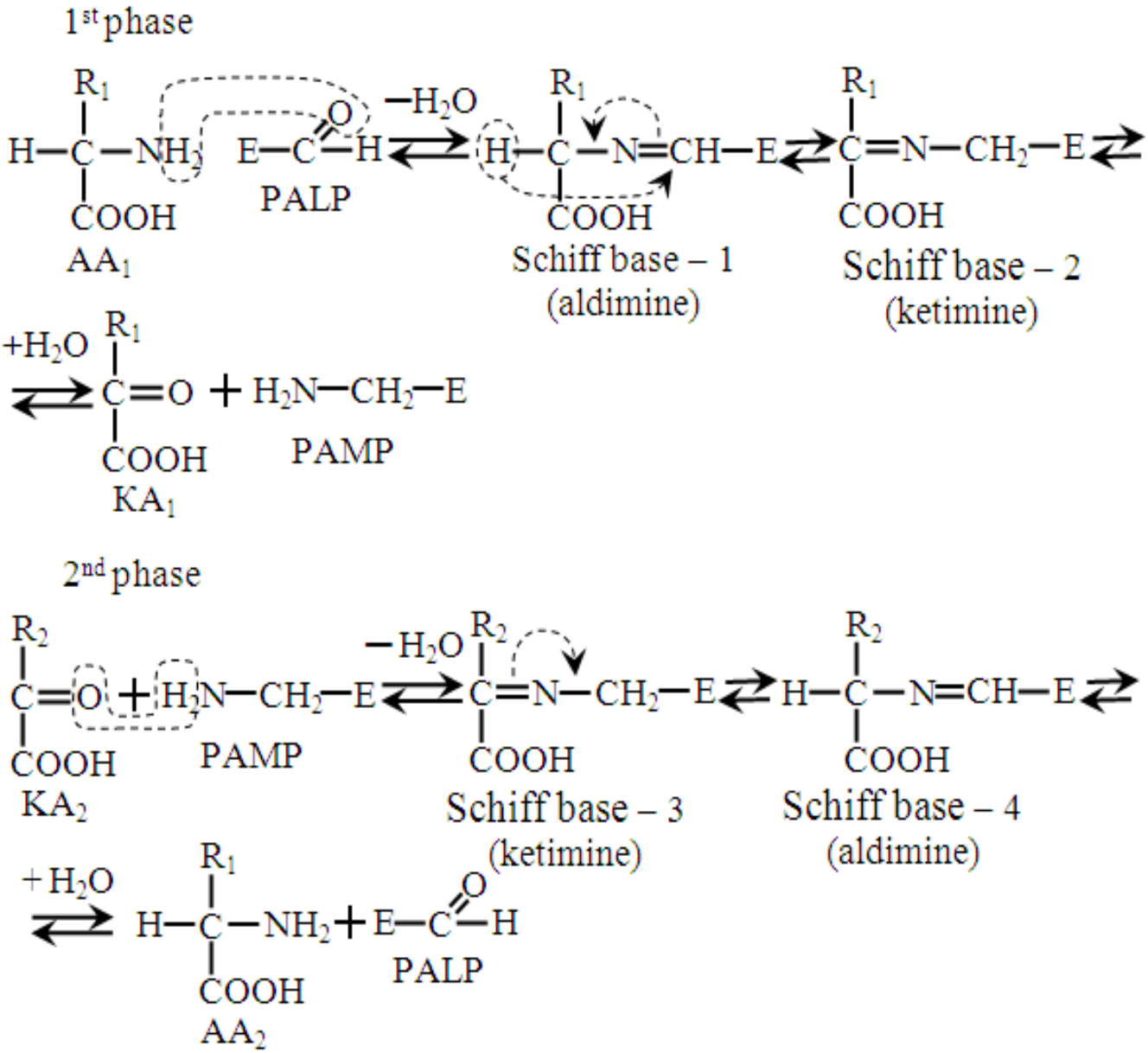
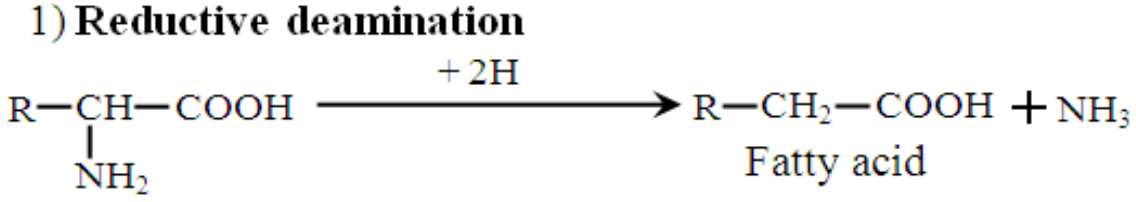


Figure 24.7 – Mechanism of transamination (scheme)

Deamination of amino acids

Deamination is the removal of the amino group from an amino acid to form ammonia. There are several types of deamination (Fig. 24.8):



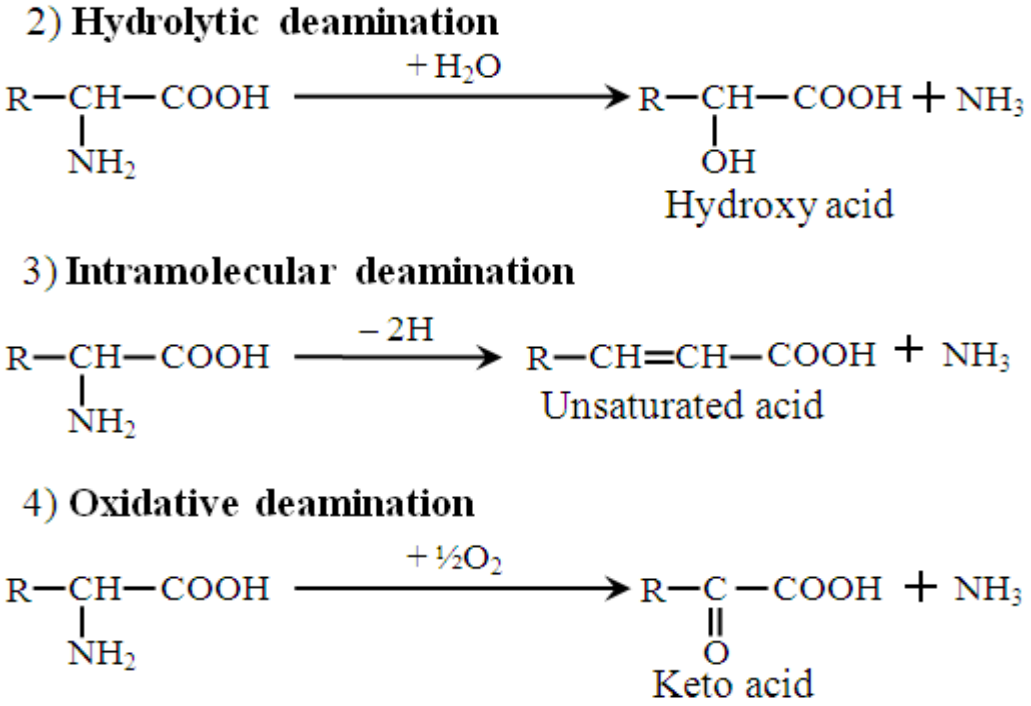
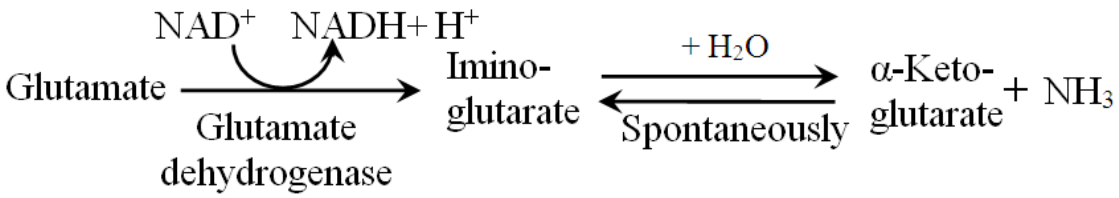


Figure 24.8 – Types of deamination

In humans, **the major type is oxidative deamination.**

Oxidative deamination

Glutamate dehydrogenase is the only enzyme involved in oxidative deamination in the body. The enzyme **directly** deaminates only glutamate.



Biological role of oxidative deamination

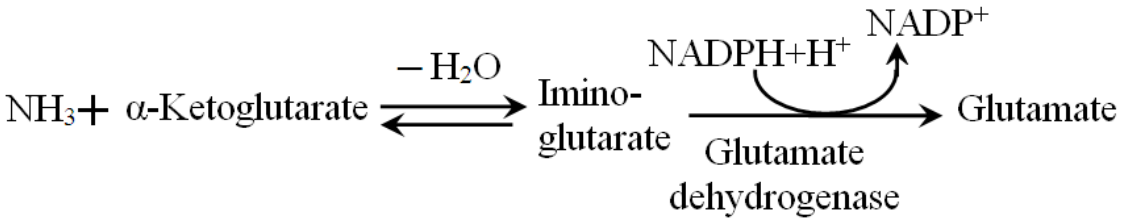
1. Deamination of excess molecules of glutamate, thus preparing them for further catabolism (degradation of carbon skeleton).

2. Glutamate dehydrogenase occupies the central position in nitrogen metabolism: the enzyme may **directly** deaminate only glutamate, but helps deaminate other amino acids by way of **indirect** deamination (transdeamination).

3. The reaction produces toxic ammonia which has to be detoxified.

Reductive amination

This is the reverse reaction of oxidative deamination with participation of $NADPH+H^+$ as a coenzyme:



Biological role of reductive amination

1. This is the way for detoxification of NH_3.
2. Due to this reaction, synthesis of the new glutamate molecules occurs.

Transdeamination

The first step in the catabolism of amino acids is the removal of amino group to form ammonia. Glutamate is the only amino acid which undergoes direct deamination, i.e. oxidative deamination by glutamate dehydrogenase. There are no enzymes for direct deamination of other amino acids.

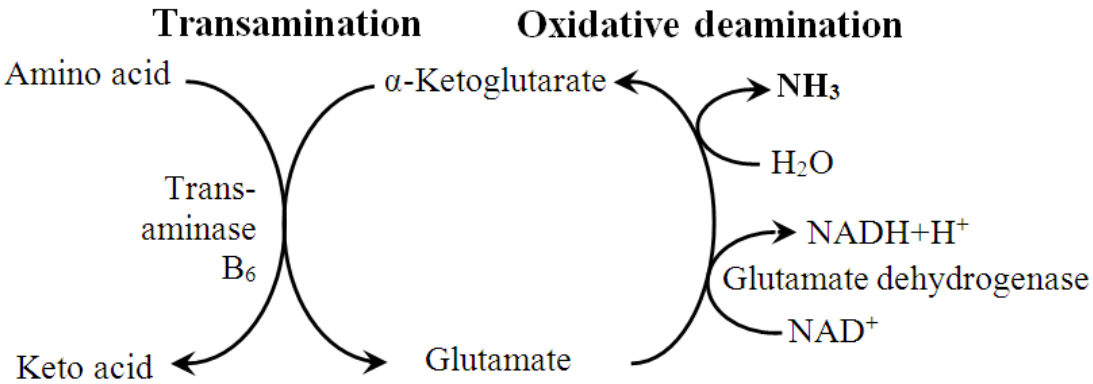


Figure 24.9 – Process of transdeamination. An amino acid first reacts with α-ketoglutarate in the transamination reaction to produce glutamate and corresponding keto acid. Glutamate thus formed is then subjected to the direct oxidative deamination by glutamate dehydrogenase.

Therefore, other amino acids (except for glutamate) may be deaminated only indirectly. That is, to be deaminated, all the other amino

acids have to undergo initially transamination with α-ketoglutarate to form glutamate. The glutamate undergoes then oxidative deamination with the release of ammonia which was previously a component part (as amino group) of the other amino acid. Thus, **transdeamination** represents combination of **transamination and oxidative deamination**:

Transdeamination = Transamination + Oxidative deamination

Schematically, transdeamination is represented in Fig. 24.9.

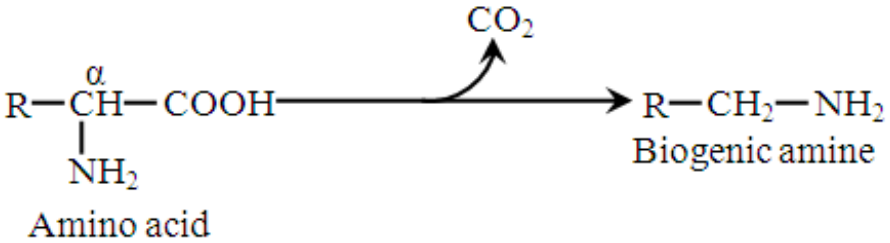
Transdeamination takes place in all the cells in the body; the amino group taken from different amino acds is transported to the liver as glutamate which is finally subject to oxidative deamination in the liver. Hence, in the process of transdeamination, the glutamate dehydrogenase reaction is the final reaction which removes amino groups of all amino acids. Thus, two steps of transdeamination proceed distantly from each other but physiologically they are coupled.

Decarboxylation of amino acids

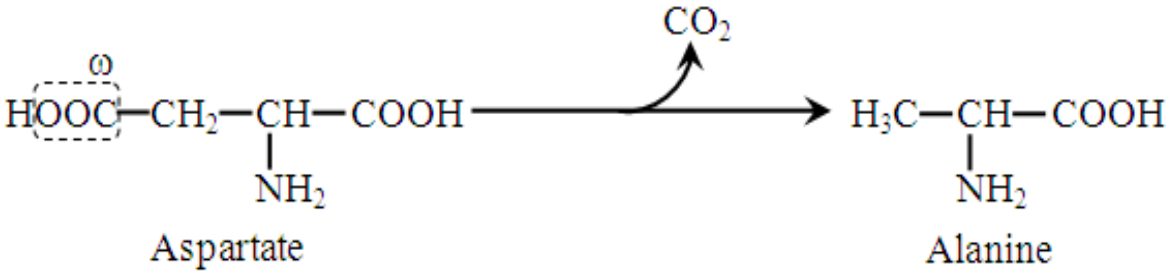
The removal of the carboxyl group from an amino acid with the release of CO_2 is called **decarboxylation**. The reaction is catalyzed by decarboxylases which coenzyme is pyridoxal phosphate (PALP), the derivative of vitamin B_6.

There are four types of the amino acid decarboxylation.

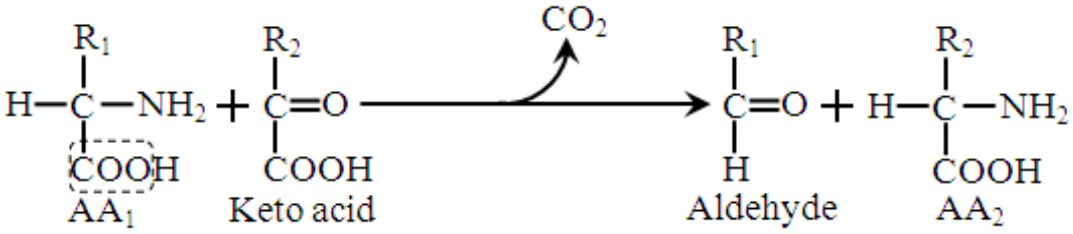
1) **α-Decarboxylation**. This type of decarboxylation is typical of animal tissues. Carboxyl group next to α-carbon of the amino acid molecule is removed. The reaction produces CO_2 and biogenic amines:



2) **ω-Decarboxylation**. This type of decarboxylation is typical of microorganism. By this pathway, a new amino acid may be produced.

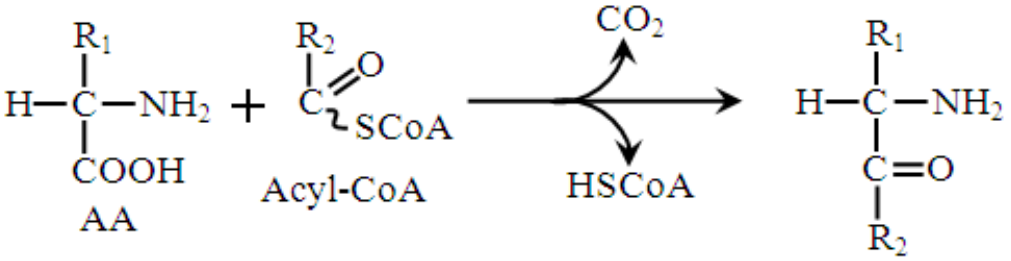


3) **Decarboxylation involving transamination reaction**.



An aldehyde and a new amino acid, corresponding to the initial keto acid, are produced by this reaction.

4) **Decarboxylation involving condensation reaction of two molecules** (takes place in synthesis of heme and sphingosine).



BIOGENIC AMINES: SYNTHESIS AND FUNCTIONS

Biogenic amines –the derivatives of tryptophan

Tryptamine is formed of tryptophan and exhibits vasoconstrictive action.

Serotonin is formed of 5-hydroxytryptophan. Serotonin is also a powerful vasoconstrictor, increases motility of gastrointestinal tract and may cause diarrhea, takes part in the regulation of blood pressure, body temperature, rate of respiration, renal filtration. Serotonin may induce sleep, may participate in the development of allergy. Serotonin is also central neurotransmitter; its excess may cause panic attacks. Serotonin level was found to be low in patients with depressive psychosis.

Biogenic amines – derivatives of tyrosine

Tyrosine is a precursor for synthesis of dopamine, norepinephrine (noradrenaline), and epinephrine (adrenaline).

Dopamine is a neurotransmitter. It controls voluntary movement. Lack of dopamine causes Parkinson's disease, which is characterized by tremor and difficulties in initiating movement; impairments in emotional responses and memory.

Norepinephrine is the major neurotransmitter in the sympathetic nervous system. Noradrenaline stimulates the heart rate, sweating, vasoconstriction in the skin, and bronchodilatation, increases the blood pressure.

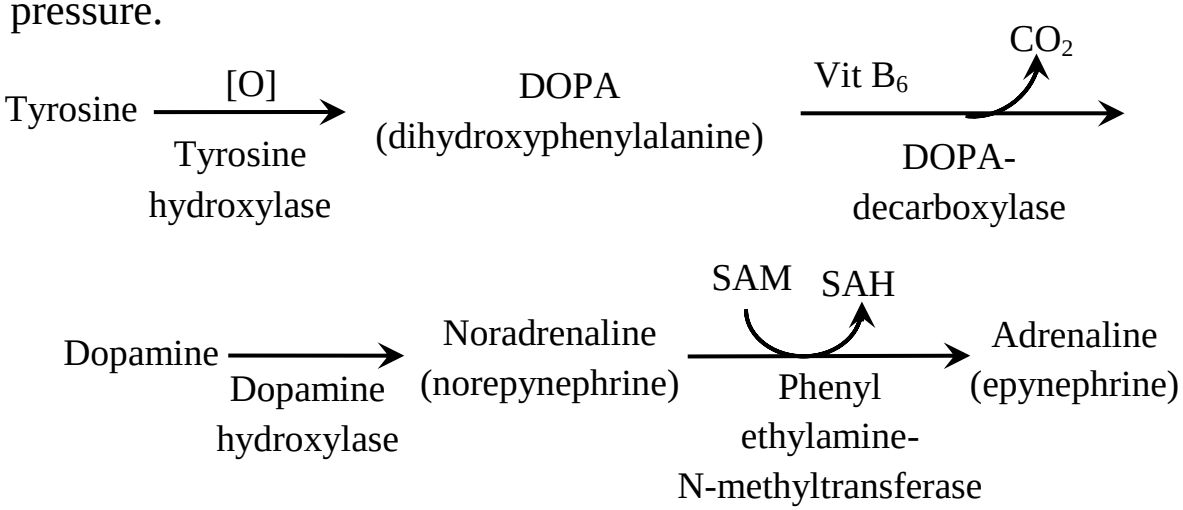


Figure 24.10 – Synthesis of dopamine, adrenaline and noradrenaline

Epinephrine is a hormone. It is produced by adrenal medulla and is secreted in response to fright, flight, and exercise. Adrenaline increases the blood pressure, stimulates the rate and force of myocardial contraction, causes relaxation of smooth muscles of bronchi (bronchodilatation), increases glycogenolysis and lipolysis.

Biogenic amine – derivative of histidine

Histamine is formed from histidine. This reaction does not require pyridoxal phosphate. Histamine exhibits wide spectrum of biological activity. It is vasodilator (causes the fall of blood pressure), mediator of pain, may be involved in the development of allergy, contracts smooth muscles of bronchi, and may cause bronchospasm, stimulates gastric secretion of HCl, is involved in inflammatory process: large amounts of histamine are generated in inflammatory foci, where this biogenic amine enhances vascular permeability and attracts leucocytes.

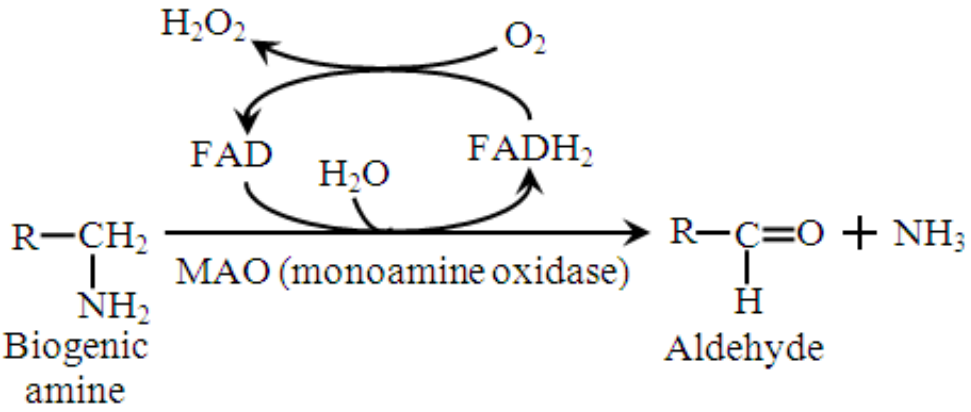


Figure 24.11 – Oxidation of biogenic amines

Biogenic amine – derivative of glutamate

Decarboxylation of glutamate produces **γ-aminobutyric asid (GABA).** GABA exerts inhibitory action on the CNS. Low level of GABA in the brain would lead to convulsions.

Oxidation of biogenic amines

Biogenic amines exert potent pharmacologic action, and their accumulation in tissues would cause unfavourable effect on the organism. In the body, there is mechanism for inactivation of biogenic amine (Fig. 24.11), resulting in the formation of aldehyde and the release of ammonia.

Catabolism of carbon skeletons of amino acids. Glucogenic or ketogenic amino acids

Katabolism of amino acids begins with their transamination.

Amino acids may be classified as glucogenic or ketogenic. Amino acids which provide their carbon skeleton for synthesis of glucose via gluconeogenesis are called **glucogenic**.

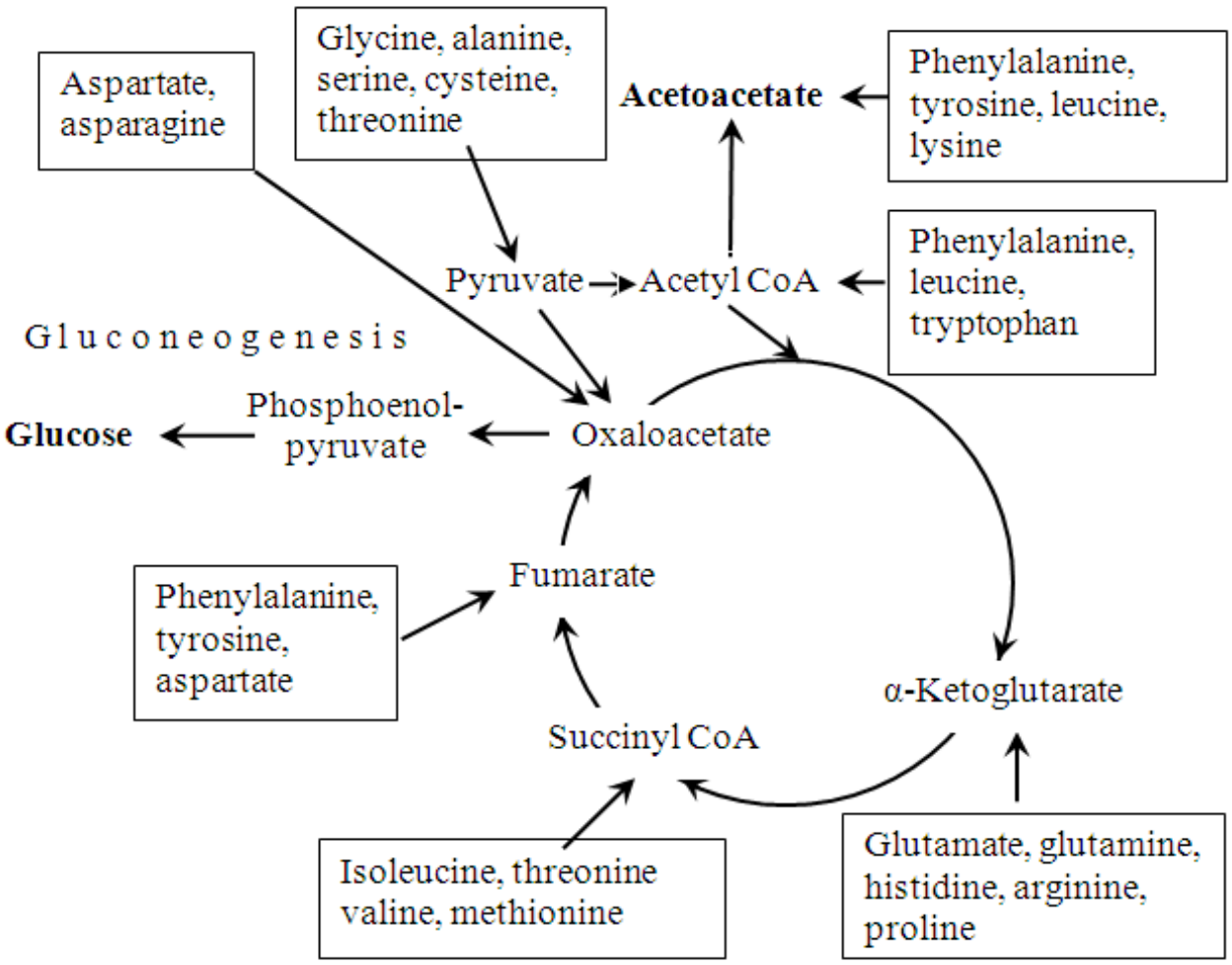


Figure 24.12 – Glucogenic and ketogenic amino acids

Those amino acids which form acetoacetate or acetyl CoA are called **ketogenic**. Many amino acids may be both glucogenic and ketogenic. Amino acid leucine is exclusively ketogenic. Schematically, glucogenic and ketogenic amino acids are shown in Fig. 25.4.

Glucogenic amino acids yield pyruvate or the TCA cycle intermediates (α-ketoglutarate, succinyl CoA, fumarate or oxaloacetate). Oxaloacetate can be then converted to phosphoenolpyruvate, and subsequently to glucose via gluconeogenesis.

Ketogenic amino acids may be converted into acetoacetate (a **ketone** body) or acetyl CoA (a precursor for synthesis of ketone bodies). The amino acids that yield acetoacetate: phenylalanine, tyrosine, leucine, and lysine. The amino acids that yield acetyl CoA: leucine, phenylalanine, and tryptophan.

CHAPTER 25

FORMATION AND DETOXIFICATION OF AMMONIA IN THE ORGANISM

Ways for formation of ammonia

In the body, there are several ways for the formation of ammonia:

1. Oxidative deamination of glutamic acid and transdeamination of other amino acids. This is the major source of ammonia in the body;
2. Deamination of amides (reactions of hydrolysis of asparagine and glutamine);
3. Oxidation of biogenic amines;
4. Deamination of the purine and pyrimidine nitrogenous bases.

Ways for detoxification of ammonia

Ammonia is highly toxic compound, especially to the central nervous system, and should be detoxified.

The **mechanisms of ammonia toxicity** are as follows:

1) Ammonia accumulating in the blood causes alkalosis (i.e. concentrational shift of hydrogen ions towards the raise in pH which promotes alcalation);

2) When concentration of ammonia is increased in the blood, ammonia diffuses into cells and across the blood/brain barrier (BBB);

3) The increase in ammonia concentration causes the increased synthesis of glutamate from α-ketoglutarate and increased synthesis of glutamine from glutamate.

When concentration of ammonia is significantly increased, ammonia depletes α-ketoglutarate pool, eliminating α-ketoglutarate from the TCA cycle and, hence, resulting in the decrease of ATP production in neurons. This is the major cause for the bizarre behaviour observed in individuals with high blood concentration of ammonia.

There are several ways for ammonia detoxification:

1) **Synthesis of carbamoyl phosphate** and its further conversion to urea which is excreted into the urine;

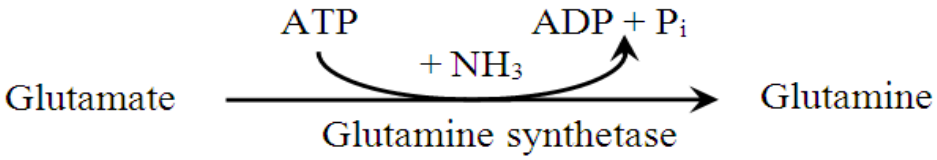
2) **Synthesis of amides**: glutamine and asparagine;

3) **Reductive amination** of α-ketoglutarate;

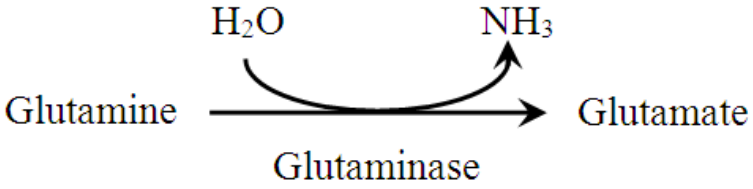
Normally, the ammonia level in the blood does not exceed 60 μmol/L.

Intracellular detoxification of ammonia

Ammonia is permanently produced by almost all cells. Ammonia intoxication is a life-threatening condition. In many tissues (brain, kidney, liver, and muscle) the intracellular ammonia immediately binds with glutamic acid to form glutamine:

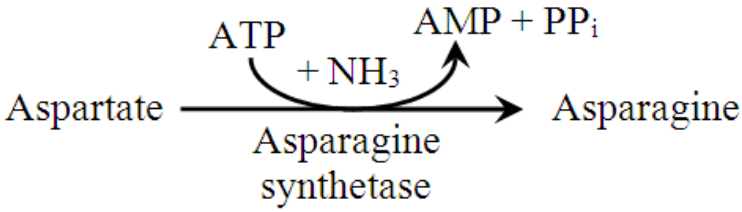


The glutamine is then transported to the liver, where the reaction is reversed by glutaminase:

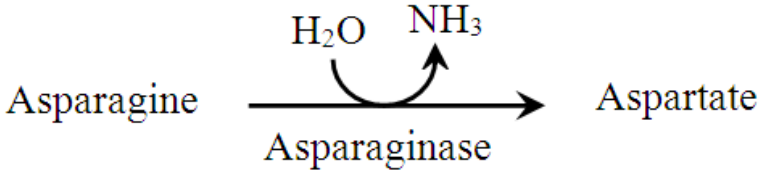


The ammonia, thus generated, is immediately detoxified in the liver by synthesis of urea.

Aspartic acid may also undergo similar conversions:



The asparagine is then transported to the liver, where the reaction is reversed by asparaginase with the release of ammonia:



Glutamine and asparagine are the major transport forms of ammonia from the brain to the liver.

Role of ammonia in the maintenance of acid-base balance in the body

Normally, excretion of ammonia into the urine is low but its excretion is increased considerably in acidosis, i.e. when the concentration of acids

(hydrogen ions) in the blood is above normal.

In acidosis, the uptake of glutamine from the blood by the kidney is increased. Also, acidosis stimulates activity of the kidney glutaminase to produce ammonia by renal tubular cells:

$$\text{Glutamine} \xrightarrow[\text{Glutaminase}]{+\ H_2O} \text{Glutamate} + NH_3$$

Non-ionized ammonia can easily diffuse through cell membrane into the tubular lumen (i.e. into the urine). Non-ionized organic acids (RH) are secreted into the lumen by tubular cells. Salts (mainly NaCl) are filtrated into the urine by renal glomeruli. In the urine, salts and organic acids dissociate to produce ions:

$$RH \rightleftharpoons R^- + H^+$$

$$NaCl \rightleftharpoons Na^+ + Cl^-$$

Ammonia binds with hydrogen ion (H^+) to form NH_4^+; the latter is neutralized by chloride-ion, and ammonium salt (NH_4Cl) is excreted through urine from the body:

$$NH_3 + H^+ \longrightarrow NH_4^+;$$

$$NH_4^+ + Cl^- \longrightarrow NH_4Cl$$

The cations (Na^+) and organic anions (R^-) remaining in the tubular lumen are reabsorbed. In the absence of ammonia, which forms NH_4^+-ion to be excreted with anion Cl^- as ammonium salt, sodium ion (Na^+) would be excreted with organic anions and would be lost from the body. The loss of Na^+ would lead to the decrease of osmotic pressure of the blood and intracellular fluid, and would result in the tissue dehydration.

Thus, due to the described mechanism, the following processes are observed to occur:

1) toxic ammonia and excess hydrogen ions are excreted from the body;

2) organic acids are saved to the body;

3) regulation of acid-base balance takes place (excretion of acidic H^+ and retention of Na^+ to increase alkaline reserve of the blood).

Biosynthesis of urea (urea cycle)

Biosynthesis of urea is a cyclic process which is also called ornithine cycle. This is the major mechanism for detoxification of ammonia in the body. Urea is the end product of amino acid metabolism. Normal concentrations of urea in the blood are 2.5-8.33 mmol/L; its excretion into the urine is 333-583 mmol/day.

Biosynthesis of urea takes place in the liver only. The 1st and the 2nd reactions of the urea cycle occur in the mitochondrial matrix, other reactions occur in the cytoplasm. Scheme of urea synthesis is shown in Fig. 25.1.

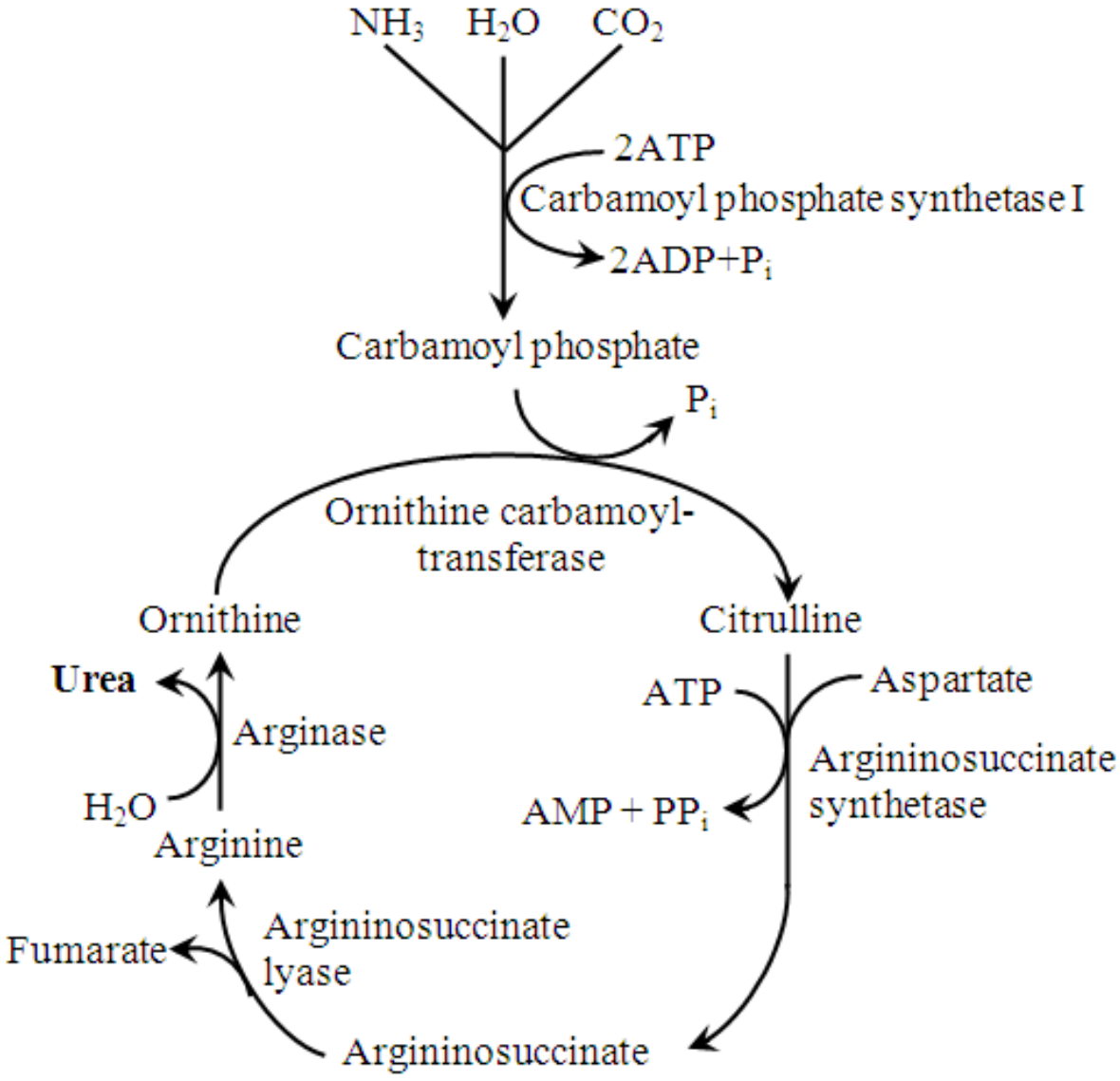


Figure 25.1 – Scheme of urea synthesis.

Disorders of urea synthesis and excretion

The liver is the only site of urea synthesis. Urea is released from hepatocytes into the blood and then is freely filtered by glomeruli. Hence, urea concentration in the blood and urine may serve as an indicator of both liver and renal functions. Urea level is decreased in the blood and urine in hepatitis, liver cirrhosis, and in genetic disorders of the urea cycle (as a result of impaired urea synthesis).

In nephritis and renal failure, when renal filtration is impaired, urea is retained and hence its concentration is increased in the blood (synthesis of urea in the liver is normal), but is decreased in the urine (renal filtration is impaired, and urea is accumulated in the blood but is not excreted into the urine).

Genetic disorders of urea cycle. Deficiency or absence of any of the urea cycle enzymes results in ammonia accumulation in the blood (**hyperammoniemia**) and tissues and increased levels of intermediates prior to metabolic block.

There are five types of the urea cycle disorders (Table 25.1).

Hyperammoniemia is the common feature of all five types of the urea cycle disorders. The brain is especially sensitive to the ammonia intoxication. Symptoms of hyperammoniemia may vary from subconscious avoidance of high-protein foods or headache occurred after protein containing meals, to severe psychoneurological manifestations (tremor, slurred speech, blurred vision, lethargy, vomiting, mental retardation, convulsions, irritability, and lost of consciousness). Severe hyperammoniemia may lead to death.

Table 25.1. – **Urea cycle disorders**

Diseases	Enzyme defect
Hyperammoniemia Type I	Carbamoyl phosphate synthetase I
Hyperammoniemia Type II	Ornithine carbamoyl transferase
Cutrullinemia	Argininosuccinate synthetase
Argininosuccinate aciduria	Argininosuccinate lyase
Hyperarginemia	Arginase

Ammonia intoxication is more severe when the metabolic block occurs at reactions 1 and 2 (in hyperammoniemia types I and II). Deficiency of enzymes catalyzing other reaction (from 3 to 5) of the urea cycle results in accumulation of intermediates which are less toxic.

Intoxication is more severe when the metabolic block occurs at reactions 1 and 2 (in hyperammoniemia types I and II ammonia concentration in the blood may be up to 530 μmol/L), because covalent binding of ammonia to carbon takes place only if citrulline is synthesized. Deficiency of enzymes catalyzing other reaction 3 to 5 of the urea cycle results in accumulation of other intermediates which are less toxic.

Management. Child may be put on a low protein diet. The minimal amount of protein as frequent small meals should be given to maintain growth and brain function. Dietary carbohydrates would decrease endogenous protein breakdown.

CHAPTER 26

METABOLISM OF METHIONINE, PHENYLALANINE AND TYROSINE

Metabolism of methionine

The major pathway of methionine metabolism is the conversion of this amino acid to **S-adenosylmethionine** (SAM), Fig. 26.1. In the molecule of SAM, the methyl group is labile, and may be transferred easily to other acceptors (substrates) with the formation of methylated substrate. The total scheme of methionine metabolism is shown in Fig. 26.2.

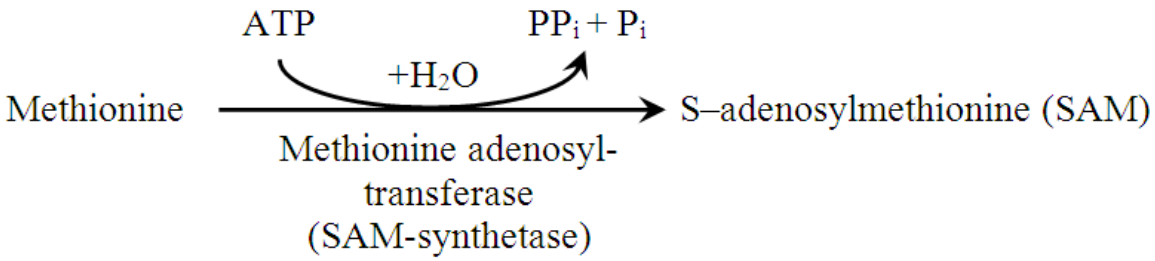
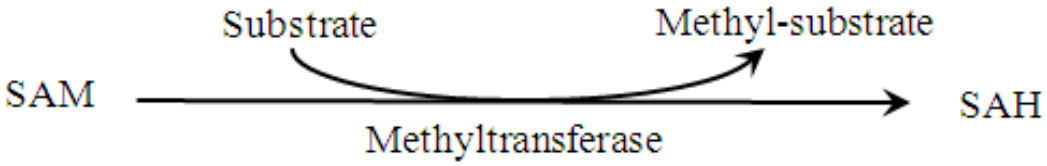


Figure 26.1 – Reaction of fomation of S-adenosylmethionine

Role of methionine in transmethylation reactions

S-adenosylmethionine (SAM) is the donor of methyl groups for transmethylation reactions. Due to these reactions, catalyzed by methyltransferases, the methyl group is transferred to an acceptor (substrate) to form methylated substrate (methyl-substrate) and S-adenosylhomocysteine (SAH):



Some examples of transmethylation reactions are represented in Table 26.1.

Table 26.1. – **Transmethylation reactions**

Acceptor of methyl group	Methylated substrate
Guanidinoacetate	Creatine
Norepinephrine (noradrenaline)	Epinephrine (adrenaline)
Phosphatidylethanolamine	Phosphatidylcholine

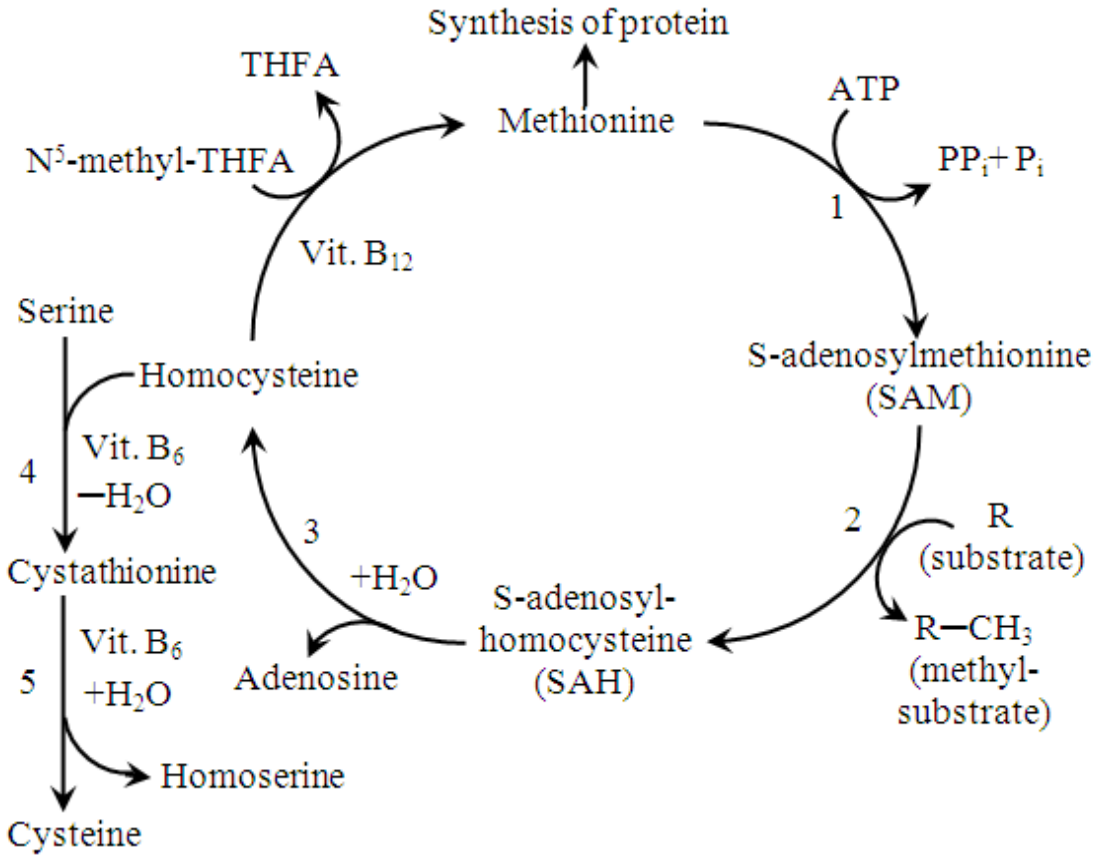


Figure 26.2 – The total scheme of methionine metabolism. Enzymes: 1 – methionine adenosyltransferase; 2 – methyltransferase; 3 – SAH-hydrolase; 4 – cystathionine synthetase; 5 – cystathionine lyase.

Synthesis of creatine

Creatine is synthesized from three amino acids: arginine, glycine, and methionine (Fig. 26.3). Creatine phosphate may be stored in muscles and serve as an immediate source of energy for ATP synthesis by way of substrate-level phosphorylation.

Creatine phosphate may be spontaneously (non-enzymatically) converted to creatinine which is excreted in the urine.

For each individual, the daily excretion of creatinine is fairly constant. The amount of creatinine excreted in the urine per day is proportionate to body muscle mass and depends on kidney function.

Normal creatinine blood levels are 0.044-0.088 mmol/L, excretion in the urine is 4.4-17.6 mmol/day. Unlike creatinine, **creatine is practically absent from the urine in adult humans**. As the creatine level in the blood

serum reaches 0.12 mmol/L, the presence of creatine is revealed in the urine.

In early childhood, physiological creatinuria takes place (due to low activity of creatine kinase at this age, creatine is not used for synthesis of creatine phosphate and is excreted in the urine).

In adults, the highest level of creatine in the urine occurs in pathologic states of muscular system, such as myopathy and progressive muscular dystrophy.

Determination of the creatine kinase activity in the blood serum is important for laboratory diagnosis of myocardial infarction.

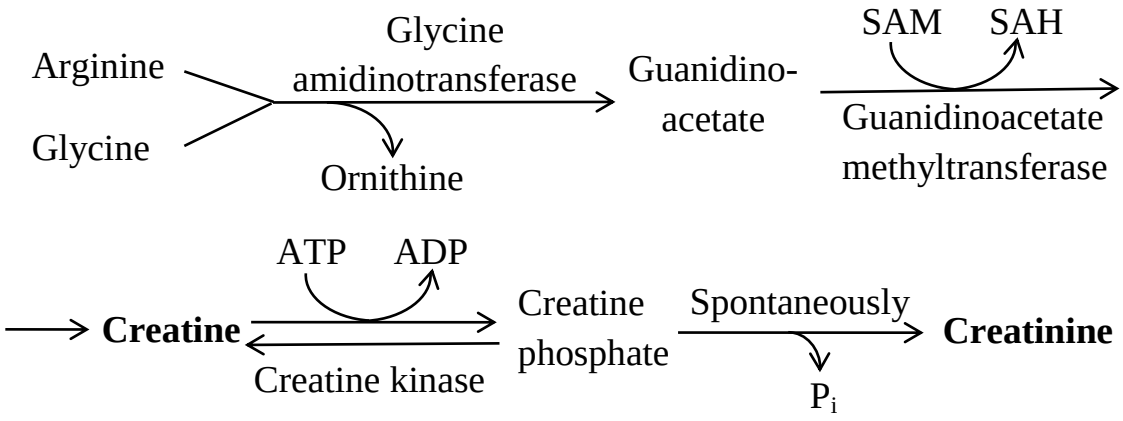


Figure 26.3 – Synthesis of creatine and creatinine

Metabolism of phenylalanine and tyrosine

Phenylalanine is an essential amino acid, but tyrosine is not considered to be essential because it is synthesized from phenylalanine. Both phenylalanine and tyrosine may undergo catabolism, or they may be also converted to catecholamines, melanin, and thyroid hormones. General scheme of the phenylalanine and tyrosine metabolism, as well as some genetic defects of the amino acids is shown in Fig. 26.4.

The major pathway for phenylalanine metabolism begins with hydroxylation of this amino acid to form tyrosine (Fig. 26.5). The reaction is catalyzed by phenylalanine hydroxylase and requires both tetrahydrobiopterin and $NADH+H^+$.

Phenylketonuria

The genetic block of **phenylalanine hydroxylase** results in phenylketonuria (PKU). Deficiency of this enzyme does not allow

phenylalanine to convert to tyrosine. As a result, phenylalanine is accumulated in the blood and is excreted into the urine at increased amounts.

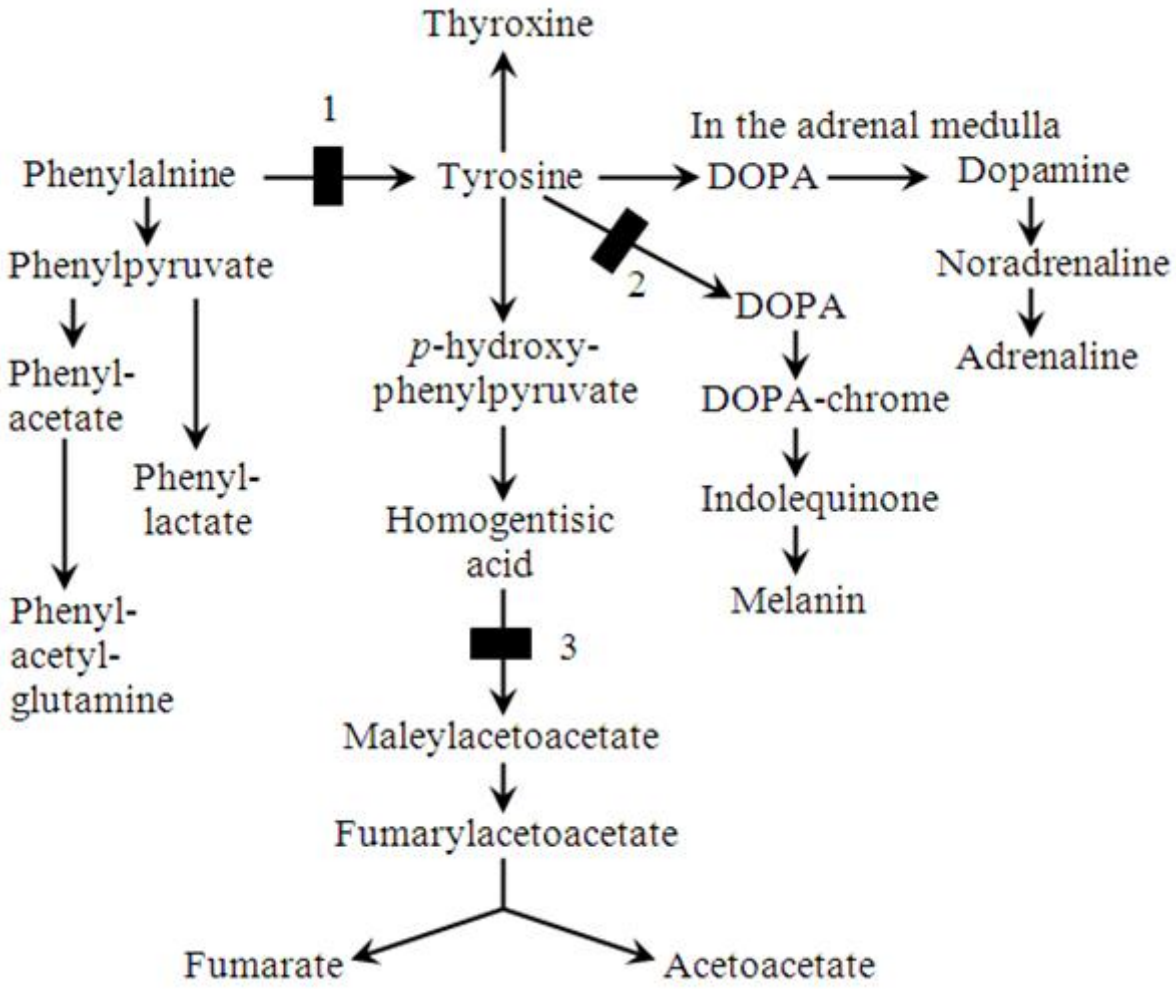


Figure 26.4 – Summary of the phenylalanine and tyrosine metabolism. The numbers indicate genetic block in diseases:
1 – phenylketonuria; 2 – albinism; 3 – alkaptonuria.

Excess of phenylalanine in the cell leads to activation of the alternative (minor) pathway of catabolism of this amino acid with the resulting formation of phenylpyruvate, phenyllactate, and phenylacetate. These products are toxic for the central nervous system, and their accumulation in the child results in severe mental retardation.

Laboratory diagnosis of PKU. Presence of phenylpyruvate in the urine may be detected by adding a drop of $FeCl_3$ to the urine. A transient blue-green colour is a positive test.

Treatment. The special diet is given with low phenylalanine content but supplemented with tyrosine.

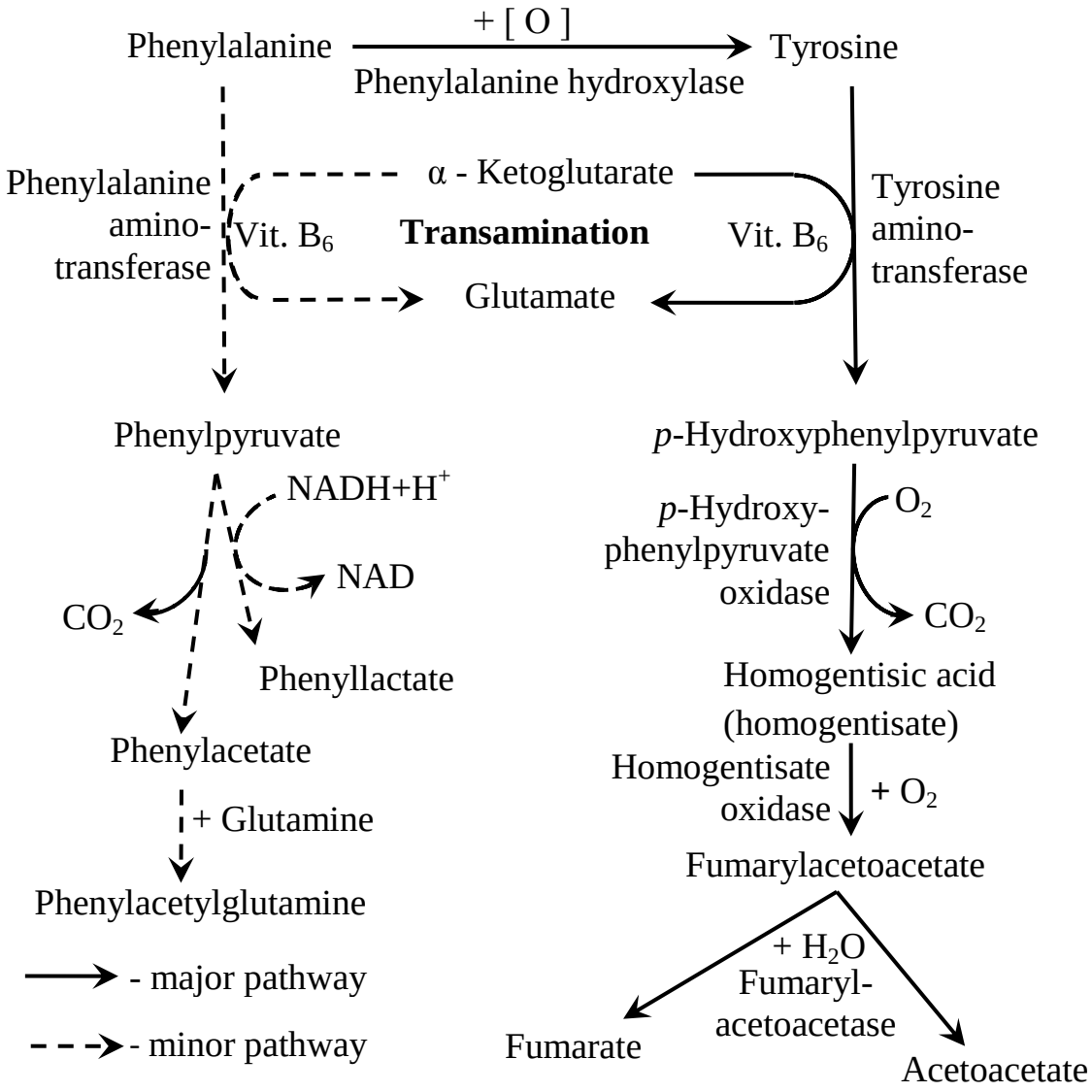


Figure 26.5 – Catabolism of phenlalanine and tyrosine

Alkaptonuria

The genetic block of **homogentisate oxydase** results in the excretion of homogentisic acid into the urine. On contact with air oxygen, homogentisic acid is oxidized to the intermediate which is then polymerized to form the pigment alkaptone of black colour. The symptom of the disease is the blackening of the urine on standing.

By the 3rd or 4th decade of life, ochronosis (connective tissue pigmentation) may be developed in the patient: alkaptone is deposited in cartilages of nose, ear and gives them dark colour. The pigment deposition

in joint cartilages leads to subsequent tissue damage causing severe arthritis. No specific treatment is required.

Synthesis of melanin

Synthesis of melanin takes place in melanocytes. Melanin is a pigment which gives black colour to the skin and hair. The enzyme tyrosinase catalyzes the two initial reactions. The remaining reactions are non-enzymatic and occur spontaneously (Fig. 26.6).

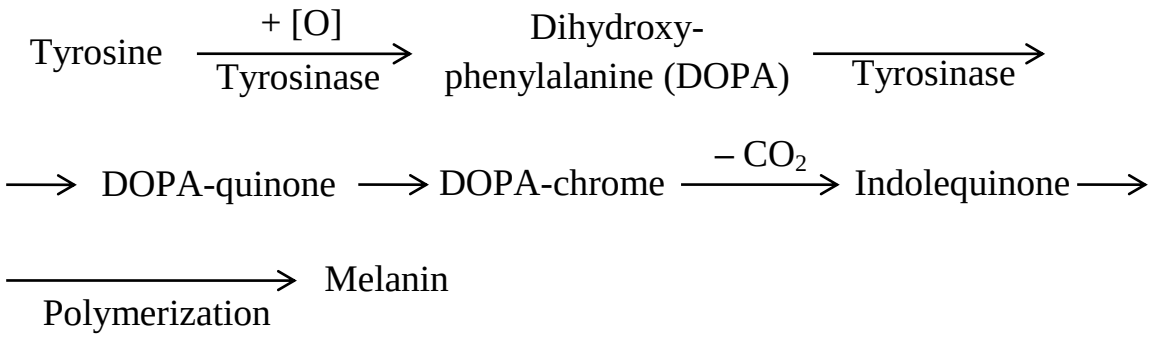


Figure 26.6 – Synthesis of melanin (scheme)

Albinism

Albinism occurs due to absence of **tyrosinase** in melanocytes, and hence melanin is absent in the skin, hair, and retina of the eye. The ocular fundus is hypopigmented, and iris may be grey. The individuals with albinism have photophobia (eyes are very sensitive to bright light). The skin has low pigmentation and is sensitive to UV rays. Hair is white. Mental ability is not affected.

CHAPTER 27

METABOLISM OF NUCLEOTIDES

Nucleotides consist of three component parts: nitrogenous bases (purine or pyrimidine), pentoses (ribose or deoxyribose), and 1-3 phosphate groups. A base bound to pentose is called nucleoside. When the nucleoside is phosphorylated, it is called nucleotide or nucleotide monophosphate. The deoxyribonucleotides or deoxyribonucleosides are denoted adding the prefix d- (deoxy-) before the nucleotide (nucleoside).

Names of nitrogenous bases, nucleosides, and nucleotides are given in the table below.

Table 27.1. – **Names of nitrogenous bases, nucleosides, and nucleotides**

Nitrogenous base	***Nucleoside***	***Nucleotide***
Purines:		
Adenine	Adenosine*	Adenosine monophosphate, AMP, adenylic acid*
	Deoxyadenosine**	Deoxyadenosine monophosphate, dAMP, deoxyadenylic acid**
Guanine	Guanosine*	Guanosine monophosphate, GMP, guanylic acid;
	Deoxyguanosine**	Deoxyguanosine monophosphate, dGMP, deoxyguanylic acid
Pyrimidines:		
Cytosine	Cytidine*	Cytidine monophosphate, CMP, cytidylic acid
	Deoxycytidine**	Deoxycytidine monophosphate, dCMP, deoxycytidylic acid
Thymine**	Thymidine**	Thymidine monophosphate, TMP, thymidylic acid**
Uracil*	Uridine*	Uridine monophosphate, UMP, uridylic acid*

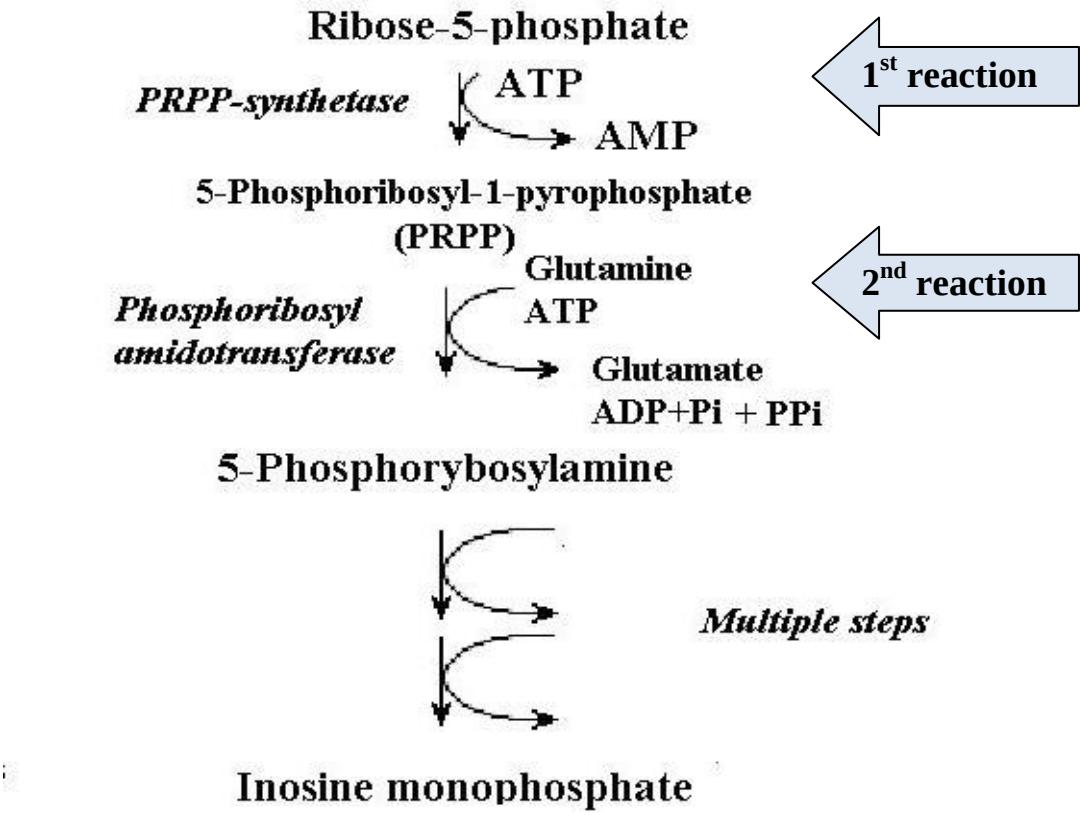
* Nucleosides and nucleotides are special for RNA; ** Nucleosides and nucleotides are special for DNA.

Biosynthesis of purine nucleotides

Purine nucleotides are synthesized in the cytoplasm from ribose-5-phosphate, a product of the pentose phosphate pathway. Both adenine and

guanine are derived from the nucleotide **inosine monophosphate** (IMP), which is the first compound in the pathway to have a completely formed purine ring system.

In the **first** reaction **5-phosphoribosyl-1-pyrophosphate (PRPP)** is synthesized from ribose-5-phosphate by ribose phosphate pyrophosphokinase (PRPP-synthetase), which is activated by inorganic phosphate and inactivated by purine ribonucleotides. PRPP is also used in pyrimidine synthesis and salvage pathways.



In the next 9 reactions, 5'-phosphoribosylamine is converted to **inosine monophosphate (IMP, inosinic acid)**.

These reactions utilize glycine, CO_2, aspartate, N^5,N^{10}-methenyl-tetrahydrofolate, N^{10}-formyl-tetrahydrofolate, and glutamine.

The origin of atoms in the purine ring is represented in the Fig. 27.1.

Inosinic acid is a precursor for synthesis of adenylic and guanylic acids (Fig. 27.2).

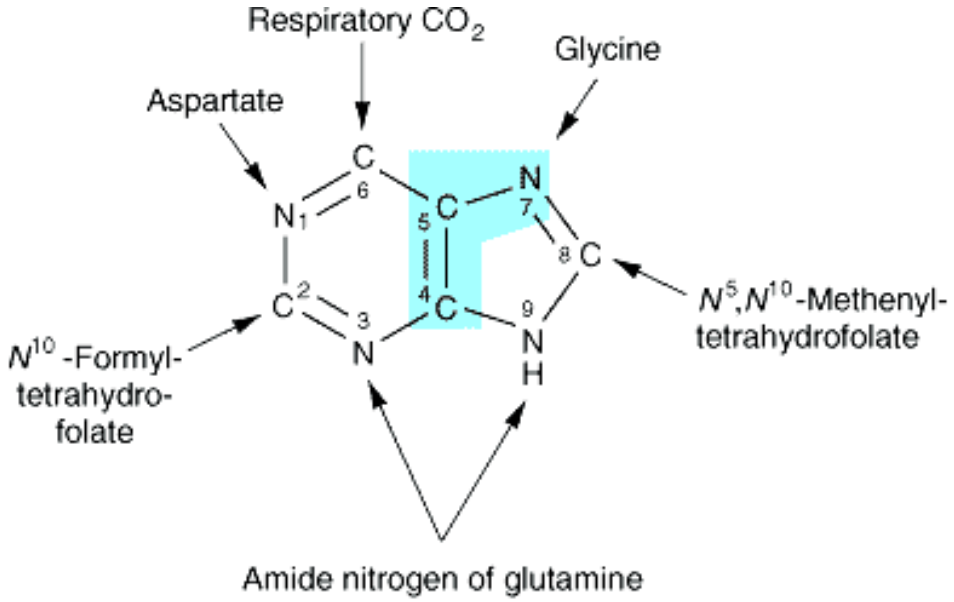


Figure 27. 1 – Origin of atoms in purine ring

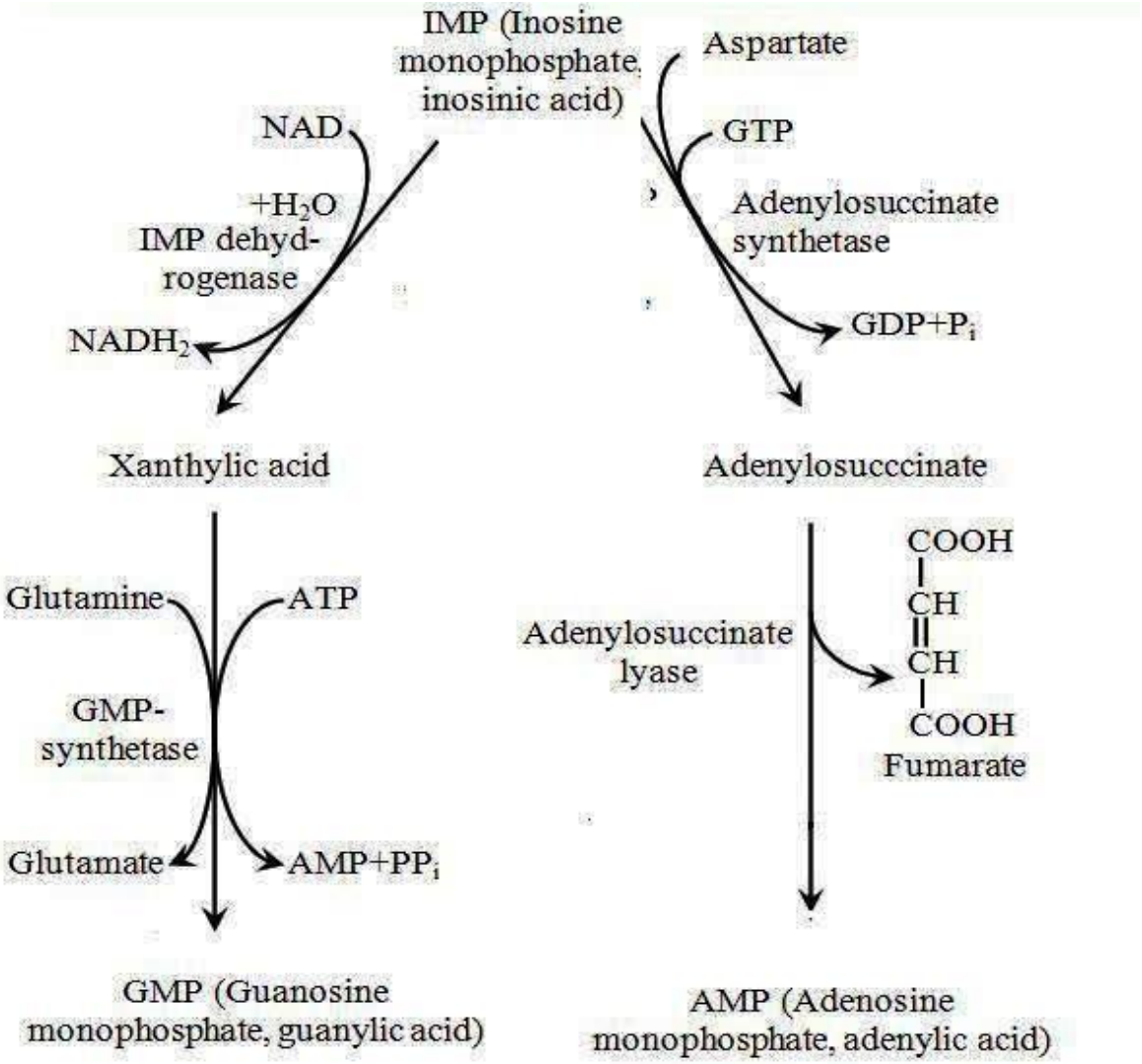


Figure 27. 2 – Synthesis of AMP and GMP

Regulation of purine synthesis

The reactions catalyzed by the enzymes **PRPP-synthetase** and **phosphoribosyl amidotransferase** are inhibited by the excessive amounts of ATP, ADP, AMP, GTP, GDP, and GMP. They act as allosteric effectors. Besides, both AMP and GMP inhibit their own formation by the feedback inhibition of **adenylosuccinate synthetase** and **IMP dehydrogenase**, respectively. GTP activates the synthesis of AMP, while ATP activates the synthesis of GMP (Fig. 27.3).

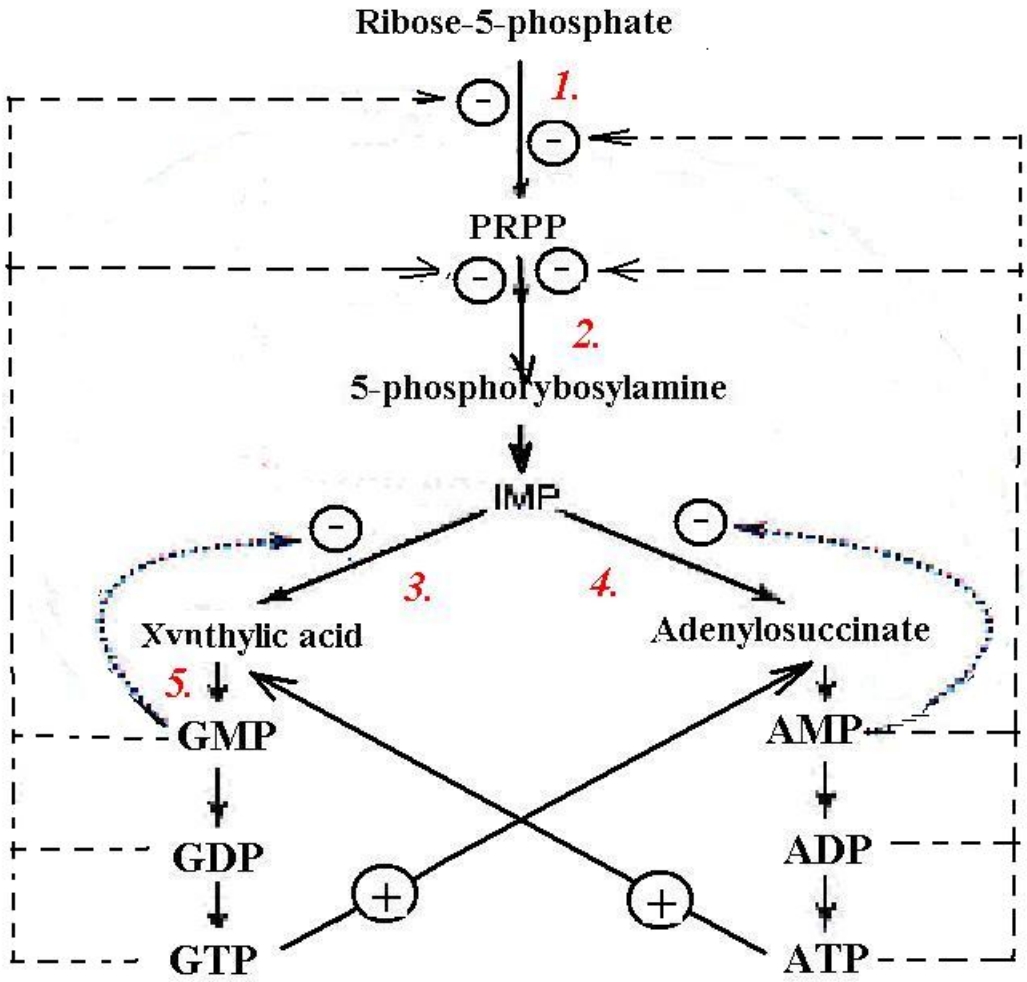


Figure 27.3 – Regulation of purine synthesis
1- PRPP-synthetase; 2 – phosphorybosyl amidotransferase;
3- IMP-dehydrogenase; 4- adenylosuccinate synthetase;
5 – GMP-synthetase.

Biosynthesis of pyrimidine nucleotides

Biosynthesis of pyrimidine nucleotides takes place in the cytoplasm. The first reaction is catalyzed by **carbamoylphosphate synthetase II (CPS II)** which is different from carbamoylphosphate synthetase I (CPS I). CPS I is located in mitochondria, takes place in urea synthesis, and uses free

ammonia for the synthesis of carbamoylphosphate. CPS II is located in the cytoplasm, takes part in the synthesis of pyrimidine nucleotides, and uses nitrogen of glutamine to form carbamoylphosphate (Fig. 27.3).

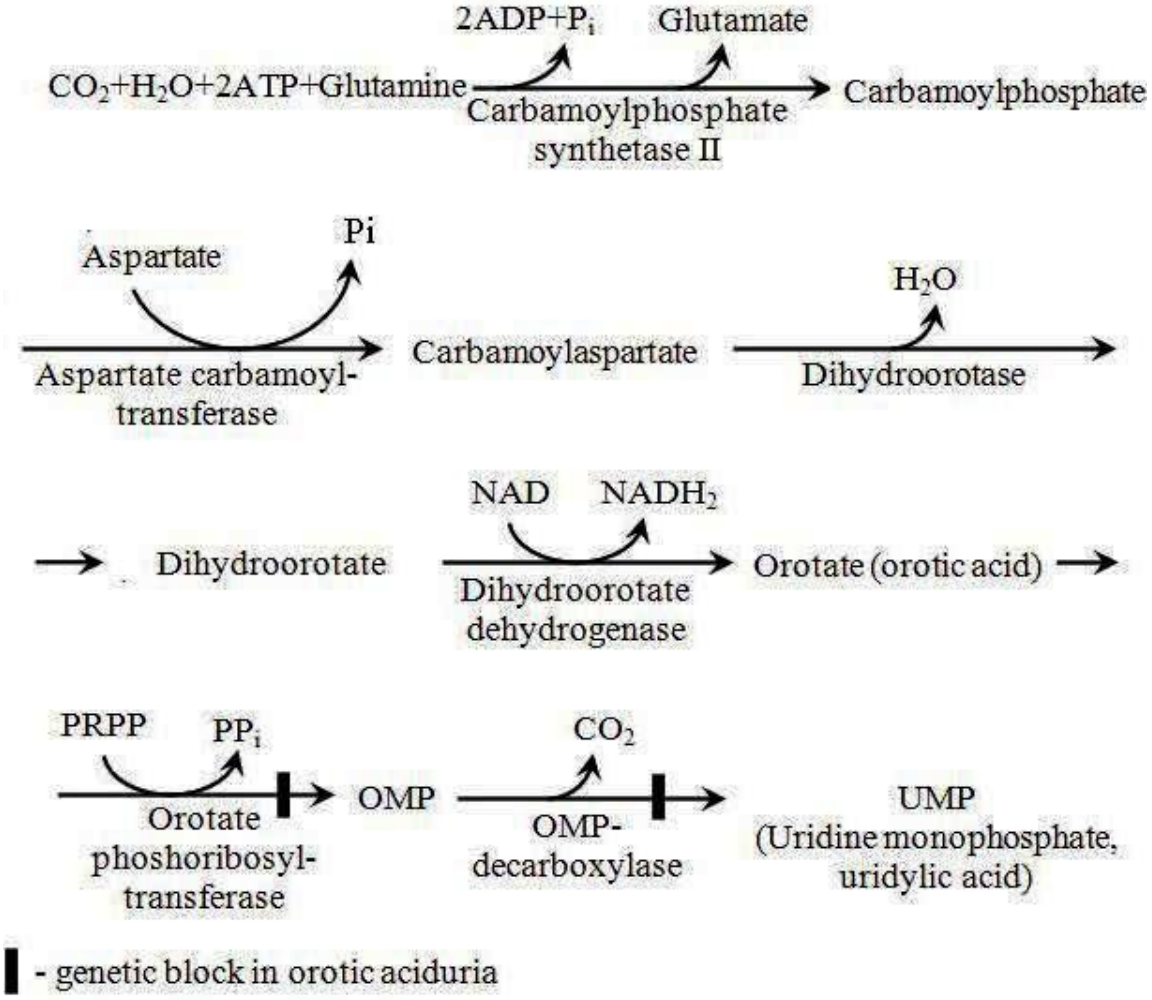


Figure 27.4 – Synthesis of pyrimidine nucleotide (OMP – orotidyl monophosphate)

Uridine monophosphate (UMP) serves as precursor in the synthesis of cytidine nucleotides.

Firstly, phosphoryltransferases (kinases) catalyze transfer of phosphoryl groups of the ATP molecules to UMP and, latter, to UDP:

UMP + ATP ↔ UDP + ADP;
UDP + ATP ↔ UTP + ADP

Cytidine triphosphate (CTP) is synthesized from UTP, is the reaction, catalyzed by CTP-synthase**:**

UTP + glutamine + ATP → CTP + glutamate + ADP + Pi

Regulation of pyrimidine synthesis

The major regulatory steps in the pyrimidine synthesis are the reactions catalyzed by **carbamoylphosphate synthetase II** and **aspartate carbamoyltransferase**. They are inhibited by UTP and CTP, respectively (Fig. 27.5).

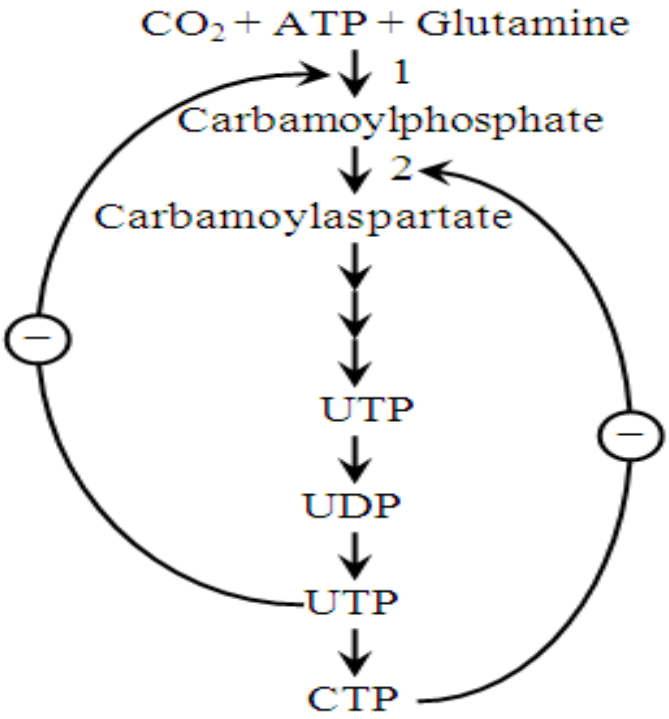


Figure 27.5 – Regulation of pyrimidine synthesis. 1 – carbamoylphosphate synthetase II; 2 – aspartate carbamoyltransferase.

Synthesis of deoxyribonucleotides

As DNA is made up of deoxyribonucleotides containing deoxyribose instead of ribose, cells require a pathway to convert ribonucleotides into their deoxy forms.

Conversion of ribose to deoxyribose takes place within ribonucleoside diphosphates. For this process, a protein **thioredoxin,** containing two HS-groups, is required (Fig. 27.6). The enzyme **ribonucleoside diphosphate reductase** removes oxygen atom from 2'-OH group of ribose to form water with the use of two hydrogen atoms from thioredoxin. As a result, deoxyribose is formed within nucleoside diphosphate.

Reduced thioredoxin is restored in the reaction catalyzed by **thioredoxin reductase** in the presence of NADPH.

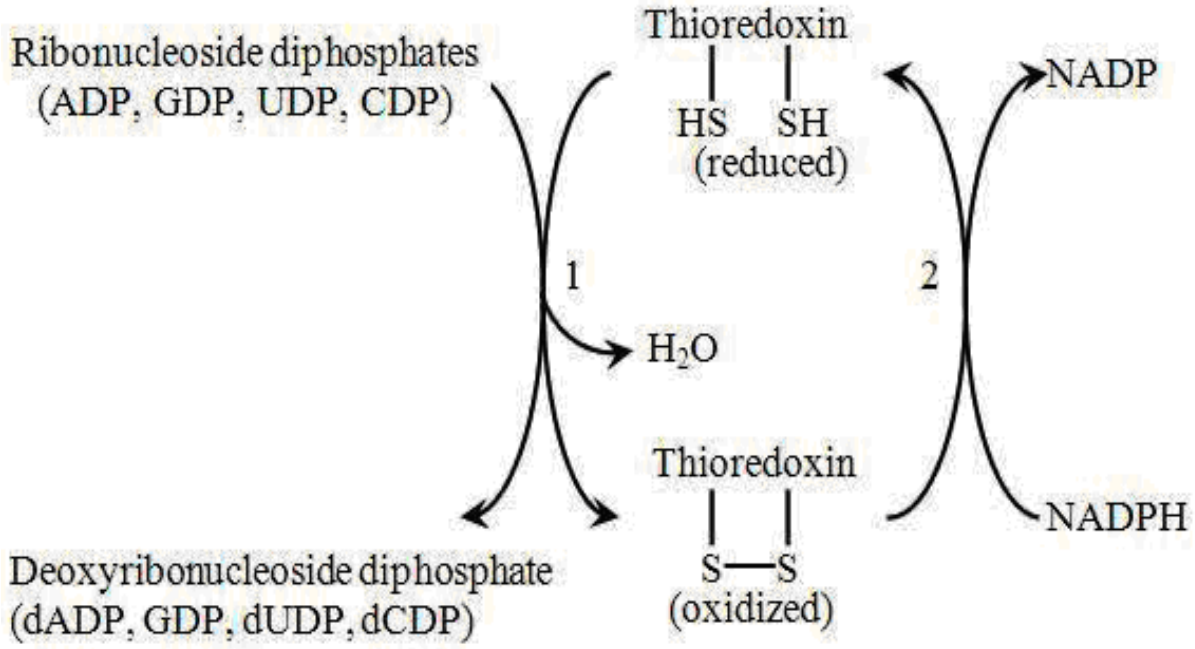


Figure 27.6 – Synthesis of deoxyribonucleotides.
1 – ribonucleoside diphosphate reductase; 2 – thioredoxin reductase.

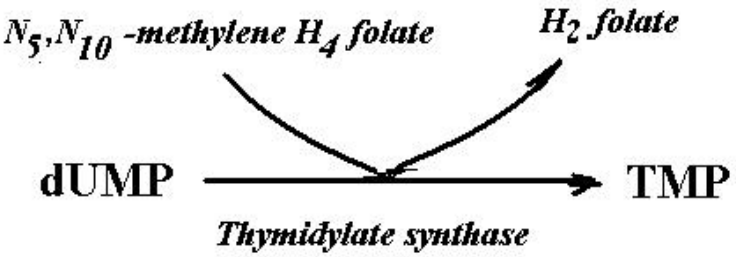
Synthesis of thymidylic acid

For synthesis of thymidylic acid (TMP), the molecule of deoxyuridine monophosphate (dUMP) is required.

First, previously formed dUDP undergoes hydrolysis to dUMP:

$$dUDP + H_2O \rightarrow dUMP + Pi$$

After that dUMP is converted to TMP by **thymidylate synthase**. The donor of methyl group for synthesis of thymidylic acid is **N^5, N^{10}-methylene-tetrahydrofolate** (N^5,N^{10}-methylene H_4 folate):



Re-utilization of nucleosides and nitrogenous bases for synthesis of nucleotides (salvage pathways)

A **salvage pathway** is a metabolic pathway in which nucleotides are synthesized from intermediates in the degradative pathway for nucleotides. Salvage pathways are used to recover nucleotides from bases and nucleosides that are formed during degradation of nucleic acids. This is important in some organs because some tissues cannot synthesize nucleotides from ribose-5-phosphate (purines), and CO_2, H_2O, glutamine, etc (pyrimidines). The salvaged bases and nucleosides can then be converted back into nucleotides.

Purine salvage pathways

Purine bases from turnover of cellular nucleic acids (or from food) can also be salvaged and reused in new nucleotides, using phosphoribosyl pyrophosphate and 2 enzymes:

- **Adenine phosphoribosyltransferase** (APRT)

  Adenine + PRPP → AMP + PPi

- **Hypoxantine-guanine phosphoribosyltransferase** (HGPRT)

  Guanine + PRPP → GMP + PPi

  Hypoxantine + PRPP → IMP + PPi

After that, IMP may be converted to the either GMP or AMP, as described above.

Pyrimidine salvage pathways

Pyrimidine bases from turnover of cellular nucleic acids (or from food) are reused in new nucleotides, using ribose-1-phosphate (or deoxyribose-1-phosphate) and pyrimidine-nucleoside phosphorylase**s.** After that, nucleoside kinase**s** phosphorylate these nucleosides into UMP and TMP, respectively.

The nucleosides cytidine and deoxycytidine can be salvaged by phosphorylation them into CMP or dCMP, respectively.

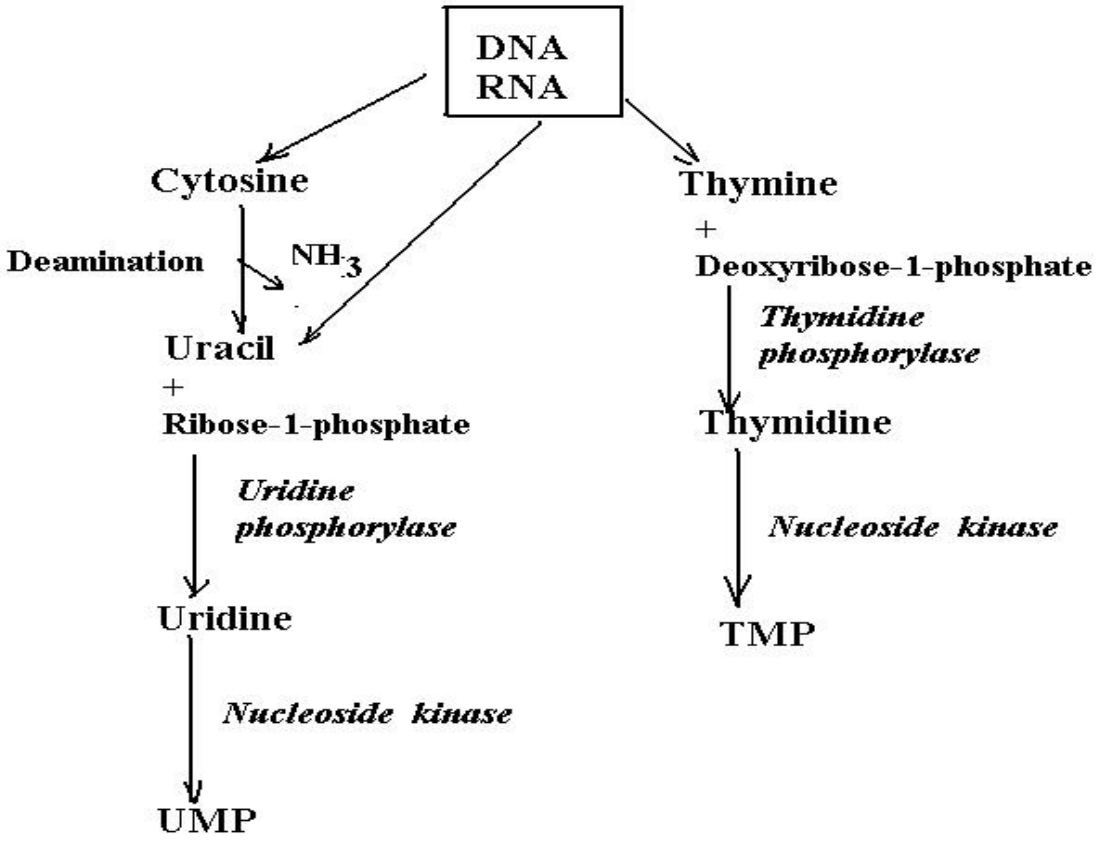


Figure 27.7 – Re-utilization of cytosine and thymine

Digestion of nucleic acids in the gastrointestinal tract

Dietary nucleic acids are ingested as nucleoproteins. In the stomach, nucleoproteins are degraded by gastric enzymes and HCl to form polypeptides and nucleic acids. Polypeptides are cleaved to form free amino acids. Degradation of nucleic acids takes place in the small intestine by DNAse and RNAse of pancreatic juice. Phosphodiesterase of the intestinal mucosa completes hydrolysis of nucleic acids to mononucleotides.

Mononucleotides may be hydrolytically cleaved by non-specific acidic and alkaline phosphatases in the intestine to form a nucleoside and phosphate; nucleosides are then absorbed into enterocytes (Fig. 27.8).

Nucleosides are cleaved inside enterocytes mainly via the **phosphorolytic** pathway. The enzyme nucleoside phosphorylase cleaves nucleoside to form a nitrogenous base and ribose 1-phosphate (deoxyribose 1-phosphate).

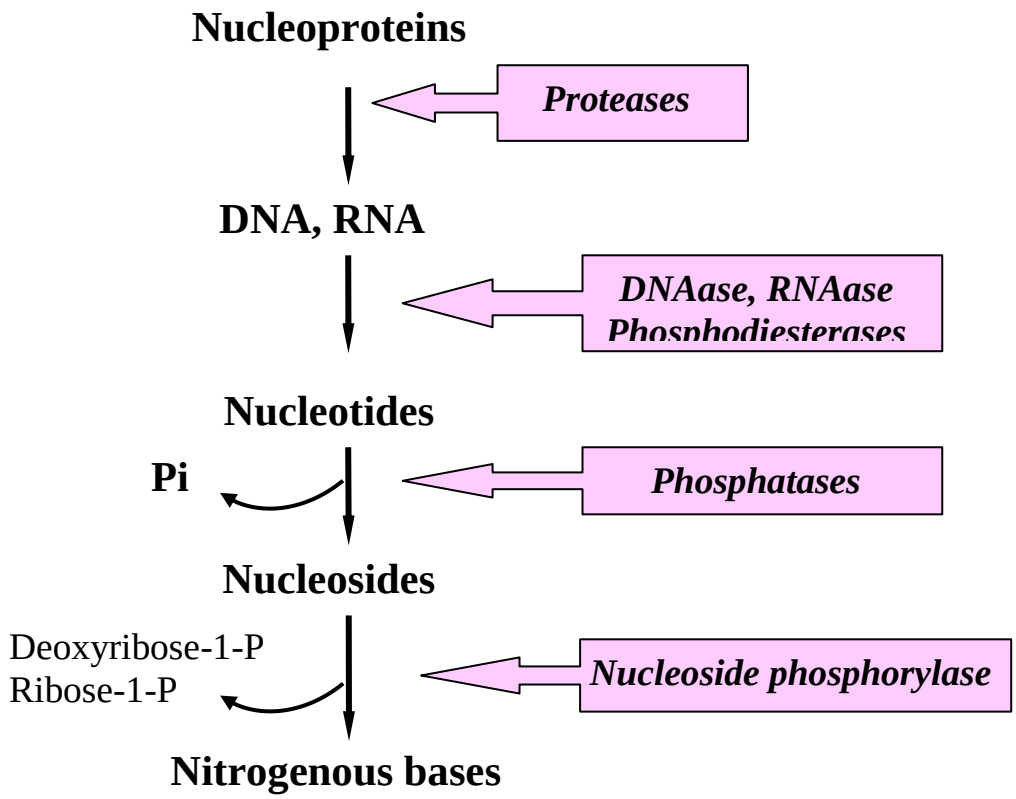


Figure 27. 8 – Digestion of nucleic acids in the gastrointestinal tract

Degradation of nucleic acids in tissues

In tissues, nucleic acids are degraded by nucleases. There are several types of nucleases:

1) **Endonucleases**. They catalyze hydrolytic cleavage of inner phosphodiester bonds of DNA or RNA to produce oligonucleotides.

2) **Exonucleases**. They catalyze hydrolytic removal of terminal mononucleotides from DNA or RNA molecule.

There are also specific nucleases involved in the breakdown of DNA or RNA molecule:

1) **Deoxyribonucleases I** (**DNases I**). They catalyze cleavage of phosphodiester bonds within one of the two strands of DNA.

2) **Deoxyribonucleases II** (**DNases II**). They catalyze cleavage of phosphodiester bonds within both DNA strands.

3) **Ribonucleases** (**RNases**). They catalyze cleavage of phosphodiester bonds within RNA.

4) **Restrictases.** They catalyze cleavage of DNA at strictly defined regions of the DNA molecule, exhibiting a palindromic structure (the same read forth and back, e.g. “madam”).

5) **Polynucleotide phosphorylase**. It catalyzes phosphorolytic breakdown of RNA by adding inorganic phosphate to a mononucleotide cleaved from RNA to produce ribonucleoside diphosphate (RDP):

$RNA + H_3PO_4 \rightarrow RNA_{(n-1)} + RDP$

6) **DNA-glycosidases** (N-glycosidases). They catalyze hydrolysis of modified nitrogenous bases in a DNA molecule. DNA-glycosidases play an important role in the repair of DNA.

Ultimately, nucleic acids in tissues undergo breakdown to mononucleotides which are hydrolyzed by **nucleases** to form free nucleosides and phosphate.

Degradation of purine nucleotides

Degradation of purine nucleotides takes place mainly in the liver. The end product of purine nucleotide catabolism is **uric acid** (urate).

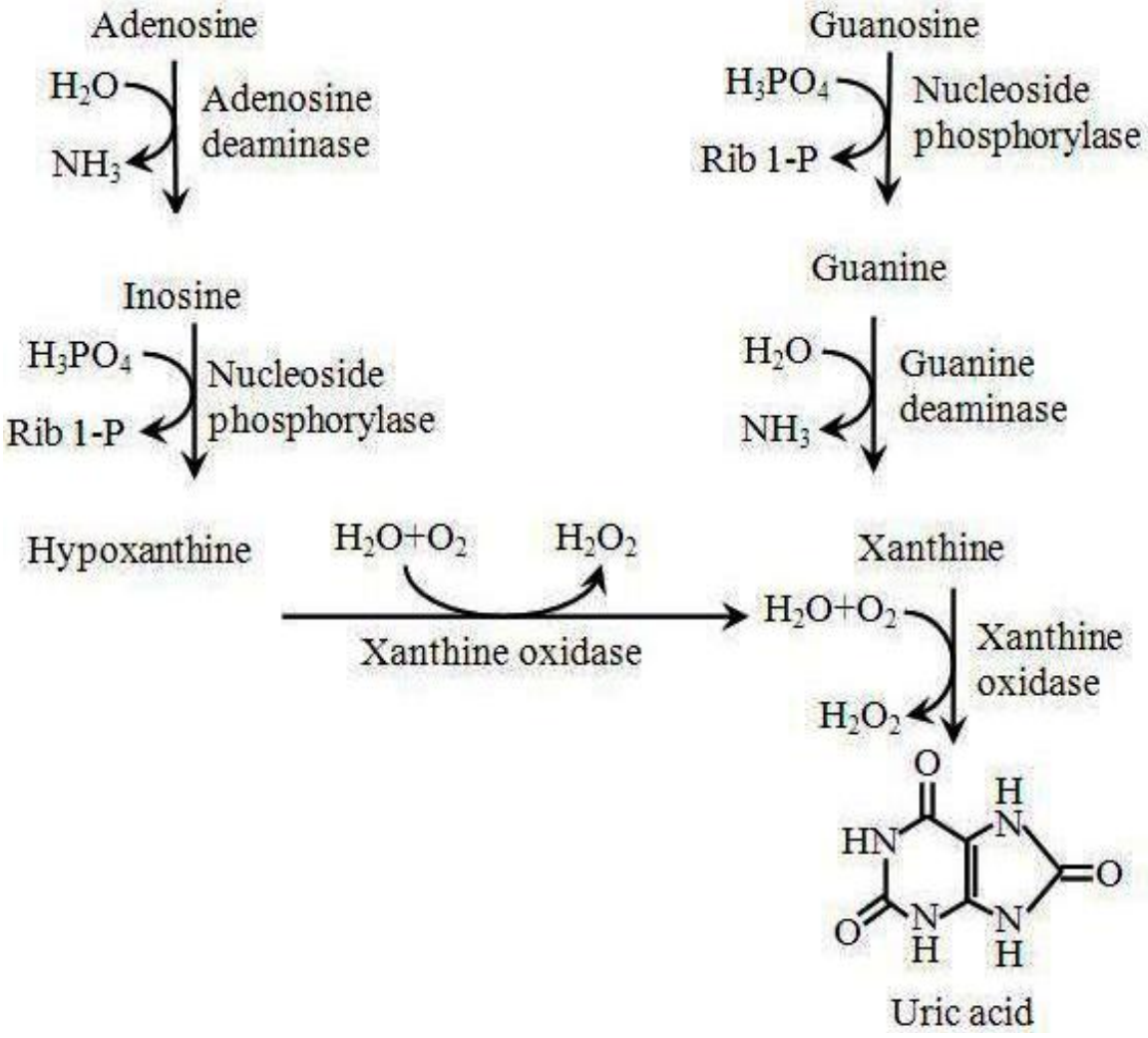


Figure 27.9 – Degradation of purine nucleosides

Each of purine nucleotides (AMP and GMP) can be converted into their corresponding nucleosides by nucleotidase. Reactions of purine nucleoside degradation are shown in the Fig. 27.9.

Normal range of uric acid in the blood plasma is 0.12-0.24 mmol/L, excretion into the urine is 2.36-5.9 mmol/day.

Degradation of pyrimidine nucleotides

The pyrimidine nucleotides are dephosphorylated by nucleotidase to form nucleosides (cytidine, uridine, and thymidine) which undergo further degradation (Fig. 27.10).

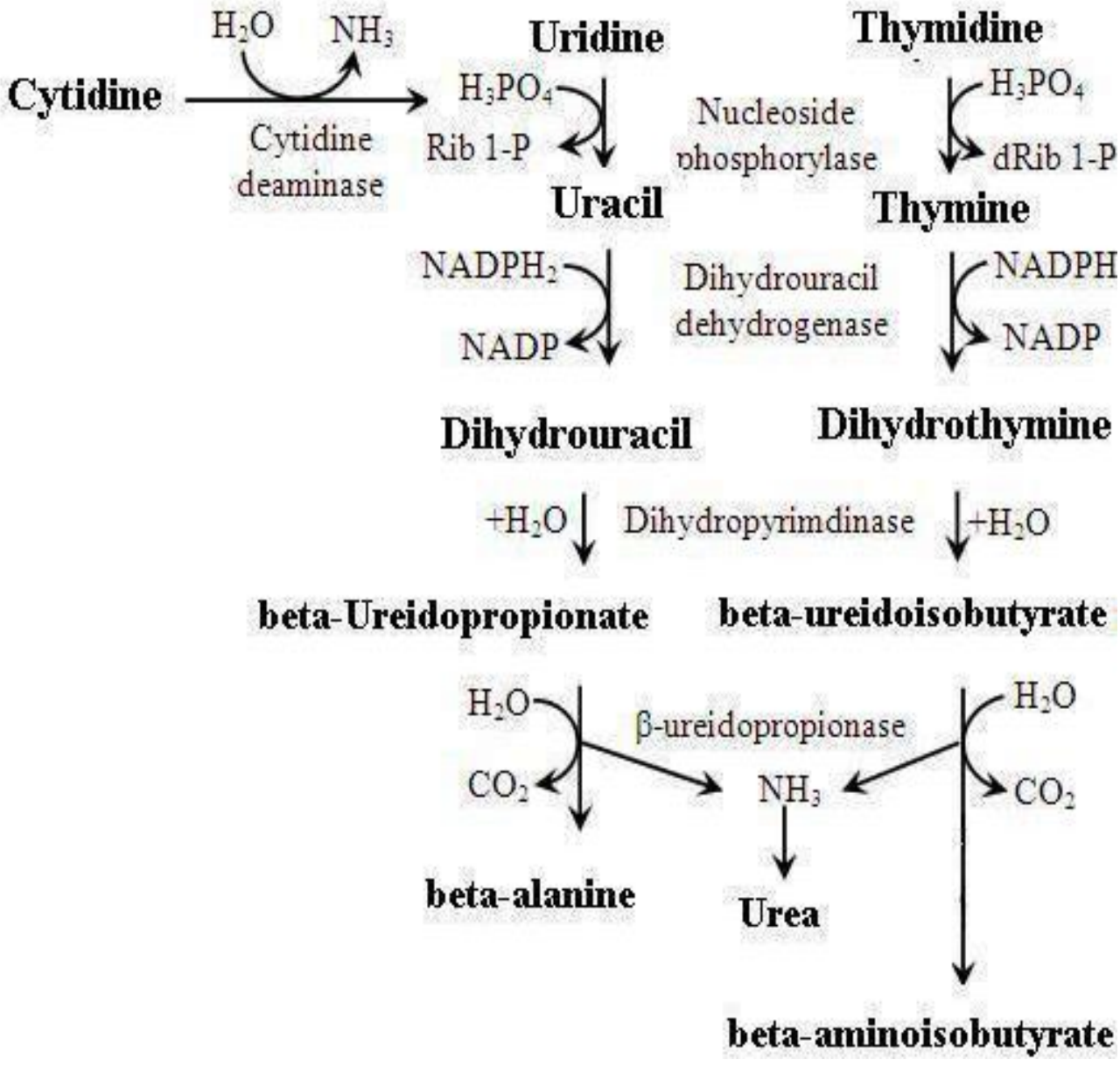


Figure 27.10 – Degradation of pyrimidine nucleosides

The first two reactions are similar to those of purine degradation (deamination of cytidine and phosphorolytic cleavage of the bond between cytosine and ribose). The next reactions include saturation (reduction) of the double bond in the pyrimidine ring and cleavage of the ring to form linear molecule. The latter is degraded to form NH_3, CO_2, and specific end products of pyrimidine catabolism: β-alanine and β-aminoisobuyric acid.

DISORDERS OF NUCLEOTIDE METABOLISM

Xanthinuria

Xanthinuria is a rare genetic disorder caused by inherited deficiency of **xanthine oxidase.** The disease is characterized by decreased production of uric acid (hypouricemia) and increased excretion of hypoxanthine and xanthine.

Type I xanthinuria can be caused by a deficiency of the enzyme converting xanthine to uric acid. Type II xanthinuria is caused by lack of one or two other enzymes in addition to xanthine oxidase. Patients have usually high concentrations of xanthine both in the blood and urine. In alkali urine, xanthinuria may result is xanthine lithiasis (formation of xanthine urinary stones).

Orotaciduria (orotic aciduria)

This rare metabolic disorder results from loss of functional **UMP-synthetase complex** that catalyzes conversion of orotate to UMP. In humans, UMP-synthase complex is composed of orotate phosphoribosyl transferase and OMP-decarboxylase (Fig. 27.4), and is coded by one gene.

The orotic acid accumulated in the organism is excreted into the urine. Higher concentration of orotic acid conducts to formation of specific needle-like crystals in the urine, but may also precipitate in the urinary tract causing its obstruction. This disorder is followed by insufficient synthesis of pyrimidines and DNA due to lack of UMP and TMP. That leads to the state of "**pyrimidine starvation**" of tissues. The disease is characterized by the **retardation of growth, impairment of mental development**, as well as **megaloblastic anemia.**

Treatment. Oral administration of UMP or uridine allows bypass the missing enzyme and provides the body with a source of pyrimidines

Gout

Gout may occur in persons with persistent elevated levels of uric acid in the blood. Uric acid and its salts (urates) exhibit low solubility in water. If concentration of uric acid is increased in the blood, the substance may precipitate to form needle-shaped sodium urate crystals which are deposited in joints.

The most common clinical manifestation of gout is repeated painful attacks of acute (sudden) joint inflammation (arthritis). The typical gouty arthritis affects the first metatarsophalangeal joint (big toe), but other joints of the foot or leg may also be involved. In the gouty attack, the joint pains are so excruciating that even touch of the linen sheet seems unbearable. Gouty attack may last for hours and repeat with intervals in few months.

Deposition of uric acid crystals in joints attracts leukocytes which engulf the crystals. Inside the white blood cell, urate salts disrupt the lysosomal membrane. Lysosomal enzymes released from the organelle digest cells, and products of cell destruction cause inflammation.

Depositions of uric salts around joints are called **tophi**. More often, tophi are located in small joints. Tophi cause deformity of joints and impair their function.

Increased excretion of uric acid may cause uric acid crystals to be deposited in the collecting tubules of kidney and lower urinary tract, leading to stone formation (**urolithiasis**). The deposition of urate crystals in renal medulla results in renal damage and impairment of renal function. Renal failure is the most frequent complication of gout.

Treatment. To reduce urate production in the organism, allopurinol is used. This structural analogue of hypoxanthine inhibits xanthine oxidase and hence decreases formation of uric acid.

CHAPTER 28

REGULATION AND INTEGRATION OF METABOLISM

For normal functioning of the body precise regulation of metabolite flow through anabolic and catabolic pathways should be performed. All related chemical processes should occur at speeds corresponding to the requirements of the organism as a whole within the environment. ATP generation, synthesis of macromolecules, transport, secretion, reabsorption, and other processes must respond to changes in the environment in which the cell, body or the whole organism exists.

The survival of multicellular organisms depends on their ability to adapt to a constantly changing environment. Intercellular communication mechanisms are necessary requirements for this adaptation.

Cellular metabolism is based on the principle of **maximal economy**. Cell consumes at any given moment such an amount of nutrients, which allows satisfying its need in energy. This high organization and coordination of metabolism is achieved by **regulatory mechanisms**. These mechanisms are quite varied and have a several levels of metabolic regulation:

- molecular;
- cellular;
- organ (tissue);
- organism.

According to the time of reaching the regulatory effect, there is fast regulation (within seconds and minutes) and slow regulation (within hours and days).

The main **regulatory mechanisms** are:

1. Regulation at the membrane level;
3. Adjusting the amount of enzymes;
4. Adjusting the enzyme activity;
5. Hormonal regulation and intracellular signal transduction pathways.

Regulation at the level of membranes can occur through several mechanisms:

- Specific ***biochemical signals*** such as neurotransmitters, hormones, and immunoglobulins bind to integral transmembrane receptor

proteins via their exposed extracellular domains, thereby transmitting information through the membranes to the cytoplasm (Chapter 13). This process, called ***transmembrane signaling*** or signal transduction, involves the generation of a number of second messenger signaling molecules, including cyclic nucleotides (**cAMP**), Ca2+, phosphoinositides (**IP_3**), and diacylglycerol (**DAG**). Many of the steps involve phosphorylation of receptors and downstream proteins.

- Selective ***permeability of membranes*** for various metabolites and ions. At the level of membranes the following regulatory factors are implemented: availability of substrates and coenzymes, removal of reaction products.
- Regulation of the ***enzyme amount***. The concentration of each enzyme is determined by correlation between the speed of its synthesis and decay. The speed of synthesis of enzymes is regulated by mechanisms common for other proteins synthesis regulation. Influence of regulatory factors may be integrally manifested in the form of repression or induction of enzyme synthesis. This mechanism concerns to a slow type of metabolic regulation.
- Regulation of ***enzyme activity***. It is one of the most diverse methods of metabolic regulation. It can be realized by a variety of mechanisms, which are detailed in Chapter 4.

Allosteric regulation of metabolic pathways

Allosteric regulators are usually of two types:

1. The final products of successive chain reactions regulating their synthesis using **back-coupling** principle.

2. ATP, ADP, AMP, NAD, and NADH + H^+. These compounds, although they are not the end products of metabolic pathways, are formed as a result of their course and have a regulatory effect on the production rate. **ATP** serves as an activator of enzymes acting in the direction of synthesis of biopolymers and the accumulation of energy and is an inhibitor of catabolic reactions. **ADP** (sometimes **AMP**) plays the opposite role – activates catabolism way, ensuring their conversion to ATP, and inhibits anabolic processes associated with the consumption of ATP. **NAD** acts like AMP, and NADH + H^+ acts like ATP.

Typically **allosteric enzymes** take place at the beginning of the reaction sequence and catalyze the stage which limits the speed of the

overall process. Usually practically irreversible reaction plays the role of such a stage. In some cases, an allosteric enzyme of one metabolic pathway specifically responds to intermediate or final products of the other. This helps achieve the necessary coordination of the different metabolic pathways, aimed at providing specific functions or processes.

For example, in muscle contraction the rate of ATP utilization increases, which is necessary for energy supply in this process. Thus, with the help of regulatory mechanisms, the rate of glycolysis becomes increased compensatory. As a result of glycolysis activation, the rate of accumulation of acetyl-CoA, being a substrate of the TCA cycle, increases. Activation of the TCA cycle leads to increased amounts of NADH + H^+, which is involved in the chain of tissue respiration, which functioning activity also increases. This leads to re-synthesis of ATP and replenishment of its pool, decreased as a result of muscle contraction.

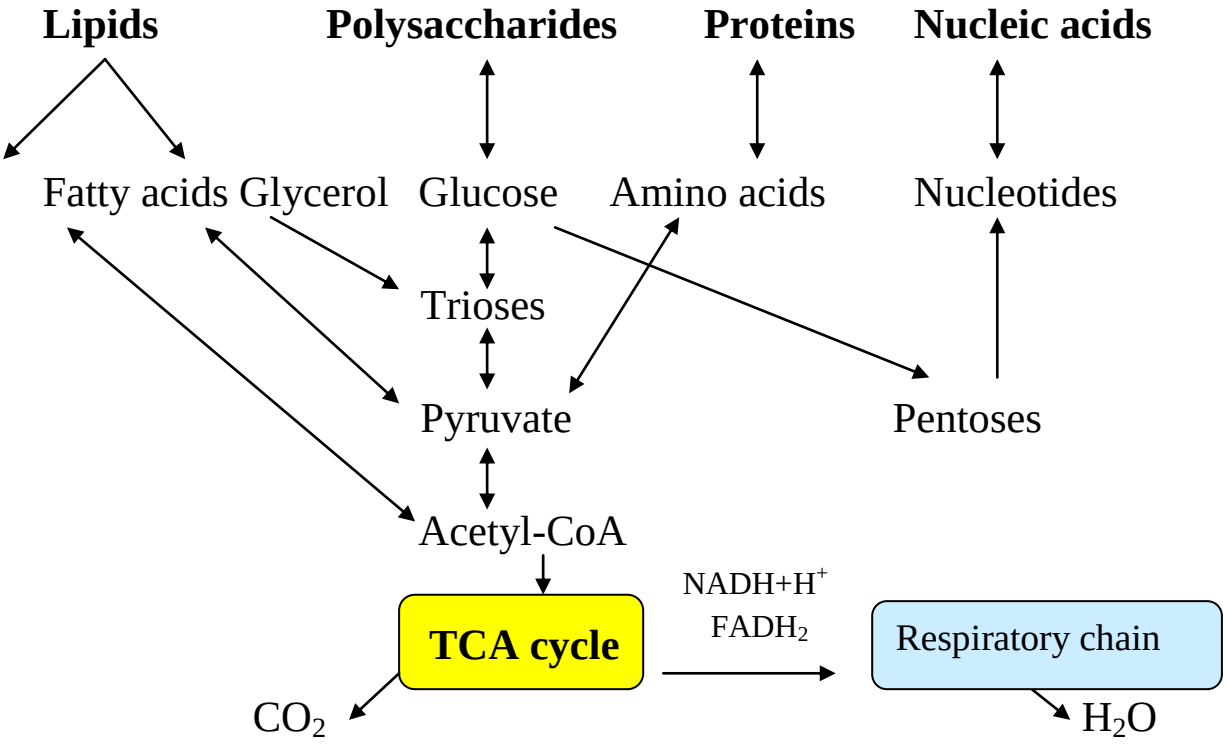


Figure 28.1 – Integration of metabolism

Integration of metabolism

In general, metabolism should not be understood as a sum of exchange of proteins, nucleic acids, carbohydrates, and lipids. In the result of interaction between the exchanges of individual classes of organic

compounds, the unified ***system of metabolic processes*** representing a qualitatively new formation occurs. Exchanges of major structural monomers of living systems – amino acids, monosaccharides (glucose), fatty acids, and mononucleotides are closely interrelated.

This interrelation is performed via key metabolites that serve as a common link on the routes of decay or fusion. Correlation of exchanges of organic compounds separate classes is particularly well expressed in the processes of their mutual transformation, though is not limited only to this. An example of such interconversion may be the weight gain due to the deposition of subcutaneous fat layer in excess consumption of carbohydrate foods. The key metabolites that carry out metabolism correlation are ***lactate***, ***pyruvate***, ***glycerophosphate***, ***acetyl-CoA***, some metabolites of the citric acid cycle (Figure 28.1).

CHAPTER 29

BIOCHEMISTRY OF THE LIVER

The liver is one of the largest organs in the human body weighing 1.2 to 2 kg, and constituting about 2-3% of body weight. The morphological and functional integrity of the liver is vital to the health of the human organism. This essentially depends upon constant maintenance of the numerous biochemical functions of the liver and the diverse metabolic processes occurring in the hepatocytes and sinusoidal cells.

Major functions of the liver

1. **Homeostatic function.** The liver collects and processes all of the gastrointestinal blood through the portal vein. It regulates the blood levels of glucose, amino acids, and other nutrients taken from food, thereby maintaining steady internal conditions in the body.
2. **Biosynthetic function.** Production and secretion of compounds for extrahepatic tissues (most blood proteins, coagulation factors, lipids, glucose, ketone bodies, etc.).
3. **Storage function.** The liver not only stores energy reserves and nutrients for the body (e.g., glycogen), but also certain mineral substances, trace elements, and vitamins, including iron, retinol, and vitamins A, D, K, folic acid, and B_{12}.
4. **Protective (detoxification) function.** The liver removes harmful substances (such as ammonia and toxins) from the blood and then breaks them down or transforms them into less harmful compounds. It also metabolizes most hormones and ingested drugs to either more or less active products. Kupffer cells in the liver ingest bacteria or other foreign material from the blood.
5. **Digestive function.** The liver takes part in digestion via synthesis of bile acids and production/secretion of and the bile.
6. **Excretory function.** Excretion of various substances with the bile (water, cholesterol, bile pigments, phospholipids, bicarbonate, and other ions).

In addition the liver plays a central role in metabolism of most nutrients taken from food, and is involved in metabolism of carbohydrates, lipids, proteins, amino acids, porphyrins etc.

Carbohydrate metabolism of the liver

The functions of the liver in carbohydrate metabolism:

- Regulation of the blood glucose level;
- Oxidative degradation of glucose either to CO_2 and H_2O, or lactate;
- Synthesis of glycogen, conversion of glycogen to glucose;
- Gluconeogenesis or synthesis of glucose from non-carbohydrate compounds;
- Conversion of glucose via pentose phosphate pathway;
- Conversion of dietary monosaccharides, e.g. fructose and galactose to glucose;
- Metabolism of glucose to glucoronic acid.

The liver is the key organ for regulation of carbohydrate metabolism and maintenance of **physiological blood glucose level** within narrow limits (3.3 – 6.4 mmol/L). The liver responds to the increase in blood glucose that follows a meal by removing about 87% of the glucose delivered from intestine by the portal vein.

On feeding, glucose taken from intestine is able to cross the plasma membrane freely and enter the liver cells independently of insulin. It is capable of taking part in metabolism only after phosphorylation to glucose-6-phosphate (by hexokinase or glucokinase) (Fig. 29.1).

Glucokinase is the hepatic enzyme which activity is regulated by high glucose concentration in the bloodstream after meal. After that, glucose-6-phosphate may be channeled into various metabolic pathways, e.g. into pentose-phosphate pathway, into glycolysis with pyruvate and/or lactate as end products, or into the citric acid cycle with degradation into CO_2, H_2O and energy (ATP). The rest of glucose is polymerized to glycogen in the process called glycogenesis. The liver is also involved in metabolism of galactose and fructose through the reactions of glycolysis.

If a blood glucose level is below the normal fasting level, the liver produces glucose both from glycogen reserves **(glycogenolysis)** and substances other than carbohydrates **(gluconeogenesis)** to compensate for any deficiency and maintain the normal glucose level in the blood.

The liver produces **glucuronic acid** and **UDP-glucoronic acid** from glucose and glucose-6-phosphate, which serve as a material in reactions of **glucuronidation**.

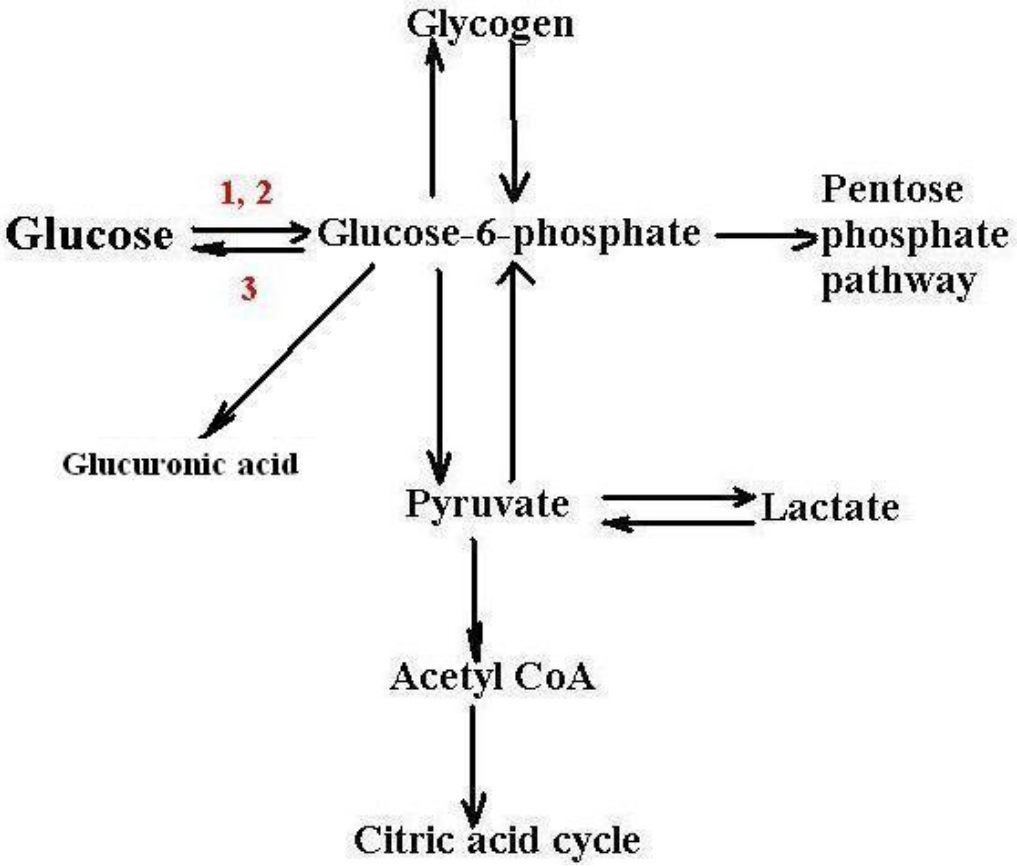


Figure 29.1 – Fate of glucose in the liver
(1 – hexokinase; 2- glucokinase; 3- glucose-6-phosphatase)

Lipid metabolism of the liver

The liver has a variety of functions in lipid metabolism:

- Uptake, oxidation of free fatty acids for energy;
- Synthesis of triacylglycerols, phospholipids, cholesterol, and esters of cholesterol;
- Formation of plasma lipoproteins (VLDL and HDL) and their catabolism, production of apo-proteins and enzymes for lipoprotein metabolism;
- Formation of ketone bodies;
- Metabolism of phospholipids;
- Synthesis of bile acids;
- Hydroxylation of the vitamin D.

The mechanism of oxidative energy generation in the liver is mainly the result of β-oxidation of fatty acids in mitochondria. This process begins with activation of free fatty acids, and followed by splitting of 2 carbon units, until the carbon chain has been completely broken down to the acetyl

CoA. The resulted acetyl-CoA can be primarily channeled into the citric acid cycle or used for synthesis of ketone bodies. The latter process is active in fasting state, when ketone bodies are required for generation of energy in extrahepatic tissues.

Synthesis of fatty acids is carried out by a mean of carboxylation of acetyl CoA to malonyl CoA in the cytoplasm and further reactions catalyzed by Fatty Acid Synthetase (FAS). The formed fatty acids are then used for synthesis of triacylglycerols and phospholipids. The liver is the major site of both synthesis and catabolism of cholesterol and phospholipids. In the liver, triacylglycerols, phospholipids, and cholesterol are packed into lipoproteins that are the major transport form of lipids in the blood. Apparently the liver is the place of synthesis of very low VLDL and HDL, and the place of destruction of cholesterol-rich HDL as well as chylomicron "remnants".

Another important function of the liver is the synthesis of bile acids from excess cholesterol, which has been synthesized or taken from food. Bile acids are then excreted with the bile to the intestine and used for digestion of dietary fats.

Fatty liver disease (or fatty liver) results from abnormal accumulation of neutral fats in hepatocytes because of elevated synthesis of triacylglycerols. Fatty liver is commonly associated with deficiency of necessary **lipotrophic factors** (choline, methionine), that leads to decreased production of phospholipids. The main causes of fatty liver are alcohol abuse, diabetes, hypertension, obesity, and some others.

Protein and amino acid metabolism of the liver

The functions of the liver in protein metabolism:

- Synthesis of the most of plasma proteins;
- Catabolism of amino acids by deamination, transamination, transdeamination, etc;
- Formation of urea, detoxification of ammonia;
- Formation of uric acid from purine bases;
- Synthesis of creatine.

The liver may synthesize approximately 120g of proteins daily. Of these proteins 70% – 80% are released by hepatocytes, so that only 20% – 30% remain available for their own use. The liver synthesizes more than 100 plasma proteins, the majority being glycoproteins. Among them are transport proteins ***(albumin, ceruloplasmin, transferrin,*** *etc),* **clotting**

factors ***(fibrinogen, prothrombin, coagulation factors V, VII, IX, X, and XI),*** and **acute-phase proteins** ***(C-reactive protein, haptoglobin, etc).*** The latter are detected only in pathological states. In addition, most of the plasma proteins are broken down in the liver.

The liver is able to convert all proteinogenic amino acids and controls levels of free amino acids in the blood plasma. Excess amino acids taken from food or delivered from other tissues undergo transamination, or deamination (transdeamination) reactions with release of ammonia. Carbon skeletons of amino acids enter the intermediary metabolism and serve for glucose synthesis or energy production.

Catabolism of amino acids generates ammonia (NH_3) and ammonium ions (NH_4^+). Ammonia produced by bacterial putrefaction in the intestine is also undergone conversions in the livers. Ammonia is toxic, particularly to the central nervous system. The liver is able to detoxify it by converting into urea in the urea cycle. The resulted urea is easily excreted via the kidneys.

Protective (or detoxification) function of the liver

Detoxification reactions are those biochemical changes in the body that convert toxic, nonpolar compounds to the less toxic and more readily extractable compounds. It should be noted, however, that in many reactions the toxicity is not completely eliminated but only lessened.

The liver metabolizes most of foreign substances (xenobiotics, drugs, ethanol), as well as steroid hormones, bilirubin, etc. These substances are inactivated and converted into highly polar extractable metabolites.

Detoxification process in the liver occurs in two phases:

phase I: the polarity of the substance is increased by oxidation or hydroxylation catalyzed by a family of microsomal cytochrome P450 oxidases (microsomal oxidation);

phase II: cytoplasmic enzymes conjugate the functional groups introduced in the first phase reactions, most often by glucuronidation, or other reactions.

Conjugation reactions:

- **Glucuronidation** is the most common type of conjugation in the liver that involves production of ester bond between a substance and glucuronic acid. For these reactions, glucuronic acid is first activated to **UDP-glucuronic acid,** which has two high-energy bonds. The energy of one of these bonds is used in the reaction. Steroids and bilirubin (Fig. 29.2),

and some antibiotics are examples of compounds which are conjugated by glucuronic acid.

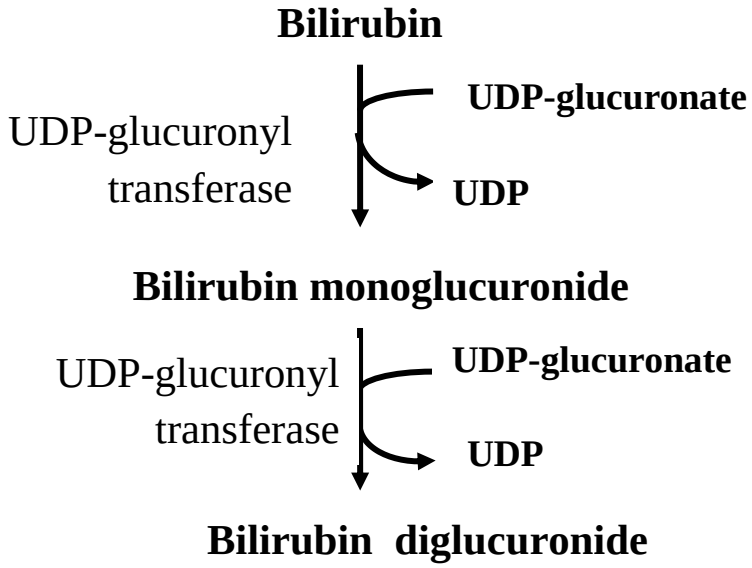


Figure 29.2 – Conjugation of bilirubin in the liver

- **Conjugation with glycine.** Glycine is conjugated with such compounds as benzoic acid, nicotinic acid, para-aminobenzoic acid, etc.
- **Sulfation reaction** involves a sulfotransferase enzyme catalyzing the transfer of a sulfo group from 3'-phosphoadenosine-5'-phosphosulfate, to a substrate molecule's hydroxyl or amine (Fig. 29.3). This reaction is used in the detoxification of indole and skatole that are toxic derivatives of tryptophan formed in bacterial putrefaction.

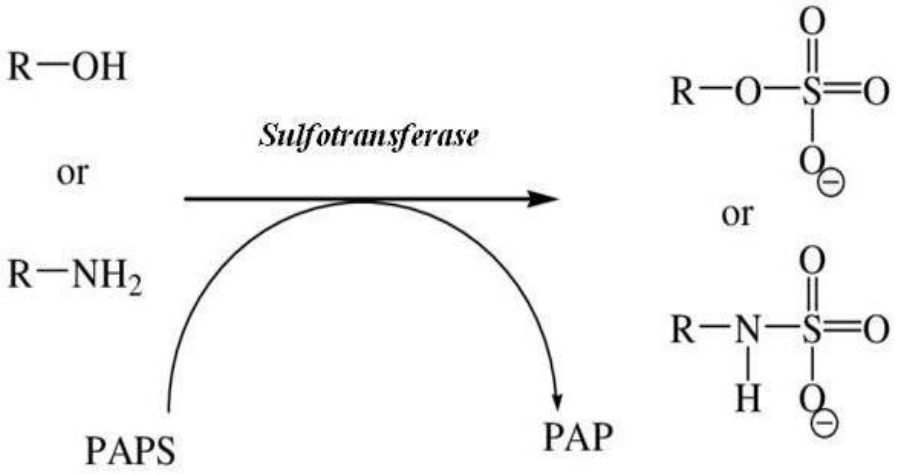


Figure 29.3 - Scheme of sulfation reaction in the liver.
(PAPS – 3'-phosphoadenosine-5'-phosphosulfate; PAP — 3'-phosphoadenosine-5'-phosph*ate)*

- **Methylation reactions.** Examples are methylation of pyridine and nicotinic acid.
- **Acetylation reactions** are used in detoxification of sulfonamide drugs.

$$X + \text{Acetyl CoA} \rightarrow \text{acetyl-}X + CoA$$

- **Microsomal oxidation** conducts to hydroxylation of a non-polar substance R-H into the polar water soluble product R-OH. The process requires molecular oxygen, reduced coenzyme NADPH, and cytochrome P450.

$$R\text{-}H + O_2 + NADPH + H^+ \rightarrow R\text{-}OH + H_2O + NADP^+$$

Besides, foreign substances may undergo **hydrolysis** and **reduction reactions**.

Bilirubin metabolism

Bilirubin is the breakdown product of the heme moiety of hemoglobin, and other hemoproteins (myoglobin, cytochromes, catalase, peroxidase, etc.). In humans about 85% of bilirubin is derived from the hemoglobin of senescent red blood cells that are phagocytosed by mononuclear cells of the spleen, bone marrow, and liver. The remaining reminders 15% come from catabolism of the precursors of erythrocytes and heme-containing proteins. An average person produces about 4 mg/kg of total bilirubin per day. The daily output of bilirubin is 250 – 350 mg.

- Bilirubin is the orange-yellow pigment derived from senescent red blood cells;
- It is a toxic waste product in the body;
- It is extracted and biotransformed mainly in the liver, and excreted in the bile and urine;
- It is a bile pigment;
- Elevations in serum and urine bilirubin levels are normally associated with jaundice;
- Determination of the levels of total bilirubin, indirect and direct bilirubin, bile pigments is used for diagnosis of liver diseases;
- Metabolism of bilirurin takes place in the cells of reticuloendotelial system, the liver, and intestine.

Erythrocytes become “old” as they lose their flexibility and become

pikilocytes (spherical), increasingly rigid and fragile. Their useful life span is 100 to 120 days, after which they become trapped and fragment in smaller circulatory channels, particularly in those of the spleen. For this reason, the spleen is sometimes called the "red blood cell graveyard".

In cells of reticuloendothelial system, the ring structure of the heme is open, and the iron atom is removed by the action of heme oxygenase and cytochrome P_{450} to produce the green-colored intermediate biliverdin. The reaction consumes 3 molecules of O_2 and the 1 molecule of NADPH with release of carbon monoxide. Subsequent reduction by biliverdin reductase converts biliverdin to indirect bilirubin, which has a red-orange color *(*Fig. 29.4*).*

Indirect (or unconjugated) bilirubin is a toxic, water-insoluble substance. It is bound to serum albumin and transported to the sinusoidal membrane of the liver cells as a **bilirubin-albumin complex**.

In the liver, bilirubin undergoes modification to increase its water solubility so that it can be excreted more easily. Bilirubin is conjugated to two molecules of glucuronic acid, creating **bilirubin diglucuronide (or direct, conjugated bilirubin)** (Fig. 29.2).

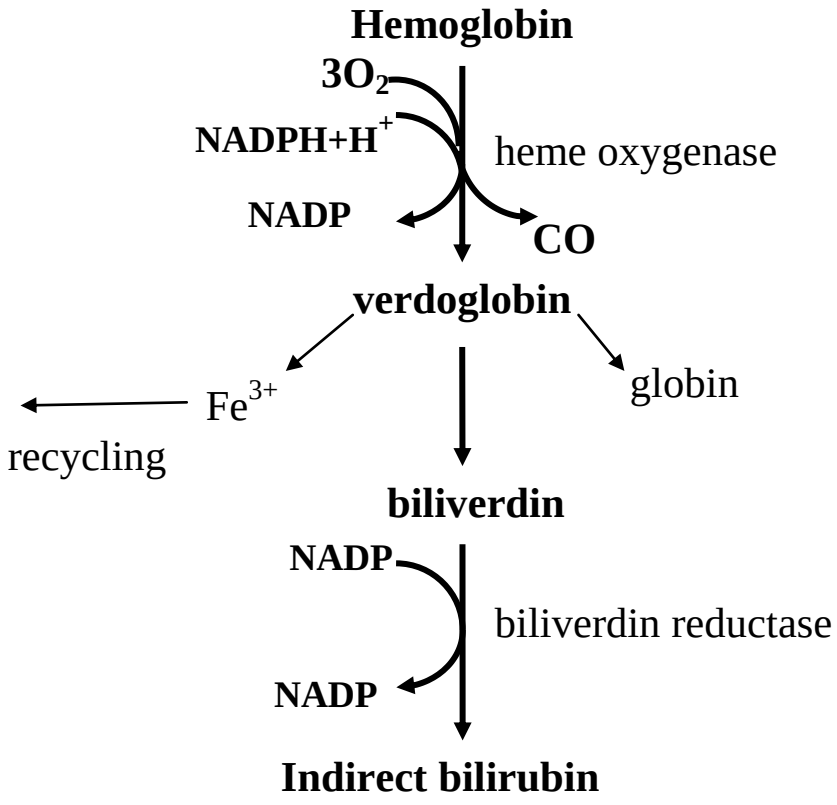


Figure 29.4 – Formation of indirect bilirubin in the cells of reticuloendotelial system

Conjugated bilirubin is a water-soluble, less toxic substance, which is subsequently eliminated via the bile and urine. Conjugated form of bilirubin is normally present in the blood in 3 – 10% of the total serum bilirubin.

The liver secretes conjugated bilirubin into the biliary canaliculi and finally to the small intestine (Fig. 29.5).

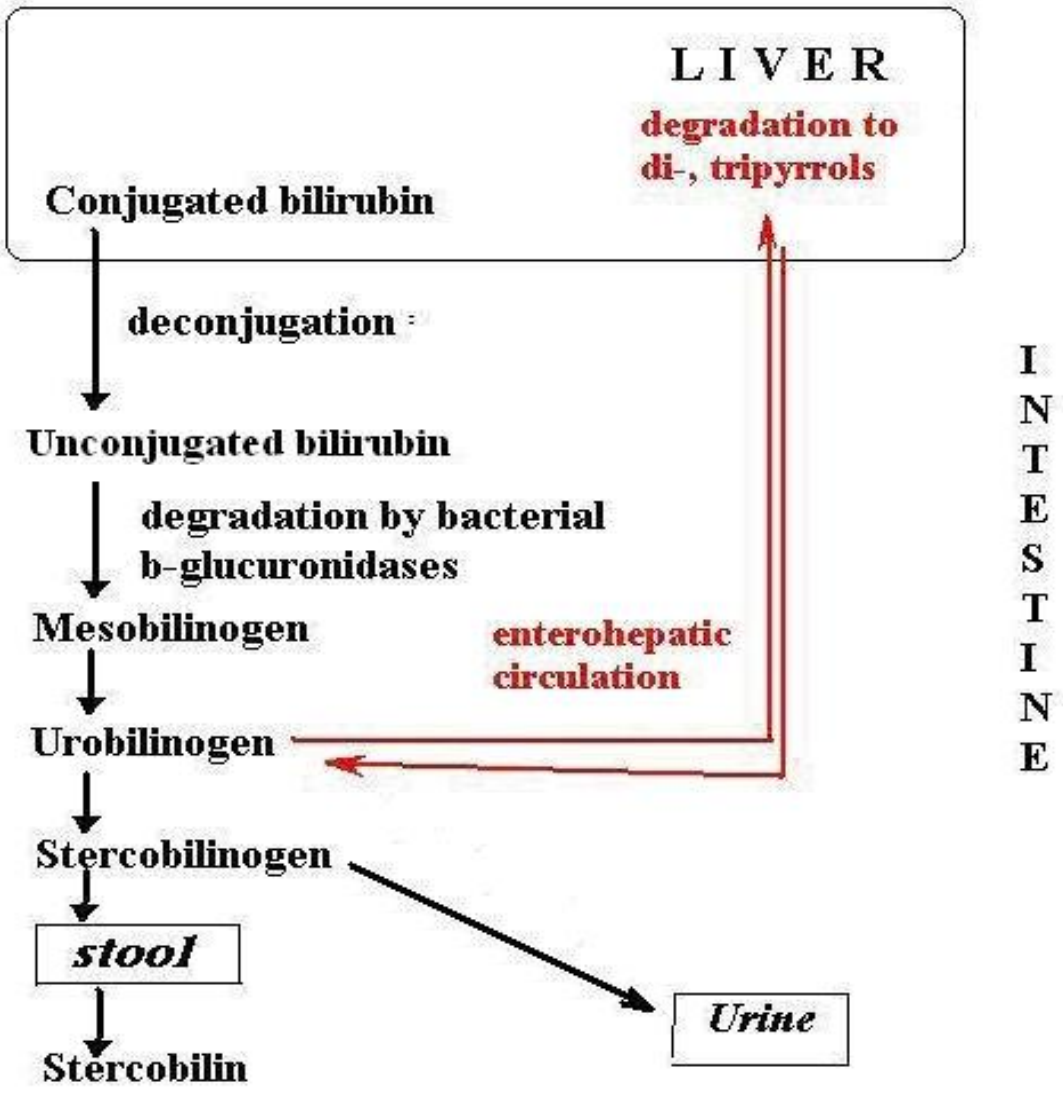


Figure 29.5 – Metabolism of bilirubin and bile pigments in the liver and intestine

In the intestine, anaerobic flora mediate further catabolism of bilirubin into bile pigments:

1. Hydrolysis of conjugated bilirubin to unconjugated bilirubin by bacterial β-glucuronidases in the distal ileum and colon;
2. Multistep hydrogenation (reduction) of unconjugated bilirubin to the colored bile pigments: **mesobilinogen, urobilinogen,** and **stercobilinogen;**

3. Up to 80% of **urobilinogen** produced daily is reduced to **stercobilinogen.** The rest 20% of urobilinogen is reabsorbed from the intestine and enters the enterohepatic circulation. The liver breaks down about 5 % of this urobilinogen to **di- and tri-pyrrole compounds**, which are excreted in the urine. The rest of the reabsorbed urobilinogen comes back to the intestine with the bile.

4. In the colon, the **stercobilinogen** spontaneously oxidized to **stercobilin**, which is colored; most stercobilin is excreted in the feces and responsible for the color of feces. A small fraction of stercobilinogen, (2% – 5%) enters the general circulation and appears in the urine.

Normal ranges of bile pigments in biological fluids are following:
total bilirubin – 5.0 – 20.5 mkmol/L(blood);
indirect bilirubin – 1.7 – 17.1 mkmol/L (blood);
direct bilirubin – 1.0 – 7.5 mkmol/L (blood);
stercobilinogen – 4 mg/day (urine);
stercobilin – 250 mg/day (feces).

Abnormalities in bilirubin metabolism are important pointers in the diagnosis of liver disease. In excess, bilirubin imparts a yellow color to the skin. This is called jaundice.

Jaundice

Hyperbilirubinemia or increased plasma concentrations of bilirubin occurs when there is an imbalance between its production and excretion. Jaundice becomes clinically evident **when the serum bilirubin level exceeds 27 - 34 mkmol/L.**

The main symptoms of the jaundice are:

- Icterus (yellow discoloration of the skin, sclerae, and mucous membrane);
- Itching due to deposits of bile salts on the skin;
- Changes in the color of stool;
- Deep orange and foamy urine.

The causes of jaundice are conventionally classified as:

I. **Prehepatic (hemolytic):** increased production of bilirubin;
II. **Intrahepatic (hepatocellular):** impaired hepatic uptake, conjugation, or secretion of bilirubin;
III. **Posthepatic (obstructive):** obstruction to biliary drainage.

1. Prehepatic (or hemolytic) jaundice results from excess production of bilirubin after hemolysis or a genetic abnormality in the hepatic uptake of unconjugated bilirubin. Commonly, hemolysis is the result of immune disease, structurally abnormal red cells, or breakdown of extravagated blood. Intravascular hemolysis releases hemoglobin into the plasma, where it is either oxidized to methemoglobin or complexed with haptoglobin. More commonly, red cells are hemolyzed extravascularly, within phagocytes, and hemoglobin is converted to bilirubin; so bilirubin is unconjugated.

Prehepatic jaundice is diagnosed **by high plasma concentrations of indirect bilirubin and unchanged direct bilirubin.** Increased level of unconjugated bilirubin causes overproduction of urobilinogen and stercobilinogen in the intestine. That results in the **increased concentration of urine stercobilinogen** and **of fecal stercobilin** (dark brown stool).

2. Intrahepatic jaundice reflects a generalized hepatocyte dysfunction and is also called hepatocellular. This kind of jaundice arises from liver disease, either inherited or acquired. The liver dysfunction impairs conjugation of bilirubin that leads to the spilling over both types of bilirubin into the blood. Therefore, hyperbilirubinemia is accompanied **by increased levels of both indirect and direct bilirubin.** In addition, **urobilinogen is elevated in the urine** because of impaired reduction of it to the di- and tri-pyrrol end products. The urine is dark because of **appearance of direct bilirubin.**

3. Posthepatic jaundice is caused by obstruction of the biliary duct by a gallstone or a tumor (usually of the head of the pancreas). Therefore the second name of this jaundice is obstructive. This prevents passage of the bile into intestine and leads to appearance of most of direct bilirubin in the blood. Obstructive jaundice is diagnosed by the predominant **elevation of direct bilirubin in the blood**, as well as other biliary metabolites, such as bile acids.

The clinical features are pale-colored stools, caused by the **absence of fecal bilirubin and stercobilin,** and **dark urine** as a result of the **presence of the water-soluble direct bilirubin**.

Neonatal “Physiological Jaundice”

This type of jaundice in newborn children results from the unconjugated hyperbilirubinemia due to accelerated hemolysis and immature hepatic system for the uptake, conjugation, and secretion of

bilirubin. In this transient condition, the glucuronation of indirect bilirubin, and probably synthesis of UDP-glucoronate, are reduced. When the plasma level of indirect bilirubin exceeds that which can be tightly bound by albumin, bilirubin can penetrate the blood-brain barrier.

Jaundice in the first 24 hours of life is abnormal and requires investigation to exclude hemolysis. Jaundice that is presentlater – after 10 days – is always abnormal, and is likely to indicate an inborn error of metabolism or structural defects of the bile ducts. If left untreated, the resulted hyperbilirubinemic toxic encephalopathy, or kernicterus, can result in mental retardation. Exposure of jaundiced neonates to blue light (phototherapy) promotes hepatic excreation of indirect bilirubin by converting it to water-soluble derivatives, which are excreted in the bile.

Genetic causes of jaundice

There are a number of genetic disorders that impair bilirubin conjugation or secretion. **Gilbert's syndrome**, affecting up to 5% of the population, causes a mild unconjugated hyperbilirubinemia that is harmless and asymptomatic. It is due to a modest impairment in ur UDP-glucuronyl transferase activity.

Other inherited diseases of bilirubin metabolism are rare. **Crigler-Najjar syndrome**, which is the result of a complete absence or marked reduction in bilirubin conjugation, causes a severe unconjugated hyperbilirubinemia that is present at birth; when the enzyme is completely absent, the condition is fatal. The **Dubin-Johnson** and **Rotor's syndromes** impair the biliary secretion of conjugated bilirubin, and therefore cause a conjugated hyperbilirubinemia, which is usually mild.

HEME SYNTHESIS IN THE LIVER

The heme moieties represent the prosthetic groups of hemoproteins (hemoglobin, myoglobin, cytochromes, oxygenases, catalases, etc). Synthesis of heme mainly takes place in the bone marrow (80 – 85%) and in the liver (15%). Two-third of the heme synthesized in the liver is required for the formation of cytochrome P 450.

Daily production of the heme is about 300 mg, of which only 1% is excreted unused in the urine and stools. Heme synthesis begins in the mitochondria, then progresses through a series of cytosolic reactions before being completed again in the mitochondria (Fig. 29.6).

The porphyrinogens represent colourless intermediate products. Each step in this process is enzymatically controlled. The most crucial enzyme is **δ-aminolaevulinic acid synthase**, whose activity is subject to negative feedback control by the end product **heme,** therefore heme controls its own rate of synthesis.

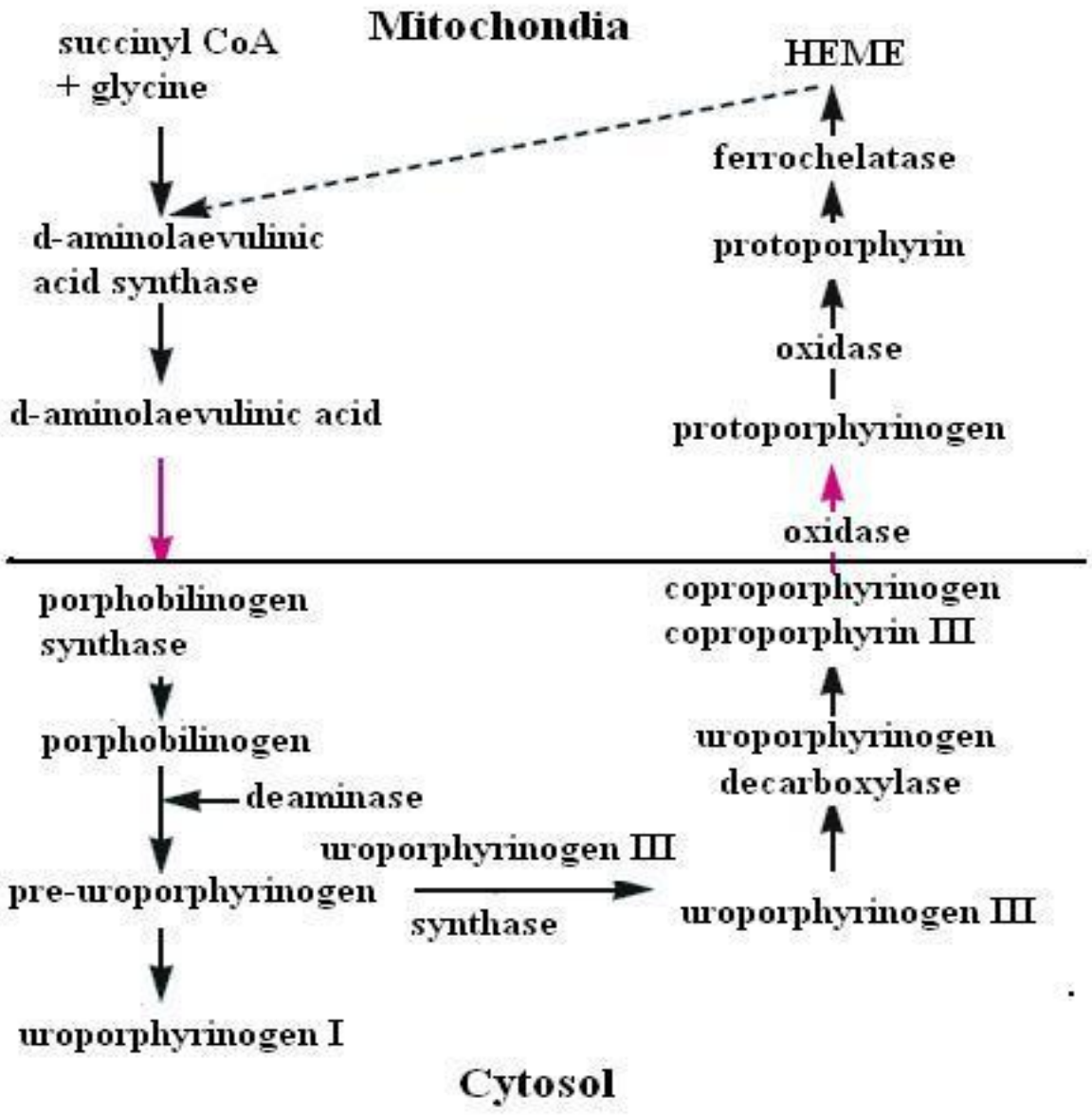


Figure 29.6 – Synthesis of heme in the liver

Porphyrins are irreversible oxidation products. With the exception of protoporphyrin, they are not utilized for other purposes, but are excreted. Under pathological conditions, they are stored in cells.

Porphyrias are diseases resulted from deficiency of some enzymes involved in the synthesis of heme and accumulation of porphyrinogens. Porphyrias may be termed **erythropoetic** or **hepatic** base on the most

affected organs. The common symptoms of porphyrias include photosensitivity and severe disfigurement, due to which patients were suggested as prototypes of so-called werewolves.

Biochemical mechanisms of hepatic failure and hepatic coma

Liver failure or hepatic failure is the inability of the liver to perform its normal synthetic and metabolic functions as part of normal physiology. Two forms are recognized, acute and chronic.

Acute liver failure is defined as "the rapid development of hepatocellular dysfunction, specifically coagulopathy and mental status changes (encephalopathy) in a patient without known prior liver disease". Hepatocellular disease may alter protein synthesis both quantitatively and qualitatively. Low plasma albumin concentrations occur commonly in liver disease, but a better index of hepatocyte synthetic function is the production of the coagulation factors II, VII, IX, and X.

The diagnosis of acute liver failure is based on physical exam, laboratory findings, patient history, and past medical history to establish mental status changes, coagulopathy, rapidity of onset, and absence of known prior liver disease respectively.

Chronic liver failure usually occurs in the cirrhosis as the result of many possible causes, such as excessive alcohol intake, hepatitis B or C, autoimmune, hereditary, and metabolic causes (such as iron or copper overload, steatohepatitis or non-alcoholic fatty liver disease).

Main causes of the liver failure:

- acute viral hepatitis;
- cirrhosis (alcoholic or non-alcoholic);
- excessive injuries or traumas;
- sepsis;
- poisonings by hepatotrophic venoms and medicines.

Signs of liver failure:

- hyperbilirubinemia;
- low total serum protein and albumin level;
- coagulopathy and hemorrhage because of impaired synthesis of clotting proteins;
- low levels of potassium, sodium, and calcium in the blood;
- high levels of toxic phenol and indol derivatives, aromatic, branched, and sulfur-containing amino acids in the blood.

Hepatic coma is a state of unconsciousness when the patient cannot

be aroused, even by powerful stimuli. Hepatic coma accompanies cerebral damage resulting from degeneration of liver cells especially that associated with cirrhosis of the liver.

Liver function tests

The liver has a substantial reserve metabolic capacity; mild liver disease may cause no symptoms, and be detected only as biochemical changes in the blood. However, the patient with severe liver disease has a yellow pigmentation of the skin, bruises readily, may bleed profusely, has an abdomen distended with fluid (ascites), and may be confused or unconscious (hepatic encephalopathy).

Liver function tests (**LFTs** or **LFs**) are groups of blood tests that provide information about the state of a patient's liver. A panel of biochemical measurements is routinely performed in the clinical laboratories on plasma or serum specimens.

The standard liver panel includes determination of:

- **total serum protein and albumin** (low plasma albumin is detected in acute and chronic liver diseases);
- **total bilirubin** and **its direct and indirect forms**, other **bile pigments;**
- **blood ammonia** (elevated in cirrhosis of the liver and disorders of the urea cycle);
- activities of **alanine aminotransferase (AlAT)** and **aspartate aminotransferase (AsAT)** (higher increases in the AlAT activity compared to the AsAT activity);
- activity of **alkaline phosphatase (ALP)** (increases in cholestasis);
- **gamma-glutamyl transferase (GTT)** (increases in alcohol abuse and hepatitis).

Other liver tests:

- **coagulation test.** Determination of **prothrombin time** and its derived measures of **prothrombin ratio (PR)** and **international normalized ration (INR);**
- Determination of ceruloplasmin, serum glucose, and some other indices.

CHAPTER 30

WATER AND ELECTROLYTE BALANCE

Body water compartments

The average adult body contains about 40-45 L of fluids, which accounts for 2/3 of the total body weight. Most of these fluids are compartmentalized in two regions of the body:

1. **Intracellular fluid (ICF)** (30-40% of the body weight) is located inside the cells and accounts for 2/3 of all body fluids.
2. **Extracellular fluid (ECF)** (20-25% of the body weight, or 1/3 of all body fluids) denotes all body fluids outside the cells and includes the interior of cells, tissue spaces between cells (including the lymph vessels), and the blood vessels**.**

Extracellular fluids, in turn, are divided into **three compartments**:

1. **Blood plasma** or the intravascular fluid makes up about 7% of the total.
2. **Interstitial fluid** fills the space between tissue cells and moves in lymph vessels. It constitutes about 26% of the total body fluid
3. **Transcellular fluid** is formed from the transport activities of cells, and is the smallest component of extracellular fluid. Examples of this fluid are cerebrospinal fluid, pericardial and pleural fluids, aqueous humor in the eye, serous fluid in the serous membranes lining body cavities, perilymph and endolymph in the inner ear, and joint fluid.

Dissolved substances

The body fluids contain two types of dissolved compounds:

1. **Non-electrolytes** (organic compounds, such as glucose, urea, creatinine, bilirubin, etc.)
2. **Electrolytes.** Electrolytes in living systems include sodium, potassium, chloride, bicarbonate, calcium, phosphate, magnesium, copper, zinc, iron, manganese, molybdenum, copper, and chromium. In terms of body functioning, six electrolytes are most important: sodium, potassium, chloride, bicarbonate, calcium, and phosphate (Table 30.1).

Major extracellular (plasma) cations: Na^+ and Ca^{2+}. Major plasma anions: Cl^- and HCO_3^-. The sum of anions and cations is equal, and the whole system remains electrically neutral.

The capillary wall, which separates plasma from the interstitial fluid, is freely permeable to water and electrolytes; therefore these compartments

have similar distribution of ions.

Table 30.1. – **Approximate concentration of major electrolytes in body water compartments (expressed in milliequivalent per liter, mEq/L)**

Solutes	Extracellular fluid		Intracellular fluid
	Plasma	Interstitial fluid	
Na^+	140	140	10
K^+	4	4	150
Ca^{2+}	5	2,5	0
Cl^-	105	115	2
PO_4^{3-}	2	2	35
HCO_3^-	27	30	10

However, capillary wall is impermeable to proteins; therefore concentration of proteins is 4 times greater in plasma than in the interstitial fluid.

Major intracellular cations: K^+ and Mg^{2+}. **Major intracellular anions:** proteins and phosphates. Concentration of proteins in the ICF is 4-5 times higher than that of plasma.

Osmolality and volume of body fluids

Osmolality of body fluids is defined as the number of all solutes that contribute to the blood osmotic pressure. Osmolality is measured in milliosmoles of the solute per kilogram of fluid (mOsm/kg).

1 mOsm= 6 x 10^{20} dissolved particles in solution

Physiological osmolality of the blood plasma typically equals the sum of doubled concentration of sodium ions and concentrations of glucose and urea.

Physiological osmolality = 2[Na^+] + [glucose] + [urea] = 280-300 mOsm

Increase in the blood osmolality points to dehydration and thickening of blood. Decrease in the blood osmolality is associated with overhydration. In normal people, increased osmolality in the blood will stimulate secretion of antidiuritic hormone (ADH). This will result in increased water reabsorption, more concentrated urine, and less concentrated blood plasma. A low serum osmolality will suppress the release of ADH, resulting in decreased water reabsorption and more concentrated plasma. Part of the osmotic pressure created by proteins (albumin) is called **oncotic pressure**

Volume of body fluids typically depends on the amount of water in the compartment. Normally, osmolality of ECF and ICF is the same, i.e. concentrations of all dissolved molecules are the same. Any change in concentration of dissolved substances in any water compartment causes movement of water between compartments. Water moves from a compartment of low osmolality to the compartment of high osmolality until the osmotic pressure becomes identical in both of them. Thus, volume of both ECF and ICF depends on the amount of the dissolved substances in these compartments.

There are the following volumes of body fluids in an average adult organism:

1. Intracellular fluid – 27 L;
2. Extracellular fluid – 15 L (interstitial fluid – 11 L, plasma – 3 L, transcellular fluid – 1 L).

Functions of water in the body. Water balance

The body is 60-70% water. The amount of water in the body depends on a number of factors, including age, sex, and the amount of body fat. In males water constitutes 60-65% of the body weight. In females it constitutes 55-60%, because of higher amounts of body fat. In general, the percentage of water increases as fat content decreases. The amount of water varies with the individual, as it depends on the condition of the subject, the amount of physical exercise, and on the environmental temperature and humidity. A constant supply is needed to replenish the fluids lost through normal physiological activities, such as respiration, perspiration, and urination.

Functions of water in the body:

1. It dissolves and transports water-soluble compounds.
2. All chemical reactions in the body require water medium.
3. Metabolic function. Water is created inside a living organism in hydrolytic reactions and cellular respiration in mitochondria. Animal metabolism produces about 100 grams of water per 100 grams of fat, 40 grams of water per 100 g of protein, and 55 grams of water per 100 g of carbohydrate. Water also takes part in hydration reactions.
4. Structural function or water refers to its ability to attract to polar groups of biological membranes and stabilize their structure.
5. Water maintains active volume of cells and extracellular space.
6. Regulation of body temperature (thermogenesis).

Daily water requirement for an adult person is about 40 ml per 1 kg of

body weight, or about 2500 ml. An important and obvious principle of fluid balance is that fluid output and intake must be equal (see the table 30.2). Water from food and drink is absorbed into the body from the digestive tract. Besides this well-known source, water is also derived within the cells from food metabolism. This byproduct water enters the bloodstream along with the water that is absorbed from the digestive tract.

Table 30.2. – Typical daily intake and output of water

Intake		***Output***	
Water in foods	1000 ml	Urine	1400 ml
Ingested liquids	1200 ml	Expired air	500 ml
Metabolic water	300 ml	Sweat	400 ml
		Feces	100 ml
Total	2500 ml	Total	2500 ml

A constant supply is needed to replenish the fluids lost through normal physiological activities, such as respiration, perspiration, and urination. Water balance depends on nutrition, physical activity, climate, normal and pathological conditions. Water normally leaves the body through the kidneys (urine), lungs (water vapor in expired air), skin (by diffusion and perspiration), and intestines (feces). Abnormally high fluid losses and, possibly, dehydration can be caused by hyperventilation, excessive sweating, vomiting or diarrhea.

Regulation of volume and osmolality of the ECF

Water metabolism and Na^+ metabolism are interrelated. Regulation of volume and osmolality of the ECF implies regulation of Na^+ and water balance. Sodium and water balance is regulated by several hormones.

Vasopressin

Vasopressin, also called **antidiuretic hormone (ADH) ,** exerts its action by affecting the permeability of the renal tubules to water. Vasopressin is secreted by hypothalamus in response to increase in osmolality of plasma and stimulation of hypotalamical osmoreceptors. ADH is then transported to the posterior lobe of hypophysis and secreted into the blood. Vasopressin increases the water reabsorption by renal tubules.

In the absence of vasopressin, less water is reabsorbed into the bloodstream, and a large volume of light yellow, dilute urine results. In the

presence of vasopressin, the tubule becomes more permeable to water that is drawn into the capillaries, thus increasing blood volume. The reabsorption of water from the tubules produces a low volume of dark yellow, concentrated urine. With the help of vasopressin, a healthy person can vary the intake of water widely and yet preserve a stable overall concentration of substances in the blood.

Aldosterone

When the water level of the body gets dangerously low, from not drinking enough water or excessive water loss caused by diarrhea or excessive perspiration, *zona glomerulosa* of adrenal cortex secretes the steroid hormone aldosterone.

At the kidneys, aldosterone stimulates the reabsorption of Na^+ from the renal tubes into the blood. Chloride ions follow the sodium ions to maintain electrical neutrality, and water follows the sodium chloride. Thus, aldosterone secretion conserves both salt and water in the body. When the fluid level of the body returns to normal, the amount of aldosterone secreted decreases. Aldosterone is also referred to as sodium-retaining hormone. It stimulates Na^+ reabsorption from the urine in the distal tubules and thus increases concentration of Na^+ in the blood.

Renin-angiotensin system

This system is involved in the regulation of blood pressure and electrolyte metabolism (through production of aldosterone). The primary hormone involved in these processes is **angiotensin II,** an octapeptide hormone made from **angionensinogen.**

Angiotensinogen is a large α2-globulin made in the liver and released into the bloodstream. *The juxtaglomerular cells* of the renal afferent arterioles produce the enzyme renin, which acts upon the substrate angiotensinogen to form the decapeptide angiotensin I. Angiotensin-converting enzyme is glycoprotein found in lung, endothelial cells and plasma, removes two carboxyl terminal amino acid residues to produce angiotensin II. In some species (including humans) angiotensin II is converted to heptapeptide angiotensin III. Both peptides are rapidly inactivated by angiotensinases to degradation products.

Action of angiotensin II and angiotensin III:

In the CNS: stimulates production and secretion of vasopressin.

In adrenal glands: initiates secretion of aldosterone.

In the kidneys: increase in reabsorption of sodium ions, decreases the rate of filtration and water retention.

In blood vessels: arteriolar vasoconstriction, increase in blood pressure.

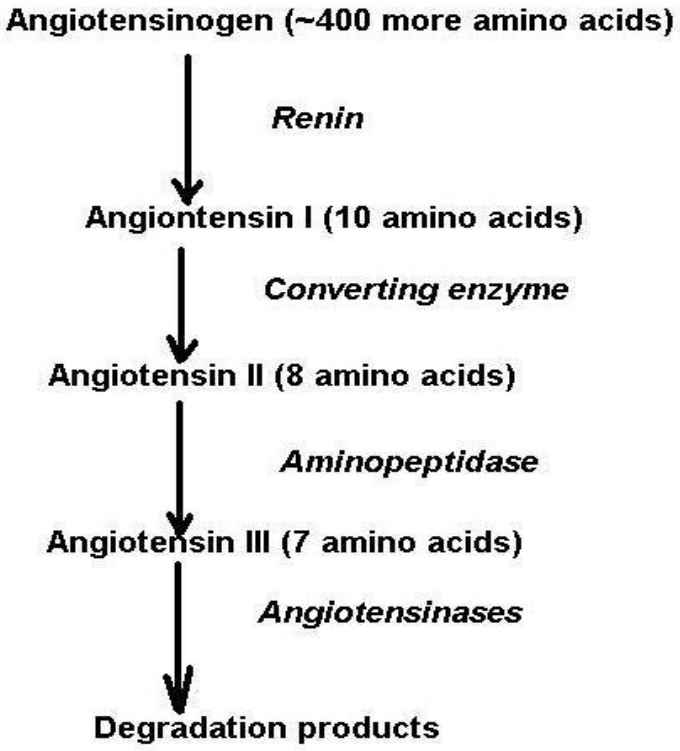


Figure 30.1 – Activation of the renin-angiotensinogen system

Atrial natriuretic peptide (ANP)

ANP is synthesized, and secreted by cardiac muscle cells in the walls of the atria in the heart. ANP is released in response to increased pressure in the atria (increased blood volume)

The main functions of ANP are:

- to oppose the renin-angiotensin-aldosterone system;
- to stimulate excretion of sodium and H_2O in the urine;
- to suppress renin level;
- to decrease the release of aldosterone and vasopressin;
- to reduce vascular resistance by causing vasodilatation.

DISTURBANCES IN WATER-MINERAL AND ACID-BASE BALANCE

Dehydration

Dehydration is a disturbance of water balance in which the output exceeds the intake, causing a reduction of body water below the normal level (**hypovolemia).** Although the term implies loss of body fluid, the clinical state to which it refers is more than this; because, characteristically, there is an accompanying disturbance of electrolytes. Dehydration can cause **hypernatremia** (high levels of sodium ions in the blood) and is

distinct from **hypovolemia** (loss of blood volume, particularly blood plasma).

Dehydration may be the result of:

1. Abnormal loss of fluids through the skin, kidneys, gastrointestinal tract;
2. Low or lack of water intake;
3. Transfer of water to the pleural or peritoneal cavities with formation of ascites.

Significant decrease in the volume of interstitial fluids may contribute to **hypovolemic shock**, or sudden loss of blood volume. There are 3 types of dehydration:

1) **isotonic dehydration** refers to the retention of both water and electrolytes (Na^+);

2) **hypertonic dehydration** occurs when the loss of water exceeds the loss of salts (vomiting, diarrhea, excessive perspiration);

3) **hypotonic dehydration** occurs when the loss of salts exceeds the loss of water.

Edema

Edema (or swelling), is an abnormal accumulation of interstitial fluid in tissues, for instance, on legs, ankles, or arms. Edema forms when the volume of interstitial fluid exceeds the ability of lymphatic vessels to return it to the circulation.

If the concentration of plasma proteins is markedly diminished (eg, due to severe protein malnutrition), fluid is not attracted back into the intravascular compartment and accumulates in the extravascular tissue spaces, a condition known as edema**.** Edema has many causes; protein deficiency is one of them.

The main causes of edema:

1. Decreased concentration of albumin in the blood plasma;
2. Increased production/secretion of vasopressin, aldosterone that causes retention of water, sodium in the body fluids;
3. Increased capillary permeability;
4. Increase capillary hydrostatic blood pressure;
5. Excess of sodium in the body;
6. Impaired blood circulation (for example, heart failure).

Acidosis and alkalosis

Blood pH normally remains within the rather narrow range of 7.35 – 7.45. A decrease in pH, called acidosis, or increase is called alkalosis, is serious and requires prompt attention.

Blood pH depends on the relative concentrations of carbonic acid (H_2CO_3) and bicarbonate ion (HCO_3^-). When blood pH is normal and a state of acid-base balance exists, components of the bicarbonate buffer are present in plasma in a ratio of 20 parts of HCO_3^- to 1 part of H_2CO_3. When respiratory and urinary systems work together to maintain the blood at pH 7.4, they do so primarily by maintaining the HCO_3^-/H_2CO_3 ratio at 20:1. Any change in the ratio produces a change in pH. An increase in the ratio of bicarbonate to carbonic acid causes the pH to increase (alkalosis); and a decrease, which is much more common, causes the pH to decrease (acidosis).

Depending on causes acidosis and alkalosis may by respiratory and metabolic.

Respiratory alkalosis is caused by **hyperventilating** — breathing rapidly and deeply. Hysteria, anxiety, or prolonged crying may result in hyperventilation in which too much carbon dioxide is exhaled, which disturbs the blood's normal carbon dioxide/carbonic acid ratio. In these conditions the reaction below shifts to the right as it attempts to restore the equilibrium:

$$H_2CO_3 \rightarrow CO_2\uparrow + H_2O$$

The equilibrium shift reduces the amount of carbonic acid present and rapidly increases the HCO_3^-/ H_2CO_3 ratio. As a result, blood pH rises to 7.54 or higher within a few minutes. A corresponding loss of carbon dioxide also occurs, but it is not large enough to maintain the HCO_3^-/ H_2CO_3 ratio at 20:1.

Respiratory acidosis is sometimes the result of slow breathing **(hypoventilation)** caused by an overdose of narcotics or barbiturates. Lung diseases, such as emphysema and pneumonia, or an object lodged in the windpipe can also result in hypoventilation. In respiratory acidosis, too little carbon dioxide is exhaled, and its concentration within the blood increases. The carbon dioxide/carbonic acid equilibrium shifts to the left to restore the equilibrium:

$$H_2CO_3 \leftarrow CO_2 + H_2O$$

As a result of the shift, the concentration of carbonic acid in the blood increases, the HCO_3^-/H_2CO_3 ratio decreases to less than 20:1, and respiratory acidosis sets in.

Metabolic acidosis. Various body processes produce acidic substances that liberate H^+ in solution. The diffusion of these substances into the bloodstream causes a shift in the carbonic acid-bicarbonate equilibrium in direction of formation of carbonic acid:

$$H^+ + HCO_3^- \rightarrow H_2CO_3$$

The shift decreases the concentration of bicarbonate ions and increases the concentration of carbonic acid. As a result, the HCO_3^-/ H_2CO_3 ratio decreases, as does the blood pH decrease.

Metabolic acidosis is often a serious problem in uncontrolled diabetes mellitus because of excessive production of ketone bodies. The state produced is called ketoacidosis.

In **metabolic alkalosis**, the body has lost acid in some form, perhaps from prolonged vomiting of the acidic stomach contents, or there has been an ingestion of alkaline substances. Excessive use of sodium bicarbonate (baking soda), for example, a common home remedy for upset stomach, increases the concentration of bicarbonate ions in the blood. A decrease in acid concentration within the body (from vomiting) or an increase in HCO_3^- levels (from excessive $NaHCO_3$) causes the HCO_3^-/H_2CO_3 ratio to rise above 20:1, and measurable alkalosis is observed. The respiratory center responds to the higher blood pH by slowing the breathing (hypoventilation). Other symptoms may include numbness, tingling, and headache.

MINERAL COMPONENTS OF TISSUES

In the body, the elements classified as minerals are never used in elemental form, but rather in the form of ions or compounds. They are classified as macrominerals (major minerals) and microminerals (trace minerals) on the basis of the amount present in the body.

1. **Macrominerals** are elements found in the body in quantities greater than 10^{-2} moles): **Na, K, Ca, Mg, Cl, P.**
2. **Microminerals** are trace elements found in the body in concentrations between 10^{-2} to 10^{-6} moles): **Fe, I, Cu, Zn, Mo, Cr, Se, F.**

The functions of minerals are consistent with their classification as major or trace and with the amount required daily in the diet. For example, compounds of some major minerals (Ca and P) are the primary inorganic structural components of bones and teeth. Other major minerals (Na, K, Cl, and Mg) form principal ions that are distributed throughout the body's

various fluids. Some trace minerals are components of vitamins (Co), enzymes (Zn and Se), hormones (I), or specialized proteins (Fe and Cu). Thus, though trace minerals are required in small quantities, their involvement in critical enzymes, hormones, and the like makes them equally as important for good health as the major minerals.

Sodium

Normal range of Na^+ in the blood is **130 – 155 mmol/L.**

Dietary sources: most food except fruit**.**

Functions: Na^+ ions form principal ions that are distributed throughout the body's various fluids. It maintains osmotic pressure of the ECF, acid-base and water balance, participates in generation of membrane potential and nerve impulse, assists in the Na^+/K^+-dependent active transport of glucose, amino acids through cellular membranes.

Metabolism. The human body contains about 100 g of sodium, which is distributed predominantly in the ECF. Sodium comes from food in the amount of 4-5 g per day and is absorbed in the proximal small intestine. Sodium is excreted from the body with urine (3.3 g / day), then (0.9 g / day), feces (0.1 g / day).

Regulation of sodium metabolism. Aldosterone increases reabsorption of Na^+ from the urine, estrogens exhibit the same action. Atrial natriuretic peptide and progesterone stimulate Na^+ excretion into the urine.

Potassium

Normal concentration of K^+ in the blood is **3.2 – 5.6 mmol/L**.

Dietary sources: meat, milk, many fruits, cereals, legumes**.**

Functions: Potassium's role in the body is the same as for Na^+. It also participates in generation of membrane potential and nerve impulse, assists in the Na^+/K^+-dependent active transport of glucose. Maintenance of normal K^+ concentration in the blood is important for heart contraction.

Metabolism. Potassium is the main intracellular cation. Human body contains 140 g of potassium. About 3-4 g of potassium is daily ingested, which is absorbed in the proximal small intestine. Potassium is excreted in the urine (3 g / day), feces (0.4 g / day), then (0.1 g / day).

Regulation of potassium metabolism. Adrenaline, aldosterone, and insulin increase intake of potassium by cells. Aldosterone increases excretion of K^+ into the urine.

Calcium

Normal concentration of Ca^{2+} in the blood **is 2.25–2.75 mmol/L**.

Dietary sources: dairy foods, dark green vegetables**.**

Functions. Ca^{2+} is a structural component of bones and teeth. It participates in blood coagulation, muscle contraction, nerve impulses transmission. Ca^{2+} increases the activity of digestive enzymes (amylase, lipase), is used as second messenger for peptide hormone signal transduction into the cell.

Metabolism. The body contains about 1 kg of calcium, mostly in the bones. The ECF contains about 14 g of calcium. Average daily intake is about 1 g per day, and only 0.3 g / day is absorbed in the intestine.

In blood plasma, calcium is contained in two forms:

1. Biologically inactive, albumin-bound calcium (40%);
2. Diffusible calcium, consisting of 2 fractions:
   - Ionized (free) calcium - 50%;
   - Calcium associated with anions (phosphate, citrate, carbonate) - 10%.

All forms of calcium are in dynamic reversible equilibrium. Physiological activity has only ionized calcium. Calcium is excreted from the body: with feces (0.7 g/day), with urine (0.2 g/day), and with sweat (0.03 g / day).

Regulation of calcium metabolism. Parathyroid hormone increases the serum concentration of Ca^{2+} through its effects on bone, kidney, and the intestine. It facilitates absorption of Ca^{2+} from the intestine and kidneys, enhances release of Ca^{2+} from bones.

Calcitonin decreases concentration of both Ca^{2+} and P in the blood, through increase of their excretion by the kidneys and inhibition of osteoclast activity in bones. Estrogens and androgens increase ossification (Ca^{2+} deposition) of epiphyses (growth zones of the bones).

Active form of vitamin D_3 also participates in regulation of Ca^{2+} and P metabolism.

Phosphorus

Dietary sources: dairy foods, meat, fish grain, legumes, nuts.

Functions. Phosphate is the second major intracellular anion. It also serves as a structural component of bones, component of many important substances (nucleic acids, phospholipids, high-energy compounds, coenzymes (NAD^+, FAD, pyridoxal phosphate), active forms of carbohydrates (glucose 6-P, etc.). Plasma phosphates ($H_2PO_4^-$ and HPO_4^{2-}) are components of phosphate buffer system. Phosphorylation reactions provide the regulation of metabolism through activation/inactivation of key

enzymes and other proteins.

Metabolism. The body contains about 650 g of phosphorus, of which 8.5% is in the skeleton, 14% in soft tissue cells, and 1% in the ECF. The daily intake phosphorus is about 2 grams, of which up to 70% is absorbed in the intestine. Phosphorus is excreted: with urine (1.5 g / day), with feces (0.5 g / day), and with sweat (about 1 mg / day).

Regulation of phosphorus metabolism. Parathyroid hormone decreases the phosphate level in the blood. Calcitonin decreases concentration of both Ca^{2+} and P in the blood.

Characteristics of **the trace elements** are given in the Table 30.3.

Table 30.3. – **Main characteristics of the trace elements**

Mineral	Content in the body	Functions	Deficiency conditions
Iron	4.5 g	Component of hemoglobin, myoglobin, cytochromes, cytochrome oxidase, oxidase enzymes (catalase, peroxidase), transferrin, ferritin, and hemosiderin.	Iron-deficient anemia.
Copper	100 mg	Component of cytochrome oxidase, and ceruloplasmin (plasma protein).	Anemia, fragility of arteries.
Cobalt	1.5 mg	Component of vit B_{12}.	Vitamin B_{12}-dependent anemia.
Iodine	15 mg	Component of thyroid hormones.	Hypothyroidism, goiter.
Magnesium	180 g	Activation of enzymes (especially kinases), protein synthesis, component of bones.	Inhibited growth, weakness, spasms.
Zinc	1.5 g	Component of numerous enzymes (carbonic anhydrase, lactate dehydrogenase, etc), normal functioning of pancreas and testis.	Poor growth, lack of sexual maturation, loss of appetite, abnormal glucose tolerance.
Molybdenum	10 mg	Coenzyme of xanthine oxidase (catabolism of purines).	Deficiencies are rare.
Selenium	15 mg	Antioxidant action through participation of glutathione metabolism. Cancer protecting agent.	Deficiencies are rare.
Chromium	15 mg	Enhances insulin action.	Reduced ability to metabolize glucose.

CHAPTER 31

BIOCHEMISTRY OF THE BLOOD

Blood – is a body fluid in humans and other animals that delivers necessary substances (nutrients and oxygen) to the cells and transports metabolic waste products away from those same cells. The blood is the most important transport medium in the body. It serves to keep the homeostasis, and it plays a decisive role in defending the body against pathogens.

Human blood constitutes about 8% of the body's weight. It consists of cells and cell fragments, and proteins in an aqueous medium, the **blood plasma**. The proportion of cellular elements, known as **hematocrit**, in the total volume is approximately 45%.

Blood plasma is separated from the blood by spinning a tube of fresh blood containing an anticoagulant in a centrifuge until the blood cells fall to the bottom of the tube. Blood plasma without clotting factors is called **serum.** Most biochemical tests are done on serum.

General characteristics of the blood

The average blood volume is **5.2 l** in males and **3.9 l** in females. Normal range of pH is **7.36-7.42**. The relative density of whole blood is **1.050-1.065**, of blood plasma **1.024-1.030**.

The blood is a rather viscous fluid, owing the high contents of proteins and erythrocytes in it, its viscosity is 4-5-fold that of water. At a body temperature of 37°C, the blood plasma osmotic pressure is about **7.6 atm**. Blood plasma contains ~ **92%** water, ~ **8%** blood plasma proteins.

Biological functions of the blood

1. **Transport.** The gases oxygen and carbondioxide are transported in the blood. The blood mediates the exchange of substances between organs and takes up metabolic end products from tissues in order to transport them to the lungs, liver, and kidney for excretion. The blood also distributes hormones throughout the organism.
2. **Homeostasis.** The blood ensures that a balanced distribution of water is maintained between the vascular system, the cells (intracellular space), and the extracellular space. The acid-base balance is regulated by the blood in combination with the lungs, liver, and kidneys. The regulation

of body temperature also depends on the controlled transport of heat by the blood.

3. **Defense.** The body uses both non-specific and specific mechanisms to defend itself against pathogens. The defense system includes the cells of the immune system and certain plasma proteins.
4. **Self-protection.** To prevent blood loss when a vessel is injured, the blood has systems for stanching blood flow and coagulating the blood (hemostasis). The dissolution of blood clots (fibrinolysis) is also managed by the blood itself.

Blood cells

The blood cells are **erythrocytes** (red blood cells, **RBC**), **leukocytes** (white blood cells, **WBC**), and **platelets** (**thrombocytes)**. They are suspended in plasma and have several specialized functions such as transport of oxygen, destruction of external agents, and clotting of blood.

One microliter of blood contains:

erythrocytes – **4.7-6.1** million (male), **4.2 - 5.4** million (female). RBC contain the blood's hemoglobin and distribute O_2. The RBC membrane contains glycoproteins that define the different **blood groups and types**;

leukocytes - 4,000–11,000. WBC are part of the body's **immune system**; they destroy and remove old or aberrant cells and cellular debris, as well as attack infectious agents (pathogens) and foreign substances;

platelets/thrombocytes – **200 000–500 000**, they take part in blood clotting (coagulation). Fibrin from the coagulation cascade creates a mesh over the platelet plug.

Erythrocytes

Erythrocytes are not true cells, as they do not possess nuclei and intracellular organelles. Erythrocytes are the end-product of erythropoiesis in the bone marrow, which is under control of **erythropoietin** produced by the kidney.

The main functions of erythrocytes are the transport of oxygen and the removal of carbon dioxide and hydrogen ion. As they lack cellular organelles, they are not capable of protein synthesis and repair. As a result, erythrocytes have a finite life span of 60-120 days before being trapped and broken down in the spleen.

Specific features of the metabolism of erythrocytes

- The erythrocyte is unique among all cells in the body – it uses glucose

and **glycolysis** as its sole source of energy. The RBC is highly dependent upon glucose as its energy source, for which its membrane contains high-affinity glucose transporters (**GLUT1**, *glucose permease*). It is not dependent upon **insulin**.

- Because RBCs lack mitochondria there is no production of ATP by oxidative phosphorylation. Glycolysis, producing lactate, is the mode of production of ATP.
- The RBC has a variety of transporters that maintain ionic and water balance.The ATP formed during glycolysis serves mainly to supply Na^+/K^+-ATPase, which maintains the erythrocytes' membrane potential.
- In the RBC, 10-20% of the glycolytic intermediate, **1,3-bisphosphoglycerate**, is diverted to the synthesis of **2,3-bisphosphoglycerate**, an allosteric regulator of the O_2 affinity of Hb (**Luebering-Rapoport pathway**).
- The pentose phosphate pathway (PPP), accounts for about **10%** of glucose metabolism in the RBC. In erythrocyte this pathway has a special role in protection against oxidative stress. Hemolytic anemia due to a deficiency of the activity of glucose-6-phosphate dehydrogenase is common.
- RBC also have systems that can inactivate reactive oxygen species (ROS), - ***superoxide dismutase***, ***catalase***, glutathione (GSH). The PPP supplies $NAD(P)H+H^+$, which is needed to regenerate reduced **glutathione** (GSH) from oxidized state with the help of ***glutathione reductase***. GSH, the most important antioxidant in the erythrocytes, serves as a coenzyme for ***glutathione peroxidase***. This selenium-containing enzyme destroys H_2O_2 and hydroperoxides, which arise during the reaction of ROS with unsaturated fatty acids in the RBC membrane.
- The reduction of nonactive **methemoglobin** (Hb Fe^{3+}) to active form Hb (Hb Fe^{2+}) is carried out by **GSH** or **ascorbate** by a non-enzymatic pathway, and also by NAD(P)H dependent ***Met-Hb reductases***.
- While biosynthesis of glycogen, fatty acids, protein, and nucleic acids does not occur in the RBC, some lipids (eg, **cholesterol**) in the red cell membrane can exchange with corresponding **plasma lipids**.
- The RBC contains certain enzymes of nucleotide metabolism (eg, ***adenosine deaminase***, ***pyrimidine nucleotidase***, and ***adenylyl kinase***). Deficiencies of these enzymes are involved in some cases of hemolytic anemia.

- When RBCs reach the end of their lifespan:
  - the **globin** is degraded to amino acids,
  - the **iron** is released from heme and reutilized, and
  - the **tetrapyrrole** component of **heme** is converted to **bilirubin**, which is mainly excreted into the bowel via the bile.

Leukocytes

Leukocytes are cells, the main function of which is to protect the body from infection. Most leukocytes are produced in the ***bone marrow***, some are produced in the ***thymus***, and others mature within several tissues. Mammalian stem cells differentiate into several kinds of blood cell within the bone marrow. This process is called **haematopoiesis**. The formation of **lymphocytes** is known as **lymphopoiesis**. All lymphocytes originate during this process from a common lymphoid progenitor before differentiating into their distinct lymphocyte types. **B cells** mature into **B lymphocytes** in the ***gut-associated lymphoid tissue***, which is located in the intestine, while **T cells** migrate to and mature in the **thymus**. In order to function correctly, leukocytes have the ability to migrate out of the bloodstream into surrounding tissues.

Leukocytes include various types of granulocyte, monocyte, and lymphocyte. All of these have immune defense functions.

- The neutrophil granulocytes, monocytes, and the macrophages can ingest and degrade pathogens.
- Eosinophilic and basophilic granulocytes have special tasks for defense against animal parasites.

The major biochemical features of leukocytes:

- active synthesis of proteins and nucleic acids;
- active aerobic glycolysis;
- active pentose phosphate pathway;
- moderate oxidative phosphorylation;
- rich in degradative enzymes.

Lymphocytes. They are the main type of cell found in lymph. Lymphocytes include:

- **natural killer** (NK) cells – which function in cell-mediated, cytotoxic innate immunity. NK cells are activated in response to a family of cytokines called ***interferons***. Activated NK cells release ***cytotoxic granules*** which then destroy the altered cells.

- **T cells** (thymus cells) – for cell-mediated, cytotoxic adaptive immunity. Regulate the immune response and destroy virus infected cells and tumor cells.
- **B cells** (bone marrow-derived cells) – for humoral, antibody-driven adaptive immunity.

The function of T cells and B cells is to recognize specific antigens. In response to pathogens, some T cells, called **T helper cells**, produce ***cytokines*** that direct the immune response, while other T cells, called **cytotoxic T cells**, produce toxic granules that contain powerful enzymes which induce the death of pathogen-infected cells.

The lymphocytes involved in ***adaptive immunity*** (i.e. B and T cells) differentiate further after exposure to an antigen; they form **effector** and **memory** lymphocytes.

Effector lymphocytes function to eliminate the antigen, either by releasing antibodies (in case of B cells), cytotoxic granules (cytotoxic T cells) or by signaling to other cells of the immune system (helper T cells).

Memory T cells remain in the peripheral tissues and circulation for an extended time ready to respond to the same antigen upon future exposure. They live from weeks to several years, which is very long compared to other leukocytes.

Thrombocytes/platelets

Thrombocytes (platelets) are cell fragments that arise in the bone marrow from large precursor cells, the **megakaryocytes** (in response to ***thrompoietin***). Like red blood cells, platelets lack a nucleus, but they possess **mitochondria**, **lysozymes**, and a **tubular network** that forms an **open canalicular system**. This honeycomb of channels increases the surface area of the platelets, which are spheroidal at rest, thereby facilitating the secretion of various endocrine and coagulation factors upon stimulation.

These factors are stored inside the platelets within densely packed secretory vesicles, called **dense granules**, which contain Ca^{2+}, ***ADP*** and ***serotonin***, and **α-granules**, which contain ***fibrinogen***, ***fibronectin***, ***platelet derived growth factor*** (**PDGF**), ***von Willebrand factor*** (**VWF**), other coagulation factors.

While platelets derive the majority of their energy from metabolizing ***glucose***, their mitochondria enable them to generate ATP via the β-oxidation of fatty acids.

They have a key role in the process of blood clotting. Platelets

normally circulate in a disk-shaped form. During hemostasis or thrombosis, platelets become activated and help form ***hemostatic plugs*** or ***thrombi***. Three major steps are involved to the process:

1. adhesion to exposed ***collagen*** in blood vessels;
2. release (exocytosis) of the contents of their storage granules;
3. aggregation.

Platelets adhere to collagen via specific receptors (including glycoprotein ***integrin***) on the platelet surface. The binding of receptors to collagen is mediated via ***von Willebrand factor***. Platelets change shape and spread out on the subendothelium. These adherent platelets release the contents of their storage granules.

Collagen-induced activation of a platelet cytosolic ***phospholipase A_2*** by increased levels of intracellular Ca^{2+} results in liberation of ***arachidonic acid*** from platelet membrane phospholipids, leading to the formation of **thromboxane A_2**.

Activated platelets, besides forming a platelet aggregate, accelerate the activation of ***factor X*** and ***prothrombin*** by exposing the anionic phospholipid ***phosphatidylserine*** on their membrane surface.

Molecules of divalent ***fibrinogen*** or multivalent ***von Willebrand factor*** then link adjacent activated platelets to each other, forming a **platelet aggregate**. Von Willebrand factor-mediated platelet aggregation occurs under conditions of high shear stress. Some agents, including ***epinephrine***, ***serotonin***, and ***vasopressin***, exert synergistic effects with other aggregating agents.

Human hemoglobin

In adults, hemoglobin (Hb) is a heterotetramer consisting of two α-globin and two β-globin subunits: α2β2. Each subunit carries a heme group, with a central bivalent iron ion. Hemoglobin can bind up to four molecules of O_2.

Derivatives of hemoglobin

Hemoglobin interacts with different ligands, forming derivatives:

Deoxyhemoglobin – HHb hemoglobin not combined with oxygen, formed when oxyhemoglobin releases its oxygen to the tissues.

Oxyhemoglobin – $HHbO_2$ the oxygen-carrying hemoglobin.

Carbhemoglobin (carbaminohaemoglobin) – $HHbCO_2$ is a compound of hemoglobin and carbon dioxide, and is one of the forms in which carbon

dioxide exists in the blood.

Carboxyhemoglobin (COHb) is a stable complex of carbon monoxideand hemoglobin that forms in red blood cells upon contact with carbon monoxide (CO). Large quantities of CO hinder the ability of Hb to deliver oxygen to the body.

Methemoglobin – MetHb is a form of hemoglobin, in which the iron in the heme group is in the Fe^{3+} state. Methemoglobin cannot bind oxygen. In human blood a trace amount of methemoglobin is normally produced spontaneously. The proportion of MetHb is kept low by reduction and usually amounts to only 1-2%.

Variants of hemoglobin in ontogenesis

Embryonic hemoglobin with the structure 2α2ε globin chain is formed in the first three months of embryonic development. Then it predominates.

Fetal Hb, HbF; its subunits are α-globin and γ-globin. HbF predominates in the fetus during the second and third trimesters of gestation and in the neonate. The most striking functional difference between HbF and HbA is its decreased sensitivity to 2,3-bisphosphoglycerate. Embryonic and fetal hemoglobins have higher O_2 affinities than HbA, as they have to take up oxygen from the maternal circulation.

HbF is gradually replaced by HbA during the first few months of life. Over 95% of the Hb found in adult humans is **HbA**, with the α2β2 globin chain composition. **HbA_2** accounts for 2-3% of the total and has an α2δ2 polypeptide composition. HbA_2 is elevated in β-thalassemia, a disease characterized by a deficiency in β-globin biosynthesis. Functionally, these two adult Hbs are indistinguishable.

Hemoglobinopathies

Hemoglobinopathies are a group of inherited single-gene disorders that result in abnormal structure of one of the globin chains of the hemoglobin molecule. Hemoglobinopathies are classified according to the type of structural change, altered function, and the resulting clinical characteristics. They can be categorized into two major groups:

- thalassemias;
- structural variants of hemoglobin.

Thalassemias result from the partial or total absence of one or more α

or β chains of hemoglobin. If the synthesis of α chains is inhibited, α thalassemia develops, reduced production of β subunits leads to β thalassemia. Pathology is most common among people of Italian, Greek, Middle Eastern, South Asian, and African descent. The variable phenotypic expression within and among the thalassemia syndromes results in a wide spectrum of anemia, which may require periodic or regular transfusions to sustain life. Because there is no physiologic means of excreting iron, transfusion therapy leads to *progressive iron overload* and ultimately multiple endocrinopathies, and potentially, lethal hepatic and/or cardiac complications.

More than 600 mutations in the genes encoding the α- and β-globin polypeptides have been documented, not all of which are harmful.

Sickle cell anemia, the most common genetic blood disorder in the world, has high clinical variability, negatively impacts quality of life and contributes to early mortality. Sickled erythrocytes cause blood flow obstruction, hemolysis, and several hemostatic changes that promote coagulation. These events, in turn, induce chronic inflammation, characterized by elevated plasma levels of pro-inflammatory markers, which aggravates the already unfavorable state of the circulatory system.

Sickle cell anemia is caused by an inherited structural abnormality in the β-globin polypeptide. The mutation is Glu 6 β→Val, it leads to formation of HbS. Clinically, an individual with sickle cell anemia presents with intermittent episodes of hemolytic and painful vaso-occlusive crises, the latter leading to severe pain in bones, chest, and abdomen. Common side effects include impaired growth, increased susceptibility to infections, and multiple organ damage. Sickle cell anemia has a prevalence of 40% in some regions of equatorial Africa; among black Americans.

HbA remains a true solute at rather high concentrations and nonreactive with nearby Hb molecules. In contrast, HbS, when deoxygenated, is less soluble. It forms long, filamentous polymers that readily precipitate, distorting erythrocyte morphology to the characteristic sickle shape. Sickled erythrocytes exhibit less deformability; they no longer move freely through the microvasculature and often block blood flow, especially in the spleen and joints. Moreover, these cells lose water, become fragile, and have a considerably shorter life span, leading to hemolysis and anemia.

Except during extreme physical exertion, the heterozygous individual appears normal. For reasons that remain to be elucidated, heterozygosity is

associated with an increased resistance to malaria, specifically growth of the infectious agent *Plasmodium falciparum* in the erythrocyte.

Plasma proteins

Plasma contains many proteins broadly classified into **albumins** and **globulins** (predominantly immunoglobulins).

Changes in the concentration of plasma proteins give important clinical information.

Albumin, the predominant plasma protein, is present normally at a concentration of **35-45 g/L**. In addition to its functions as a protein reserve in nutritional depletion and as an osmotic regulator, is a major transport protein for several ligands – free fatty acids, bilirubin, trace metals, and hormones.

Other proteins are more specialized: they bind specific ligands, e.g. ***ceruloplasmin*** binds Cu^{2+}, and ***thyroid binding globulin*** binds thyroid hormones.

Immunoglobulins are unique molecules that participate in the defense against antigens that may enter or attempt to enter the body. They have a common structure and five classes of immunoglobulin exist. Immunoglobulins are produced in response to foreign substances (antigens). They are a uniquely diverse group of molecules, recognizing and reacting with a wide range of specific antigenic structures. Serum and urine protein electrophoresis is an important way of identifying the presence of **monoclonal immunoglobulins**.

The **acute phase response** is a nonspecific response to tissue injury or infection; it affects several organs and tissues. During the acute phase response, there is a characteristic pattern of change in certain proteins in the plasma concentration**.** An increase in the synthesis of proteins such as proteinase inhibitors (**α1-antitrypsin**), coagulation proteins (**fibrinogen, prothrombin**), complement proteins, and ***C-reactive protein*** is of obvious clinical benefit.

The production of these proteins is stimulated by **pro-inflammatory cytokines** released by macrophages, and of these ***interleukin-1*** (IL-1), ***interleukin-6*** (IL-6), and ***tumor necrosis factor*** (TNF) have a central role in the induction of the acute phase response.

The acute phase proteins have a number of different functions in the response to inflammation:

- Binding proteins, ***opsonins***, such as ***C-reactive protein*** (**CRP**), bind

to macromolecules released by damaged tissue or infective agents and promote their phagocytosis.

- ***Complement factors*** promote the phagocytosis of foreign molecules. Over 30 proteins and protein fragments make up the ***complement system***, including serum proteins, and cell membrane receptors. They account for about 10% of the globulin fraction of blood serum.
- ***Protease inhibitors***, such as α1-antitrypsin and α1-antichymotrypsin inhibit proteolytic enzymes. These latter two acute phase proteins also promote fibroblast growth and the production of connective tissue required for the repair and resolution of the injury.

The synthesis of albumin and transferrin decreases during the acute phase response.

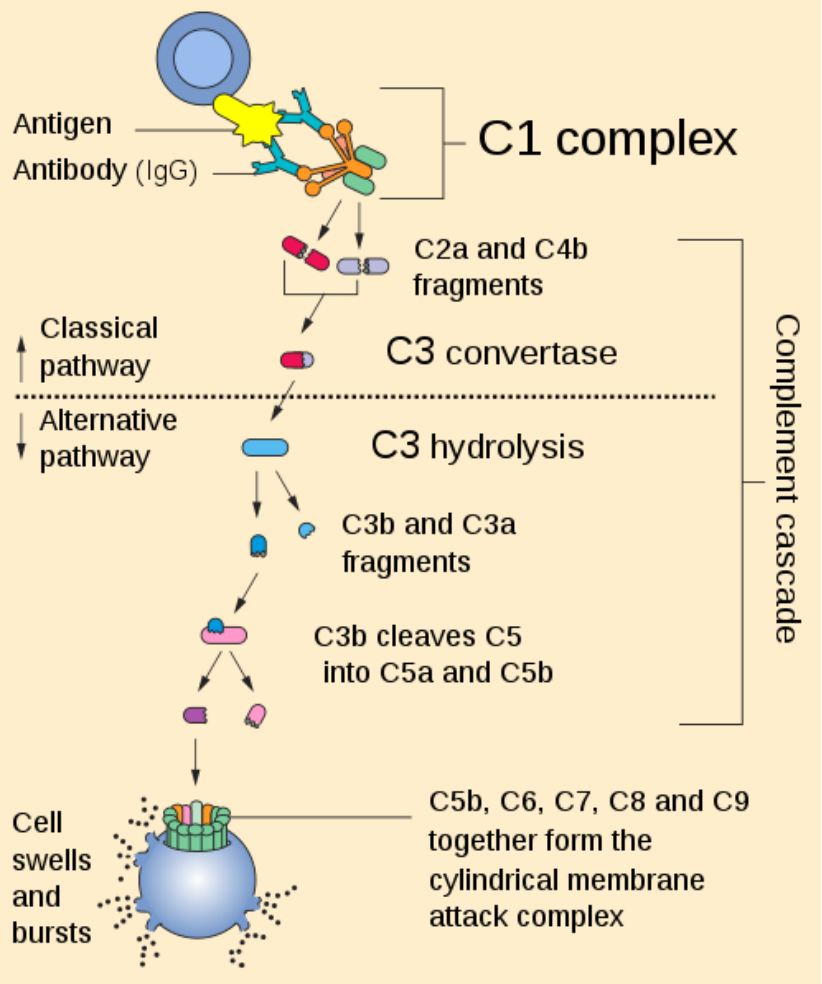


Figure 31.1 – Scheme of the complement system (This is a file from the Wikimedia Commons).

The **complement system** (Fig. 31.1) consists of a number of small proteins that are synthesized by the liver, and circulate in the blood as

inactive precursors. When stimulated by one of several triggers, proteases in the system cleave specific proteins to release cytokines and initiate an amplifying cascade of further cleavages. The end result of this *complement activation* or *complement fixation* cascade is stimulation of phagocytes to clear foreign and damaged material, inflammation to attract additional phagocytes, and activation of the cell-killing **membrane attack complex**.

Iron metabolism

Iron (Fe) is quantitatively the most important trace element. The human body contains 4-5g of iron. The vast majority of body iron (at least 2.1 g in humans) is distributed in the hemoglobin of red blood cells and developing erythroid cells and serves in oxygen transport. Significant amounts of iron are also present in macrophages (up to 600 mg) and in the myoglobin of muscles (~300 mg), whereas excess body iron (~1 g) is stored in the liver. Other tissues contain lower, but not negligible, quantities of iron. About 1% of the iron is bound in iron-sulfur clusters, which function as transporters in the respiratory chain, and in other redox chains.

Mammals lose iron from sloughing of mucosal and skin cells or during bleeding, but do not possess any regulated mechanism for iron excretion from the body. Therefore balance is maintained by the tight control of dietary iron absorption in the duodenum.

Iron can only be resorbed by the bowel in bivalent form (Fe^{2+}). Incoming iron in the Fe^{3+} state is reduced to Fe^{2+} by a **ferric reductase** (*duodenal cytochrome b*) present on the surface of enterocytes. Vitamin C in food is also preferable.

The uptake of nutritional iron involves the subsequent transport of Fe^{2+} across the apical membrane of enterocytes by **DMT1** (***divalent metal transporter 1***), a member of the SLC (**solute carrier**) group of membrane transport proteins. Dietary **heme** can also be transported across the apical membrane lumen into enterocyte and **by heme carrier protein 1** (**HCP1**) and subsequently metabolized in the enterocytes by HO-1 (***haemoxygenase 1***) to liberate Fe^{2+}. Free haemoglobin is scavenged by ***haptoglobin***, a liver-derived plasma protein, which in turn is internalized by macrophages upon binding to the receptor. Free heme is also scavenged by plasma ***haemopexin*** and undergoes endocytosis in macrophages upon binding to the receptor.

Directly internalized or heme-derived from the degradation of

haemoglobin following direct haemoglobin uptake or erythrophagocytosis, is degraded by ***hemoxygenase 1*** (**HO-1**), yielding Fe^{2+}, which is released into the plasma through **ferroportin**. The ferroportin-mediated efflux of Fe^{2+} is coupled by its re-oxidation to Fe^{3+}, catalysed by the *membrane-bound ferroxidase-**hephaestin***- that physically interacts with **ferroportin**, and possibly also by its plasma homologue - **ceruloplasmin**.

Exported iron is scavenged by **transferring** (**Tf**), which maintains Fe^{3+} in a redox-inert state and delivers it into tissues. The total iron content of **Tf** (~3 mg) corresponds to less than 0.1% of body iron, but it is highly dynamic and undergoes more than ten times daily turnover to sustain **erythropoiesis**. It is released from the **transferrin** into tissues after it has bound to **specific cell transferrin receptors - TfR1**.

The binding of **ferric ions** to **transferrin** protects against the toxic effects of these ions. In inflammatory reactions, the **iron-transferrin complex** is degraded by the reticuloendothelial system without a corresponding increase in the synthesis of either of its components; this results in low plasma concentrations of transferrin and iron.

The **transferrin** iron pool is replenished mostly by iron recycled from effete red blood cells and, to a lesser extent, by newly absorbed dietary iron. Senescent red blood cells are cleared by reticuloendothelial macrophages, which metabolize haemoglobin and haem, and release iron into the bloodstream. By analogy to intestinal enterocytes, macrophages export **Fe^{2+}** from their plasma membrane via **ferroportin**, in a process coupled by re-oxidation of Fe^{2+} to Fe^{3+} by **ceruloplasmin** and followed by the loading of Fe^{3+} to **transferrin.**

Iron-loaded **holo-transferrin** binds with high affinity to **TfR1** (*transferrin receptor*) on the surface of cells, and the complex undergoes *endocytosis*. A proton pump promotes acidification of the endosome to pH 5.5,triggering the release of Fe^{3+} from **Tf** that remains bound to TfR1. The *ferrireductase* reduces Fe^{3+} to Fe^{2+}, which is transported across the endosomal membrane by *divalent metal transporter 1* **(DMT1)** to the cytosol or directly to mitochondria in erythroid cells. Following the release of iron, the affinity of **transferrin** to **TfR1** drops~500-fold, resulting in its dissociation. In the final step of the cycle, **apo-transferrin** is secreted into the bloodstream to recapture Fe^{3+}.

Ferroportin is expressed in many iron-exporting cells, including placental syncytiotrophoblasts, and plays a fundamental role in the release of iron from tissues into the bloodstream, but also in maternal iron transfer

to the fetus. The complete disruption of mouse **ferroportin** is embryonic lethal, whereas its conditional inactivation leads to iron retention and accumulation in enterocytes, macrophages and hepatocytes.

The ferroportin-mediated efflux of Fe^{2+} from enterocytes and macrophages into the plasma is critical for systemic iron homoeostasis. This process is negatively regulated by **hepcidin**, a liver-derived **peptide hormone** that binds to **ferroportin** and promotes its phosphorylation, internalization and lysosomal degradation.

Hepcidin accumulates following iron intake and under inflammatory conditions, resulting in decreased dietary-iron absorption and iron retention in macrophages; during infection, this very probably serves to deprive invading bacteria from iron that is essential for growth. Conversely, **hepcidin** levels drop in iron deficiency, hypoxia or phlebotomy-induced anaemia, and this response promotes intestinal iron absorption and iron release from macrophages. The disruption of **hepcidin** is associated with systemic iron overload (**haemochromatosis**), whereas pathological elevation of **hepcidin** levels contributes to the development of the *anaemia of chronic disease* and accounts for the phenotype of hereditary *iron-refractory iron deficiency anaemia*.

Dietary-iron absorption can be induced independently of the **hepcidin** pathway by transcriptional activation of **DMT1** and **Dcytb** in duodenal enterocytes.

Ferritin is a universal intracellular protein that stores iron and releases it in a controlled fashion.Ferritin is the major iron storage protein found in almost all cells of the body. It acts as the reserve of iron in the liver and bone marrow.

Ferritin is a conserved protein consisting of 24 **H** (heavy) and **L** (light) subunits, encoded by distinct genes. **Ferritin** assembles into a shell-like structure with a cavity of ~8 nm that provides storage space for up to 4500 Fe^{3+} ions. The incorporation of iron into **holo-ferritin** also requires the *ferroxidase* activity of H-ferritin, whereas L-ferritin chains provide a nucleation centre. Excess iron is incorporated into **ferritin** (a universal intracellular protein)and stored in this form in the **liver** and other organs.

In addition to **ferritin**, there is another storage form, **hemosiderin -** a structure consisting of ferritin degradation products and iron oxide clusters. **Hemosiderin** is a derivative of ferritin and is found in the liver, spleen, and bone marrow. It is insoluble in aqueous solutions, and forms aggregates that slowly release iron when deficiency exists.

Mitochondria contain a nuclear-encoded ferritin isoform.

A secreted glycosylated isoform of predominantly **L-ferritin** circulates in the bloodstream. It contains very low amounts of iron, suggesting that it does not play an essential role in iron storage or traffic, but it is used as a **clinical marker** for body iron stores.

How iron crosses the **blood–brain barrier**? It appears that the endothelial cells at the luminal site of blood capillaries express **transferrin receptors** and take up iron from plasma **transferrin**. The release of iron from endothelial cells (but also neurons or astrocytes) into the brain interstitium involves **ferroportin** in conjunction with a ***ferroxidase*** activity. The *ferroxidase* activity can be provided by *ferroportin-interacting proteins* such as:

- ***hephaestin***,
- **glycosylphosphatidylinositol–ceruloplasmin** - a membrane associated ceruloplasmin isoform**,** or
- **β-amyloidprecursor protein (**β-APP).

Inside the interstitial fluid, iron is captured by brain **transferrin** that is secreted from oligodendrocytes.

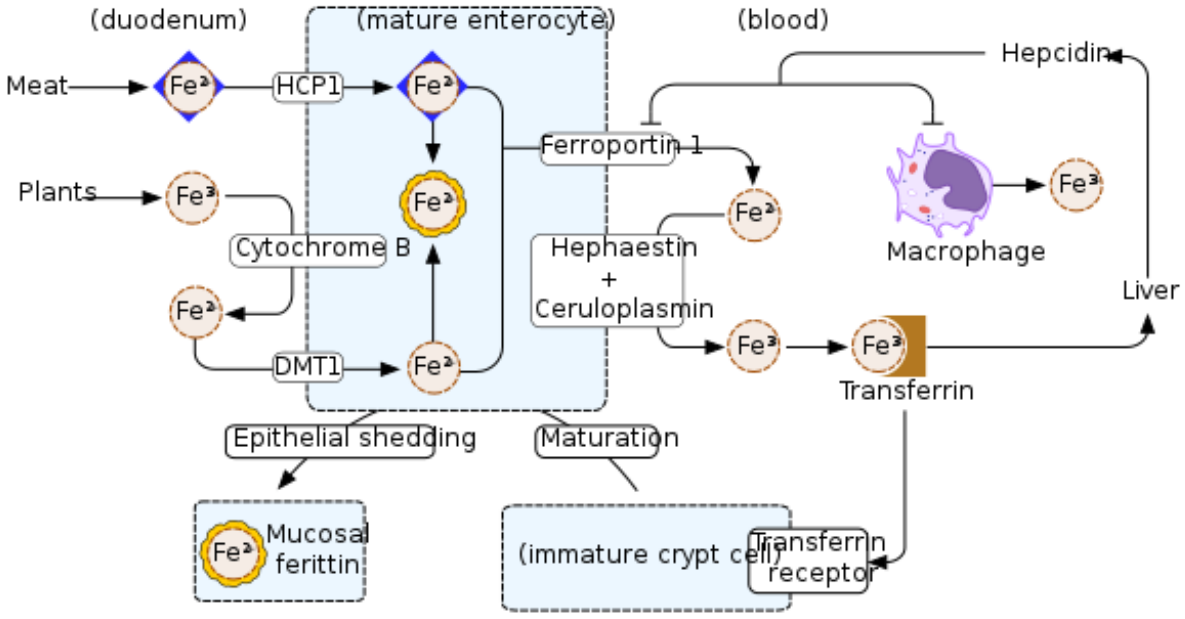


Figure 31.2 – Hormonal regulation of iron efflux from duodenal enterocytes and reticuloendothelial macrophages by hepcidin.

Enterocytes absorb inorganic or haem iron from the diet and macrophages phagocytose iron-loaded senescent red blood cells or acquire

iron by other mechanisms. Both cell types release Fe^{2+} into the plasma via **ferroportin**, which is incorporated into **apo-transferrin** following oxidation to Fe^{3+} via **hephaestin** or **ceruloplasmin**. Hepatocytes generate the iron-regulatory hormone **hepcidin** in response to high iron or inflammatory signals, which inhibits the efflux of iron via **ferroportin** and promotes its retention within enterocytes and macrophages. **DMT1**- *divalent metal transporter 1*; **HCP1 -** *haem carrier protein 1*.[Christensen ZP. 2018].

Human homeostatic iron regulator protein (HFE protein)

The HFE protein is a protein which regulates circulating iron uptake by regulating the interaction of the **transferrin receptor** with transferrin. HFE protein is prominent in small intestinal absorptive cells, gastric epithelial cells, tissue macrophages, and blood monocytes and granulocytes, and the syncytiotrophoblast, an iron transport tissue in the placenta.

The iron storage disorder **hereditary hemochromatosis** is an autosomal recessive genetic disorder that usually results from defects in the gene encoding HFE protein. About 1/200 of people of Northern European origin have two copies of this variant; they, particularly males, are at high risk of developing hemochromatosis.

Hereditary haemochromatosis is characterised by accumulation of iron in parenchymal organs, and clinical manifestation classically includes liver disease (cirrhosis), rheumatoid arthritis, diabetes, and skin color changes. Hereditary haemochromatosis was also associated with coronary artery disease and atrial fibrillation, osteoporosis, tiredness, and susceptibility to infection, but diagnosis is now commonly triggered by chronic fatigue or arthropathy.

Iron-deficiency anemias

Disturbances of the iron metabolism are frequent and can lead to severe disease pictures. Iron deficiency is usually due to blood loss, or more rarely to inadequate iron uptake. During pregnancy, increased demand can also cause iron deficiency states. In severe cases, reduced hemoglobin synthesis can lead to **anemia** (“iron-deficiency anemia”). In these patients, the erythrocytes are smaller and have less hemoglobin.

Hemostasis

Coagulation, also known as **clotting**, is the process by which blood

changes from a liquid to a gel, forming a **blood clot**. It potentially results in **hemostasis**, the cessation of blood loss from a damaged vessel, followed by repair. The mechanism of coagulation involves:

- *activation*, *adhesion* and *aggregation of platelets*, as well as
- *deposition and maturation of fibrin*.

Hemostasis means "the arrest of bleeding". After tissue injury that ruptures smaller vessels (including everyday trauma, injections, surgical incisions, and tooth extractions), a series of interactions between the vessel wall and the circulating blood normally occurs, resulting in cessation of blood loss from injured vessels within a few minutes.

Hemostasis results from effective sealing of the ruptured vessels by a **hemostatic plug** composed of **blood platelets** and ***fibrin***. ***Fibrin*** is derived from circulating *fibrinogen*, whereas platelets are small cells that circulate in the blood and have an important role in the initiation of hemostasis. Hemostasis requires the effective, coordinated function of:

- blood vessels,
- platelets,
- coagulation factors and
- the fibrinolytic system.

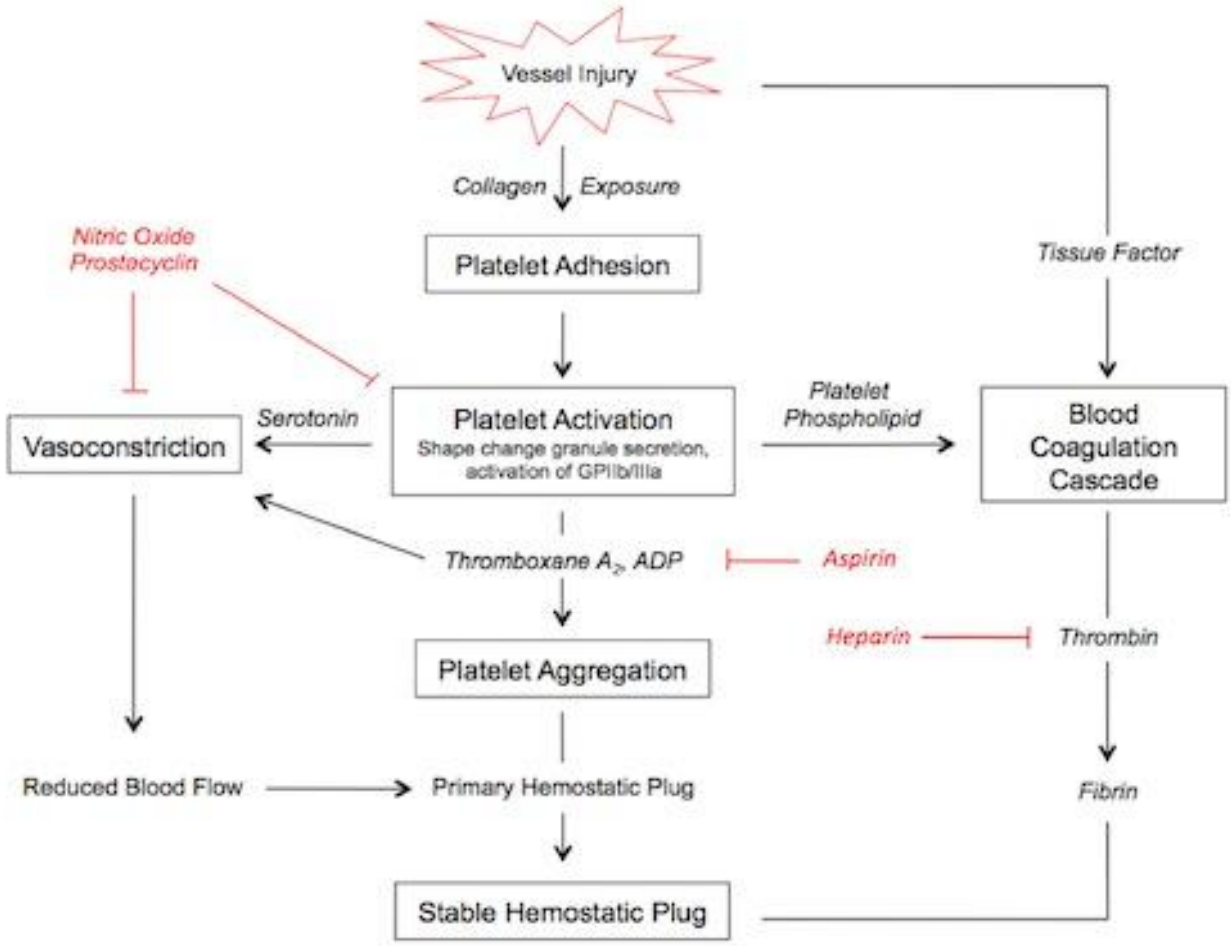


Figure 31.3 – Hemostasis is maintained in the body via three mechanisms [Dorman K. 2009].)

Hemostasis is maintained in the body via three mechanisms (Fig. 31.3):

- **Vascular spasm** (vasoconstriction) - is produced by vascular smooth muscle cells, and is the blood vessel's first response to injury. *Collagen* is exposed at the site of injury, the *collagen* promotes platelets to adhere to the injury site. Platelets release **cytoplasmic granules** which contain *serotonin*, *ADP* and *thromboxane A2*, all of which increase the effect of vasoconstriction.
- **Platelet plug formation (**primary hemostasis**)**- Platelets adhere to damaged endothelium to form a platelet plug (**primary hemostasis**) and then degranulate. Plug formation is activated by a glycoprotein called **Von Willebrand factor** (**vWF**), which is found in plasma. Platelets express certain receptors, some of which are used for the adhesion of platelets to ***collagen***. When platelets are activated, they express glycoprotein receptors that interact with other platelets, producing aggregation and adhesion. Platelets release **cytoplasmic granules** such

as *adenosine diphosphate* (ADP), *serotonin* and *thromboxane A2*.

- **Clot formation (s**econdary hemostasis**)** - Once the platelet plug has been formed by the platelets, the clotting factors (a dozen proteins that travel along the blood plasma in an inactive state) are activated in a sequence of events known as **'coagulation cascade' (**Fig. 31.4) which leads to the formation of *Fibrin* from inactive *fibrinogen* plasma protein. **Coagulation**, also known as **clotting**, is the process by which blood changes from a liquid to a gel, forming a blood clot.

The role of thrombin in hemostasis

The pathways of hemostasis are simultaneously activated following injury, resulting in increasing thrombin production, and the end product of a stabilized **fibrin clot**.

In **primary hemostasis**, circulating platelets adhere to the exposed extracellular matrix in close proximity to ***tissue factor*** (**TF**)-bearing cells. **Secondary hemostasis** involves a series of enzyme-mediated coagulation events, which begin on the **TF**-bearing cells (**initiation**) and transfer to the platelet surface during the thrombin amplification and propagation stages.

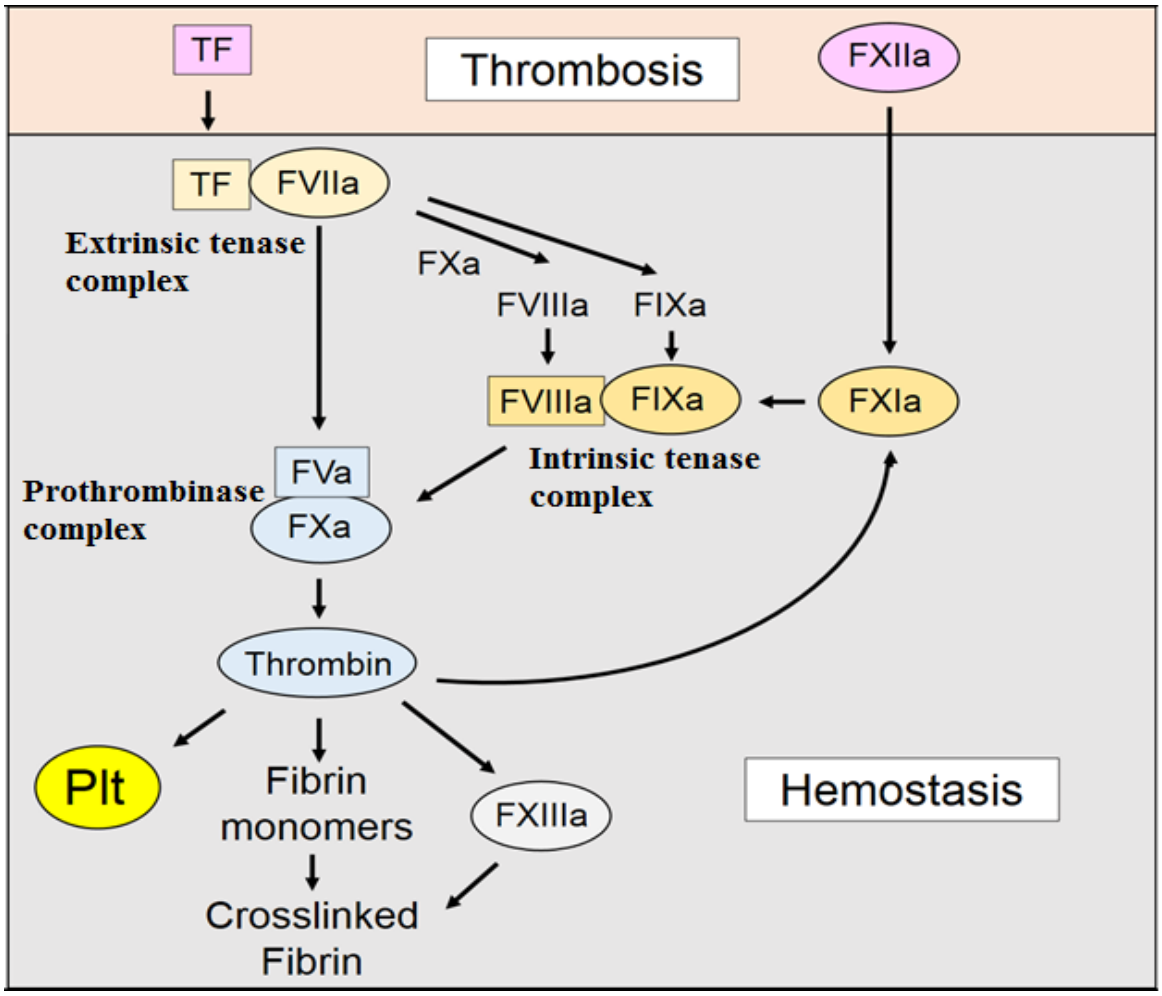


Figure 31.4 – Simplified scheme of blood coagulation reactoions.

Exposure of tissue factor (TF) upon vascular damage leads to complex formation with factor VIIa (**extrinsic tenase complex**). Factors IX and FX are subsequently activated. Factor IXa combines with the protein cofactor FVIIIa to form the **intrinsic tenase complex** that efficiently activates factor FX. Factor Xa combines with the protein cofactor FVa to form the **prothrombinase complex**, which activates *prothrombin* into *thrombin*. [Mackman Lab. 2019].

The ***coagulation cascade*** of secondary hemostasis has two initial pathways which lead to ***fibrin*** formation. These are:

- the contact activation pathway**(intrinsic pathway**), and
- the tissue factor pathway (**extrinsic pathway**),

which both lead to the same fundamental reactions that produce ***fibrin***.

Extrinsic pathway. During the **initiation phase**, activated factor

(**F**).The **extrinsic tenase complex** is formed from membrane-anchored **tissue factor** (**TF**) and active factor **VIIa** from membrane or from solution and subsequently activates **factor X** (**FX→ FXa**) and **factorFIX (FIX → FIXa)** via ternary complex formation.

The factor **FXa** formed by factor **VIIa**/**TF** binds to **FVa** on the **TF**-bearing cell (**prothrombinase complex**) and converts a small amount of ***prothrombin*** (**FII**) to ***thrombin*** (Fig 31.4).

In the **amplification phase**, the ***thrombin*** generated on **TF**-bearing cells further activates the platelets adhered to the site of injury. ***Thrombin*** formed during this stage also activates cofactors **V** and **VIII**, in addition to **XI** on the platelet surface.

During **propagation phase** on the activated platelet, ***thrombin*** is generated following proteolytic activation of its precursor, ***prothrombin*** (**FII**), as a result of two-site cleavage by ***prothrombinase***; formation of the ***prothrombinase complex*** requires the membrane-dependent interaction of **FXa** with the cofactor protein **FVa**. The factor **FIXa** in the propagation phase is generated during the initiation stage.

This production of a large amount of ***thrombin*** (**thrombin burst**) is sufficient to cleave soluble ***fibrinogen*** and generate insoluble ***fibrin fibers*** that form a **mesh** around the initial platelet plug. This ***fibrin clot*** is further stabilized by the ***thrombin*** activation of factor **FXIII** which cross-links the *fibrin* fibers.

During this process some red and white blood cells are trapped in the mesh which causes the primary hemostasis plug to become harder: the resultant plug is called as '**thrombus**' or '**Clot**'.

During clotting, ***fibrinogen*** is converted to ***fibrin*** as a result of proteolytic cleavage by ***thrombin***, and so a major difference between plasma and serum is the absence of fibrinogen in serum.

Intrinsic pathway. Factor **XII** becomes activated when it comes into contact with negatively charged surfaces and undergoes a conformational change, which generates small amounts of activated factor **FXIIa** (Fig.31.4). The plasma contact system drives proinflammatory and procoagulant pathways and is composed of plasma proteases, substrates, and inhibitors produced and secreted by the liver. It consists of the *serine proteases* factors **XII** (FXII), **FXI**, *plasma prekallikrein* (**Pre-K**) zymogens, the non-enzymatic cofactor *high molecular weight kininogen* (**HMWK**).

Factor XIIa cleaves *plasma prekallikrein* (**Pre-K**) to form

plasmakallikrein (**Kal**), which reciprocally activates **FXII** and generates a positive feedback loop of **FXII** activation. **Pre-K** subsequently digests **HMWK** to release *bradykinin* (**BK**), a potent proinflammatory peptide and the end product of the **kallikrein-kinin pathway**. *Bradykinin* activates signaling pathways resulting in increased:

- vascular permeability,
- vasodilation, and
- chemotaxis of neutrophils.

Factor **XIIa** also activates the **intrinsic coagulation cascade** via factor **XI** cleavage to generate activated **factor XIa**. Factor **XIa** initiates a series of Ca^{2+}-dependent sequential proteolytic cleavage events that lead to *thrombin* generation, *fibrin* formation, and production of a **fibrin clot** in plasma.

Inorganic polyphosphate found in platelets activates factor **XII** *in vivo* and can induce coagulation in pathological thrombus formation.

The contact system acts as the cross-road of inflammation, coagulation, and innate immunity.

Regulation of blood clotting.

To prevent the coagulation reaction from becoming excessive, the blood contains a number of anticoagulant substances, including proteinase inhibitors. For example, ***antithrombin*** binds to various proteinases in the cascade and thereby inactivates them. **Heparin**, an anticoagulant, potentiates the effect of ***antithrombin***. ***Thrombomodulin***, which is located on the vascular endothelia, also inactivates thrombin.

Thrombin generation is attenuated by ***TF pathway inhibitor*** (**TFPI**) in a process that involves reversible inhibition of **tenasecomplex**(**TF/FVIIa), FXa**, and activated ***protein C*** (**APC**) pathways, and proteolytic inhibition of the cofactors **FVa** and **FVIIIa**; in the normal process of clot formation to achieve hemorrhagic control, this **TFPI** process regulates the initial production of **FXa** that leads to the first small amount of ***thrombin*** generation during the initiation phase.

Anticoagulant ***antithrombin*** (**AT**) may also inhibit ***thrombin*** generation in plasma by inhibiting ***thrombin*** directly and also by inactivating any uncomplexed **FXa** that dissociates into the blood during all stages of coagulation. ***Fibrin***-bound ***thrombin*** appears to be protected from inactivation by **AT**.

Thrombin itself also binds to the transmembrane receptor

thrombomodulin on endothelial cells, which results in activation of **APC**; **APC** modulates the activity of the procoagulant **FVa** and **FVIIIa**, in conjunction with cofactor ***protein S*** and factor **FV**, thus blocking further ***thrombin*** propagation.

Table 31.1. **– Coagulation factors and related substances**

Number and/or name	Function
Coagulation factors	
I (**fibrinogen**)	Forms clot (fibrin)
II (**prothrombin**)	Its active form (IIa) activates I, V, VII, VIII, XI, XIII, protein C, platelets
III (**tissue factor** or tissue thromboplastin)	Co-factor of VIIa (formerly known as factor III)
IV (**calcium**)	Required for coagulation factors to bind to phospholipid (formerly known as factor IV)
V (**proaccelerin**)	Co-factor of X with which it forms the **prothrombinase complex**
VII (**proconvertin**)	Activates IX, X
VIII (**Antihemophilic factor A**)	Co-factor of IX with which it forms the **tenase complex**
IX (**Antihemophilic factor B** or **Christmas factor**)	Activates X: forms **tenase complex** with factor VIII
X (**Stuart-Prower factor**)	Activates II: forms **prothrombinase complex** with factor V
XI (**plasma thromboplastinantecedent**)	Activates factor IX
XII (**Hageman factor**)	Activates factor XI, VII and prekallikrein
XIII (**fibrin-stabilizing factor**)	Cross-links fibrin
von Willebrand factor	Binds to VIII, mediates platelet adhesion
prekallikrein (**Fletcher factor**)	Activates XII and prekallikrein; cleaves HMWK
high-molecular-weight kininogen (**HMWK**) (**Fitzgerald factor**)	Supports reciprocal activation of XII, XI, and prekallikrein
Anticoagulant substances	
antithrombin	Inhibits IIa, Xa, and other proteases
heparin cofactor II	Inhibits IIa, cofactor for heparin and

	dermatan sulfate ("minor antithrombin")
protein C	Inactivates Va and VIIIa
protein S	Cofactor for activated protein C (APC),
protein Z	Mediates thrombin adhesion to phospholipids and stimulates degradation of factor X by ZPI
protein Z-related protease inhibitor (**ZPI**)	Degrades factors X (in presence of protein Z) and XI (independently)
plasminogen	Converts to plasmin, lyses fibrin and other proteins
Tissue plasminogen activator (**tPA**)	Activates plasminogen
urokinase	Activates plasminogen
Plasminogen activator inhibitor-1/2 (**PAI1/2**)	Inactivates tPA & urokinase (endothelial PAI)

Fibrinolysis

The fibrin thrombus resulting from bloodclotting is dissolved again by ***plasmin***, a proteinase found in the blood plasma. For this purpose, the precursor ***plasminogen*** first has to be proteolytically activated by enzymes from various tissues. This group includes the ***plasminogen activator*** from the kidney (***urokinase***) and tissue plasminogen activator (**t-PA**) from vascular endothelia. By contrast, the plasma protein ***antiplasmin***, which binds to active ***plasmin*** and thereby inactivates it, inhibits fibrinolysis.

Urokinase, **t-PA**, and ***streptokinase***, a bacterial proteinase with similar activity, are used clinically to dissolve thrombi following heart attacks. All of these proteins are expressed recombinantly in bacteria.

Hemophilia

Hemophilia is a group of hereditarygenetic disorders that impair the body's ability to control blood clotting or coagulation, which is used to stop bleeding when a blood vessel is broken.

Hemophilia A (clotting **factor VIII** deficiency) is the most common form of the disorder.

Hemophilia B (**factor IX** deficiency) occurs rarely.

Like most recessive sex-linked X chromosome disorders, hemophilia

is more likely to occur in males than females. Females are almost exclusively asymptomatic carriers of the disorder. Hemophilia lowers blood plasma clotting factor levels of the coagulation factors needed for a normal clotting process. Thus when a blood vessel is injured, a temporary scab does form, but the missing coagulation factors prevent fibrin formation, which is necessary to maintain the blood clot. A hemophiliac does not bleed more intensely than a person without it, but can bleed for a much longer time. In severe hemophiliacs even a minor injury can result in blood loss lasting days or weeks, or even never healing completely. In areas such as the brain or inside joints, this can be fatal or permanently debilitating.

Disseminated intravascular coagulation

Disseminated intravascular coagulation (DIC), also known as *disseminated intravascular coagulopathy* is a pathological activation of coagulation mechanisms that happens in response to a variety of diseases. DIC leads to the formation of small blood clots inside the blood vessels throughout the body. As the small clots consume coagulation proteins and platelets, normal coagulation is disrupted and abnormal bleeding occurs from the skin (e.g. from sites where blood samples were taken), the gastrointestinal tract, the respiratory tract and surgical wounds. The small clots also disrupt normal blood flow to organs (such as the kidneys), which may malfunction as a result. DIC can occur acutely but also on a slower, chronic basis. It is common in the critically ill, and may participate in the development of multiple organ failure, which may lead to death.

CHAPTER 32

BIOCHEMISTRY OF THE KIDNEY AND URINE

Biochemical functions of the kidney

The kidneys are bean-shaped organs that serve several essential regulatory roles in vertebrates. The kidney's parenchyma is divided into the outer part renal cortex, and the inner part renal medulla, and consists of about 1 million filtering units termed nephrons (basic structural and functional unit).

The major functions of the kidney are as follows:

1. **Excretory function:** formation of the urine, excretion of metabolic waste products such as urea, uric acid, creatinine, ammonia, as well as various drugs and toxins from the body;
2. **Reabsorption of vital nutrients** (glucose, amino acids, sodium, etc from the primary urine);
3. **Regulation of water and electrolyte balance** (sodium, potassium, other electrolytes);
4. **Maintenance of the acid-base balance** (**pH**) (renal mechanisms);
5. **Endocrine function**: production of renin (a key part of the renin-angiotensin-aldosteron system), erythropoietin (stimulates production of erythrocytes). 1-α, 25-dihydroxycholecalcipherol (regulator of calcium homeostasis).
6. **Metabolic function**: synthesis of specific substances, catabolism of lipids, amino acids, carbohydrates; higher rate of energy metabolism, etc.

The most active metabolic pathways are:

- Aerobic glycolysis;
- Gluconeogenesis;
- β-oxidation of fatty acids;
- Utilization of ketone bodies;
- Transamination and deamination of amino acids;
- Energy dependent transport of ions, waste products, etc.

Carbohydrate metabolism in the kidney

Glucose recycling in the kidney is an energy dependent process. Glucose uptake occurs as compulsatory cotransport with Na^+ ions, and is driven by the concentration gradient of Na^+ between the urine and interior of the cells (secondary active transport)

Intensity of glucose metabolism differs in the renal cortex and medulla. Aerobic processes prevail in the renal cortex, while the renal medulla was shown to be the place of anaerobic glycolysis.

The kidney in humans is capable of producing glucose from glutamine, lactate, and glycerol in the process called gluconeogenesis. These substrates are obtained from the blood plasma, and glutamine is the main substrate for gluconeogenesis. The kidney is responsible for about half of the total gluconeogenesis in fasting humans. The regulation of glucose production in the kidney is achieved by action of insulin, catecholamines, and other hormones. Renal gluconeogenesis takes place in the renal cortex, while the renal medulla is incapable of producing glucose due to absence of necessary enzymes.

Energy metabolism in the kidney

The physiological processes that occur in the kidney require a large supply of energy generated by metabolic reactions. Most metabolic processes in the kidney are aerobic, and oxygen consumption in the renal tissue is very high. High metabolic activity of oxidative metabolism is required to maintain tubular reabsorption.

The kidney consumes **about 10% of all oxygen in the body;** about 70% of it used to generate energy for the active sodium transport which in turn determines reabsorption of glucose and amino acids.

The partial pressure of oxygen (pO_2) is greater in the kidney cortex than in the medulla. As pO_2 is low in the medulla, but the activity of Na^+,K^{+-}-ATPase is high there, cells of the distal segments of nephrons are especially sensitive to the decreased oxygen supply.

About 90% of ATP is derived from β-oxidation of fatty acids and utilization of ketone bodies. The rest 10% of ATP is derived from glucose, lactate, pyruvate, glycerol, citrate, and AA absorbed from the blood. About 80% of all ATP available in the renal tubular cells is used for functioning of the sodium pump (Na^+,K^+-ATPase), that determines reabsorption of glucose and amino acids.

Main features of lipid metabolism in the kidney

From all lipids the kidneys phospholipids and cholesterol constitute about 80-85% of the total amount of lipids. The remainders 10-15% of lipids are triacylglycerols. Higher rate of β- oxidation of fatty acids and utilization of ketone bodies provides the kidney's need for energy. The

kidneys are also the place of utilization of plasma lipoproteins.

Metabolism of amino acids and proteins in the kidney

The kidney has an important role in amino acid metabolism and in **the regulation of plasma concentrations of many amino acids.** This role is dependent on a supply of amino acids to maintain the physiology and integrity of the kidney, allow the uptake, degradation, synthesis, and release of amino acids, and maintain acid-base homeostasis.

About 70g of amino acids per day, derived from the diet and metabolism in the liver, muscle and other tissues, are filtered from the arterial blood by the kidney. Most of these amino acids are actively reabsorbed in the proximal tubules and after metabolism leave the kidney by the renal vein. The most metabolized amino acids in the kidney are **glutamine, glutamate, aspartate**, and **glycine.** In normal kidney **glutamine** is used for maintenance of acid-base homeostasis. Higher activity of aminotransferases and glutamate dehydrogenase provides removal of ammonia from amino acids in the transdeamination process.

Besides, the kidney is a major site for the **catabolism of both circulating and kidney peptides and proteins**, because of the high activity of peptidases in the proximal tubular brush border. Of circulating proteins the kidney breaks down **small and medium-sized blood plasma proteins** (below 6000 Da), such as **insulin** and **peptide hormones.**

The kidneys are the place for synthesis of some regulatory proteins, such as renin, and erythropoietin**.** They also produce the **glutaminase** enzyme, which catalyzes removal of ammonia from glutamine and assists in the maintenance of acid-base balance. Other proteins produced in the kidneys are **urokinase, proteins of fibrinolysis,** and **complement system.**

The kidneys play a role in the synthesis of **creatinine**. The first reaction in this process (production of guanidine acetate from arginine and glycine) takes place in the kidneys.

Biological active compounds synthesized in the kidney

Renin is the part of the renin–angiotensin system that regulates blood pressure and fibrinolysis. It is synthesized by the juxtaglomerular cells in the kidneys from prorenin, already present in the blood. The renin is then secreted into the circulation and acts on conversion of angiotensinogen into angiotensin-I.

Erythropoietin is a polypeptide hormon that is formed predominantly

by the kidney (also by the liver). It controls the differentiation of the bone marrow stem cells. The release is stimulated by hypoxia (low pO2). The hormon ensures that the bone marrow cells are converted to erythrocytes, so that their concentration in the blood increases (erythropoiesis)

1-α, 25-dihydroxycholecalcipherol is a steroid-related hormon involved in calcium homeostasis. It is an active form of vitamin D_3 (calcitriol) increases the level of Ca^{2+} in the blood . The main functions of 1-alpha, 25-dihydroxycholecalcipherol: (1) to increase the uptake of Ca^{2+} from the gut into the blood; (2) increase reabsorption of Ca^{2+} by the kidneys

MAINTENANCE OF ACID-BASE HOMEOSTASIS (pH BALANCE) BY THE KIDNEY

The kidneys maintain acid-base homeostasis by regulating the pH of the blood plasma. During the normal metabolism, the organism produces large quantities of both organic and inorganic acids. The acids produced are divided into volatile acids and nonvolatile acids.

Volatile acids are represented by **carbonic acid**. It is derived from CO_2 produced in metabolism. CO_2 dissolves in water and forms carbonic acid (H_2CO_3) which in turn dissociates into hydrogen ion (H^+) and bicarbonate ion (HCO_3^-):

$$CO_2 + H_2O \leftrightarrow H_2CO_3 \leftrightarrow H^+ + HCO_3^-$$

Thus, CO_2 secondarily generates large amounts of hydrogen ions. As the total reaction is reversed, the carbonic acid may be eliminated as CO_2 by the lungs.

Nonvolatile acids are represented by **sulphuric acid** derived from sulphur-containing substances (amino acids, proteoglycans, sulpholipids), **phosphoric acid** (derived from phospholipids, phosphoproteins, and nucleic acids), **lactic acid** (produced from glucose), **ketone bodies** (acetoacetate and β-hydroxybutyrate) and, to less extent, other organic acids such as pyruvic acid, citric, etc. By definition, nonvolatile acids cannot be removed through the lungs, and must be excreted via the kidney.

These endogenously generated acids are donors of H^+ and hence tend to shift the pH of the extracellular fluid. The production of basic compounds in the body is negligible. Any disturbance in the pH of one fluid compartment leads to the change in the pH of other fluid compartments because the cell membranes are permeable to H^+ or OH^- .

The increase or decrease of pH affects the ionization of proteins and, consequently, the functions of proteins and activity of many enzymes. The body is very sensitive to its pH. Outside the range of pH that is compatible with life, proteins are denatured and digested, enzymes lose their ability to function, and the body is unable to sustain itself.

As hydrogen ion concentration affects living processes, the regulation of pH is an essential property of a living organism. **Hydrogen ion concentration (pH) of blood plasma is normally 7.35-7.45.** Life is threatened when the pH is lowered below 7.25 or increased above 7.55. Death occurs when pH is below 7.0 or above 7.6.

The acid-base balance implies the relative constancy of the hydrogen ion concentration (pH value) in the internal medium of the organism.

Mechanisms for regulation of pH

The acid-base balance in the blood and extracellular fluids is maintained by cooperative interaction of three main mechanisms:

- **buffer systems of the blood;**
- **respiratory mechanisms,**
- **renal mechanisms.**

The effect of buffer systems of the blood is manifested within 30 seconds. The lungs require a period of 1-3 min to normalize pH in the blood. Kidney needs 10-20 h to restore a disturbed acid-base balance.

Buffers can respond immediately to addition of acid or base, but they do not eliminate the acid from the body. Buffers are also unable to replenish the alkali reserve of the body. For the elimination of acids and replenishment of the alkali reserve, the respiratory, and renal regulatory mechanisms are essential.

Buffer systems of the blood

Three major buffer systems of the blood are bicarbonate buffer system and the hemoglobin buffer, phosphate buffer, and the plasma proteins. In extracellular fluids pH is maintained by bicarbonate and phosphate buffer systems, as the ECF do not contain proteins.

1. Bicarbonate buffer system.

Carbon dioxide produced in tissues diffuses through cell membranes into the plasma and then to the RBC. In the RBC, the enzyme carbonic anhydrase binds CO_2 to form carbonic acid. The latter dissociates to give H^+ and HCO_3^- :

$$CO_2 + H_2O \leftrightarrow H_2CO_3 \leftrightarrow H^+ + HCO_3^-$$

The HCO_3^- leaves the RBC and enters the plasma. Bicarbonate (HCO_3^-) represents the alkali reserve of the blood.

The kidney can supply the plasma with HCO_3^-: the organ regulates bicarbonate tubular reabsorption and regeneration of HCO_3^- by the renal tubular cells.

When H^+ concentration is getting increased in the blood plasma, bicarbonate reacts with H^+ to form H_2CO_3:

$$H^+ + HCO_3^- \rightarrow H_2CO_3$$

The H_2CO_3 concentration in the blood plasma is determined by the pCO_2 (CO_2 partial pressure) in alveolar gas mixture. The decrease of H_2CO_3 concentration in the blood plasma is due to release of CO_2:

$$H_2CO_3 \rightarrow CO_2 + H_2O$$

Excess of CO_2 is then expired through the lungs.

2. Hemoglobin buffer system. This is another most powerful buffer system of the blood. There are both hemoglobin buffer system (HHb/Hb^-) and oxyhemoglobin buffer system ($HHbO_2/HbO_2^-$).

3. Protein buffer system. Plasma proteins regulate pH by attaching/detaching of protons to the charged groups of amino acid residues.

Respiratory mechanism for regulation of pH

The respiratory system helps control the acidity of blood by regulating the elimination of carbon dioxide and water molecules. These molecules, exhaled in every breath, come from carbonic acid as follows:

$$H^+ + HCO_3^- \leftrightarrow H_2CO_3 \leftrightarrow CO_2\uparrow + H_2O$$

The more CO_2 and H_2O are exhaled, the more carbonic acid is removed from the blood, thus elevating the blood pH to a more alkaline level. Due to respiration, CO_2 is eliminated from the blood. Removal of CO_2 is essential for maintenance of the blood pH.

A significant increase in the CO_2 of arterial blood or decrease below about 7.38 of arterial blood pH, causes breathing to increase both in rate and depth, resulting in **hyperventilation**. Increased ventilation eliminates more CO_2, reduces carbonic acid and protons concentrations, and increases

the blood pH back toward the normal level.

In the opposite situation, an increase in blood pH above normal causes **hypoventilation,** a reduced rate of respiration. Less carbon dioxide is exhaled, and the higher concentration of carbonic acid remaining in the blood lowers the pH back to normal.

Renal regulation of pH

Since the pH of urine varies from 4.5-8.0, this indicates kidney has ability to excrete various amounts of acids and bases to maintain the normal pH of blood. This ability makes the kidney the final defense mechanism against change in body pH.

There are three mechanisms due to which the kidney regulates pH of the blood plasma:

I. Reabsorption of bicarbonate by the renal tubular cells;
II. Excretion of H^+ as $H_2PO_4^-$;
III. Excretion of H^+ as ammonium ion (NH_4^+).

I. Reabsorption of bicarbonate by the renal tubular cells

The bicarbonate (and Na^+) reabsorption takes place in the proximal convoluted tubules. At the same time, H^+ is secreted into the lumen of the tubule in exchange for the Na^+ reabsorbed (Fig.32.1).

Mitochondria of tubule cells produce CO_2 and H_2O in the electron transport chain and TCA cycle. The brush boarder of tubular cell contains the enzyme **carbonic anhydrase** that combines CO_2 and H_2O molecules to form carbonic acid (H_2CO_3). Carbonic acid easily dissociates to give HCO_3^- and H^+. Protons from the blood and tubular cells are secreted to the tubular lumen in exchange for Na^+ ions, and bicarbonate ion HCO_3^- is reabsorbed in plasma along with sodium ion.

In tubular lumen, hydrogen ions combine back with bicarbonate to give carbonic acid, which dissociates to CO_2 and water. Most of this carbon dioxide is taken by the cells and converted by intracellular carbon anhydrase. Water molecules are excreted in the urine.

This mechanism serves to increase the alkali reserve of the blood plasma and prevent the loss of bicarbonate with the urine.

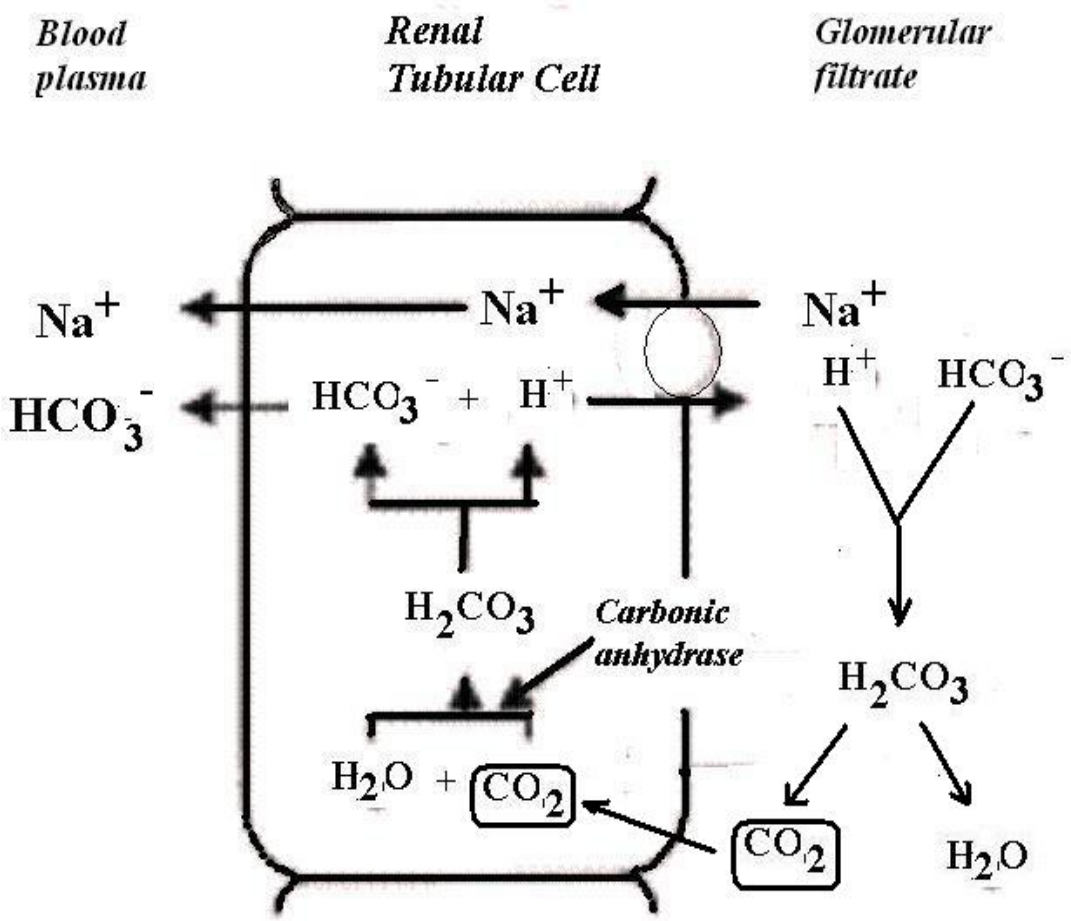


Figure 32.1 – Reabsorption of bicarbonate by the renal tubular cells

II. Excretion of H^+ as $H_2PO_4^-$

The fate of protons in the distal tubules differs from the fate of H^+ in the proximal tubules. Normally, there is no bicarbonate available in the distal tubule lumen, because it has already been reabsorbed in the proximal tubule.

The renal filtrate, produced in the glomeruli, contains sufficient amount of **phosphates**: **hydrogen phosphate ion (*HPO_4^{2-}; basic phosphate)*** and **dihydrogen phosphate ion *($H_2PO_4^-$; acid phosphate).*** As the tubular fluid passes down the renal tubules, more and more H^+ is secreted from the tubule cells into the tubule lumen in exchange for sodium ion. In the glomerular filtrate, Na_2HPO_4 is converted to NaH_2PO_4 to be excreted into the urine. This process helps excrete (eliminate) acids from the bod, and allows to reabsorb and save sodium ions.

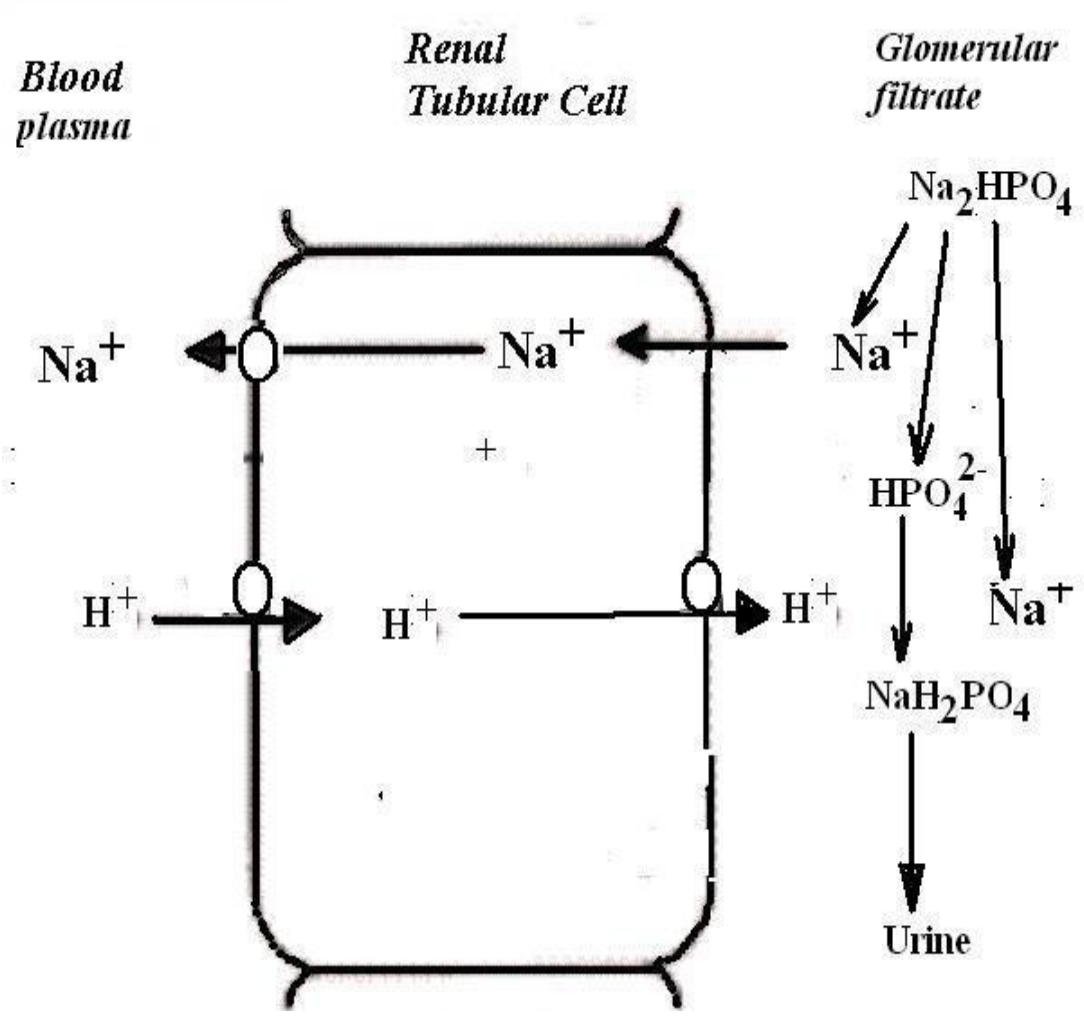


Figure 32.2 – Excretion of H^+ as $H_2PO_4^-$

III. Excretion of H^+ as ammonium ion (NH_4^+)

This process occurs in the **distal** tubules and does not represent direct excretion of hydrogen ions. **Glutaminase** and **Glutamate dehydrogenase** are present in renal tubular cells that catalyze deamination of glutamine and glutamate with release of **ammonia (NH_3)**. Ammonia diffuses readily down its concentration gradient into the tubular lumen, where it forms ammonium ions. Ammonium ions cannot cross cell membrane and thus are trapped in tubular urine. They combine with anions like chloride, and then excreted into the urine. This mechanism also allows reabsorbing and saving sodium ions avoiding dehydration.

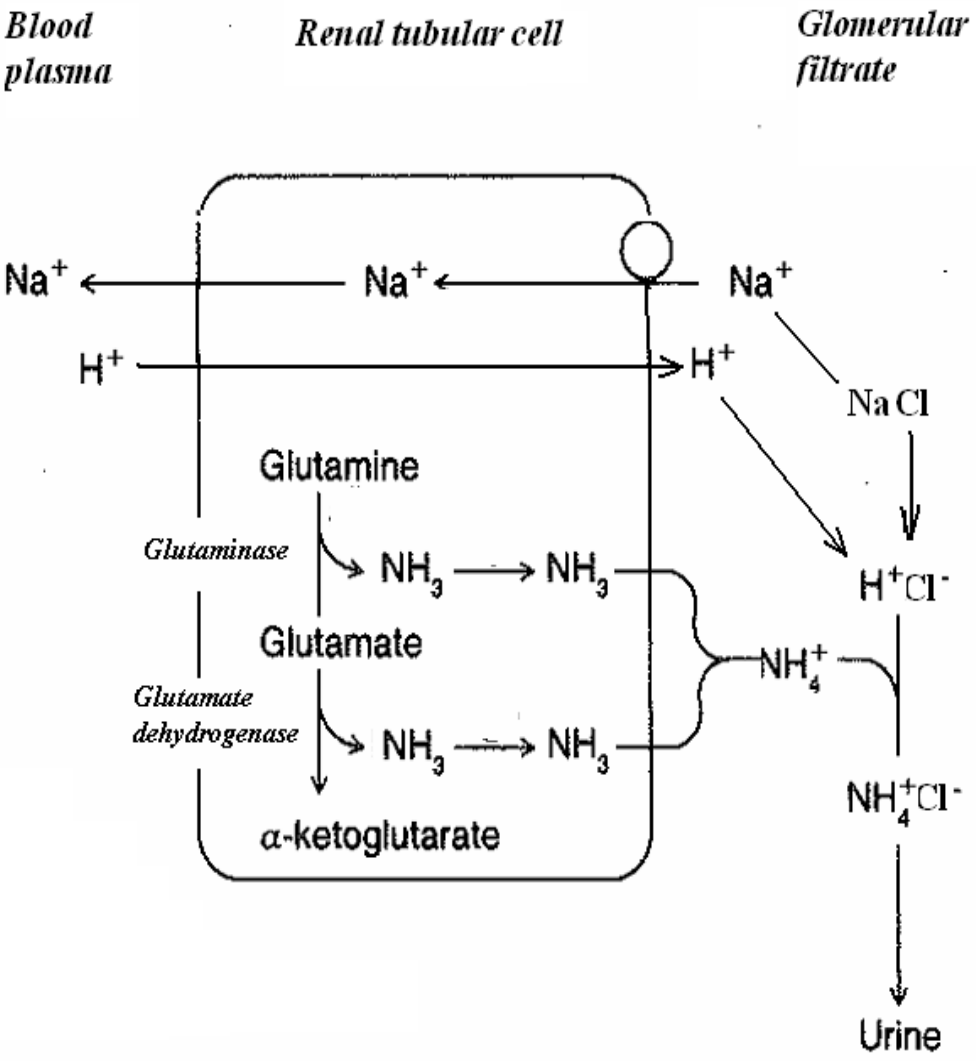


Figure 32.3 – Excretion of H^+ as ammonium ion (NH_4^+)

PHYSICAL CHARACTERISTICS OF URINE

Physical characteristics of urine include appearance, color, turbidity (transparency), smell (odor), pH (acidity - alkalinity), and density. Many of these characteristics are notable and identifiable by vision alone, but some require laboratory testing.

Volume

The average quantity of urine under ordinary dietary conditions is normally 1,500 ml per day for men and 1,200 ml for women.

Polyuria is excessive or an abnormally large production or passage of urine (greater than 2.5 litres per day in adults). The most common cause of polyuria is uncontrolled **diabetes mellitus,** in which the blood glucose levels are so high that glucose is excreted in the urine. In the absence of

diabetes mellitus, the most common causes may be excessive fluid intake, the dietary intake of food stimulating diuresis (e.g. melon, water melon, cucumber, pumpkin), decreased secretion of aldosterone due to adrenal cortical tumor, **diabetes insipidus** (due to lack of antidiuretic hormone the volume of the urine excreted may amount to 15 litres per day or more).

Oliguria is the low output of urine, specifically more than 80 ml/day but less than 400ml/day. The decreased output of urine may be a sign of dehydration caused by deficient liquid intake. Pathological cases include urinary tract infections, hypovolemic shock, and others.

Anuria is clinically defined as less than 50mL urine output per day up to complete nonpassage of urine. It is often caused by failure in the function of kidneys, because of the mercury poisoning, severe obstruction like kidney stones or tumours. It may occur with end stage renal disease. Acute anuria, where the decline in urine production occurs quickly, is usually a sign of obstruction or acute renal failure. Acute renal failure can be caused by factors not related to the kidney, such as heart failure, mercury poisoning, infection, and other conditions that cause the kidney to be deprived of blood flow.

Specific gravity

Density of the urine is also known as "specific gravity". This is the ratio of the weight of a volume of the urine compared with the weight of the same volume of distilled water.

In adults specific gravity of the urine varies in the range of 1.015 to 1.029. Increases in specific gravity may be associated with increased concentration of solutes in the urine due to dehydration, diarrhea, excessive sweating, urinary tract/bladder infection, glucosuria, renal artery stenosis, hepatorenal syndrome, decreased blood flow to the kidney (especially as a result of heart failure), and excess of antidiuretic hormone caused by Syndrome of inappropriate antidiuretic hormone. In neonates, normal urine specific gravity is 1.003. Hypovolemic patients usually have a specific gravity >1.015.

Decreased specific gravity (or decreased concentration of solutes in urine) may be associated with renal failure, pyelonephritis, diabetes insipidus, acute tubular necrosis, interstitial nephritis, and excessive fluid intake (e.g., psychogenic polydipsia). **Color**

Normally, the urine is straw-yellow or yellow-amber due to pigment urochrom, which is the product of break down of a heme. Color of the urine may vary according to recent diet and the concentration of the urine. Drinking more water generally tends to reduce the concentration of urine, and therefore causes it to have a lighter color. Dark urine may indicate dehydration. Red urine indicates red blood cells within the urine, a sign of kidney damage and disease.

Appearance (transparency)

The normal urine is perfectly clear and transparent. The cloudy appearance of the urine may be due to presence of salts (oxalates), pus (infections of urinary tract), protein, blood, cell elements, bacteria, mucus, or fat.

Smell

The smell of urine may provide health information. For example, urine of diabetics may have a sweet or fruity odor due to the presence of ketones (organic molecules of a particular structure) or glucose. Generally fresh urine has a mild smell but aged urine has a stronger odor similar to that of ammonia.

pH

The pH of normal urine is generally weakly acidic and in the range from 5.3 to 6.5. Much of the variation occurs due to diet. For example, high protein diets result in more acidic urine, but vegetarian diets generally result in more alkaline urine.

Severe shifts in the values of pH may be caused by metabolic abnormalities. For example, distinctly acidic urine reaction is observed in diabetes mellitus, starvation, and fever due to presence of ketone bodies (ketonuria). In persons with hyperuricosuria, acidic urine can contribute to the formation of stones of uric acid in the kidneys, ureters, or bladder.

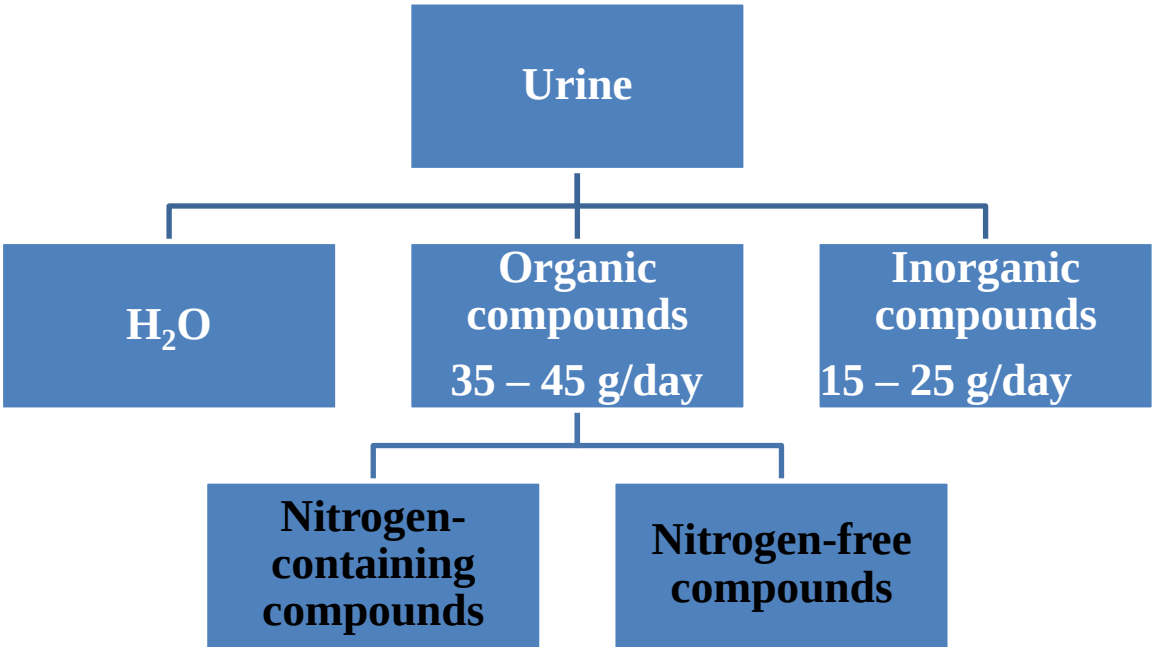
The alkaline urine occurs in cystitis and and pyelitis: microorganisms produce the enzyme urease which can degrade urea to form ammonia (alkaline solution NH_4OH) in the bladder.

Abnormalities in any of these of physical characteristics may indicate disease or metabolic imbalances. These problems may seem superficial or minor on their own, but can actually be the symptoms for more serious diseases, such as diabetes mellitus or a renal pathology.

Chemical composition of urine

Urine is a sterile fluid composed of: water (95%), organic (35-45 g/day), and inorganic constituents (15-25 g/day)

Organic constituents are divided into nitrogen-containing and nitrogen-free compounds.



Nitrogen-containing compounds are represented by urea, uric acid, creatine, creatinine, amino acids, bilirubin, indican. Urea is the largest constituent of the solids, constituting more than 80% of all nitrogen-containing compounds. Most of them are products of nitrogen metabolism (metabolism of amino acids and nitrogenous bases); bilirubin is a product of heme breakdown.

Nitrogen-free compounds include various organic acids, such as oxalic, lactic, citric, valeric, ketone bodies (β-hydroxybutyric and acetoacetic acids).

Besides, the urine may contain pigments, hormones, and their metabolites. The exact proportions of these constituents vary with individuals and with factors such as diet and health. In healthy persons, urine contains very little protein; an excess is suggestive of illness.

Major inorganic (mineral) components of the urine. The urine contains practically all the mineral components present in the blood plasma: sodium, potassium, calcium, magnesium, chloride, phosphates, sulphate, and ammonia (ammonium salts).

PATHOLOGICAL COMPONENTS OF THE URINE

Pathological components of the urine are normally absent in the normal urine, or present only in trace amounts and increase in diseases. They are proteins (proteinuria), blood (hematuria), glucose (glucosuria), ketone bodies (ketonuria), and bile pigments.

Proteins

The presence of protein in the urine is called **proteinuria**. The condition is often **a sign of kidney disease**. Healthy kidneys do not allow a significant amount of protein to pass through their filters. But filters damaged by kidney disease may let proteins such as albumin leak from the blood into the urine. Kidney disease often has no early symptoms. One of its first signs may be proteinuria that's discovered by a urine test done during a routine physical exam. Blood tests will then be done to see how well the kidneys are working. Since serum proteins are readily reabsorbed from urine, the presence of excess protein indicates either an insufficiency of absorption or impaired filtration.

There are three main mechanisms to cause proteinuria:

1. Due to disease in the glomerulus **(renal proteinurea).** The acute inflammation of the kidney (**acute nephritis**) results in damage to the glomerular basement membrane and the size of the pores of glomerular filter is getting increased. Hence, the glomeruli become more permeable, and plasma proteins appear in the urine.
2. Due to low reabsorption at proximal tubule **(extrarenal proteinurea)** due to inflammation of lower urinary tract (cystitis, pyelitis, urethritis, prostatitis).
3. Because of increased quantity of proteins in the blood serum **(overflow proteinuria).** Proteins can be present in the urine of patients with the increased blood pressure (arterial hypertension) when plasma proteins are extruded through the glomerular filter into the urine. Proteins can appear in the urine because of the reduced blood flow in the glomeruli (this takes place in heart decompensation).

Proteinuria can also be caused by certain biological agents used in cancer treatment or by excessive fluid intake (drinking in excess of 4 litres of water per day).

Blood in the urine

In the urine, the blood may occur either in the form of red blood cells (**hematuria**) or as dissolved hemoglobin (**hemoglobinuria**).

Hematuria is divided into renal and extrarenal hematuria. The **renal**

hematuria is the main symptom of **acute hephritis**. The **extrarenal hematuria** occurs in inflammation of urinary tract (**cystitis**), traumata of its epithelium (e.g. in **urolithiasis**), as well as in **cancer of the bladder**.

Hemoglobinuria. In hemolysis, erythrocytes release hemoglobin into the plasma thus resulting in hemoglobinemia which leads to hemoglobinuria. Hemoglobin has molecular weight less than that of albumin and can pass through glomerular filter to appear in the urine.

Hematuria and hemoglobinuria may be distinguished by cytological method (**microscopically**), i.e. by examining the urine sediment through the microscope when red blood cells become visible in case of hematuria.

The simple **benzidine test** is used to detect occult blood in the urine. Hemoglobin exhibits low peroxidase activity due to which cleaves H_2O_2 to release the atom oxygen. The latter oxidizes benzidine to form the colored derivative. Developing blue-green color indicates the presence of blood or blood pigments.

Glucose

If blood glucose level exceeds approximately 10 mmol/L, glucose is not completely reabsorbed by the kidneys and appears in the urine. The blood glucose level at which this occurs is called the **renal threshold**, and the condition in which glucose appears in the urine is called **glucosuria**.

The main causes of **glucosuria** are elevated blood glucose level in diabetes mellitus and renal glucosuria, due to an intrinsic problem with glucose reabsorption within the kidneys themselves.

Ketone bodies

The increased concentration of ketone bodies in the urine is called **ketonuria**. The main causes of ketonuria are **diabetes mellitus, renal glucosuria, starvation,** or low-carbohydrate diet. Ketonuria is also observed in the increased utilization of carbohydrates in the organism caused by **hyperthyroidism**.

Bile pigments

Direct (conjugated) **bilirubin** is present in the urine both in obstruction of biliary tract (obstructive jaundice) and in lesion of hepatocytes (hepatocellular jaundice). Unconjugated (indirect) bilirubin, bound with albumin, cannot pass through the renal filter. **Urobilinogen** is present in the urine in hepatocellular jaundice when the liver loses its capacity to degrade urobilinogen absorbed from the intestine. In hemolytic jaundice, the concentration of **stercobilinogen** is sharply increased in the

urine.

The absence of stercobilinogen (along with the presence of direct bilirubin) in the urine is characteristic of obstructive jaundice. Stercobilinogen is absent in the urine due to the interrupted supply of bile into the intestine because of obstruction of the bile duct.

CHAPTER 33

METABOLISM IN NERVOUS TISSUE

The human brain is the most complicated of all known living structures. Nervous system and, primarily, the brain play a major role in the coordination of behavioral, biochemical, and physiological processes in the body. With the nervous system, body perceives changes in the environment and reacts to them.

Nerve tissue is composed of several cell types. The cells of the nervous system can be divided into two broad categories: **nerve cells** (or **neurons**), and **supporting cells** called **neuroglia** (or simply glia).

Nerve cells are specialized for electrical signaling over long distances.

The most obvious sign of neuronal specialization for communication via electrical signaling is the extensive branching of neurons (dendritic branches). Dendrites arise from the neuronal cell body and are the primary target for synaptic input from other neurons.

The synaptic contacts made on dendrites comprise a special elaboration of the secretory apparatus found in most polarized epithelial cells. Typically, the presynaptic terminal is immediately adjacent to a postsynaptic specialization of the target cell. For the majority of synapses, there is no physical continuity between these pre- and postsynaptic elements. Instead, **pre-** and **postsynaptic** components communicate via secretion of molecules from the presynaptic terminal that bind to receptors in the postsynaptic specialization. These molecules must traverse an interval of extracellular space between pre- and postsynaptic elements called the **synaptic cleft**. The synaptic cleft is the site of extracellular proteins that influence the diffusion, binding, and degradation of molecules secreted by the presynaptic terminal. The number of synaptic inputs received by each nerve cell in the human nervous system varies from 1 to about 100,000.

The information conveyed by synapses on the neuronal dendrites is integrated and “read out” at the origin of the **axon**, the portion of the nerve cell specialized for signal conduction to the next site of synaptic interaction. The **axon** is a unique extension from the neuronal cell body that may travel a few hundred micrometers. The axons that run from the human spinal cord to the foot are about a meter long.

The electrical event that carries signals over such distances is called the **action potential**, which is a self-regenerating wave of electrical activity

that propagates from its point of initiation at the cell body to the terminus of the axon where synaptic contacts are made. The **target cells** of neurons include other nerve cells in the brain, spinal cord, and autonomic ganglia, and the cells of muscles and glands throughout the body.

Nerve cells generate **electrical signals** to convey information over substantial distances and transmit it to other cells by means of synaptic connections. These signals ultimately depend on changes in the **resting electrical potential** across the neuronal membrane. A resting potential occurs because nerve cell membranes are permeable to one or more ion species subject to an electrochemical gradient. A negative membrane potential at rest results from a net efflux of K^+ across neuronal membranes that are predominantly permeable to K^+. In contrast, an **action potential** occurs when a transient rise in Na^+ permeability allows a net flow of Na^+ in the opposite direction across the membrane that is now predominantly permeable to Na^+. A depolarizing stimulus—a synaptic potential or a receptor potential in an intact neuron—locally depolarizes the axon, thus opening the voltage-sensitive Na^+ channels in that region. The opening of Na^+ channels causes inward movement of Na^+, and the resultant **depolarization** of the membrane potential generates an action potential at that site.

Local current generated by the action potential will then flow passively down. The brief rise in membrane Na^+ permeability is followed by a secondary, transient rise in membrane K^+ permeability that **repolarizes** the neuronal membrane and produces a brief undershoot of the action potential. As a result of these processes, the membrane is **depolarized** in an **all-or-none** fashion during an action potential. When these active permeability changes subside, the membrane potential returns to its resting level because of the high resting membrane permeability to K^+. The local depolarization triggers an action potential in this region, which then spreads again in a continuing cycle, until the end of the axon is reached. Action potentials propagate along the nerve cell axons initiated by the voltage gradient between the active and inactive regions of the axon by virtue of the **local current flow**.

Node of Ranvier (myelin sheath gaps) is a short **unmyelinated** areas - periodically interleaved segments of the myelin sheath of axons. Action potentials effectively "jump" from node to node, with a very high speed of propagation.

Together, transporters and channels provide a reasonably

comprehensive molecular explanation for the ability of neurons to generate electrical signals. **Ion transporters** and **channels** have complementary functions. The primary purpose of transporters is to generate **transmembrane concentration gradients**, which are then exploited by ion channels to generate electrical signals.

Ion channels are responsible for the **voltage-dependent** conductances of nerve cell membranes. The channels underlying the action potential are integral membrane proteins that open or close ion-selective pores in response to the membrane potential, allowing specific ions to diffuse across the membrane.

The flow of ions through single open channels can be detected as tiny electrical currents, and the synchronous opening of many such channels generates the macroscopic currents that produce **action potentials**. Molecular studies show that such voltage-gated channels have highly conserved structures that are responsible for features such as ion permeation and voltage sensing, as well as the features that specify ion selectivity and toxin sensitivity.

Other types of channels are sensitive to **chemical signals**, such as neurotransmitters or second messengers, or to heat or membrane deformation.

A large number of ion channel genes create channels with a correspondingly wide range of functional characteristics, thus allowing different types of neurons to have a remarkable spectrum of electrical properties. Ion transporter proteins are quite different in both structure and function.

The **energy** needed for ion movement against a concentration gradient (e.g., in maintaining the resting potential) is provided either by the hydrolysis of ATP or the electrochemical gradient of co-transported ions.

The **Na^+/K^+ pump** produces and maintains the transmembrane gradients of Na^+ and K^+, while other transporters are responsible for the electrochemical gradients for other physiologically important ions, such as Cl^-, Ca^{2+}, and H^+.

The chemical and electrical process by which the information encoded by action potentials is passed on at synaptic contacts to the next cell in a pathway is called **synaptic transmission**. Two different types of synapse—**electrical** and **chemical**—can be distinguished on the basis of their mechanism of transmission. At electrical synapses, current flows through

gap junctions, which are specialized membrane channels that connect two cells.

Presynaptic terminals (also called *synaptic endings*, or *axon terminals*) and their postsynaptic specializations are typically **chemical synapses**, the most abundant type of synapse in the nervous system. The space between the pre- and postsynaptic neurons is substantially greater at chemical synapses than at electrical synapses and is called the **synaptic cleft**.

Chemical synapses enable cell-to-cell communication via the secretion of **neurotransmitters**; these chemical agents released by the presynaptic neurons produce secondary current flow in postsynaptic neurons by activating specific **receptor** molecules. The total number of neurotransmitters is of more than 100, which can be classified into two broad categories:

- small-molecule neurotransmitters (acetylcholine (ACh);
- neuropeptides.

Small-molecule neurotransmitters mediate rapid synaptic actions, whereas neuropeptides tend to modulate slower, ongoing synaptic functions.

Many types of **neurons synthesize and release two or more different neurotransmitters**. When more than one transmitter is present within a nerve terminal, the molecules are called **co-transmitters.** Because different types of transmitters can be packaged in different populations of synaptic vesicles, co-transmitters need not be released simultaneously. Most small-molecule neurotransmitters are packaged in vesicles 40 to 60 nm in diameter. Neuropeptides are packaged into synaptic vesicles that range from 90 to 250 nm in diameter.

When peptide and small-molecule neurotransmitters act as **co-transmitters** at the same synapse, they are differentially released according to the pattern of synaptic activity: **low-frequency activity** often releases only small neurotransmitters, whereas **high-frequency activity** is required to release neuropeptides from the same presynaptic terminals. As a result, the chemical signaling properties of such synapses change according to the rate of activity.

Virtually all neurotransmitters undergo a similar cycle of use:

- synthesis and packaging into **synaptic vesicles**;
- release from the presynaptic cell;

- binding to postsynaptic receptors;
- rapid removal and/or degradation.

The process is initiated when an action potential invades the terminal of the presynaptic neuron. The change in membrane potential caused by the arrival of the action potential leads to the opening of **voltage-gated calcium channels** in the presynaptic membrane. The secretion of neurotransmitters is triggered by a transient increase in Ca^{2+} concentration within the presynaptic terminal (the external Ca^{2+} concentration is ~ 10^{-3} M, whereas the internal Ca^{2+} concentration is ~ 10^{-7} M). The opening of channels causes a rapid influx of Ca^{2+} into the presynaptic terminal. The rise in Ca^{2+} concentration causes synaptic vesicles to fuse with the presynaptic plasma membrane and release their contents into the **synaptic cleft**.

The secretory organelles in the presynaptic terminal of chemical synapses are **synaptic vesicles**, which are generally spherical structures filled with neurotransmitter molecules. The positioning of synaptic vesicles at the presynaptic membrane and their fusion to initiate neurotransmitter release is regulated by a number of proteins (**synaptotagmin, clathrin, dynamin, synapsin**) either within or associated with the vesicle. The neurotransmitters released from synaptic vesicles modify the electrical properties of the target cell by binding to **neurotransmitter receptors**, which are localized at the postsynaptic membranes.

The action of a transmitter drives the postsynaptic membrane potential for the particular ion channels being activated. The general principle is that transmitter binding to postsynaptic receptors produces a **postsynaptic conductance change** as ion channels are opened. The postsynaptic conductance is increased if—as at the neuromuscular junction—channels are opened, and decreased if channels are closed. This conductance change typically generates an electrical current, the **postsynaptic current**, which in turn changes the postsynaptic membrane potential to produce a **postsynaptic potential (**PSP**)**. PSPs are called **excitatory** (EPSPs) if they increase the likelihood of a postsynaptic action potential occurring, and **inhibitory** (IPSPs) if they decrease this likelihood. Examples are the EPSP produced at neuromuscular synapses by **ACh**, EPSPs produced at certain **glutamatergic** synapses, and IPSPs produced at certain **GABAergic** synapses.

Importantly, a given transmitter may activate both ionotropic and metabotropic receptors to produce both fast and slow PSPs at the same synapse.

Two Families of Postsynaptic Receptors

There are two major classes of **receptors**:

- **ionotropic receptors / ligand-gated ion channels.** These receptors contain two functional domains: an *extracellular site* that binds neurotransmitters, and a *membrane-spanning domain* that forms an ion channel - combine transmitter-binding and channel functions into a single molecular entity. Such receptors are multimers made up of at least four or five individual protein subunits, each of which contributes to the pore of the ion channel.
- **metabotropic receptors / G-protein-coupled receptors**. Metabotropic receptors are monomeric proteins with an extracellular domain that contains a neurotransmitter binding site and an intracellular domain that binds to **G-proteins**. Neurotransmitter binding to metabotropic receptors activates G-proteins, which then dissociate from the receptor and interact directly with ion channels or bind to other effector proteins, such as enzymes, that make intracellular messengers that open or close ion channels. Thus, **G-proteins** can be thought of as transducers that couple neurotransmitter binding to the regulation of postsynaptic ion channels.

Whether the postsynaptic actions of a particular neurotransmitter are **excitatory** or **inhibitory** is determined by the ionic permeability of the ion channel affected by the transmitter, and by the concentration of permeant ions inside and outside the cell.

After a neurotransmitter has been secreted into the synaptic cleft, it must be removed to enable the postsynaptic cell to engage in another cycle of synaptic transmission.

The removal of neurotransmitters involves **diffusion** away from the postsynaptic receptors, in combination with **reuptake** into nerve terminals or surrounding glial cells, **degradation** by specific enzymes, or a combination of these mechanisms. **Specific transporter proteins** remove most small-molecule neurotransmitters (or their metabolites) from the synaptic cleft, ultimately delivering them back to the presynaptic terminal for reuse.

Neurotransmitters

Neuropeptides are relatively large transmitter molecules composed of 3 to 36 amino acids:

- Methionine enkephalin (*Tyr–Gly–Gly–Phe*–Met);
- Leucine-enkephalin (*Tyr-Gly-Gly-Phe*-Leu);
- α-Endorphin (*Tyr–Gly-Gly-Phe-Met*-Thr-Ser-Glu-Lys-Ser-Gln-Thr-Pro-Leu–Val-Thr);
- Oxytocin (Cys-Tyr-Ile-Gln-Arg-Cys-Pro-Arg/Lys-Gly).

Small-molecule neurotransmitters:

- individual amino acids (glutamate, aspartate, glycine, GABA);
- transmitters - acetylcholine (Ach);
- biogenic amines (Chapter):
  - catecholamines: *dopamine, norepinephrine, epinephrine*;
  - indoleamine – *serotonin*;
  - imidazoleamine - *histamine*.

Acetylcholine (Ach) is a neurotransmitter:

- at skeletal neuromuscular junctions;
- as the neuromuscular synapse between the vagus nerve and cardiac muscle fibers;
- at synapses in the ganglia of the visceral motor system;
- at a variety of sites within the central nervous system.

Acetylcholine is synthesized in nerve terminals from the precursors **acetyl coenzyme A** (acetyl CoA, which is synthesized from glucose) and choline (vitamin B_4), in a reaction catalyzed by *choline acetyltransferase*. Choline is present in plasma at a high concentration (~ **10 mM**) and is taken up into cholinergic neurons by a high-affinity **Na^+/choline transporter**.

After synthesis in the cytoplasm of the neuron, a vesicular Ach transporter loads approximately 10,000 molecules of ACh into each cholinergic vesicle. In contrast to most other small-molecule neurotransmitters, the postsynaptic actions of ACh at many cholinergic synapses (the neuromuscular junction in particular) is not terminated by reuptake but by a powerful hydrolytic enzyme, ***acetylcholinesterase*** (AChE).This enzyme is concentrated in the synaptic cleft, ensuring a rapid decrease in ACh concentration after its release from the presynaptic terminal. AChE has a very high catalytic activity (about 5000 molecules of ACh per AChE molecule per second) and hydrolyzes Ach into **acetate** and

choline. The choline produced by ACh hydrolysis is transported back into nerve terminals and used to resynthesize ACh. Among the many interesting drugs that interact with cholinergic enzymes are the **organophosphates**. This group includes some potent chemical warfare agents.

Many of the postsynaptic actions of ACh are mediated by the **nicotinic ACh receptor** (nAChR), so named because the CNS stimulant, **nicotine**, also binds to these receptors. Nicotine consumption produces some degree of euphoria, relaxation, and eventually **addiction**.

A second class of ACh receptors is activated by muscarine, a poisonous alkaloid found in some mushrooms, and, thus, they are referred to as **muscarinic ACh receptors** (mAChRs). MAChRs are metabotropic and mediate most of the effects of ACh in brain. Muscarinic ACh receptors are highly expressed in the striatum and various other forebrain regions, where they exert an inhibitory influence on dopamine-mediated motor effects. They mediate peripheral cholinergic responses of autonomic effector organs—such as heart, smooth muscle, and exocrine glands—and are responsible for the inhibition of heart rate by the **vagus nerve**.

Glutamate is the most important transmitter in normal brain function.

Nearly all excitatory neurons in the central nervous system are glutamatergic, and it is estimated that over half of all brain synapses release this agent.

Glutamate plays an especially important role in clinical neurology because elevated concentrations of extracellular glutamate, released as a result of neural injury, are toxic to neurons.

Glutamatergic neurotransmission requires close metabolic interaction between brain astrocytes and glutamatergic neurons. During neurotransmission, the glutamate concentration in the synaptic cleft rapidly increases to levels as high as 1 mM. High affinity glutamate transporters (***glutamate aspartate transporter***, (GLAST) and ***glutamate transporter 1***, (GLT1) in astrocytes remove glutamate to maintain the low resting concentration of ~1-10μM.

Glutamate taken up into astrocytes is oxidized for energy or converted to ***glutamine*** which is released into the extracellular milieu and taken up by neurons to be used for energy or formation of neurotransmitter glutamate.

Glutamate is a nonessential amino acid that does not cross the **blood-brain barrier** and therefore must be synthesized in neurons from local precursors. The most prevalent precursor for glutamate synthesis is

glutamine, which is released by glial cells. Once released, glutamine is taken up into presynaptic terminals and metabolized to ***glutamate*** by the mitochondrial enzyme ***glutaminase***. Glutamate can also be synthesized by transamination of ***2-oxoglutarate***, an intermediate of the tricarboxylic acid cycle.

Once released, glutamate is removed from the synaptic cleft by the ***excitatory amino acid transporters*** (EAATs). Glutamate taken up by glial cells is converted into glutamine by the enzyme ***glutamine synthetase***; glutamine is then transported out of the glial cells and into nerve terminals (**glutamate-glutamine cycle**).

Several types of **glutamate receptors** have been identified. Three of these are ionotropic receptors:

- NMDA receptors;
- AMPA receptors;
- kainate receptors.

These receptors are named after the agonists that activate them: **NMDA** (N-methyl-D-aspartate), **AMPA** (α-amino-3-hydroxyl-5-methyl-4-isoxazolepropionic acid), and **kainic acid**.

All of the ionotropic glutamate receptors are nonselective cation channels similar to the nAChR, allowing the passage of Na^+ and K^+, and in some cases small amounts of Ca^{2+}. Hence AMPA, kainate, and NMDA receptor activation always produces **excitatory postsynaptic responses**. Recent electrophysiological studies show that the hyperexcitability in brain is due to altered functioning of kainite and AMPA receptors that lead to enhanced glutamatergic neurotransmission.

In addition to these **ionotropic glutamate receptors**, there are three types of **metabotropic glutamate receptors** (mGluRs). These receptors, which modulate postsynaptic ion channels indirectly, differ in their coupling to intracellular signal transduction pathways and in their sensitivity to pharmacological agents. Activation of many of these receptors leads to inhibition of postsynaptic Ca^{2+} and Na^+ channels.

GABA and Glycine. Most inhibitory synapses in the brain and spinal cord use either GABA (γ-aminobutyric acid) or glycine as neurotransmitters.

The predominant precursor for GABA synthesis is glucose, which is metabolized to glutamate by the tricarboxylic acid cycle enzymes (lactate and glutamine can also act as precursors). The enzyme *glutamic acid*

decarboxylase (GAD), which is found almost exclusively in GABAergic neurons, catalyzes the conversion of glutamate to GABA. Once GABA is synthesized, it is transported into synaptic vesicles via a **vesicular inhibitory amino acid transporter.**

GAD requires a cofactor, **pyridoxal phosphate**, for activity. Because **pyridoxal phosphate** is derived from vitamin B_6, a B_6 deficiency can lead to diminished GABA synthesis. The significance of this became clear after a disastrous series of infant deaths was linked to the omission of vitamin B_6 from infant formula.

The mechanism of GABA removal is similar to that for glutamate: Both neurons and glia contain high-affinity transporters for GABA, termed **GAT**s. Most GABA is eventually converted to **succinate**, which is metabolized further in **the tricarboxylic acid cycle** that mediates cellular ATP synthesis. The enzymes required for this degradation, *GABA transaminase* and *succinic semialdehyde dehydrogenase*, are mitochondrial enzymes.

Metabotropic GABA receptors ($GABA_B$) are also widely distributed in brain. Like the **ionotropic $GABA_A$ receptors**, $GABA_B$ receptors are inhibitory.

About half of the inhibitory synapses in the spinal cord use **glycine**; most other inhibitory synapses use GABA.

Glycine is synthesized from serine by the mitochondrial isoform of *serine hydroxymethyltransferase*, and is transported into synaptic vesicles via the same vesicular inhibitory amino acid transporter that loads GABA into vesicles.

Once released from the presynaptic cell, glycine is rapidly removed from the synaptic cleft by the plasma membrane **glycine transporters**. Mutations in the genes coding for some of these enzymes result in **hyperglycinemia**, a devastating neonatal disease characterized by lethargy, seizures, and mental retardation.

The Biogenic Amines (Chapter)

Dopamine is produced by the action of *DOPA decarboxylase* on DOPA. Following its synthesis in the cytoplasm of presynaptic terminals, dopamine is loaded into synaptic vesicles via a **vesicular monoamine transporter**.

Dopamine action in the synaptic cleft is terminated by reuptake of

dopamine into nerve terminals or surrounding glial cells by a **Na^+-dependent dopamine transporter**, termed **DAT**. Cocaine apparently produces its psychotropic effects by binding to and inhibiting DAT, yielding a net increase in dopamine release from specific brain areas. Amphetamine, another addictive drug, also inhibits DAT. The two major enzymes involved in the catabolism of dopamine are ***monoamine oxidase*** (MAO) and ***catechol O-methyltransferase*** (COMT). Both neurons and glia contain mitochondrial MAO and cytoplasmic COMT.

Once released, dopamine acts exclusively by activating **G-protein-coupled receptors**. These are mainly dopamine-specific receptors, although **β-adrenergic receptors** also serve as important targets of norepinepherine and epinephrine.

Norepinephrine (noradrenaline) is used as a neurotransmitter in the locus coeruleus, a brainstem nucleus that projects diffusely to a variety of forebrain targets and influences sleep and wakefulness, attention, and feeding behavior. Perhaps the most prominent noradrenergic neurons are **sympathetic ganglion cells**, which employ norepinephrine as the **major peripheral transmitter** in this division of the visceral motor system.

Norepinepherine, as well as epinephrine, acts on **α-** and **β-adrenergic receptors**. Both types of receptor are G-protein-coupled.

Norepinephrine synthesis requires ***dopamineβ-hydroxylase***, which catalyzes the production of norepinephrine from dopamine. Like dopamine, norepinepherine is degraded by MAO and COMT.

Epinephrine (adrenaline) is found in the brain at lower levels than the other catecholamines, and is also present in fewer brain neurons than other catecholamines. The enzyme that synthesizes epinephrine, ***phenylethanolamine-N-methyltransferase***, is present only in epinephrine-secreting neurons. Epinephrine acts on both α- and β-adrenergic receptors.

Histamine is found in neurons in the hypothalamus. The central histamine projections mediate arousal and attention, similar to central ACh and norepinephrine projections. Histamine also controls the reactivity of the vestibular system. Histamine is produced from the amino acid histidine by a **histidine decarboxylase.**

Serotonin, or 5-hydroxytryptamine (5-HT), was initially thought to

increase vascular tone by virtue of its presence in serum (hence the name serotonin). Serotonin is found primarily in groups of neurons in the raphe region of the pons and upper brainstem, which have widespread projections to the forebrain and regulate sleep and wakefulness. 5-HT is synthesized from the amino acid **tryptophan**, which is an essential dietary requirement.

Most 5-HT receptors are **metabotropic**. These have been implicated in behaviors, including the emotions, circadian rhythms, motor behaviors, and state of mental arousal. Only one group of serotonin receptors, called the **5-HT$_3$ receptors**, are ligand-gated ion channels. These are **non-selective cation channels** and therefore mediate excitatory postsynaptic responses.

Neuroglial cell (glial cells or glia) are quite different from nerve cells. **Neuroglia** is a system of cells surrounding the nerve cells of the brain and spinal cord and not directly involved in the specific functions of the nervous tissue. The population of glial cells in the CNS exceeds more than 10 times the number of neurons. In respect of neurons, neuroglia fulfills auxiliary functions: supporting, trophic, insulating, secretory, protective, absorption of chemical mediators, participation in the restoration and regeneration (glial cells retain the ability to divide throughout the life of the organism).

There are three types of glial cells in the mature central nervous system: **astrocytes**, **oligodendrocytes**, and **microglial** cells.

Astrocytes are characteristic star-shaped glial cells in the brain and spinal cord. The astrocyte proportion varies by region and ranges from **20**% to **40**% of all glia. They perform many functions, including:

- **structural** - they are involved in the physical structuring of the brain;
- biochemical support of endothelial cells that form the **blood–brain barrier**,
- **glycogen** fuel reserve buffer. Astrocytes contain glycogen and are capable of **gluconeogenesis**. The astrocytes are next to neurons in the frontal cortex, and hippocampus store and release glucose.
- provision of nutrients to the nervous tissue. They provide neurons with nutrients such as **lactate.**
- **glucose sensing**. Normally associated with neurons, the detection of interstitial glucose levels within the brain is also controlled by astrocytes. Astrocytes in vitro become activated by low glucose, and in vivo this activation increases gastric emptying to increase

digestion.

- the **secretion or absorption of neural transmitters**. Astrocytes express ***plasma membrane transporters*** such as ***glutamate transporters*** for several neurotransmitters, including glutamate, ATP, and GABA. Astrocytes **release glutamate** or **ATP** in a vesicular, Ca^{2+}-dependent manner.
- electrical activity in **neurons** causes astrocytes to release ATP, - as an important stimulus for ***myelin*** to form. However, the ATP does not act directly on oligodendrocytes. Instead, it causes astrocytes to secrete cytokine ***leukemia inhibitory factor*** (LIF), a regulatory protein that promotes the myelinating activity of **oligodendrocytes**.
- **maintenance of extracellular ion balance**. Astrocytes express ***potassium channels*** at a high density. Abnormal accumulation of extracellular potassium is well known to result in **epileptic** neuronal activity.
- **modulation of synaptic transmission.** In the hippocampus, astrocytes suppress synaptic transmission by releasing ATP, which is hydrolyzed by ***ectonucleotidases*** to yield adenosine. Adenosine acts on neuronal ***adenosine receptors*** to inhibit synaptic transmission.
- may serve as intermediaries in neuronal regulation of **blood flow**.
- Astrocytes alone are sufficient to drive the **molecular oscillations** in the SCN and **circadian behavior** in mice, and thus can autonomously initiate and sustain complex mammalian behavior.
- repair and scarring process of the brain and spinal cord following traumatic injuries.

Oligodendrocytes lay down a laminated, lipid-rich wrapping called ***myelin*** around some, but not all, axons. ***Myelin*** has important effects on the speed of the transmission of electrical signals. In the peripheral nervous system, the cells that elaborate myelin are called **Schwann cells**.

Microglial cells are derived primarily from hematopoietic precursor cells. They share many properties with macrophages found in other tissues, and are primarily **scavenger cells** that remove cellular debris from sites of injury or normal cell turnover. In addition, microglia, like their macrophage counterparts, secrete signaling molecules—particularly a wide range of **cytokines** that are also produced by cells of the immune system—that can modulate local inflammation and influence cell survival or death. Following brain damage, the number of microglia at the site of injury increases

dramatically. Some of these cells proliferate from microglia resident in the brain, while others come from **macrophages** that migrate to the injured area and enter the brain via local disruptions in the cerebral vasculature.

To maintain the normal functioning of the neuron, there are two mechanisms:

1. Transversal transport of substances – the metabolism of the extracellular space.
2. Longitudinal transport – a continuous exchange of substances between the body and the neuron spikes, concerns mainly reproductions neuroplasm.

Role of axoplasmal flow

1. Continuous replenish of neuron components in normal and pathological conditions.
2. The liberation of substances from neuron and synaptic transport.
3. Transport substances in the body of neuron.
4. Transferring information between compartments of neuron.

In axonal transport involved as intracellular organelles (mitochondria, lysosomes, synaptic vesicles, neurofilaments) and certain metabolites (lipids, nucleotides, glycoproteins, free amino acids and other).

The synthesis of small-molecule neurotransmitters occurs locally within presynaptic terminals. The enzymes needed to synthesize these transmitters are produced in the neuronal cell body and transported to the nerve terminal cytoplasm at **0.5–5 millimeters a day** by a mechanism called **slow axonal transport**. Peptide-filled vesicles are transported along an axon and down to the synaptic terminal via **fast axonal transport**. This process carries vesicles at rates up to 400 mm/day along cytoskeletal elements called **microtubules**.

Microtubules are long, cylindrical filaments, 25 nm in diameter, present throughout neurons and other cells. Peptide-containing vesicles are moved along these microtubule “tracks” by ATP-requiring “motor” proteins such as ***kinesin***.

Blood-brain barrier (BBB)

The **blood–brain barrier** is a highly selective semipermeable border that separates the circulating blood from the brain and extracellular fluid in the central nervous system (CNS).

Morphological basis of BBB is brain **vascular endothelium**,

perivascular base membrane, and the plasma membrane of glial cells.

The astrocyte end-feet encircling endothelial cells were thought to aid in the maintenance of the **blood–brain barrier**, but recent research indicates that they do not play a substantial role; instead, it is the **tight junctions** and **basal lamina** of the cerebral **endothelial cells** that play the most substantial role in maintaining the barrier.

This system allows the passage of water, some gases, and lipid-soluble molecules by passive diffusion, as well as the selective transport of molecules such as **glucose** and **amino acids** that are crucial to neural function. The BBB restricts the passage of **pathogens**, the diffusion of **solutes** in the blood, and **large** or **hydrophilic molecules** (**non-essential fatty acids** do not cross BBB) into the cerebrospinal fluid (CSF) (**non-essential fatty acids** do not cross BBB), while allowing the diffusion of hydrophobic molecules (O_2, CO_2, hormones) and small polar molecules. **Ketone bodies** can pass BBB - these are important fuels for brain during starvation.

Cells of the barrier actively transport some vitamins (**B_9**) and metabolic products (**glucose**) across the barrier using specific **transport proteins**. The relative amounts of monocarboxylic acid transporters (MCT) that mediate ketone body and lactate uptake, and the blood-brain barrier and cell-specific glucose transporters change during development in human.

The intensity of penetration of a number of substances through the BBB into the brain is determined not only by the state of the BBB, but the intensity of the central nervous system functioning and metabolism. BBB plays a role in protecting the brain against endogenous and exogenous toxins, circulating in the blood, and it prevents "escape of" neurotransmitters, and other active compounds from the blood into the interstitial fluid. However, the most important function of the BBB apparently is to preserve a special internal environment of the brain.

Specialized structures participating in sensory and secretory integration within **neural circuits** – the **circumventricular organs** and **choroid plexus** – do not have a blood–brain barrier.

General features of nervous tissue metabolism

1. High intensity as compared with other tissues.

2. Surprisingly high level of exchange persists in the absence of a large functional activity – even during sleep.

3. Metabolism in peripheral nerve fibers is different from exchanges nerve cells themselves.

4. The overall intensity in the metabolism of the nerve fibers is low.

Exchange of free amino acids in the brain

Amino acids play an important role in the metabolism and functioning of the CNS. This is not only because of an exclusive role of amino acids as a source of synthesis of a large number of important biological compounds such as proteins, peptides, lipids, some, several hormones, vitamins, biologically active amines. Amino acids and their derivatives are involved in synaptic transmission, in implemention of interneuronal connections as neurotransmitters and neuromodulators. Their energy significance is also very important for ***glutamic amino group*** is directly linked to the citric acid cycle.

The exchange of free amino acids in the brain:

1. Greater ability of nerve tissue to maintain levels of amino acids relative constancy.

2. Content of free amino acids in the brain is 8-10 times higher than in plasma.

3. The existence of high amino acid concentration gradient between the blood and the brain by selectively active transport across the BBB.

4. High concentrations of ***glutamate***, ***glutamine***, ***asparagine***, ***N-acetylasparagine*** and ***GABA***. They constitute **75%** of the pool of free amino acids in the brain.

5. Expressed regional amino acid content in different parts of the brain.

6. Existence of separated amino acids content in different subcellular structures of nerve cells.

7. Aromatic amino acids are of particular value as precursors of catecholamines and serotonin.

Neuropeptides

Neuropeptides are small protein-like molecules (peptides) used by neurons to communicate with each other. They are neuronal signaling molecules that influence the activity of the brain and the body in specific ways. Different neuropeptides are involved in a wide range of brain functions, including analgesia, reward, food intake, metabolism, reproduction, social behaviors, learning, and memory.

In recent years has been discovered a large number of peptides capable even in low concentrations to affect the nervous tissue, acting as

modulators of a number of functions, as well as actions of ***neurotransmitters***, ***hormones***, ***pharmacological agents***.

Considering the preferential localization of these peptides in the central nervous system they are called **neuropeptides**: ***β-endorphin***, ***dynorphin, enkephalin, neuropeptide Y, ghrelin, growth-hormone releasing hormone, neurotensin, α-melanocyte-stimulating hormone somatostatin, oxytocin***, ***vasopressin*** etc.

The human genome contains about 90 genes that encode precursors of neuropeptides. At present about 100 different peptides are known to be released by different populations of neurons in the mammalian brain.

Neuropeptides are small and medium size peptides, usually linear, containing from 2 to 40-50 amino acid residues. Part of neuropeptides is modified by terminal amino acids. Neuropeptides are synthesized by proteolysis of larger precursor peptide in neurons and concentrated in vesicles of nerve endings. Shelf-life of most neuropeptides varies from minutes (for oligopeptides) to hours (average peptide size).

Neuropeptides signals play a role in information processing that is different from that of conventional neurotransmitters. They can affect ***gene expression***, ***local blood flow***, ***synaptogenesis***, and ***glial cell morphology***. Peptides tend to have prolonged actions, and some have striking effects on behavior. For example, oxytocin and vasopressin have specific effects on ***social behaviors***, including maternal behavior and pair bonding.

There is a complex ***hierarchical system*** in which some neuropeptides induce or inhibit the output of other neuropeptides. Neuropeptides modulate neuronal communication by acting on cell surface receptors.

Some neurons make several different peptides. ***Vasopressin*** co-exists with ***dynorphin*** and ***galanin*** in magnocellular neurons of the supraoptic nucleus and paraventricular nucleus. ***Oxytocin*** in the supraoptic nucleus co-exists with ***enkephalin***, ***dynorphin***, ***cocaine-and amphetamine regulated transcript*** (CART) and ***cholecystokinin***.

Energy metabolism in the neural tissue

Adult brain has an absolute requirement for glucose as an energy substrate. In contrast, the developing brain can utilize glucose as well as several alternative substrates including ketone bodies, glycerol, lactate, amino acids, and fatty acids for energy and for the biosynthesis of lipids, and proteins required for brain development.

Metabolism is necessary for providing the energy required to support

all cellular functions including: cell division, ATP synthesis, maintaining ionic gradients, synaptogenesis, the synthesis of membrane lipids, proteins, carbohydrates, nucleic acids, the synthesis, release, and transport/uptake of neurotransmitters, and maintaining redox status and the level of reduced glutathione.

Characteristic features of the energy metabolism in the brain are:

1. Its high intensity in comparison with other tissues.

2. Large rate of oxygen consumption and glucose from the blood. The human brain, which accounts for 2% of body weight, consumes up to 20% of oxygen used by the body at rest.

3. Oxygen consumption by gray matter is 30-50% higher than by a white one. Peripheral nerves use 30 times less oxygen than equivalent amount by weight of the CNS tissue.

4. Different rate of oxygen consumption by certain regions of the CNS: cerebral cortex > cerebellum > midbrain > midbrain and medulla > spinal cord.

5. Neurons have more intense respiration than glial cells. In the cerebral cortex 70% of the total oxygen uptake is accounted for neurons and 30% – for glial cells.

6. Substrates released by astrocytes including ***glutamine*** and ***ketones*** can be utilized by neurons and other cells. ***Glutamate*** released by neurons is preferentially used by astrocytes for energy.

7. Approximately 70% of the ATP produced in the brain is spent on maintaining ionic gradients between the contents of the nerve cells and the environment.

8. ***Lactate*** can be released by both neurons and astrocytes and used for energy by neurons, astrocytes and possibly other cell types.

Features of the carbohydrate metabolism in the brain tissue

1. The functional activity of the brain is most affected by the metabolism of carbohydrates.

2. The brain as an energy material uses ***glucose*** almost exclusively. Glucose taken up across the BBB for adult brain is used for energy. Metabolism of glucose via the pentose phosphate pathway (PPP) is essential for providing reduced nicotinamide adenosine dinucleotide phosphate (**NADPH**), which is an essential cofactor for lipid biosynthesis and is also needed for maintaining the level of reduced glutathione. The PPP also provides ribose-5-phosphate which is needed for the synthesis of

nucleotides. Metabolism of glucose via the PPP is higher in developing brain than in adults.

3. Dominant mode of glucose metabolism in nervous tissue is aerobic glycolysis.

4. Existence of a single functional complex of two glycolytic enzymes – hexokinase and phosphofructokinase, synchronously controlled by pool of adenine nucleotides.

5. The ***tricarboxylic acid cycle*** (TCA) activity is essential for both energy production and for providing precursors for many key compounds including neurotransmitters.

6. The TCA cycle is very important for providing precursors for the synthesis of many compounds including fatty acids and sterols, amino acids, purines, pyrimidines, porphyrins, and heme. The TCA cycle intermediate α-ketoglutarate is the direct precursor for glutamate, the main excitatory neurotransmitter in brain. The main inhibitory neurotransmitter gamma-aminobutyric acid (GABA) is formed by the decarboxylation of glutamate in GABAergic neurons.

6. The ***malate-aspartate shuttle*** is the most important shuttle in brain

Adult brain relies on ***glucose*** as an energy substrate, whereas developing brain can utilize alternative substrates as well as glucose for energy and for the biosynthesis of lipids and proteins required for brain development. Use of ***lactate*** is high in the perinatal period and ketone body utilization is high throughout the suckling period and declines thereafter.

Astrocytes have the capability to store some energy through synthesis of the macromolecule ***glycogen*** from glucose. Glycogen turnover provides a small but readily available amount of energy that is thought to have a role in ***glutamatergic neurotransmission.***

The uptake of substrates is mediated by specific transporters on the blood-brain barrier and brain cells. The relative amounts of ***monocarboxylic acid transporters*** (MCT) that mediate ketone body and lactate uptake, and the BBB and cell-specific ***glucose transporters*** change during development in human brain.

Lipid metabolism in neural tissue

Brain is an eminent organ of the human body; it comprises more than 100 billion nerve cells which communicate by well-known structures called **synapses**.

Blood–brain barrier inhibits entry of certain fatty acids and lipids into

CNS. However, ***short-chain fatty acids***: butyric acid, propionic acid, acetic acid, and the ***medium-chain fatty acids*** – octanoic acid, heptanoic acid - can cross the BBB and be metabolized by brain cells.

The nervous system is enriched with important classes of lipids; ***glycerophospholipids***, ***sphingolipids,*** and ***cholesterol*** are considered to be present in almost equal ratios.

Cholesterol. The brain contains 25% of total body cholesterol. Cholesterol is the most important component and fundamental functional unit of the mammalian cell membrane. Most of the body cholesterol resides in brain in the form of **myelin** which contains almost 80% of cholesterol found in adult brain.

Cholesterol is required for cellular processes e.g., glial cell proliferation, neurite outgrowth, microtubules stability, synaptogenesis, and myelination.

The **blood brain barrier** (BBB) hinders the passage of plasma lipoproteins to CNS, therefore, cholesterol requirement of CNS is met with locally synthesized cholesterol. The neurons can synthesize only a minute quantity of cholesterol themselves and mostly rely on cholesterol-containing lipoproteins secreted by astrocytes.

In CNS, the transport of cholesterol is carried by special lipoproteins such as ***Apolipoprotein-E*** (Apo-E) that are secreted by astrocytes. The cholesterol-Apo-E complex accelerates axonal extension when applied to distal end but not to the cell body of neurons.

Sufficient availability of cholesterol for synapse formation is a critical step in the structural and functional development of our nervous system.

Cholesterol plays a crucial role in the **regeneration of nerve** after injuries both in CNS and PNS. Local availability of cholesterol at nerve damage is necessary for nerve regeneration. The cholesterol-rich transporter ***lipoprotein Apo-E*** has been reported to accumulate at the site of injury after nerve crush. The Apo-E is synthesized by macrophage and accumulated at the site of regenerating axon and it increases following an injury.

The lysosomal dysfunction that occurs in **lysosomal storage diseases** may impair mitochondrial function and brain energy metabolism. Intramitochondrial cholesterol accumulation in brain and mitochondrial dysfunction plays a role in oxidative stress observed in ***lysosomal storage diseases*** (eg. Niemann-Pick type C disease). Accumulation of cholesterol within the mitochondria impairs reduced ***glutathione*** (GSH) transport into

mitochondria, decreasing mitochondrial GSH pool. Mitochondrial dysfunction leads to caspase-9 activation and ***apoptosis***.

Genetic disorders of the cholesterol biosynthetic pathway - are associated with myelination defects but also include complex craniofacial malformations:

- lacking of ***7-dehydrocholesterol reductase*** - human Smith-Lemli-Opitz-syndrome (**SLOS**): microcephaly, autistic behaviours, micrognathia (mandibular hypoplasia), heart defects and/or renal, pulmonary, liver, and eye abnormalities.
- lacking of ***24-dehydrocholesterol reductase*** - **desmosterolosis**: loss of white matter, brain abnormalities, delayed speech and motor skills, muscle stiffness, heart defects.

Both cholesterol and sphingolipids are embedded in the microdomains of membrane - **lipid rafts** - are functional units of the neuronal cell membrane - and compose the major portion of the brain particularly in the form of **myelin -** a form of membrane acquiring maximum lipids among entire biological membranes.

Maintenance of functional activities of the nervous system depends on the unique lipid contents found in the different membrane regions (**lipid rafts**) of neuronal cells.

Mammalian brain is very rich in lipids (60-65%) comprising **saturated**, **mono-** and **polyunsaturated** (PUFA). In mammals, the major fatty acids in sphingolipids are ***palmitic acid*** (C16:0), ***lignoceric acid*** (C24:0) and ***nervonic acid*** (C24:1).

Nervonic acid is the major omega-9 PUFAs in human brain accounts for ~ **40%** total fatty acid **insphingolipids**.

Docosahexaenoic acid (DHA, 22:6n-3) accounts for ~ **10%–20%** of total fatty acid composition of prefrontal cortex (PFC) in adulthood.

The lipids act as signaling molecules, source of energy, for contributing to synaptogenesis, neurogenesis, impulse conduction and many others. Furthermore, **lysophospholipids**, **endocannabinoids**, and **sphingolipids** are involved in cellular signaling, including regulation of numerous ion pumps, channels, and transporters.

Sphingolipids (SP)**.** Synthesis of SP requires Palmitoyl-CoA and L-serine. Although, L-serine is not classified as an essential amino acid, but its external supply is vital for the synthesis of ***phosphatidylserine*** (PS) and

sphingolipids in the specific types of neurons. Being an important part of membrane **lipid rafts**, sphingolipids play a vital role in the life cycle of cells. There are different types of sphingolipids that exist in the brain and contribute structurally in membrane microdomains formation. Sphingolipids are involved in:

- neuronal differentiation;
- synaptic transmission;
- neuronal-glial connections;
- associated with myelin stability.

Pathological changes in the normal metabolism of sphingolipids and their homeostasis are the common factors leading to progression of **schizophrenia, metabolic syndrome,** and development of various neurological diseases.

There are four classes of **lipid transportation proteins** involved in lipid synthesis and transportation in adult brain, including:

- ***fatty acid translocase*** (FAT/CD36),
- ***caveolin-1***,
- ***fatty acid binding proteins*** (FABPs),
- ***long chain acyl-coA synthase*** (ACS),
- ***fatty acid transportation proteins*** (FATPs), and
- newly identified (**Mfsd2a**) as a ***DHA transporter in brain***.

Expression levels of these proteins change with age and greatly impact the cerebral concentration of fatty acids, especially polyunsaturated fatty acids.

In mammals, the major fatty acids (FA) in sphingolipids of brain are C16:0 FA (**palmitic acid**), C18:0 (**stearic acid**) and very long-chain fatty acids (VLCFAs) - C24 FAs (C24:0 - **lignoceric acid** and C24:1 - **nervonic acid**). Patients with multiple sclerosis have decreased levels of nervonic acid in the brain.

For example, C24 sphingomyelin reflects **~25**% of the total **sphingomyelin** in brain. For β-oxidation of VLCFAs such as C24:0, C26:0 are necessary **peroxisomes** - single membrane bounded key metabolic organelles contribute to cellular lipid metabolism, e.g. the **β-oxidation of fatty acids** and the synthesis of **myelin sheath lipids**.

Myelin sheaths contain comparatively high concentrations of ***plasmalogens /ether*** lipids synthesized in peroxisomes.

Peroxisome deficits may facilitate alterations in neuronal migration, a progressive demyelination of neurons or inflammatory activation of microglia and the development of neurodegenerative disorders.

The **myelin sheath** is deposited around the axon by **Schwann cells** in the following manner: The membrane of a Schwann cell first envelops the axon. The Schwann cell then rotates around the axon many times, laying down multiple layers of Schwann cell membrane containing the lipid substance ***sphingomyelin***. This substance is an excellent electrical insulator that decreases ion flow through the membrane about 5000-fold.

At the juncture between each two successive Schwann cells along the axon, a small uninsulated area only 2 to 3 micrometers in length remains where ions still can flow with ease through the axon membrane between the extracellular fluid and the intracellular fluid inside the axon. This area about once every 1 to 3 millimeters along the length of the myelin sheath is called the **node of Ranvier**.

The velocity of action potential conduction in nerve fibers from as little as 0.25 m/sec in small unmyelinated fibers to as great as **100 m/sec** (more than the length of a football field in 1 second) in large myelinated fibers.

Lipid metabolism in the nervous tissue has the following characteristics:

1. The brain has a high ability to synthesize fatty acids.
2. In the brain there is practically no β-oxidation of fatty acids in mitochondria.
3. **Dietary** conditions significantly influence the content of long chain PUFAs in the central nervous system.
4. DHA and ***arachidonic acid*** (AA, 20:4n-6) can be derived directly from diet or through metabolic conversion of their ***α-linolenic acid*** (18:3n-3), ***linoleic acid*** (18:2n-6). Very long-chain fatty acids - ***nervonic acid*** - can synthesize only fromessential precursor - ***oleic acid*** (18:1n-9).
5. AA-derivatives, such as ***endocannabinoids***, have been shown to act as retrograde messengers in hippocampal **long-term potentiation,** to be involved in cortical **neuron migration**, regulate specific molecular events related to **neural development,** are implicated in the development of **synaptic connectivity** in brain maturation.
6. At early post-natal life before weaning, human brain greatly accumulates DHA. DHA is very selectively up-taken through the **blood–**

brain barrier during this period, considering that its cerebral increase represents 50% of total body amount. Deficiency of **DHA** during the perinatal period leads to **increased cytokine production** by altering microglia phenotype, thus resulting in neuroinflammatory profile.

7. Gestational **DHA deficiency** has been shown to potentially contribute to the etiopathogenesis of **Schizophrenia** and **Autistic Spectrum Disorders**. Omega-3 and -6 fatty acids act in a competitive manner to generate mediators for energy metabolism, influencing feeding behavior, neural plasticity, and memory during aging.

8. Constant composition of lipids in the mature brain confirms low speed of their renewal. The lipid fatty acid composition and fluidity of brain membranes change in an age-dependent manner.

9. ***Phosphatidylcholine*** and ***phosphatidylinositol*** are restored quickly in the brain tissue.

10. Rate of ***cholesterol*** synthesis in the brain is high in the period of CNS formation and at this neonatal stage, any interruption in its synthesis and provision can lead to the development of neurodegenerative disorders. With age, the activity of this process reduces.

11. Synthesis of ***cerebrosides*** and ***sulfatide*** occurs most actively during myelination.

12. ***Phosphathidylserine*** regulates many important enzymes, such as *protein kinase C,* which controls the function of structural proteins involved in neurite outgrowth and neurotransmitter release.

In the mature brain 90% of ***cerebrosides*** are the myelin sheath, while ***gangliosides*** are the components of a typical neuron.

Neurochemical basis of memory

Memory is a complex and not yet sufficiently studied process involving phases of capturing, storing, and retrieving the information received. All these phases are closely related, and often it is very difficult to distinguish between them in the analysis of memory functions.

Experiments in lower animals have demonstrated that **memory** traces can occur at all levels of the nervous system. Most memory that we associate with **intellectual processes** is based on memory traces in the cerebral cortex. (746 (см706)

Learning is the name given to the process by which new information is acquired by the nervous system and is observable through changes in behavior. ***Memory*** refers to the encoding, storage, and retrieval of learned

information. Equally fascinating (and important) is the normal ability to forget information. Pathological forgetfulness, or ***amnesia***, has been especially instructive about the neurological underpinnings of memory; amnesia is defined as the inability to learn new information or to retrieve information that has already been acquired.

Types of biological memory:

1. Genetic;
2. Epigenetic;
3. Immunological;
4. Neurological (sometimes called psychic or individual).

Currently, neurological memory has three main stages of formation that correspond to **three types of memory**:

1. ***Immediate memory*** (duration from several milliseconds to seconds) - is the routine ability to hold ongoing experiences in mind for fractions of a second. The capacity of immediate memory is very large and each sensory modality (visual, verbal, tactile, and so on) appears to have its own memory register (***short-term memory***).
2. ***Working memory*** - is the ability to hold information in mind for seconds to minutes once the present moment has passed. (***short-term memory***).
3. ***Long-term memory*** - the retention of information in a more permanent form of storage for days, weeks, or even a lifetime.

Evidence for a continual transfer of information from working memory to long-term memory, or ***consolidationis,*** apparent in the phenomenon of priming.

Humans have at least two qualitatively different systems of information storage, which are generally referred to as:

- **declarative memory** - is the storage (and retrieval) of material that is available to consciousness and can be expressed by language (ability to remember a telephone number, a song, or the images of some past event)
- **nondeclarative memory** (procedural memory) - Such memories involve skills and associations that are, by and large, acquired and retrieved at an unconscious level.

Neurological memory system has a complex organization and has no strict localization in certain regions of the brain. According to modern

concepts, memory traces (***engrams***) are locked in the brain in the form of changes in the status of the synaptic apparatus, which results in preferential conduction of excitation in certain nerve pathways.

After information perception in the process of its capturing and fixing in the brain is successively changing, neurochemical processes occur. In the early stages, in a ***short-term memory*** the changing of "fast" synapse functions occur. It is associated with the release and shift of concentration of "classical" and peptide mediators. Subsequently, during a period from a few seconds to several days, involving a wide spectrum of neurochemical processes, occur involving changes in composition and structure of neurospecifical proteins, in particular changes in the degree of phosphorylation, as well as modification of RNA synthesis.

For the formation of a lifelong ***long-term memory*** persistent synthesis of new biopolymers is necessary. It may be done in case of stable rearrangement in some genome parts functioning. The latter can occur either as a result of structural changes in the DNA or the formation of stable cycles for continuous synthesis of repressors or derepressors of transcriptions. It is also possible that the formation of long-term memory embraces participation of immunological mechanisms due to which antibody-like compounds are synthesized in the brain. These compounds are able to modify the activity of synapses in certain nerve pathways for a long time. In the mechanisms of memory formation "classic" neurotransmitters participate as well as a large number of neuropeptides acting as neurotransmitters and neuromodulators.

Formation of **long-term potentiation** (LTP) and **long-term depression** (LTD) by the synapse of **hippocampus** is a mechanism of **learning** and **memory**. LTP is divided into:

- an **early stage** (E-LTP) lasting ~1 hr that does not require gene transcription and
- **alate-phase** (L-LTP, 1–4 hr) that is dependent on gene transcription, particularly mediated by the transcription factor ***cAMP response element-binding protein*** (CREB). Deficits in CREB-dependent transcription could account for their **long-term memory** impairments.

The ***calmodulin-dependent protein kinase II*** (CaMKII) gene was one of the first to be implicated in learning and memory and also appears to modulate **memory consolidation**. Decreases in the levels of ***calmodulin-dependent protein kinase IV*** (CaMKIV**)** impair LTP and memory, overexpression of this molecule can enhance LTP and memory.

The observations of patients and animal studies show that **long-term declarative memories** are widely stored in the **cerebral cortex**. The lexicon that links speech sounds and their symbolic significance is located in the association cortex of the **superior temporal lobe**, and damage to this area typically results in an inability to link words and meanings. The inability of patients with temporal lobe lesions to recognize objects and/or faces suggests that such memories are stored there.

Although forgetting is a normal and apparently essential mental process, it can also be pathological, a condition called ***amnesia***. An inability to establish new memories following neurological insult is called ***anterograde amnesia***, whereas difficulty retrieving memories established prior to the precipitating neuropathology is called ***retrograde amnesia***.

Mechanisms that can cause sleep

Sleep has been postulated to serve many functions, including:

1) neural maturation,
2) facilitation of learning or memory,
3) cognition,
4) clearance of metabolic waste products generated by neural activity in the awake brain,
5) conservation of metabolic energy.

The principal value of sleep is to restore natural balances among the neuronal centers.

Sleep is caused by an active inhibitory process. A center located below the **midpontile level** of the **brain stem** appears to be required to cause sleep by inhibiting other parts of the brain. The most conspicuous stimulation area for causing almost natural sleep is the **raphe nuclei** in the **lower half of the pons** and in the **medulla**.

Many nerve endings of fibers from raphe neurons secrete **serotonin**. When a drug that blocks the formation of serotonin is administered to an animal, the animal often cannot sleep for the next several days.

Discrete lesions in the raphe nuclei lead to a high state of **wakefulness**. This phenomenon is also true of bilateral lesions in the **medial rostral suprachiasmal** area in the anterior hypothalamus. In both instances, the excitatory reticular nuclei of the mesencephalon and upper pons seem to become released from inhibition, thus causing **intense wakefulness**.

The cerebrospinal fluid and the blood or urine of animals that have been kept awake for several days contain a substance or substances that will cause sleep when injected into the brain ventricular system of another animal. One likely substance has been identified as ***muramyl peptide***, a low molecular-weight substance that accumulates in the cerebrospinal fluid and urine in animals kept awake for several days.

Orexin (***hypocretin***) is produced by neurons in the hypothalamus that provide excitatory input to many other areas of the brain where there are ***orexin receptors***. Orexin neurons are most active during waking and almost stop firing during **slow wave** and **rapid eye movement** sleep.

Loss of ***orexin*** signaling as a result of defective ***orexin receptors*** or destruction of ***orexin-producing neurons*** causes **narcolepsy**, a sleep disorder characterized by overwhelming daytime drowsiness and sudden attacks of sleep that can occur even when a person is talking or working.

Glymphatic system

The increased clearance of metabolic waste during sleep occurs via increased functioning of the ***glymphatic system*** (2012). The glymphatic system (or ***glymphatic clearance pathway***) is a functional waste clearance pathway for the vertebrate CNS.

The pathway consists of a para-arterial influx route for cerebrospinal fluid (CSF) to enter the brain parenchyma, coupled to a clearance mechanism for the removal of interstitial fluid (ISF) and extracellular solutes from the interstitial compartments of the brain and spinal cord.

Exchange of solutes between CSF and ISF is driven by ***arterial pulsation*** and regulated during sleep by the expansion and contraction of brain extracellular space.

Spinal fluid (cerebrospinal fluid, CSF)

In the brain ventricles, the epithelial cells of the choroid plexus are the major site for the production of CSF. The CSF is not an ultrafiltrate of the plasma but is actively secreted by the choroid plexus. The epithelium of the choroid plexus mediates the secretion of Na^+, Cl^-, and HCO_3 into the CSF, as well as the net absorption of K^+ from the CSF into the blood. Na/K-ATPase is located in the apical membrane of choroid plexus cells and plays a pivotal role in the active ouabain-sensitive transport of Na^+ from epithelial cells to CSF and of K^+ from CSF to choroid plexus cells. Hyponatremia causes important CNS symptoms (e.g., ***amnesias***, ***headaches***, ***epilepsy***).

Table 32.1. **The composition of the cerebrospinal fluid**

volume	100-150 ml
color	Colorless
transparency	transparent
water	99 %
solid precipitate	1%-10 g/l
organic compounds	2-2.4 g/l
total protein	0.15-0.33 g/l
albumin	0.12-0.26 g/l
globulins	0.03-0.06 g/l
glucose	2.50-4.15 mmol/l
inorganic compounds	7.6-8.0 g/l
sodium	135-150 mmol/l
potassium	2.3-4.3 mmol/l
chlorides	120-130 mmol/l
calcium	1.2-1.6 mmol/l

The total amount of liquor in the adult is 100-150 ml, in children it is 80-90 ml. The rate of formation of CSF ranges at about 350-750 ml/day. Liquor renews 3-7 times a day.

Distribution of liquor:

- lateral ventricles – 20-30 ml;
- 3 and 4 of the ventricles – 5.3 ml;
- subarachnoid space of the brain – 20-30 ml;
- subarachnoid space of the spinal cord – 50-70 ml.

Functions of CSF:

1. Mechanical protection of the brain.
2. Excretory function – removal of metabolites from the brain.
3. Transport of various biologically active substances.
4. Environmental monitoring of the brain:
   - buffer role in the rapid changes of the blood ;
   - regulation of the optimal concentration of ions and pH to ensure the normal excitability of the central nervous system;
   - a special protective immunobiological barrier.

CHAPTER 34

BIOCHEMISTRY OF MUSCLE TISSUE

Muscle is the major biochemical transducer that converts potential (chemical) energy into kinetic (mechanical) energy.

Muscle, the largest single tissue in the human body, makes up - less than 25% of body mass at birth, more than 40% in the young adult, and somewhat less than 30% in the aged adult. About 40 percent of the body is skeletal muscle, and perhaps another 10 percent is smooth and cardiac muscle.

There are three types of muscle tissue:

- skeletal muscle;
- heart muscle;
- smooth muscle.

Both skeletal and cardiac muscles appear striated upon microscopic observation.

Striated muscles include: skeletal; muscles of the tongue and upper third of the esophagus; external muscles of the eyeball, and others. Striated muscle is composed of multinucleated ***muscle fiber cells*** surrounded by plasma membrane, the ***sarcolemma***.

Smooth muscle is a part of the visceral muscles of gastrointestinal tract, bronchi, urinary tract, blood vessels.

Morphologically, myocardium refers to the striated muscles, but in some other respects it is intermediate between smooth and striated muscles.

All skeletal muscles are composed of numerous fibers ranging from 10 to 80 μm in diameter. In most skeletal muscles, each fiber extends the entire length of the muscle. Except for about 2% of the fibers, each fiber is usually innervated by only one nerve ending, located near the middle of the fiber.

Skeletal Muscle Fiber. The ***sarcolemma*** consists of a true cell plasma membrane and an outer coat made up of a thin layer of ***polysaccharide*** material that contains numerous thin ***collagen fibrils***. At each end of the muscle fiber this surface layer of the sarcolemma fuses with a ***tendon fiber***. The tendon fibers in turn collect into bundles to form the ***muscle tendons*** that then connect the muscles to the bones.

Myofibrils. Each muscle fiber contains several hundred to several thousand myofibrils. Each of which is composed of about 1500 adjacent ***myosin filaments*** and 3000 ***actin filaments***, which are large polymerized

protein molecules that are responsible for the actual muscle contraction.

The light bands of myofibrils contain only actin ***filaments*** and are called **I bands** (they are ***isotropic*** to polarized light). The dark bands contain ***myosin filaments*** and are called **A bands** because they are ***anisotropic*** to polarized light.

Actin filaments are attached to a **Z disk**. From this disc, these filaments extend in both directions to interdigitate with the ***myosin filaments***. The Z disk, which is composed of filamentous proteins different from the actin and myosin filaments, passes crosswise across the myofibril and also crosswise from myofibril to myofibril.

These bands give skeletal and cardiac muscle their **striated appearance**.

The portion of the myofibril that lies between two successive Z disks is called a **sarcomere**. When the muscle fiber is contracted the length of the sarcomere is about 2 μm. At this length, the actin filaments completely overlap the myosin filaments.

The spaces between the myofibrils are filled with intracellular fluid called **sarcoplasm**, containing large quantities of potassium, magnesium, and phosphate, plus multiple protein enzymes. Also present are tremendous numbers of **mitochondria** that lie parallel to the myofibrils. These mitochondria supply the contracting myofibrils with large amounts of energy in the form of **ATP** formed by the mitochondria.

Proteins of muscle tissue

There are three groups of proteins:

I. Myofibrillar proteins – 45%;
II. Sarcoplasmic proteins – 35%;
III. Stroma proteins – 20%.

I. **Myofibrillar proteins**.

This group includes:

- ***Myosin.*** Myosins constitute a family of proteins, with at least 12 classes having been identified in the human genome, contributes 55% of muscle protein by weight and forms the thick filaments. The functional myosin molecule is composed of six polypeptide chains: two identical ***heavy chains*** (M = 230 kDa) and two each of two kinds of ***light chains*** (M = 20 kDa). One L chains called the ***essential light chain*** and the other - the ***regulatory light chain***. A long ***fibrous tail***

consisting of two identical intertwined helices. Each helix has a globular head portion attached at one end. Between each head domain and tail domain is a **flexible**.

Myosin exhibit ***ATPase*** activity, which is accelerated 100- to 200-fold by complexing with ***F-actin***. F-actin greatly enhances the rate at which myosin ATPase releases its products - ADP and P_i. Thus, although ***F-actin*** does not affect the hydrolysis step per se, its ability to promote release of the products produced by the ***ATPase*** activity greatly accelerates the overall rate of catalysis.

- ***Actin***. Monomeric G-actin (43 kDa; G, globular) makes up 25% of muscle protein by weight. At physiologic ionic strength and in the presence of Mg^{2+}, the binding of ATP by a ***G-actin*** monomer leads to polymerization. ***G-actin*** polymerizes noncovalently to form an insoluble double helical filament called ***F-actin***. The ATP is hydrolyzed, but the ADP and P_i is retained in the ***actin***. The F-actin fiber is 6–7 nm thick and has a repeating structure every 35.5 nm. Within ***F-actin filaments***, the G-actin monomers are arranged in a two-strand helix.

- ***Titin***. ***Titin filamentous*** molecules keep the myosin and actin filaments in place. Each titin molecule has a molecular weight of about 3 million (**38 138 amino acides**), which makes it one of the largest protein molecules in the body. Because it is filamentous, it is very ***springy*** and acts as a framework that holds the myosin and actin filaments in place so that the contractile machinery of the sarcomere will work. One end of the ***titin*** molecule is elastic and is attached to the ***Z disk***, acting as a spring and changing length as the sarcomere contracts and relaxes. The other part of the titin molecule tethers it to the ***myosin thick filament***.

The ***titin*** molecule also appears to act as a ***template*** for initial formation of portions of the contractile filaments of the sarcomere, especially the myosin filaments.

REGULATORY PROTEINS:

- ***tropomyosin*** - a fibrous protein that exists as elongated dimers lying along, or close to, the groove in the F-actin helix;
- ***troponin*** or the ***troponin complex***, is a complex of three regulatory proteins:

- ***troponin*** T - binds to tropomyosin, interlocking them to form a ***troponin-tropomyosin complex***;
- ***troponin*** C - binds to ***calcium ions*** to produce a conformational change in ***troponin*** I. Playing the main role in Ca^{2+} dependent regulation of muscle contraction;
- ***troponin*** I - binds to ***actin*** in thin myofilaments to hold the troponin-tropomyosin complex in place and inhibits ATP-ase activity of acto-myosin.

The presence of ***tropomyosin*** and the ***troponins*** inhibits the binding of myosin heads to actin unless Ca^{2+} is present at a concentration of about 10^{-5} M. Smooth muscle does not have troponin.

Certain subtypes of ***troponin*** (cardiac I and T) are sensitive and specific indicators of damage to the myocardium. They are measured in the blood to differentiate between unstable angina and ***myocardial infarction*** in people with chest pain or acute coronary syndrome.

II. **Sarcoplasmic proteins**. They are soluble in salt solutions of low ionic concentration. Sarcoplasmic proteins include: respiratory pigment myoglobin, a variety of enzymes (glycolysis, respiration and oxidative phosphorylation, nitrogen, and lipid metabolism) etc.

III. **Stroma proteins.**

It is represented mainly by **collagen** and **elastin**. **Miostromin** protein is involved in the formation of the sarcolemma and line Z.

Extractive compounds of muscles:

- adenine nucleotides (ATP, ADP, AMP);
- glycogen – alternate source of energy;
- creatine, creatine – standby power for resynthesis of ATP;
- free amino acids;
- carnosine, anserine – specific nitrogenous substances, increase the amplitude of muscle contraction, reduced by fatigue;
- inorganic salts.

Biochemical mechanisms of muscle contraction and relaxation

Biochemical cycle of muscle contraction consists of five stages:

- 1-2-3 – contraction stages;
- 4-5 – relaxation stages.

Stage 1 – in the resting stage myosin "head" can hydrolyze ATP to ADP and P_i, but does not provide release of the hydrolysis products. A stable complex "***myosin – ADP – P_i***" is formed.

Stage 2 – stimulation of motor nerve causes the release of **Ca^{2+}** from the sarcoplasmic reticulum of muscle fibers. Ca^{2+} ions bind to ***troponin C***. As a result of this interaction, the conformation of the troponin molecule changes as well as tropomyosin. Due to this, the sites of myosin binding open in actin. ***Myosin "head" binds to F- actin***, forming with the axis of the fibril angle of about 90°.

Stage 3 – Accession of actin to myosin provides ***release of ADP*** and Pi from the actin-myosin complex. This leads to a change in the conformation of this complex, and the angle between actin and myosin "head" varies from 90° to 45°. As a result of changes in the angle the actin filaments are drawn between myosin filaments – they slide towards each other. Sarcomeres become shortened, ***muscle fibers contract***.

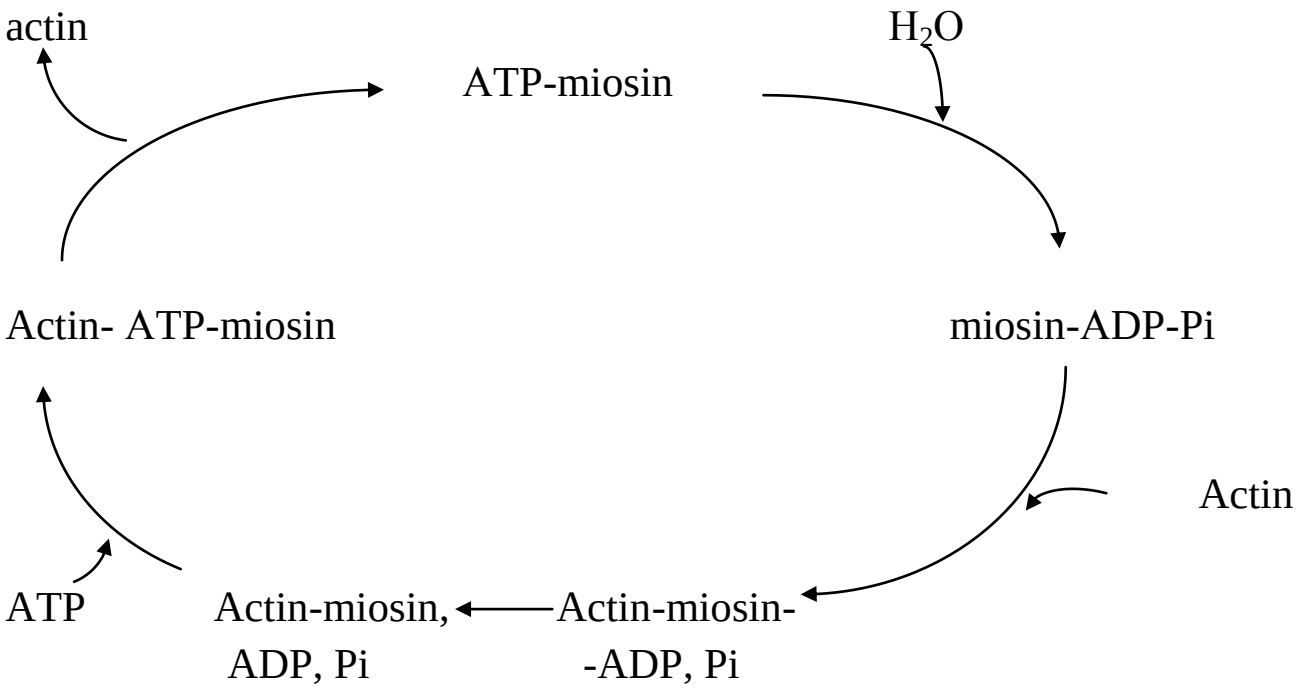


Figure 34.1. Cycle of muscle contraction

Stage 4 – the ***new ATP*** molecule binds to the actin-myosin complex.

Stage 5 – myosin-ATP complex has low affinity for actin, and myosin "head" separates from F-actin. Filaments are returned in its original condition, the muscle relaxes. Then the cycle resumes.

The driving force of muscle contraction is the energy released by the hydrolysis of ATP.

Role of calcium in the regulation of muscle contraction

The key role in the regulation of muscle contraction belongs to calcium ions (Ca^{2+}). The myofibrils have the ability to interact with ATP and a contract only in the presence of certain concentrations in the medium of calcium ions. In resting muscle, Ca^{2+} concentration is maintained below the threshold, with the participation of Ca^{2+}-dependent ATPase. In the resting state, the system of active transport of calcium accumulates in sarcoplasmic reticulum and T- tubule system.

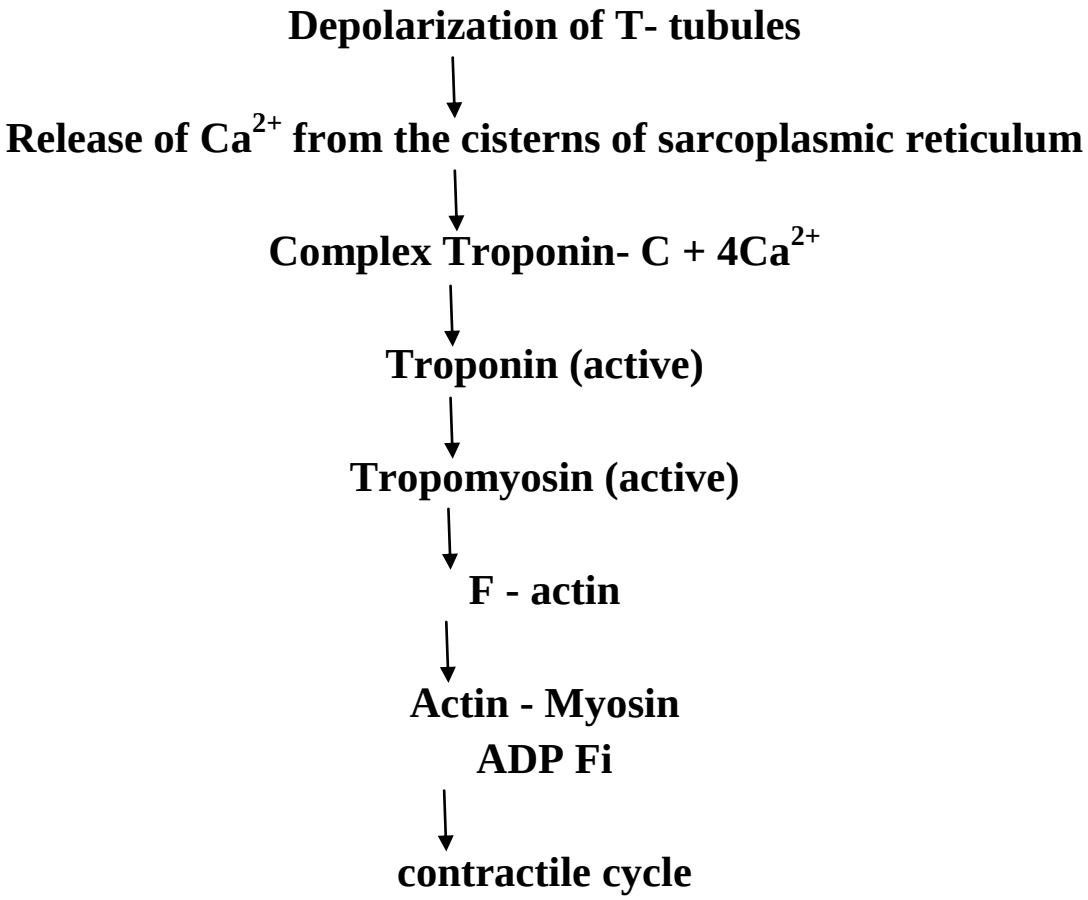


Fig. 34.2. Role of calcium ions in muscle contraction

Muscle contraction is initiated by the arrival of nervous impulse at the end plate of the motor nerve. ***Acetylcholine*** is released into the synapse and interacts with postsynaptic receptors of the muscle fiber. Next, the ***potential of action*** spreads along the sarcolemma to the transverse tubules of the T system and the signal to sarcoplasmic reticulum is transmitted. Calcium

releases from sarcoplasmic reticulum into sarcoplasm and its concentration increases from $\mathbf{10^{-7}}$ to $\mathbf{10^{-5}}$mmol/l. Calcium binds to troponin C, which causes conformational changes to tropomyosin and, further, actin. Previously closed centers in actin open for binding with myosin. Actin interacts with myosin, which initiates contraction of the muscle fiber.

Upon termination of the motor impulse, Ca^{2+} via calcium-dependent ATPase is pumped from the cytoplasm into the sarcoplasmic reticulum cisterns. Removing of calcium from complex Troponin-C leads to a shift of tropomyosin and closing of the active sites of actin. Myosin "head" is detached from actin. Muscle relaxes.

Calcium is an allosteric modulator of muscle contraction.

Biochemistry of muscle fatigue

Fatigue is a body condition that occurs due to prolonged muscle load and is characterized by a temporary decrease in performance.

A central role in the development of the fatigue belongs to the nervous system. In a state of fatigue, concentration of ATP decreases in the nerve cells, the synthesis of acetylcholine at synapses becomes disrupted as well as transfer of motor impulses to the muscle.

Biochemical changes in the working muscle during fatigue are:

– decrease of ATP concentration, phosphocreatine, glycogen;

– reduced activity of Ca^{2+} - ATPase actomyosin, which leads to a reduction in the cleavage rate of ATP myofibrils and decrease in the intensity of the fulfilled workload;

– decrease in the activity of enzymes of aerobic oxidation of substrates and violation of oxidation conjugation with ATP synthesis;

– increased glycolysis, accompanied by the accumulation of lactic acid and a decrease of pH in blood (up to 7.25-7.15);

– acidification of the blood leads to disturbances of homeostasis, aching muscles, nausea, dizziness;

– development of intracellular metabolic acidosis and inhibition of key enzymes of glycolysis.

Fatigue is a protective reaction of the body to protect it from functional exhaustion.

Smooth muscle

Despite the relatively few myosin filaments in smooth muscle, and despite the slow cycling time of the cross-bridges, the maximum force of contraction of smooth muscle is often greater than that of skeletal muscle—as great as 4 to 6 kg/cm2 cross-sectional area for smooth muscle, in comparison with 3 to 4 kilograms for skeletal muscle. This great force of smooth muscle contraction results from the prolonged period of attachment of the myosin cross-bridges to the actin filaments.

The energy consumed to maintain contraction is often minuscule, sometimes as little as 1/300 the energy required for comparable sustained skeletal muscle contraction. This mechanism is called the "***latch" mechanism***. The importance of the latch mechanism is that it can maintain prolonged tonic contraction in smooth muscle for hours with little use of energy.

Smooth muscle does not contain troponin, the regulatory protein that is activated by calcium ions to cause skeletal muscle contraction. In place of troponin, smooth muscle cells contain a large amount of another regulatory protein called ***calmodulin***. Calmodulin initiates contraction by activating the myosin cross-bridges.

1. ***Calcium*** concentration in the cytosolic fluid of the smooth muscle increases as a result of the influx of calcium from the extracellular fluid through calcium channels and/or release of calcium from the sarcoplasmic reticulum.

2. The calcium ions bind reversibly with ***calmodulin***.

3. The calmodulin-calcium complex then joins with and activates ***myosin light chain kinase***, a phosphorylating enzyme.

4. One of the light chains of each myosin head, called the ***regulatory chain***, becomes phosphorylated in response to this myosin kinase. When this chain is not phosphorylated, the attachment-detachment cycling of the myosin head with the actin filament does not occur. However, when the regulatory chain is phosphorylated, the head has the capability of binding repetitively with the actin filament.

Although the contractile process in smooth muscle, as in skeletal muscle, is activated by calcium ions, the source of the calcium ions differs. Most of the calcium ions that cause contraction enter the smooth muscle cell from the ***extracellular fluid*** at the time of the action potential. The concentration of Ca^{2+} in the extracellular fluid is greater than 10^{-3} M, in comparison with less than 10^{-7} M inside the smooth muscle cell; this

situation causes rapid diffusion of the Ca^{2+} into the cell from the extracellular fluid when the calcium channels open. The force of contraction of smooth muscle is usually highly dependent on the extracellular fluid calcium ion concentration.

To cause relaxation of smooth muscle after it has contracted, the calcium ions must be removed from the intracellular fluids. This removal is achieved by a ***calcium pump*** that pumps calcium ions out of the smooth muscle fiber back into the extracellular fluid. This pump requires ATP and is slow acting in comparison with the fast-acting sarcoplasmic reticulum pump in skeletal muscle.

The smooth muscle cell membrane has far more voltage-gated ***calcium*** channels than skeletal muscle but few voltage-gated ***sodium*** channels. Therefore, sodium participates little in the generation of the action potential in most smooth muscle.

Relaxation of the smooth muscle requires another enzyme, ***myosin phosphatase***, located in the cytosol of the smooth muscle cell, which splits the phosphate from the regulatory light chain. Then the cycling stops and contraction ceases. The time required for relaxation of muscle contraction, therefore, is determined to a great extent by the amount of active ***myosin phosphatase*** in the cell.

When the ***myosin kinase*** and ***myosin phosphatase*** enzymes are both strongly activated, the cycling frequency of the myosin heads and the velocity of contraction are great. Then, as the activation of the enzymes decreases, the cycling frequency decreases, but at the same time, the deactivation of these enzymes allows the myosin heads to remain attached to the actin filament for a longer and longer proportion of the cycling period. Therefore, the number of heads attached to the actin filament at any given time remains large. Because the number of heads attached to the actin determines the static force of contraction, tension is maintained, or "latched," yet little energy is used by the muscle because ATP is not degraded to ADP except on the rare occasion when a head detaches.

Smooth muscle can be stimulated to contract by nervous signals, hormonal stimulation, stretch of the muscle, and in several other ways. The principal reason for the difference is that the smooth muscle membrane contains many types of receptor proteins that can initiate the contractile process. Still other receptor proteins inhibit smooth muscle contraction, which is another difference from skeletal muscle.

The most important transmitter substances secreted by the autonomic

nerves innervating smooth muscle are ***acetylcholine*** and ***norepinephrine***, but they are never secreted by the same nerve fibers. *Acetylcholine* and *norepinephrine* excite or inhibit smooth muscle by first binding with a **receptor** protein on the surface of the muscle cell membrane. Some of the receptor proteins are excitatory receptors, whereas others are inhibitory receptors. For example, norepinephrine inhibits contraction of smooth muscle in the intestine but stimulates contraction of smooth muscle in blood vessels.

The **arterioles**, **meta-arterioles**, and **precapillary sphincters** have little or no nervous supply. The smooth muscle is highly contractile, responding rapidly to changes in local chemical conditions in the surrounding interstitial fluid and to stretch caused by changes in blood pressure.

In the normal resting state, many of these small blood vessels remain contracted. However, when extra blood flow to the tissue is necessary, multiple factors can relax the vessel wall, thus allowing for increased flow. In this way, a powerful local feedback control system controls the blood flow to the local tissue area. Some of the specific control factors are as follows:

1. **Lack of oxygen** in the local tissues causes smooth muscle relaxation and, therefore, vasodilation.
2. Excess **carbon dioxide** causes vasodilation.
3. Increased **hydrogen ion** concentration causes vasodilation.

Adenosine, **lactic acid**, increased **potassium ions**, diminished **calcium ion** concentration, and increased **body temperature** can all cause local vasodilation.

Many circulating hormones in the blood affect smooth muscle contraction to some degree, and some have profound effects. Among the more important of these hormones are ***norepinephrine***, ***epinephrine***, ***angiotensin II***, ***endothelin***, ***vasopressin***, ***oxytocin***, ***serotonin***, and ***histamine***.

A hormone causes contraction of a smooth muscle when the muscle cell membrane contains ***hormone-gated excitatory receptors*** for the respective hormone. Conversely, the hormone causes inhibition if the membrane contains ***inhibitory receptors*** for the hormone rather than excitatory receptors.

Inhibition occurs when the hormone closes the sodium and ***calcium channels*** to prevent entry of these positive ions; inhibition also occurs if the

normally closed ***potassium channels*** are opened, allowing positive potassium ions to diffuse out of the cell. Both of these actions increase the degree of negativity inside the muscle cell, a state called **hyperpolarization**, which strongly inhibits muscle contraction.

The **heart** is composed of three major types of cardiac muscle: ***atrial muscle***, ***ventricular muscle***, and specialized excitatory and conductive ***muscle fibers***.

The atrial and ventricular types of muscle contract in much the same way as skeletal muscle, except that the duration of contraction is much longer.

The specialized excitatory and conductive fibers of the heart, however, contract only feebly because they contain few contractile fibrils; instead, they exhibit either automatic rhythmical electrical discharge in the form of action potentials or conduction of the action potentials through the heart, providing an excitatory system that controls the **rhythmical beating** of the heart.

Cardiac muscle is striated in the same manner as in skeletal muscle. The dark areas crossing the cardiac muscle fibers are called ***intercalated discs***; they are actually cell membranes that separate individual cardiac muscle cells from one another. At each intercalated disc the cell membranes fuse with one another to form permeable "communicating" junctions (***gap junctions***) that allow rapid diffusion of ions, so that **action potentials** travel easily from one cardiac muscle cell to the next, past the intercalated discs. Thus, cardiac muscle is a *syncytium* of many heart muscle cells in which the cardiac cells are so interconnected that when one cell becomes excited, the action potential rapidly spreads to all of them.

In cardiac muscle, the action potential is caused by opening of two types of channels:

(1) the same ***voltage-activated fast sodium channels*** as those in skeletal muscle and

(2) another entirely different population of ***L-type calcium channels*** (slow calcium channels), which are also called ***calcium-sodium channels***.

These channels differ from the fast sodium channels in that they are slower to open and, even more important, remain open for several tenths of a second. Immediately after the onset of the action potential, the permeability of the cardiac muscle membrane for $\mathbf{K^+}$ decreases about fivefold, an effect that does not occur in skeletal muscle.

CHAPTER 35

BIOCHEMISTRY OF CONNECTIVE TISSUE

Most mammalian cells are located in tissues where they are surrounded by a complex **extracellular matrix** often referred to as "**connective tissue**".

Connective tissue comprises about half of the dry weight of the body. All varieties of connective tissue are built according to common morpho-biochemical principles: **contains few cells** in comparison with other tissues. As a result, the **extracellular matrix** takes up more space than the cells and has a complex chemical composition.

The **extracellular matrix** contains three major classes of biomolecules:

1. **structural proteins** - ***collagen***, ***elastin***, and ***fibrillin***;
2. **specialized proteins** – ***fibronectin, laminin***, ***tenascin***, ***osteonectin***, etc.;
3. **proteoglycans**.

Collagen

In the extracellular matrix, collagen molecules form polymers called ***collagen fibrils***. They have a great strength and minor extensibility (may withstand a load 10 000 times exceeding their own weight). It is the most abundant protein in mammals, making **25%** to **35%** of the whole-body protein content.

Collagen molecules consist of three polypeptide chains, called ***α-chains,*** wound together to form ***triple-helices*** of elongated fibrils.

Over 20 α-chains are identified , the majority of which (***collagen type I***) incorporates 1000 amino acid residues, but the chains slightly differ in amino acid sequence. The collagen composition may include three identical or different chains.

The primary repeating structure of collagen α-chains is unusual (Gly-X-Y). Since every third amino acid in the polypeptide chain is presented by ***glycine***, about 25% – by ***proline*** or 4% – ***hydroxyproline***, about 10% – by ***alanine***.

Hydroxyproline is formed by the posttranslational hydroxylation of peptide-bound ***proline*** residues catalyzed by the enzyme ***prolyl hydroxylase***, whose cofactors are **ascorbic acid** (vitamin C) and ***α-ketoglutarate***. ***Lysines*** in the Y position may also be posttranslationally modified to ***hydroxylysine*** through the action of ***lysyl hydroxylase***, an

enzyme with similar cofactors. Some of these hydroxylysines may be further modified by the addition of galactose or galactosyl-glucose through an **O-glycosidic linkage**. In collagen such amino acids as ***cysteine*** and ***tryptophan*** are absent.

In some tissues, for example tendons, fibers associate into even larger bundles, which may have a diameter of up to 500 μm. Collagen fibers are stabilized by the formation of covalent cross-links, both within and between the triple helical units. These cross-links form through the action of ***lysyl oxidase***, a copper-dependent enzyme that oxidatively deaminates the ε-amino groups of certain lysine and hydroxylysine residues, yielding reactive aldehydes. Such aldehydes can form aldol condensation products with other lysine or hydroxylysine-derived aldehydes or form Schiff bases with the ε-amino groups of unoxidizedlysines or hydroxylysines. These reactions, after further chemical rearrangements, result in the stable covalent cross-links that are important for the tensile strength of the fibers. ***Histidine*** may also be involved in certain cross-links.

Catabolism of collagen. As any protein, collagen functions only at a certain time in the body. It belongs to the slowly exchanging proteins with half-life of about a month. Disruption of collagen fibers occurs enzymatically with the help of active oxygen forms.

Native collagen is not hydrolyzed by simple peptidase. The main enzyme of its catabolism is ***collagenase***, which cleaves peptide bonds in certain areas of collagen. Normally it is synthesized by connective tissue cells, particularly **fibroblasts** and **macrophages**. The resulting collagen fragments are soluble in water at body temperature, they spontaneously denature and become available for other actions of proteolytic enzymes.

Newly synthesized collagen undergoes extensive **posttranslational modification** before becoming a part of a mature extracellular collagen fiber.

Bone contains both organic and inorganic material. The organic matter is mainly protein. The principal proteins of bone are ***type I collagen*** comprising 90- 95% of the organic material. ***Type II collagen*** is the principal protein of **hyaline cartilage**.

A number of heritable diseases of bone (eg. osteogenesis imperfecta) and of cartilage (eg. the chondrodystrophies) are caused by mutations in the genes for ***collagen*** and proteins involved in bone mineralization and cartilage formation.

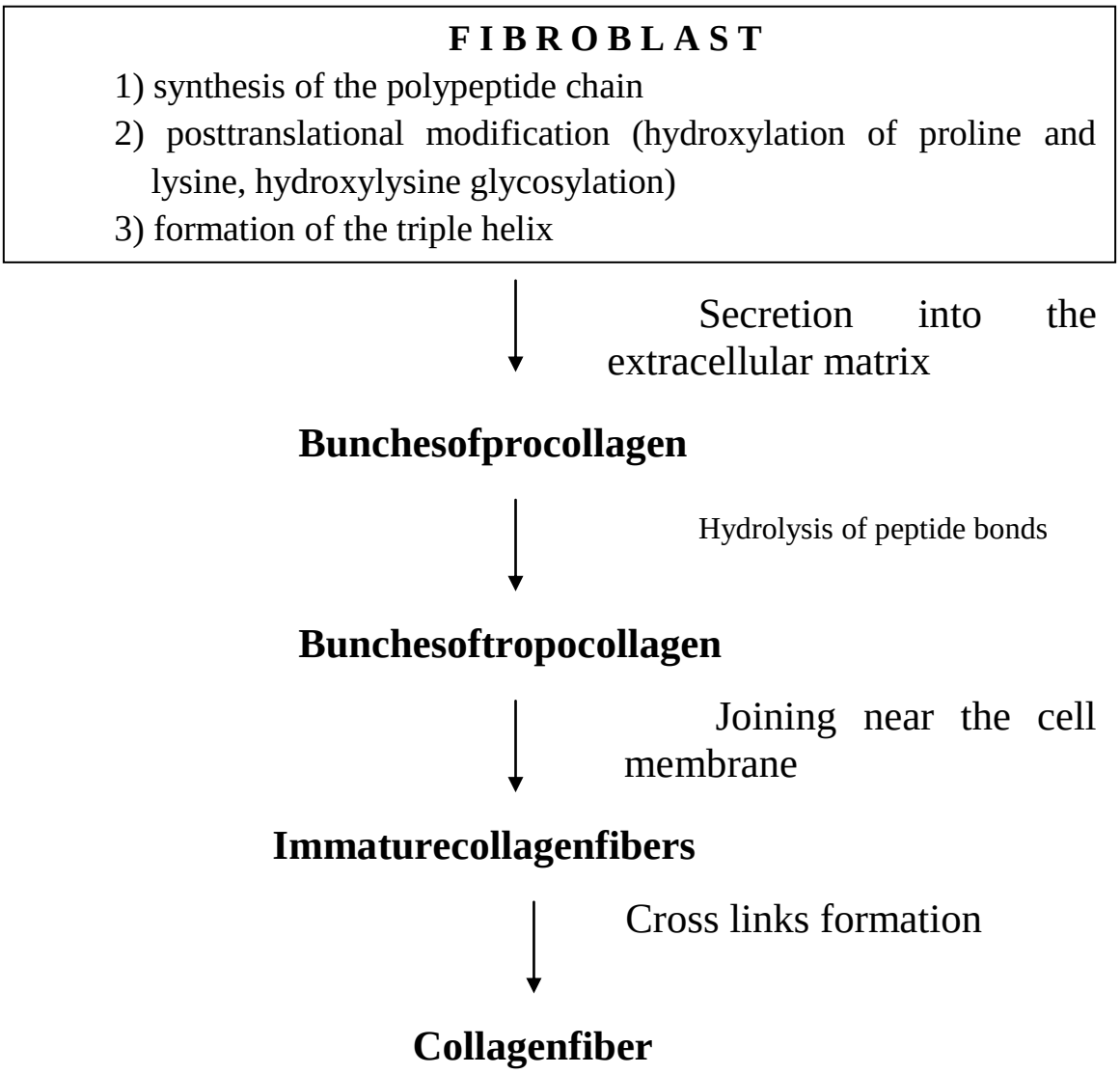


Fig. 34.1.Stages of formation of collagen fibers.

There are a number of diseases associated with disruption of the structure and synthesis of collagen. They make up a whole group of connective tissue disorders, called **collagenoses**. So about **50%** of all collagen proteins are in the tissues of the skeleton, about **40%** – in the skin and **10%** – in the stroma of inner organs, the clinical picture of the disease is extremely polymorphic. In many diseases not only osteo- articular pathology or changes in the skin are observed, but also pronounced visceral manifestations (affected intestine, kidney, lung, heart). The most common and well-studied collagenoses include **osteogenesis imperfecta**, **Ehlers – Danlos syndrome**, **Marfan syndrome**, as well as **scurvy**.

Fibronectin

Fibronectin, a large glycoprotein consists of two identical subunits (~ 440 kDa) present on cell surfaces, in the extracellular matrix, and in blood. Fibronectin binds to membrane-spanning receptor proteins called ***integrins***. Fibronectin also binds to other extracellular matrix proteins such as ***collagen***, ***fibrin***, and proteoglycans **- *heparan sulfate*** and ***chondroitin sulfate.***

Fibronectin plays a major role in **cell adhesion**, **growth**, **migration**, and **differentiation**, and it is important for processes such as wound healing and embryonic development.

Altered ***fibronectin*** expression, degradation, and organization has been associated with a number of pathologies, including cancer and fibrosis. Fibronectin is necessary for embryogenesis, and inactivating the gene for fibronectin results in early embryonic lethality. In mammalian development, the absence of fibronectin leads to defects in mesodermal, neural tube, and vascular development.

Fibronectin is also found in normal human saliva, which helps prevent colonization of the oral cavity and pharynx by potentially pathogenic bacteria.

Elastin

Elastin is a connective tissue protein that is responsible for properties of extensibility and elastic recoil in tissues. In contrast to stable fibrils of collagen, elastin has rubbery properties. Filaments of elastin contained in lung tissue, in the vessel wall, in the elastic cords, may be several times stretched as compared to their normal length. But after unloading they return to the folded conformation.

In contrast to collagen, there appears to be only **one genetic type of elastin**, although variants arise by alternative splicing.

Elastin contains in its structure about 800 amino acid residues, which are dominated by non-polar amino acid radicals: ***glycine***, ***valine***, ***alanine***. Elastin contains quite a lot of ***proline*** and ***lysine***, but only little ***hydroxyproline***, and ***hydroxylysine*** is completely absent. The presence of large amounts of hydrophobic radicals prevents the creation of a stable globules, resulting polypeptide chains not forming regular secondary and tertiary structure, and take different configurations. In connective tissue molecules form fibers and elastin fibers in which the individual peptide chains are tied by many rigid cross-linkages into a branched chain. In the

formation of these cross-links ***lysine*** residues of two, three or four peptide chains are involved. Structures formed like these are called ***desmosines***.

The presence of covalent cross-links between peptide chains with a disordered, random conformation allows the entire network of elastin fibers stretch and shrink in different directions, giving the appropriate tissue elasticity property.

Elastin is synthesized as soluble monomer of ~70 kDa, which is called "***tropoelastin***". After crosslinking, elastin acquires its final shape, which is characterized by insolubility, high stability and a very low metabolic activity.

Some of the prolines of tropoelastin are hydroxylated to hydroxyproline by ***prolyl hydroxylase***, though hydroxylysine and glycosylated hydroxylysine are not present. Unlike collagen, tropoelastin is not synthesized in a pro-form with extension peptides. Furthermore, elastin does not contain repeat **Gly-X-Y** sequences, triple helical structure or carbohydrate moieties.

After secretion from the cell, certain ***lysyl*** residues of tropoelastin are **oxidatively deaminated** to aldehydes by ***lysyl oxidase***. The major cross-links formed in elastin are the ***desmosines***, which result from the condensation of three of these lysine-derived aldehydes with an unmodified lysine to form a tetrafunctional cross-link unique to elastin.

Once cross-linked in its mature, extracellular form, elastin is highly insoluble and extremely stable and has a very low-turnover rate. Elastin exhibits a variety of random coil conformations that permit the protein to stretch and subsequently recoil during the performance of its physiologic functions.

Microfibrils are fine fiber-like strands 10-12 nm in diameter which provide a scaffold for the deposition of elastin in the extracellular matrix. ***Fibrillins*** are large glycoproteins (~ 350 kDa) that are major structural component of these fibers. They are secreted (subsequent to a proteolytic cleavage) into the extracellular matrix by fibroblasts and become incorporated into the insoluble microfibrils.

Deletions in the ***elastin gene*** have been found in approximately 90% of subjects with the **Williams-Beuren syndrome**, a developmental disorder affecting connective tissue and the central nervous system.

The mutations, by affecting synthesis of elastin, probably play a causative role in the **supravalvular aortic stenosis** often found in this condition.

Fragmentation or, alternatively, a decrease of elastin is found in conditions such as ***pulmonary emphysema***, ***cutis laxa*** (***generalized elastolysis***), and ***aging*** of the skin.

Proteoglycans and glycoproteins

Proteoglycans are high molecular compounds consisting of the protein (5-10%) and glycosaminoglycans (90-95%). They form the main substance of the extracellular matrix.

Glycosaminoglycans are heteropolysaccharides composed of repetitive disaccharide monomers which are ***hexosamines*** and ***uronic acids***. Previously they were called **mucopolysaccharides**, since they have been found in mucosal secretions. They bind large amounts of water, resulting in the gelled character of the intercellular substance. Deficiencies of enzymes that degrade glycosaminoglycans result in **mucopolysaccharidoses.**

The proteins in **proteoglycans** are presented by single polypeptide chain of different molecular weight. Proteins of proteoglycans are called ***core proteins***. Polysaccharide components from various proteoglycans are different.

Functions of proteoglycans:

- are the structural components of the extracellular matrix;
- provide turgor of different tissues;
- aspolyanions bind polications and cations;
- act as a sieve in the extracellular matrix (filtration in the kidneys);
- affect cell migration;
- resist compression forces in the extracellular matrix;
- support the transparency of the cornea;
- perform a structural role in the sclera;
- are anticoagulants;
- form the receptors on the cell surface;
- form cell-to-cell contacts;
- are part of synaptic vesicles and other cells.

Currently the structure of the **six major classes** of glycosaminoglycans is known.

1. ***Hyaluronic acid*** is found in many organs and tissues. In cartilage, it is bound to the protein and is involved in the formation of proteoglycan aggregates in some tissues (vitreous, umbilical cord, joint fluid) occurs in free form. It is thought to play an important role in permitting cell migration during morphogenesis and wound repair. The repeating disaccharide unit of

hyaluronic acid consists of **D-glucuronic acid** and **N-acetylglucosamine**.

2. ***Chondroitin sulfates*** are the most common glycosaminoglycans in the human body. They are found in cartilages, tendons, ligaments, arteries, cornea. Chondroitin sulfates constitute essential elements of ***aggrecan*** – the main proteoglycan of cartilage matrix. In humans there are two types of chondroitinsulphates: ***chondroitin-4-sulphate*** and ***chondroitin-6-sulfate***. They are constructed in the same manner: of D- glucuronic acid and N-acetyl-D-galactosamine-4-sulphate or N- acetyl-D- galactosamine-6-sulfate, respectively. Proteoglycans linked to chondroitin sulfate by the ***Xyl-Ser O-glycosidic bond*** and are prominent components of cartilage. They are found in high amounts in the extracellular matrix of the central nervous system.

3. ***Keratan sulfates I*** and ***II*** are most heterogeneous glycosaminoglycans. They differ from each other on the total carbohydrate content and distribution in different tissues. They contain ***galactose*** and ***N-acetyl-D-glucosamine -6-sulfate***. Keratan sulfate I was originally isolated from the cornea, while keratan sulfate II came from cartilage and intervertebral discs. In the eye, they lie between collagen fibrils and play a critical role in corneal transparency. Changes in proteoglycan composition found in corneal scars disappear, when the cornea heals.

4. ***Dermatan sulfates*** are typical for the skin, blood vessels, heart valves, meniscus, intervertebral discs. The disaccharide unit – ***L-iduronic acid*** and ***N-acetyl-D-galactosamine-4-sulfate*** has a repeating character.

5. ***Heparin*** is an important component of the blood anticoagulation system. It is synthesized by mast cells. The highest amount of heparin is found in the lung, liver and skin. The repeating disaccharide units are composed of ***D-glucuronate-2-sulfate*** and ***N-acetylglucosamine-6-sulfate***.

6. ***Heparansulphate*** is a part of the basal membrane proteoglycans. Disaccharide unit structure is the same as that of heparin, but has more N-acetyl groups. This proteoglycan is also found in the basement membrane of the kidney along with ***type IVcollagen*** and ***laminin***, where it plays a major role in determining the charge selectiveness of glomerular filtration.

In the extracellular matrix there are different types of **proteoglycans**. Among them, there are very large ones, for example ***aggrecan*** (~ 2 x 10^3kDa) is the major proteoglycan in **cartilage**.

Besides the extracellular matrix has a set of so-called small

proteoglycans which are widely distributed in connective tissues of different types and perform different functions therein. These proteoglycans have small core protein, to which one or two glucoseaminoglican chains are attached. Most studied are ***decorin***, ***biglycan, fibromodulin, perlecan***.

Proteoglycans differ from a large group of proteins, which are called **glycoproteins**. These proteins also contain oligosaccharide chains of varying lengths, covalently attached to the polypeptide backbone. The carbohydrate component of the glycoproteins is much smaller in mass than that of proteoglycans and not more than 40% of the total weight.

Function of **glycoproteins**:

– structural molecule;
– protective (mucins, immunoglobulins, antigens of major complex of histocompatibility, compliment, interferon);
– transporter molecules for vitamins, lipids, microelements;
– hormones: thyrotropin, human chorionic gonadotropin;
– enzymes (nucleases, blood clotting factors);
– formation of cell-cell contacts.

The metabolism of proteoglycans and glycoproteins is dependent on the rate of synthesis and degradation. Their polypeptide chains are synthesized on polyribosomes. Polysaccharide chains are attached to the protein via a linking region, including trisaccharide galactose – galactose – xylose and are connected to the serine residue of the core protein.

Polysaccharide chains are synthesized by successive addition of monosaccharides. Appropriate nucleotide sugars are generally donors of monosaccharides. Synthesis reaction is catalysed by enzymes of the family of ***transferases***, having absolute substrate specificity. These transferases are localised on the membranes of the Golgi apparatus. Here via the endoplasmic reticulum core protein comes, to which monosaccharides of binding region are attached, and then the whole carbohydrates chain builds up. Sulfation of the carbohydrate portion is done by **3-phosphoadenosine-5-phosphosulfate** (**PAPS**).

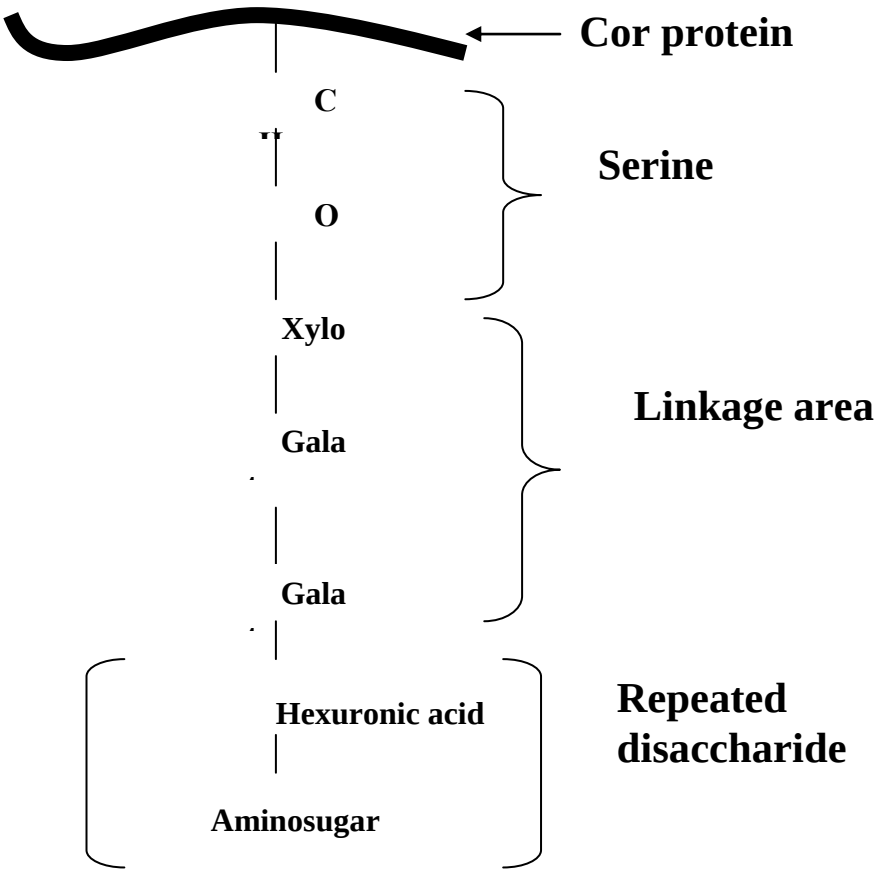


Fig. 34.2.The general scheme of the structure of glycoproteins.

Glucocorticoids affect the synthesis of glycosaminoglycans: they inhibit the formation of hyaluronic acid and sulfated glycosaminoglycans.

Destruction of the polysaccharide chains is performed due to ***exo-*** and ***endoglycosidases***, and ***sulfatase***, which include ***hyaluronidase glucuronidase***, ***galactosidase***, ***neuraminidase***, and other lysosomal hydrolases, providing a gradual splitting of monomers.

Genetically determined defect of these enzymes leads to disruption of decay of protein-carbohydrate complexes and their accumulation in lysosomes. Development of **mucopolysaccharidoses** is manifested in significant impairments in mental development, vascular lesions, corneal clouding, skeletal deformities.

CHAPTER 36

CLINICAL BIOCHEMISTRY

The science of biochemistry is fundamental to the practice of clinical medicine. Many diseases have long been known to have a biochemical basis and research in biochemistry is increasingly providing descriptions of pathological processes and explanations for disease at a molecular level.

Clinical biochemistry is a special branch of medicine dealing with measurement and interpretation of the physic-chemical condition and dynamics in healthy and diseased humans.

Clinical biochemistry encompasses the use of biochemical techniques both in the study of fundamental disease processes and in the diagnosis and management of disease.

Clinical biochemistry is the division of **laboratory medicine** that deals with the measurement of chemicals (both natural and unnatural) in blood, urine, and other body fluids. These test results are useful for detecting health problems, determining prognosis. Laboratory tests of one kind or another are essential parts of medicine. Biochemical tests can be used for:

- early diagnosis of disease. Use of plasma concentration of cardiac ***troponin I, LDH_1,*** in early diagnosis of myocardial infarction;
- investigation of the causes and mechanisms of disease. Demonstration of the nature of the genetic defect in ***cystic fibrosis;***
- as screening tests for early diagnosis of disease. Measurement of ***thyroid stimulating hormone*** (TSH) in diagnosis of congenital hypothyroidism;
- monitoring the progression of disease. Measurement of serum ***alanine aminotransferase*** (ALT) to monitor the progress of viral hepatitisfor;
- suggestion of rational treatment of disease. Elevated ***low density lipoprotein cholesterol*** is an indication for therapy with cholesterol-lowering drugs (eg, statins) in people at risk of cardiovascular disease;
- assessment of the response of disease to therapy. Measurement of TSH in patients being treated for hypo- or hyperthyroidism and the outcome of treatment.

Biochemical data are used extensively in medicine, both in the management of patients and in research. But before a test is requested, the rationale for doing this should always be considered. In common with other investigations, biochemical tests should be performed to answer specific questions; if there is no question, a test cannot provide an answer.

SPECIFIC USES OF BIOCHEMICAL TESTS

Diagnosis

Laboratory tests can provide useful information for diagnosis and treatment of disease as well as information about normal metabolism and the pathology of disease.

The ideal diagnostic test would be:

- 100% ***sensitive*** (all cases of the condition in question would be correctly diagnosed using it) and
- 100% ***specific*** (no individual without the condition would be wrongly diagnosed as having it).

With the exception of genetically determined diseases, the number of conditions in which biochemical tests alone provide a precise diagnosis is very small. There are several reasons for this. Biochemical changes are often a consequence of a pathological process which can be common to many conditions:

- tissue destruction leads to the release of intracellular enzymes into the plasma, few such enzymes are specific to any tissue;
- tissue destruction can occur for many reasons, for example, ischaemia, exposure to toxins, etc;
- a biochemical variable can be influenced by more than one type of process.

Factors affecting test results

The generation of biochemical data is subject to potential error at every stage in the process. It is essential that the sources of error are identified and understood, so that their effects can be minimized.

The sources of errors in biochemical tests are conventionally described in three categories.

1. **Pre-analytical factors,** that is, either outside or within the laboratory, but before the analysis is performed;
    - ***Technical factors***:

- correct identification of the patient and appropriate preparation of the patient where necessary;
- collection of the sample required into a container with, where necessary, the correct preservative;
- accurate labeling of the specimen container, and
- secure transport to the laboratory.

- ***Biological factors***:
  - **Age**. The reference values for many biochemical variables do not vary with age;
  - **Genetic diseases**. Plasma phenylalanine is elevated in phenylketonuria; serum ammonium is elevated in disorders of the urea cycle;
  - **Sex**. Apart from the obvious differences in plasma gonadal hormone concentrations between adult men and women, other analytcs demonstrate sex-related differences in concentration, often because their metabolism is influenced by the gonadal hormones;
  - **Food intake and changes in nutritional status**. Serum albumin and retinol binding protein are low in protein-energy malnutrition;
  - **Body mass**. Obese individuals tend to have higher plasma insulin and triglyceride concentrations than the lean;
  - **Time-dependent changes**. Rhythmic changes occur in many physiological functions and are reflected in changes in the levels of biochemical variables with time;
  - **Changes in blood pH**. Serum bicarbonate is low in ***metabolic acidosis*** (eg, diabetic ketoacidosis) and high in ***metabolic alkalosis*** (eg, severe vomiting due to pyloric stenosis);
  - **Cell injury or death (necrosis)**. Serum ***creatine kinase*** (MB isoform) is elevated in *myocardial infarction*; serum pancreatic ***amylase*** is elevated in pancreatitis;
  - **Organ failure**. Serum ***creatinine*** and ***urea*** are elevated in *renal failure*; serum ***ammonium*** and ***bilirubin*** are elevated in *liver failure*;
  - **Stress**. Concern for the patient dictates that stress should be minimized at all times, but this is particularly important in relation to blood sampling for those analytes whose level is

responsive to stress. Stress increases serum *cortisol* and *catecholamines*;

- **Drugs**. Drugs, whether taken for therapeutic, social or other purposes, can have profound effects on the results of biochemical tests. Drugs used in cancer chemotherapy increase serum *uric acid*;
- **Changes in fluid balance.** Hypernatremia (high serum sodium) in patients who are dehydrated due to excessive sweating or vomiting;
- **Exercise and various physiological conditions**. High serum and urine levels of human chorionic gonadotropin (hCG) in pregnancy; high blood lactate following strenuous exercise.
- **Trauma**. Serum myoglobin may be elevated following muscle injury.

2. Analytical factors

- **Reference range*.** For any compound that is measured (an **analyte**), there is a range of values around the average or mean that can be considered to be normal**.** This is the result of biological variations between individuals.
- **Analytical range**. The test selected must be capable of measuring the analyte in question over the whole range of concentrations.
- **Accuracy**. Accuracy reflects the ability of an assay to produce a result that reflects the true value.
- **Precision**. Precision is a reflection of reproducibility. *Imprecision* is defined as the standard deviation for a series of replicate analyses. *Analytical variation* or imprecision can be measured by performing repeated analyses on a single sample and calculating the standard deviation of the results.
- **Sensitivity**. The lowest analyte concentration that can be measured with acceptable accuracy and precision.
- **Specificity and interference**. *Specificity* is the ability of an assay to measure only the analyte it purports to measure. *Interference* is a related but separate problem, in that a substance alters the signal given by the analyte in question but does not generate a signal itself.

3. **Post-analytical factors.** That is, during data processing or transmission, or in relation to the interpretation of the data.

* - If the results obtained for a target healthy population group (depending on age, gender, and perhaps ethnicity) are statistically normally distributed (i.e., the results show a symmetrical Gaussian distribution around the mean), then the acceptable or normal range is taken to be ***± 2x standard deviation around the mean value*** for the population group under consideration. This range includes 95% of the target population, and is known as the **reference range**.

The **interpretation** of biochemical data requires adequate knowledge of all the factors which can affect the test result. These include the ***physiological***, ***biochemical,*** and ***pathological*** principles on which the test is based as well as the reliability of the analysis. It is also necessary to understand the ***statistical principles*** which relate to the distribution of data in healthy individuals and those with disease, and how the test performs in the specific circumstances in which it is used. Such knowledge is also essential for the appropriate selection of laboratory tests.

Validity of laboratory results

Diagnostic laboratories are subject to inspection and regulatory procedures to assess the validity of their results and ensure quality control of their reports. Such measures will ensure that the value of the concentration, activity or amount of a substance in a specimen reported represents the best value obtainable with the method, reagents, and instruments used, and the technicians involved in obtaining and processing the specimen.

The common samples for analysis are **blood** and **urine**. Blood is collected into tubes with or without an ***anticoagulant***, depending on whether **plasma** or **serum** is required for the estimation. Less commonly, samples of **saliva**, **cerebrospinal fluid** or **feces** may be used.

There is a difference between measurement of an analyte in a blood sample and in urine. The concentration of an analyte in blood reflects levels at the time the sample was taken, whereas a urine sample represents the cumulative excretion of the analyte over a period of time.

Apart from measurement of **blood gases**, for which arterial samples are required, blood samples are usually of venous blood.

Blood glucose is often measured in **capillary blood** from a finger prick. Some analyses use whole blood; others require either ***serum*** or ***plasma***.

- For a ***serum sample***, the blood is allowed to clot, then the red cells and fibrin clot are removed by centrifugation.
- For a ***plasma sample***, the blood is collected into a tube containing an anticoagulant, and the red cells are removed by centrifugation.

The difference between ***serum*** and ***plasma*** is that plasma contains the clotting factors including fibrinogen, prothrombin, while serum does not. Different anticoagulants are used for collection of plasma samples, depending on the assay to be performed: **citrate**, ethylenediaminetetraacetic acid (**EDTA**), or **oxalate**, all of which chelate calcium and so inhibit coagulation. **Heparin**, which acts by activating antithrombin III, is another commonly used anticoagulant.

TECHNIQUES USED IN CLINICAL CHEMISTRY

Many laboratory tests rely on production of a colored product that can be measured by absorption spectrophotometry or fluorimetry.

Absorption spectrophotometry.

Different compounds absorb light at different wavelengths; the energy of the absorbed light excites electrons to an unstable orbital. The absorbance of light at a specific wavelength in the visible or ultraviolet range is directly proportional to the concentration of the colored end product, and hence to the concentration of the analyte in the sample.

Spectrophotofluorimetry.

In ***fluorescence*** spectrophotometry the electrons return to a lower energy state in a single quantum jump, emitting light of a higher wavelength (lower energy) than the exciting light. The intensity of the fluorescence is proportional to the concentration of the ***fluorophore***, and hence the concentration of the analyte. Fluorimetry permits both greater specificity and sensitivity of the analysis. The specificity is greater than for absorption spectrophotometry because both the exciting wavelength and the emitted wavelength are specific for the fluorophore. Fluorimetry is more sensitive because it is easier to detect the emission of a small amount of light than the absorption.

High-pressure liquid chromatography (HPLC).

Many compounds can be measured by HPLC, sometimes in conjunction with mass spectrometry. The measurement of a large number of

analytes in a sample is the basis of ***metabolomics****, and of ***metabonomics*****, which is the effect of a disease, drug, or other treatment on metabolism. Multiple analytes are measured in the same sample using HPLC to separate analytes, followed by

- colorimetric,
- fluorimetric,
- electrochemical,
- mass spectrometry detection.

Atomic absorption spectrometry is a spectroanalytical procedure for the quantitative determination of chemical elements. Atomic absorption spectroscopy is based on absorption of light by free metallic ions.

* - The **metabolome** represents the complete set of ***metabolites*** in a biological cell, tissue, organ or organism, which are the end products of cellular processes.

** - the quantitative measurement of the dynamic multiparametric metabolic response of living systems to pathophysiological stimuli or genetic modification.

Enzymes in Clinical Chemistry

Enzymes are important in clinical chemistry in three different ways:

- to measure analytes in a sample,
- to measure the activity of enzymes themselves in a sample,
- as a test of vitamin nutritional status.

When an enzyme is used to detect an analyte, the limiting factor in the assay must be the **analyte** itself; the enzyme and other reagents must be present in excess. More importantly, the concentration of the analyte in the sample must be adjusted to be below the $\mathbf{K_m}$ of the enzyme, so that there is a large change in the rate of reaction with a small change in the concentration of the analyte.

When cells are damaged or die, their contents leak out into the bloodstream. The increase in enzyme activity in plasma above the normal range often indicates the degree of severity of tissue damage. The concentration of substrate added must be considerably in excess of the $\mathbf{K_m}$ of the enzyme, so that the enzyme is acting at or near $\mathbf{V_{max}}$, and even a relatively large change in the concentration of substrate does not have a significant effect on the rate of reaction.

If an enzyme has a vitamin-derived coenzyme that is essential for activity, then measurement of the activity of the enzyme in red blood cells with and without added coenzyme can be used as an index of vitamin nutritional status. Such enzyme activation assays are available for **thiamin** (vit B_1,using red cell ***transketolase***), **riboflavin** (vit B_2, using red cell ***glutathione reductase***), and pyridoxine (vit B_6, using one or the other of the red cell ***transaminases***).

Many analytes (and especially hormones) are measured by competitive binding assays, using either a naturally occurring binding protein or an **antiserum** or **monoclonal antibody** to bind the ligand. Trace amounts of high specific activity radioactive ligand, or fluorescently-labelled ligand, or binding protein are used.

For a number of assays the enzymes or antibodies and reagents can be combined on a specially designed **plastic strip**. For measurement of blood glucose, a finger-prick blood sample is placed on the test strip and the concentration of glucose is measured using a handheld device called the **glucometer**.

For urine testing, several different assays can be included as separate pellets on a plastic stick called a **dipstick**, to detect or semiquantitatively estimate levels of ***glucose***, ***ketone bodies***, ***protein***, and several other analytes at the same time. Similar dipsticks are available to detect ***human chorionic gonadotropin*** (hCG) in urine, as a home **pregnancy test**.

The enzyme-linked immunosorbent assay (ELISA).

The assay uses a solid-phase enzyme immunoassay (**EIA**) to detect the presence of a ligand (commonly a protein) in a liquid sample using antibodies directed against the protein to be measured.

In the most simple form of an ELISA, antigens from the sample are attached to a surface. Then, a matching antibody is applied over the surface so it can bind to the antigen. This antibody is linked to an enzyme, and in the final step, a substance containing the enzyme's substrate is added. The subsequent reaction produces a detectable signal, most commonly a color change.

Radioimmunoassay (**RIA**)

A radioimmunoassay is an immunoassay that uses radiolabeled molecules in a stepwise formation of immune complexes. A RIA is a very sensitive in vitro assay technique used to measure **concentrations of**

substances, usually measuring antigen concentrations (***hormone*** levels in blood) by use of **antibodies**.

I**mmunoradiometric assay** (**IRMA**)

Is an immunoassay that uses radiolabeled molecules but in an immediate rather than stepwise way.

R**adioallergosorbent test** (**RAST**)

Is an example of radioimmunoassay. It is used to detect the causative allergen for an allergy.

Laboratory Tests Measure Coagulation, Thrombolysis, and Platelet Aggregation

A number of laboratory tests are available to measure the phases of hemostasis. The tests include:

- **platelet count** - quantitates the number of platelets;
- **bleeding time/closure time**. The skin bleeding time is an overall test of platelet and vessel wall function, while the closure time measured using the platelet function analyzer PFA-100 is an in vitro test of platelet-related hemostasis;
- **platelet aggregation** - measures responses to specific aggregating agents;
- **activated partial thromboplastin time** (aPTT or PTT) - measure of the intrinsic pathway;
- **prothrombin time** (PT), measure the effectiveness of warfarin;
- **thrombin time** (TT),
- **concentration of fibrinogen**,
- **fibrin clot stability**,
- measurement of **fibrin degradation products**.