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БИОХИМИЯ

Пособие для студентов факультета иностранных учащихся
(английский язык обучения)

Под общей редакцией профессора В.В. Лелевича

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BIOCHEMISTRY

Manual for the medical faculty for international students
(in English)

Edited by professor V.V. Lelevich

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В издании, написанном в соответствии с учебной программой, представлены основные современные сведения по всем разделам биохимии. Пособие составлено с учетом различий в базовой подготовке иностранных студентов и содержит краткое изложение теоретических вопросов по соответствующим темам. В 35 главах приведена характеристика метаболизма белков, углеводов, липидов, нуклеиновых кислот в норме и при некоторых патологических состояниях. Охарактеризованы особенности метаболизма в различных органах и тканях. Изложены современные представления о молекулярных основах нарушений при ряде патологических состояний и болезней. Дана характеристика особенностей действия гормонов. Описаны некоторые прикладные аспекты биохимических и молекулярно-биологических исследований. Издание предназначено для студентов медицинских вузов, обучающихся на английском языке.

This issue contains information that will be useful in the process of studying biochemistry.

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CONTENTS

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. Although the term “biochemistry” seems to have been first used in 1882, it is generally accepted that theword "biochemistry" was first proposed in 1903 byCarl Neuberg, a German chemist.

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 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 conversion. Over the last 40 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).

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

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

Methods used in biochemistry

- *for separating and purifying of biomolecules*
  salt fractionation
  chromatography
  electrophoresis
  ultracentrifugation

- *for determining biomolecular structure*
  elemental analysis
  spectroscopy
  mass spectrometry
  X-ray crystallography
  use hydrolysis and enzymes to degrade the biomolecules

- *for determining substances concentrations*
  spectrophotometry
  colourimetry

Preparations for 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.

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 – 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 medecina"*. 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 consumed and carbon dioxide 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: 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 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.

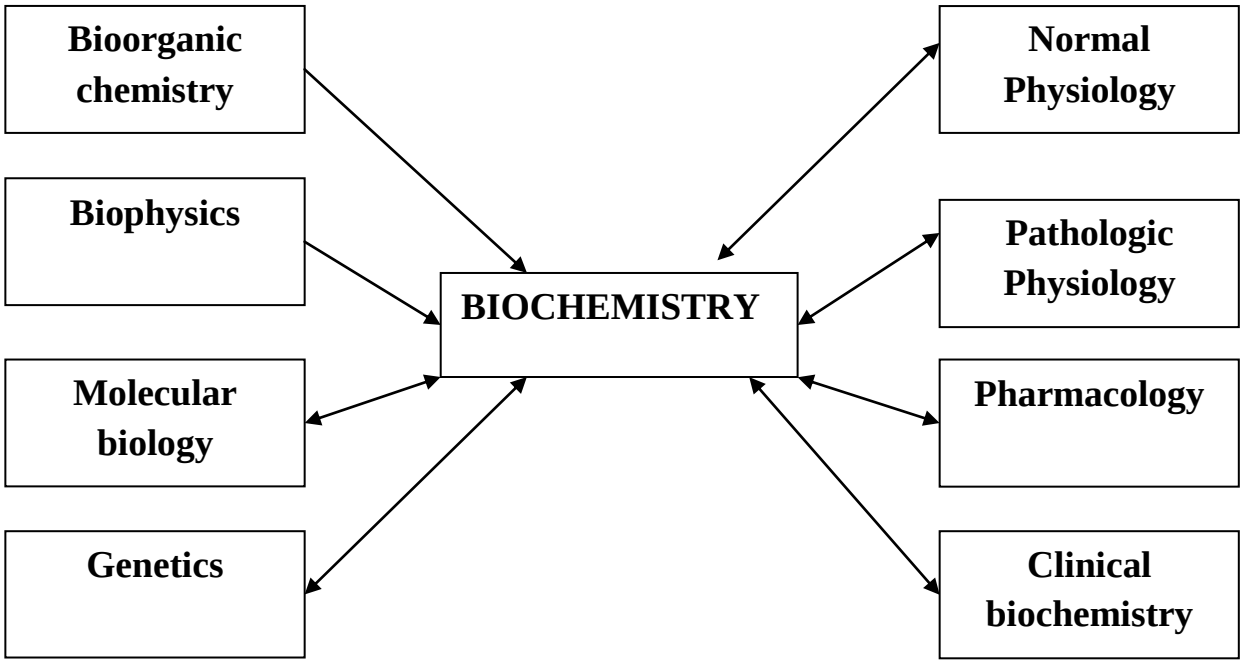


Fig. 1.1. Relations of biochemistry with other disciplines

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 to understand current therapies, which include recombinant proteins, such as human insulin or erythropoietin synthesized by bacteria. It helps to understand the action of new drugs. In the 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

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 and its role in organism

Amino acids are organic acids with at least one amino group. There are over 300 naturally occurring amino acids. In the human organism there are 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 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.

In addition to the 20 common amino acids, 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;
- 5-hydroxylysine, derived from lysine.

The former is found in plant cell wall proteins, and both are found in collagen, a fibrous protein of connective tissues.

Representatives of nonprotein amino acids ornithine and citrulline deserve special note because they 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 interchangeably.

Peptides exhibit a number of specific functions. Even the smallest peptides can have biologically important effects. 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, a 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.

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.

Table 2.1.

Occurrence of proteins in 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%

Total proteins content in a human body is 45% of dry mass.

Physico-chemical properties of proteins

- Molecular mass;
- shape and size of molecules;
- high viscosity in solution;
- low diffusion;
- pronounced swelling ability;
- optical activity;
- carge;
- mobility in electric field;
- low osmotic and high oncotic pressures;
- solubility;
- ability to absorb uv light at 280 nm;
- amphoteric;
- denaturation.

Denaturation of proteins. Destruction of three-dimensional structure of protein molecule with loss of 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.

Molecular mass of proteins varies from 6,000 Da to over 1,000,000 Da. For determination of molecular mass 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.

Protein purification stages:

- selecton of a protein source
- homogenization and solubilization

- separation:
    - salting-out
    - chromatography
    - electrophoresis
- dialysis.

Structural levels of organization of protein molecules

The *primary structure* consists of a sequence of amino acids linked together by peptide bonds and includes any disulfide bonds.

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.

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.
2. Edman degradation procedure (carried out on a machine, called a sequenator).
3. Reaction with dansyl chloride.
4. Use of aminopeptidase.

Methods for determination of C-terminal amino acids

1. Akabori's method.
2. Reaction with cyanogen bromide (CNBr).
3. Use of carboxypeptidase.

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

Secondary structure is particularly stable arrangements of amino acid residues giving rise to recurring structural patterns.The most prominent types of secondary structure are the α-helix and β-sheet.

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

Tertiary structure is the complete three-dimensional structure of a polypeptide chain. 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.

Tertiary structure is stabilized by ionic, hydrogen, covalent (disulfide) bonds and hydrophobic interactions.

Quaternary structure results from interactions between the subunits of multisubunit (multimeric) proteins or large protein assemblies. Some 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 a 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.

The association of polypeptide chains can serve a variety of functions:

- many multisubunit proteins have regulatory roles; 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 on 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 site 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. Folding for many proteins is facilitated by the action of specialized proteins. Molecular chaperones are proteins that interact with partially folded or improperly folded polypeptides, facilitating correct folding pathways or providing microenvironments in which folding can occur.

A misfolded protein appears to be the causative agent of a number of rare degenerative brain diseases in mammals.

Simple and conjugated proteins

Depending on their composition proteins can be classified as simple and conjugated. Simple proteins composed of amino acid residues only. Conjugated proteins 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. 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)
  include 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, the enzyme 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. Examples of phosphoproteins are casein in 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. A 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 called specific proteins that are part of all cells and tissues of living organisms which serve as biological catalysts.

General 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 enzymes from 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, guanylate cyclase, 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	thiamindiphosphate	transketolase, pyruvate dehydrogenase
B_2-riboflavin	FMN, FAD	Flavin-dependentdehydrogenase
B_3-pantothenic acid	coenzyme A (CoA)	reactionof acylation
B_6-pyridoxine	pyridoxal-phosphate	aminotransferase
PP-nicotinamide	NAD, NADP	NAD (NADP)-dependent dehydrogenase
Folic acid	Tetrahydrofolic acid	transferring one-carbongroups

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 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, required Zn^{2+} ions.

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 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 are 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). 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 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:

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

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.

Energy changes in chemical reactions

Any chemical reactions taking place, 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. Increase the rate of a chemical reaction can be heat. This 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.

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 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.

Active site at all stages of enzymatic catalysis can not 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, while urease synthesizes 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 aspartatdecarboxylase 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 of 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

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 a*daptive 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. Specifics:
   a) irreversible
   b) reversible:

- competitive
- noncompetitive.

Nonspecific inhibitors cause denaturation of the enzyme - is 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.

Diisopropyl fluorophosphate (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 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

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 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 dehydrogenese 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 dehydrogenese. However, cleavage of two hydrogen atoms from malonic acid 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 acetylcholinesterase, which catalyzes the hydrolysis of acetylcholine on choline and acetic acid.

When adding inhibitors, activity of acetylcholinesterase 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 anticholinesterase 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 what leads to abnormal synthesis products. Abnormal products have no functional activityand 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.

Noncompetitive inhibitor may bind either to the enzyme or to the enzyme-substrate complex to form an inactive complex. Accession 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 of 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.

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 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).

Blood plasma enzymes

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

1. Secretory enzymes 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
acid phosphatase	prostate
alkaline phosphatase	bone

Enzymopathies

The reason for many diseases is disfunction 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:

$$A \xrightarrow{E1} B \xrightarrow{E2} C \xrightarrow{E3} D \xrightarrow{E4} P$$

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 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. 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 excrets 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 various appropriate substrates detect 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 enzyme);

• 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 develop 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. Preparation of milk.

CHAPTER 5

STRUCTURE AND FUNCTION OF NUCLEIC ACIDS

Nucleic acids are a 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.

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.

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

Chemical Composition of nucleic acids

DNA: adenine, guanine, cytosine, thymine, deoxyribose, phosphoric acid.

RNA: adenine, guanine, cytosine, uracil, ribose, phosphoric acid.

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

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.

Structure and function of DNA

DNA contains and stores the genetic information.

- It is 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 the 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. The orientation of the DNA strands is anti-parallel (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.

The DNA double helix is stabilized primarily by two forces:

1. hydrogen bonds between nucleotides;
2. base-stacking interactions among the aromatic nucleobases.

Tertiary structure of DNA is different in prokaryotic and eukaryotic cells. In prokaryotesit is 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**.

The 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,
- to control gene expression and DNA replication.

There are four levels of chromatin organization (Fig. 5.1)

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. In interphase chromosomes chromatin fibers organized into **loops (3)** anchored in a scaffold. The final level of chromatin organization **is metaphase chromosome (4)**.

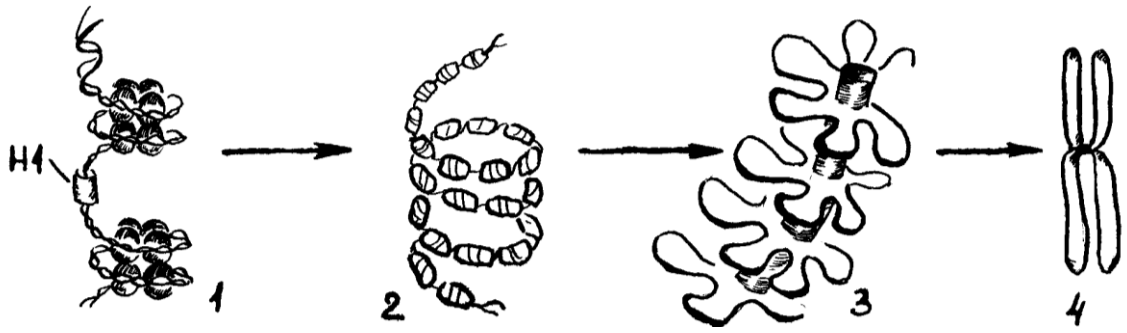


Fig. 5.1. Levels of chromatin organization

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, including intramolecular double-stranded regions that are important to their function. These secondary structures are the product of intramolecular base pairing that occurs between complementary nucleotides within a single RNA molecule. There are three general classes of RNA molecules found in prokaryotic and eukaryotic cells: ribosomal RNA (rRNA), transfer RNA (tRNA), and messenger RNA (mRNA). Each class has a distinctive size and function.

Messenger RNAs (mRNA) 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 (tRNA) 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 has been described as a clover leaf (Fig. 5.2). 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. 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.

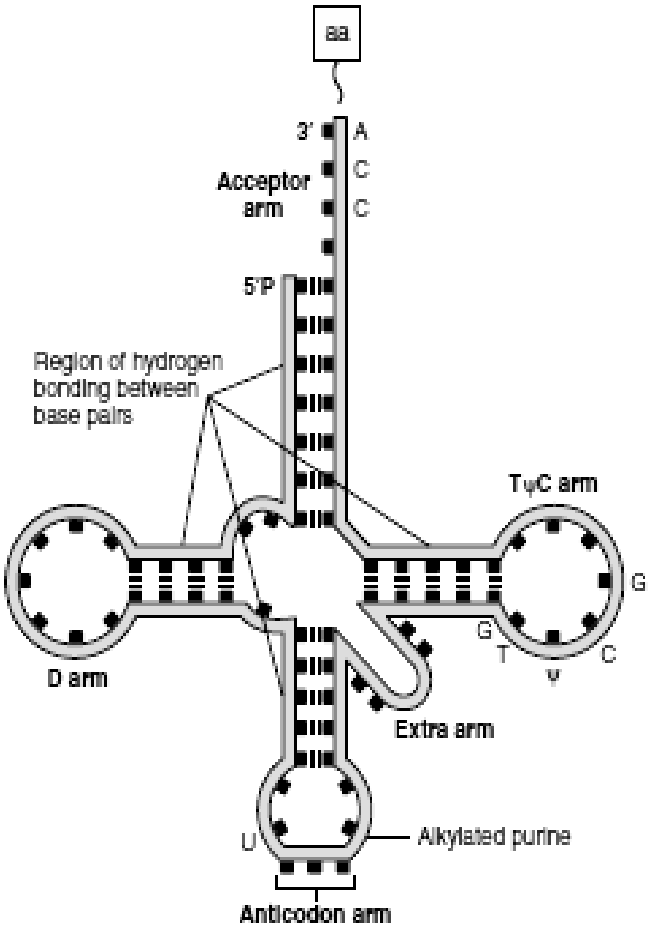


Fig. 5.2. Structure of tRNA

Ribosomal RNA (rRNA) is the structural and catalytic component of the ribosomes. rRNAfrom 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.

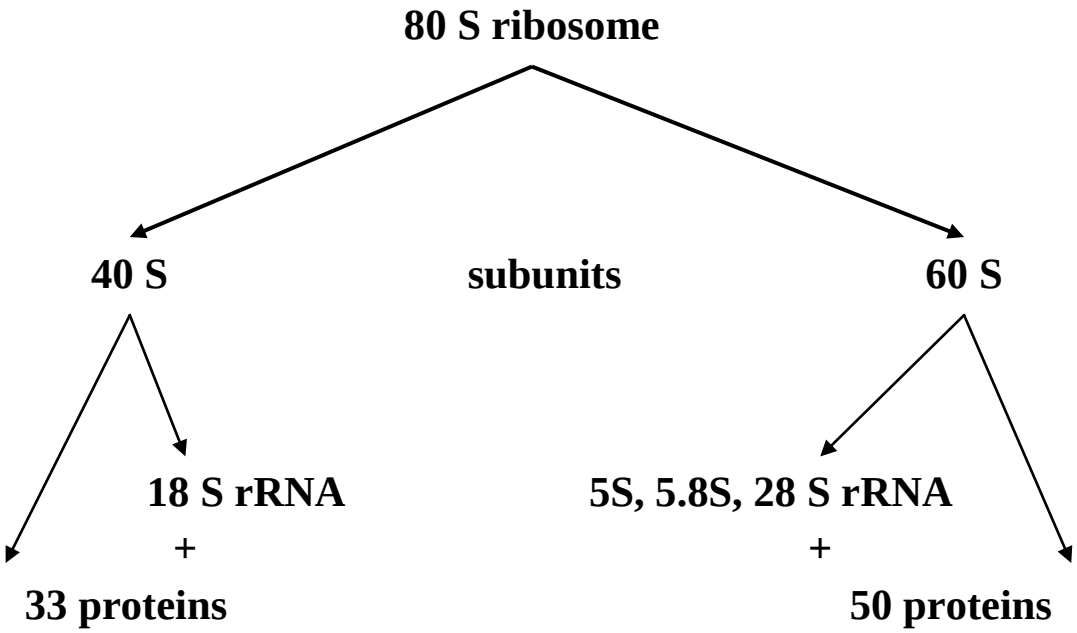


Fig. 5.3. Structure of eukaryotic ribosomes

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 pairwith one another can be used to detect similar DNA sequences in two different species or within the genomeof a single species. For example, if duplex DNAs isolated from humancells and from mouse cells are completely denatured byheating, then mixed some strands of the mouse DNA willassociate with human DNA strands to yield hybridduplexes, in which segments of a mouse DNA strandform base-paired regions with segments of a human DNAstrand. The closerthe evolutionary relationship between two species, themore extensively their DNAs will hybridize. The hybridization of DNA strands from

differentsources 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 thepresence of many other sequences, if one already hasan appropriate complementary DNA strand (usually labeledin some way) to hybridize with. Thecomplementary DNA can be from a different species orfrom the same species, or it can be synthesized chemicallyin the laboratory.

Hybridization techniques can be variedto detect a specific RNA rather than DNA. The isolationand identification of specific genes and RNAs rely onthese hybridization techniques. Applications of thistechnology make possible the identification of an individualon the basis of a single hair left at the scene of acrime or the prediction of the onset of a disease decadesbefore 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.

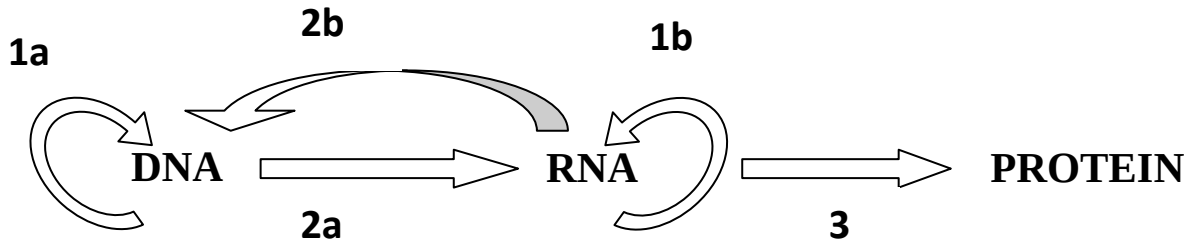


Fig. 6.1. Ways of transport of genetic information

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

Biosynthesis of DNA

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.

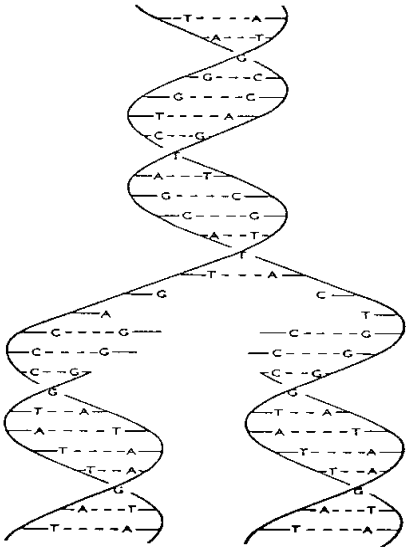


Fig. 6.2. Semiconservative replication of DNA

The site at which DNA replication is initiated is termed the "origin of replication". Eukaryotes have many origins of replication.

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.

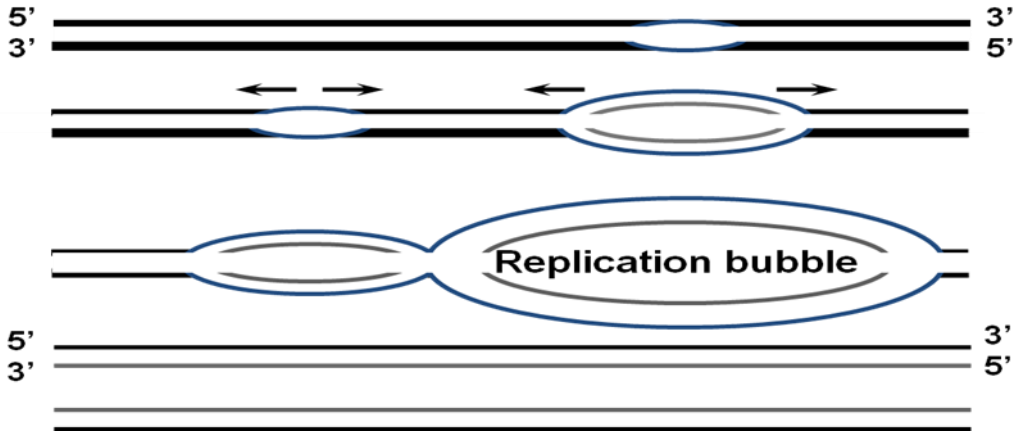


*Fig. 6.3.General sc**h**eme of replication*

One or both ends of the replication bubble are dynamic points, termed replication fork, where parent DNA is being unwound and the separated strands quickly 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 **DNApolymerase 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**.Inthe 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.

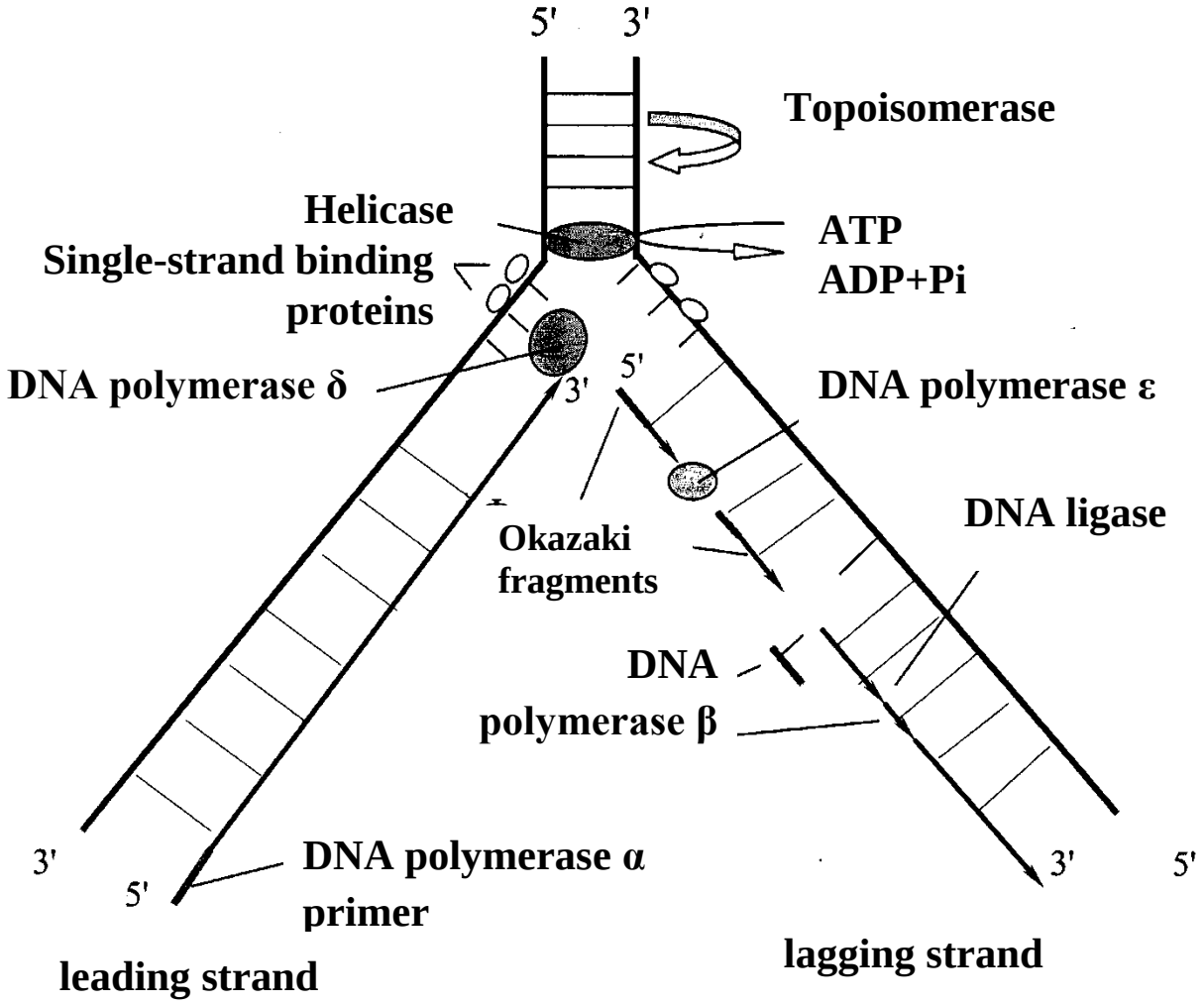


Fig.6.4. Replication fork

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.DNA polymerase δ** synthesizes the leading strand, **DNA polymerase ε –** 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

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. 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.

Types of DNA-dependent RNA polymerases:

- **RNA polymerase I** synthesizes the rRNAs;
- **RNA polymerase II** synthesizes mRNA;
- **RNA polymerase III** synthesizes tRNAs and 5S rRNA.

Transcription occurs in several stages:

- **initiation,**
- **elongation,**
- **termination**.

Initiation involves the interaction of the RNA polymerase with specific sites on the DNA, known as promoters (Fig. 6.5). Promoters are characteristic sequences of DNA, usually 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.

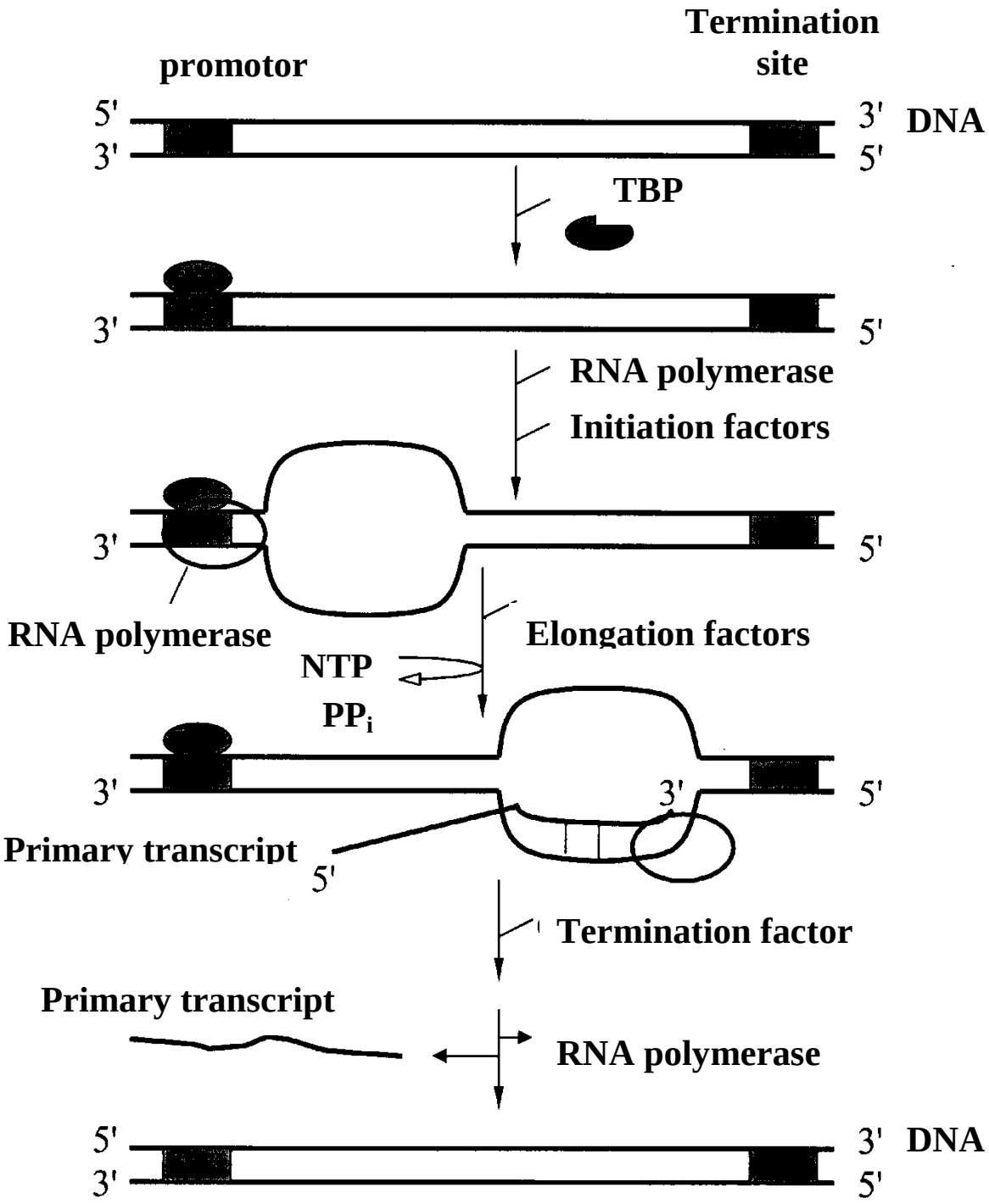


Fig. 6.5. Scheme of reverse transcription

TBP – TATA-binding protein

RNA processing

RNA molecules are usually processed before they become functional. A newly synthesized RNA molecule is called a **primary transcript**.

Most eukaryotic **mRNAs** contain specific structures that are added to their 5' and 3' ends after transcription has occurred. A 7-methyl guanine residue 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.**

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.Thesequence 5'-CCA-3' is added to the 3' end of tRNAs.After processing, specific nucleotides are modified to give the unusual complement of bases found in most tRNAs.

The eukaryotic **rRNAs** are synthesized as a single RNA transcript with a size of 45S and. 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.

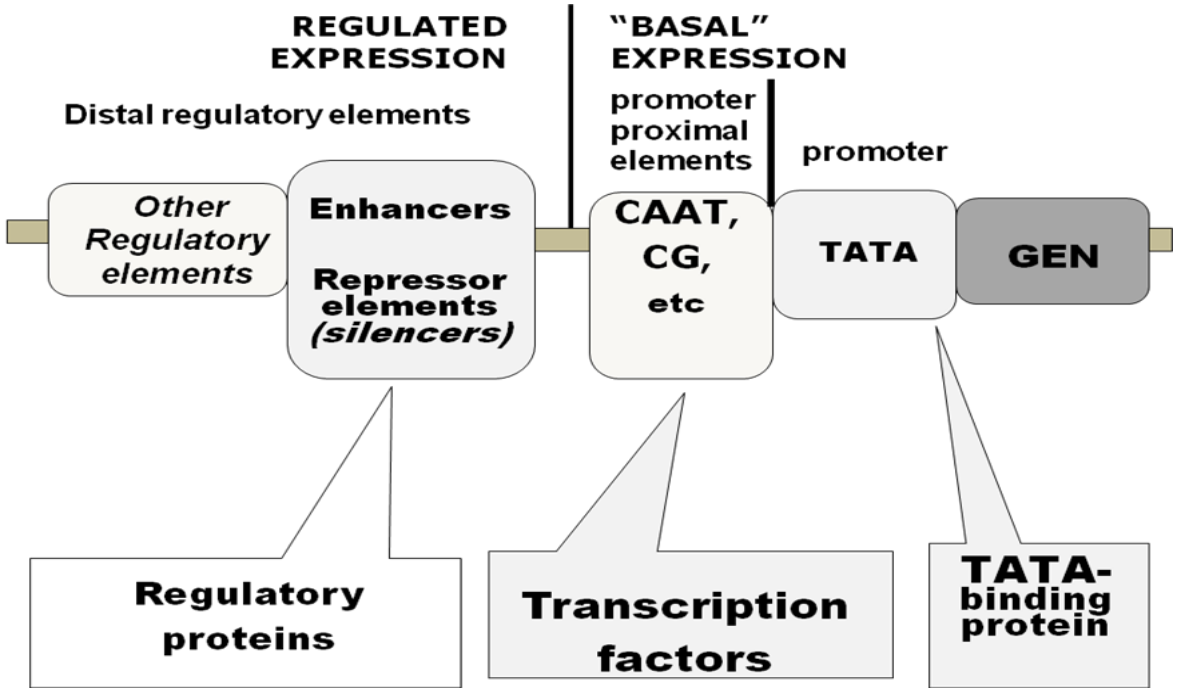


Fig. 6.5. Transcription control regions

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.

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 withinthe viral particle an RNA-dependent DNA polymerasecalled **reversetranscriptase**. On infection, the viral RNA and the enzyme enter the host cell. The reverse transcriptasefirst catalyzes the synthesis of a DNA strand complementary to the viral RNA (Fig. 6.6), 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. Some retroviruses cause cancer and AIDS.

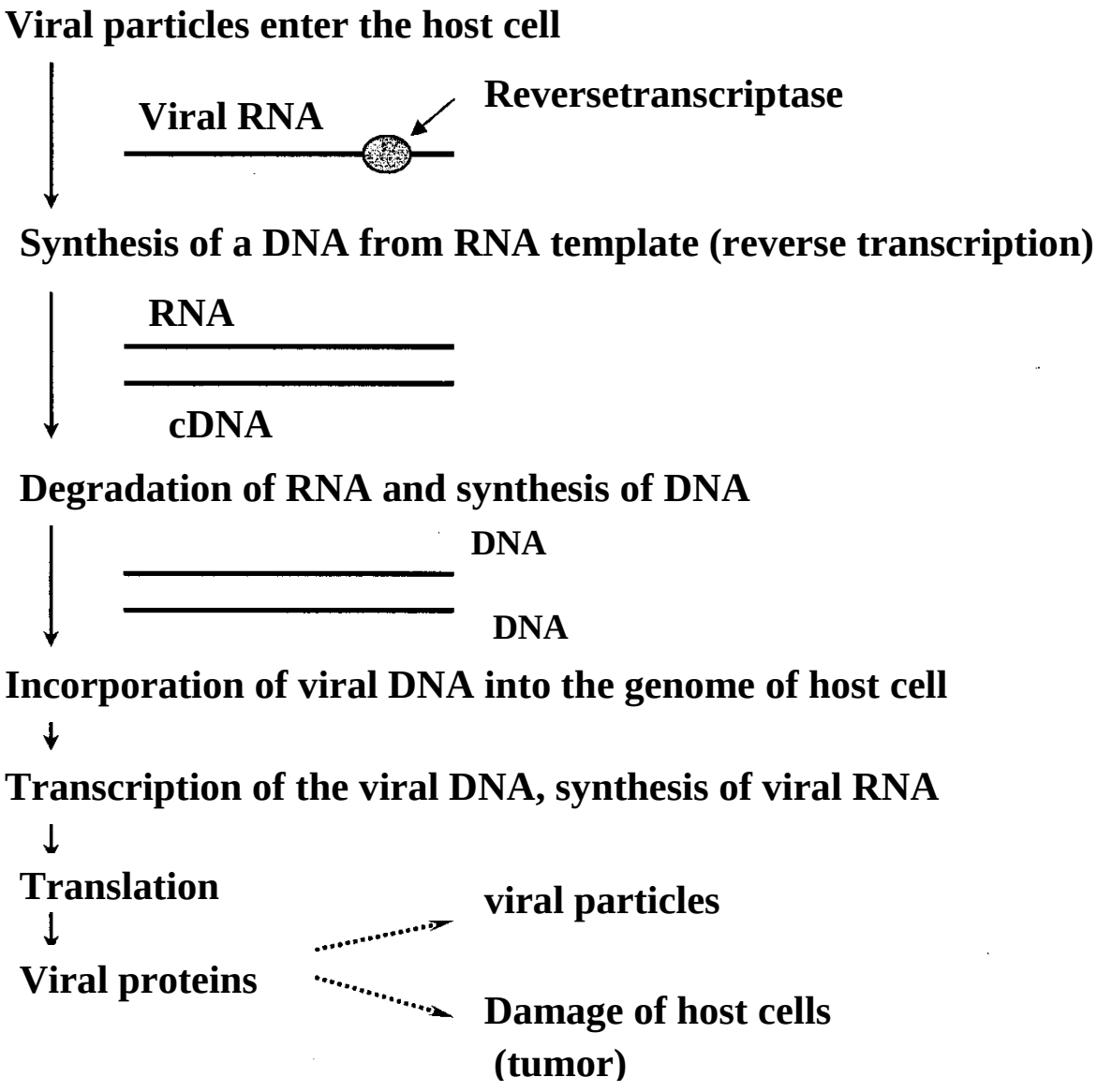


Fig. 6.6. Scheme of reverse transcription

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 – multiplecodons decode the same amino acid;
- unambiguous – given specific codon indicate only a single amino acid;
- nonoverlapping – 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 allspecies, with some minor deviations in mitochondriaand 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 massage is read in direction 5′→3′

Stages of protein synthesis:

- activation of amino acids;
- translation, which divided into three steps:
  - initiation;
  - elongation;
  - termination;
- Post-translational modificationof proteins.

Activation of amino acids

Amino acids are activated and attached to their corresponding tRNAs by highly specific enzymes aminoacyl-tRNAsynthetases. Each aminoacyl-tRNA synthetase recognizes a particular amino acid and the tRNAs specific for that amino acid. An amino acid first reactswith 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.

Translation in eukaryotes

The translation of mRNA begins near the 5' end and moves towards the 3' end, and proteins are synthesized starting with their amino-terminal ends and progressing toward the carboxy-terminal end; thus the 5' end of the RNA corresponds to the amino-terminal end of the protein; the 3' end of the RNA corresponds to the carboxy-terminal end of the protein.

Initiation of 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. 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 basepairs with this codon. After that the large ribosomal subunit (60S) joins the complex and protein synthesis is ready to begin.

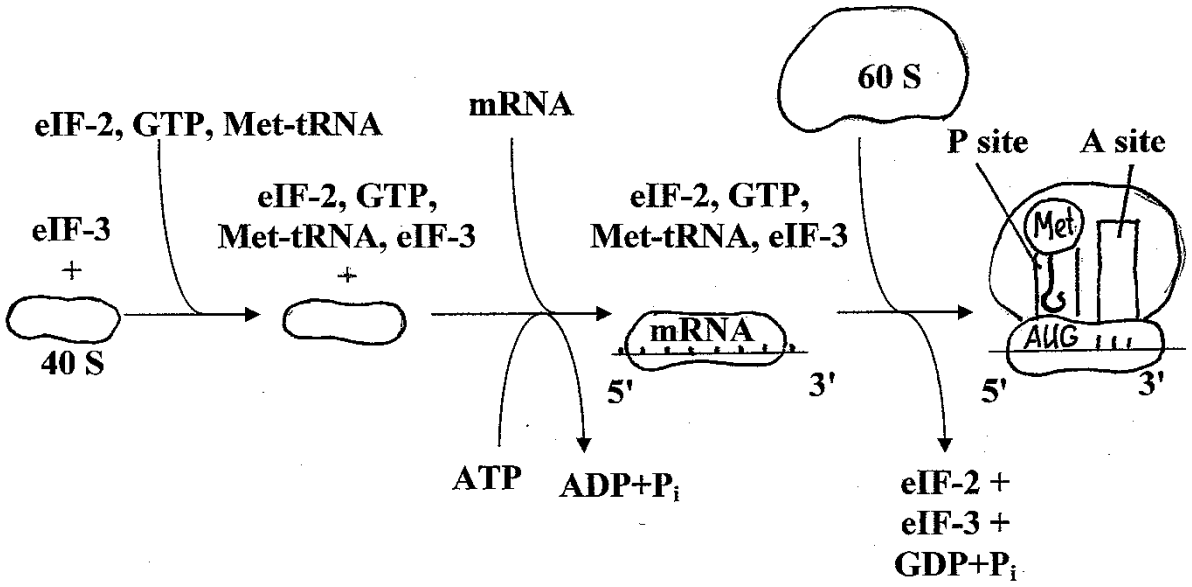


Fig. 7.1. Initiation of translation.

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.

Elongation is a cyclic process on the ribosome in which one amino acidis added to the nascent peptide chain. Elongation begins with the binding of an aminoacyl-tRNA to the A site of the ribosome (Fig. 7.2).

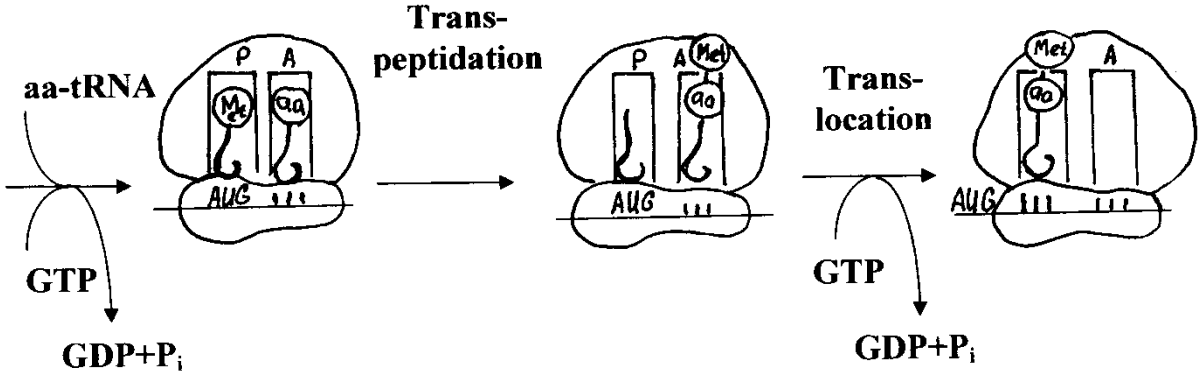


Fig. 7.2. Elongation.

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.

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 recognize these codons, and cause the protein that is attached to the last tRNA molecule in the P site to be released (Fig.7.3). 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 cyclecan be repeated.

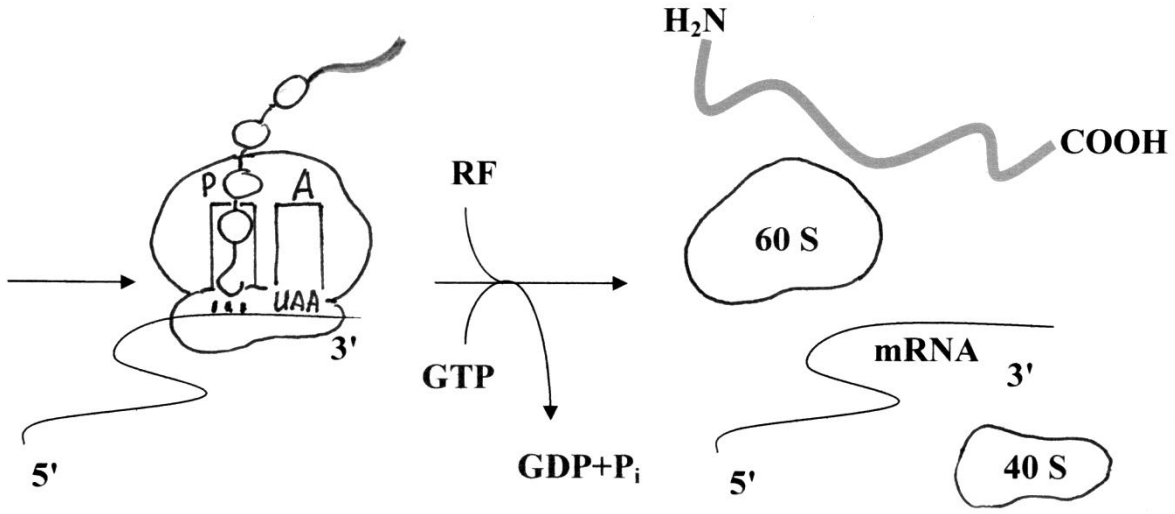


Fig. 7.3. Termination of translation.

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 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;
- nalf-life of mRNA;
- level of translation;
- posttranslational 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, and factors that effected alternative intron splicing, post-transcriptional editing of pre-mRNA, and 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 on 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.

mRNAs have different half-lives. Some are degraded more rapidly than others. Protein synthesis can be regulated at the transcriptional level. For example, iron status regulates translation of an iron carrier-protein.

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 50 S ribosome subunit, causes misreading of mRNA
Erythromycin	bacterial 50 S ribosome subunit, prevents translocation
Chloramphenicol	bacterial ribosome-inhibition of peptidyl transferase activity
Cycloheximide	eukaryotic 80 S 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** (also called 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 staggeredcuts 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 endonucleasescleave both strandsof DNA at the opposing phosphodiester bonds, leaving no unpaired bases on the ends, often called blunt ends.Sticky ends are particularly useful in constructinghybrid 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.

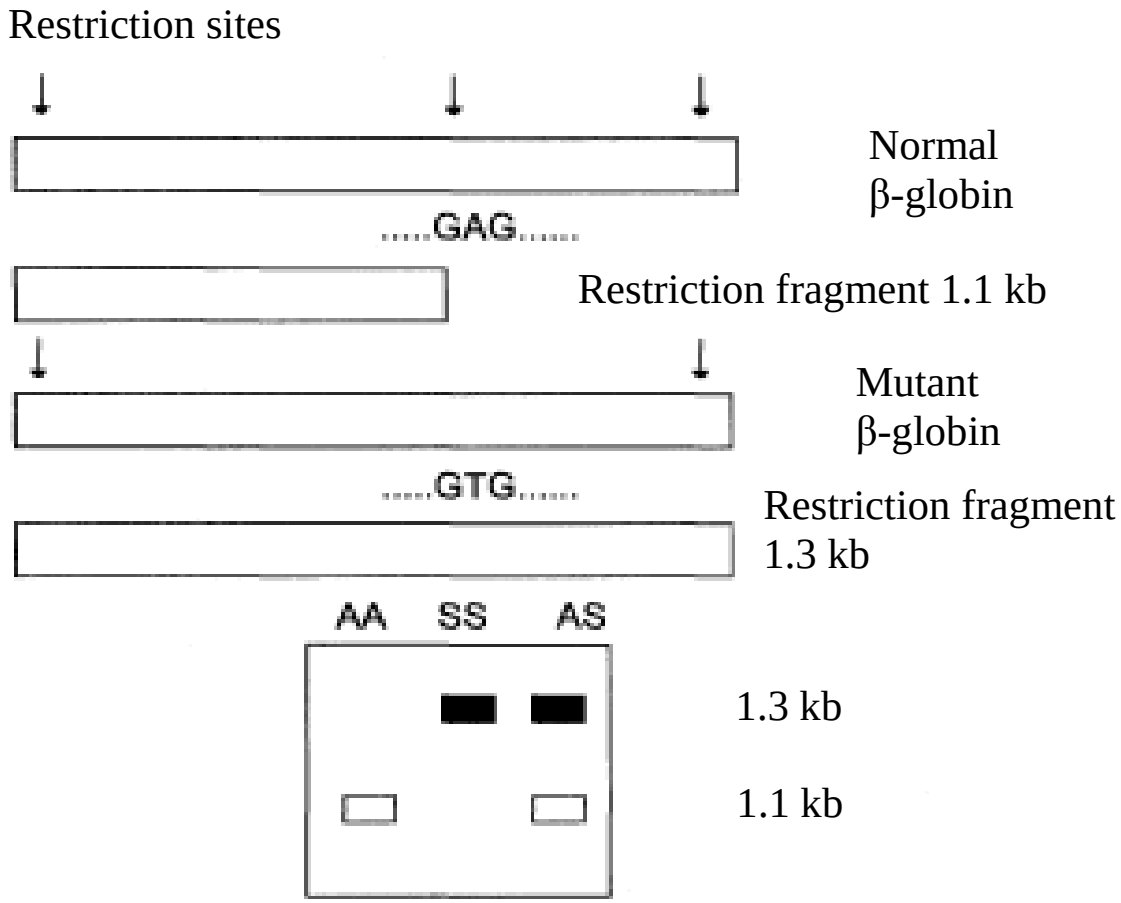


Fig. 8.1. Restriction fragment length polymorphisms

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, respective.

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, with 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 then be 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). 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.

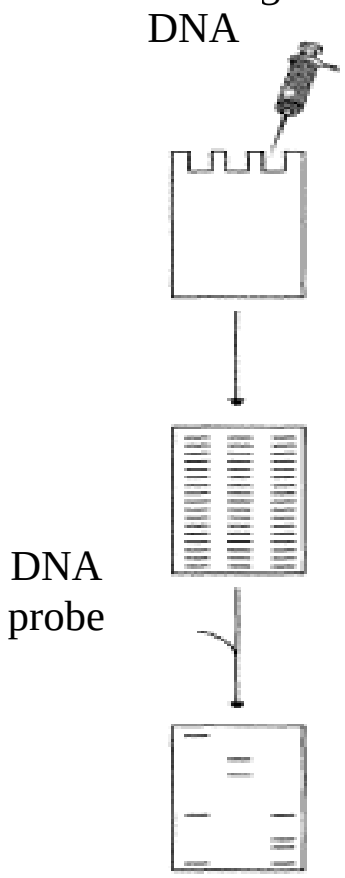


Fig. 8.2. Southern blotting

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 such that the number of copies of the desired DNA template increases simply due to the exponential increase in number of replicating host cells;
- enzyme-based DNA cloning (cell-free): this method 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 istypically 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).

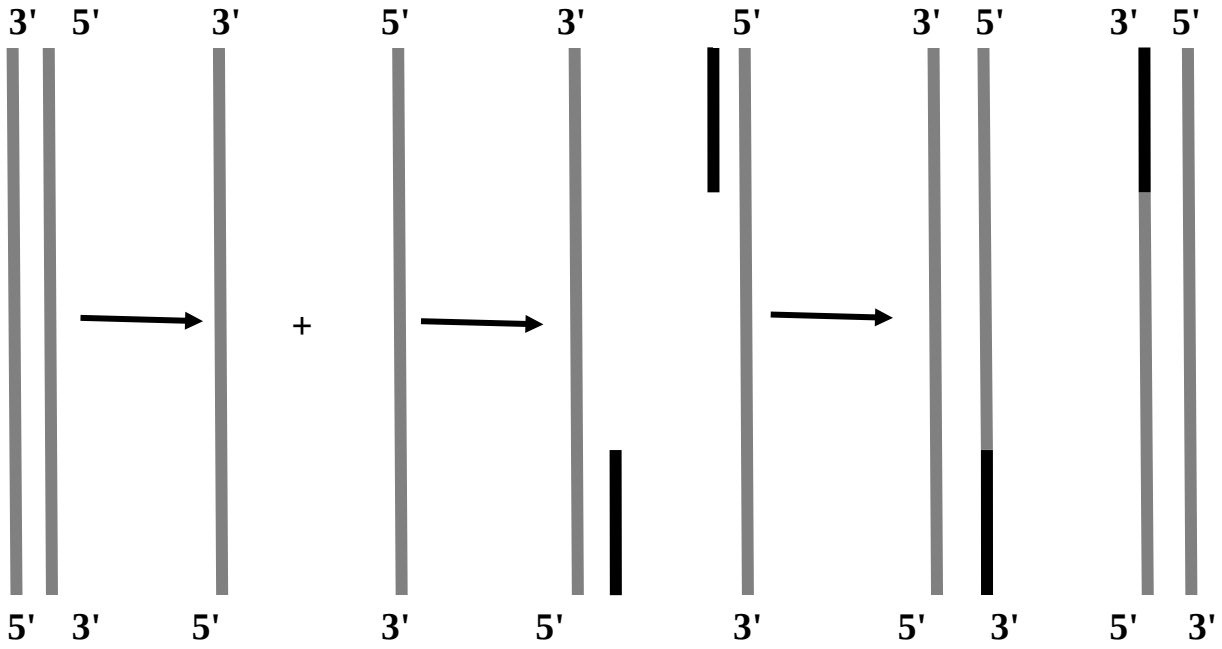


Fig. 8.3. Polymerase chain reaction

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 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'-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. This means that in any one 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.

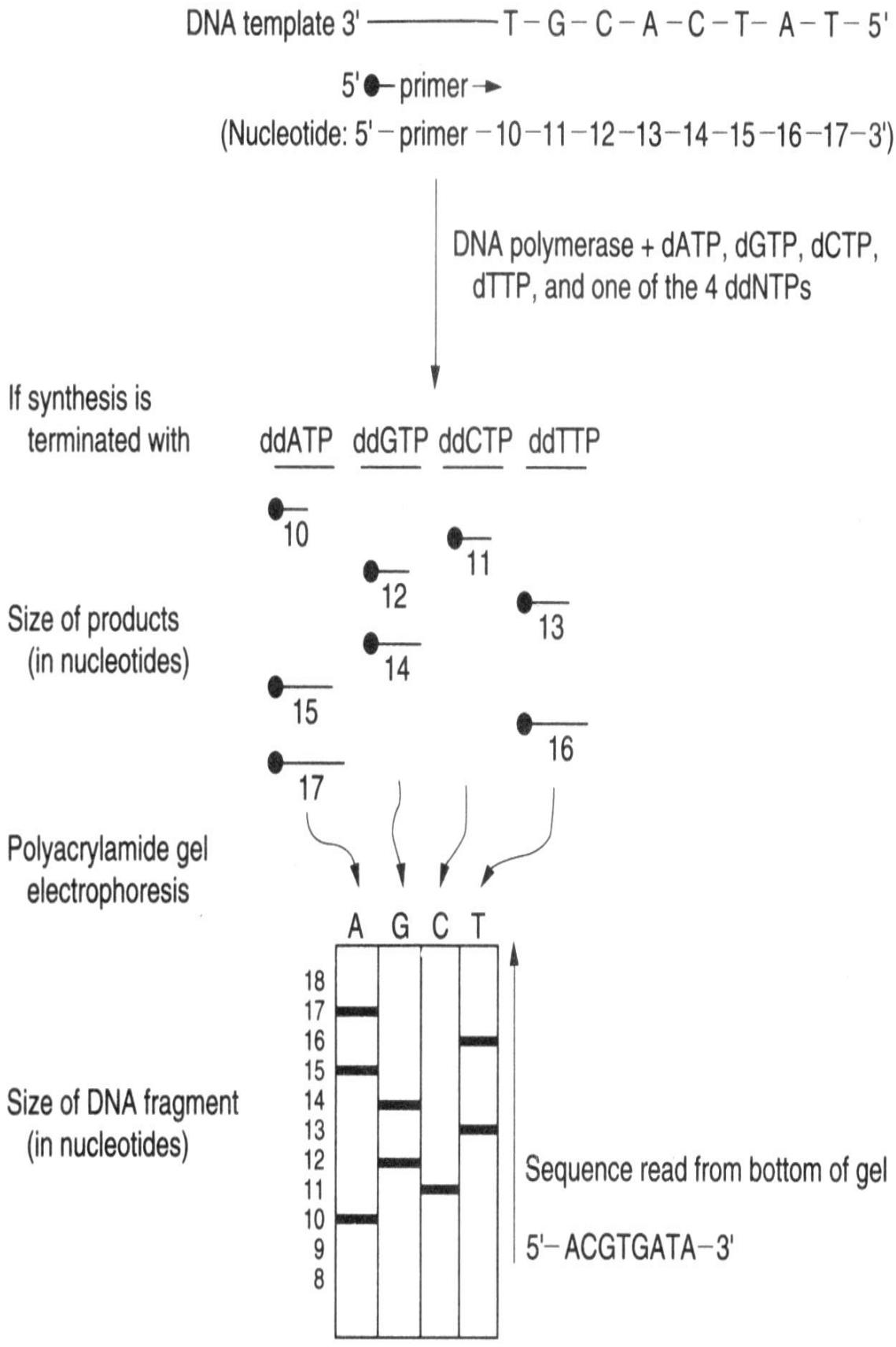


Fig. 8.4. Chain termination DNA sequencing

CHAPTER 9

INTRODUCTION INTO METABOLISM

Metabolism represents all the **enzymatic reactions and physical processes** in the body.

Metabolism includes three steps:

1) the uptake of substances from the outside (nutrition, respiration);

2) conversion of substances in the body, i.e. all chemical reactions occurring in the cell;

3) excretion of end products from the body.

Functions of metabolism

1) **synthesis** of molecules that are required for the cell structure and functioning;

2) **cleavage** of molecules to end products and their removal from the body;

3) production and utilization of **energy**.

Thus, metabolism provides the organism with materials and energy essential for its vital activity, growth and reproduction. Perpetual metabolism is the most important property of life. The cessation of metabolic processes means the cessation of life.

In the cell, different substrate molecules undergo a number of chemical changes (reactions) catalyzed by enzymes. Definite succession of such chemical conversions is referred to as **metabolic pathway**, and the intermediate products involved in this process are called **metabolites**.

There are **two sides** of metabolism: anabolism and catabolism.

Anabolism is synthesis of complex molecules from simpler components. This process requires energy.

Catabolism is degradation of complex molecules to simpler components. In this process, energy is released.

The catabolic and anabolic pathways are closely interrelated. Energy produced in catabolic processes is used for anabolic reactions.

Major end products of catabolism

The major classes of organic compounds in the body may undergo cleavage to end products (Table 9.1.).

Table 9.1.

End products of catabolism

Body substances	***End products***
Carbohydrates	CO_2, H_2O
Lipids	CO_2, H_2O
Proteins (amino acids)	CO_2, H_2O, NH_3, urea, creatinine, indican CO_2,
Nucleic acids	H_2O, NH_3, uric acid

Specific and common pathways of catabolism

The major classes of compounds in the food are carbohydrates, lipids, and proteins. They are degraded in the gastrointestinal tract, so that smaller molecules (constituents) are produced. The products of digestion are absorbed into the blood and supply tissues with nutrients which enter the cells. In the cell, the components are converted to acetyl CoA.

These steps represent **specific pathways** of catabolism (Fig.9.1). Acetyl CoA is the common product of specific pathways for catabolism not only of food components but also of tissue fuels (carbohydrates, fatty acids, and amino acids).

Then, the **common pathways** of catabolism take place. Acetyl CoA undergoes utilization in the tricarboxylic acid cycle (the TCA cycle) to produce two molecules of CO_2.

Reduced substrates (SH_2) and reduced cofactors ($NADH_2$, and $FADH_2$) are oxidized in the electron transport chain (the ETC) to produce energy a major portion of which is stored as ATP (Fig.9.1).

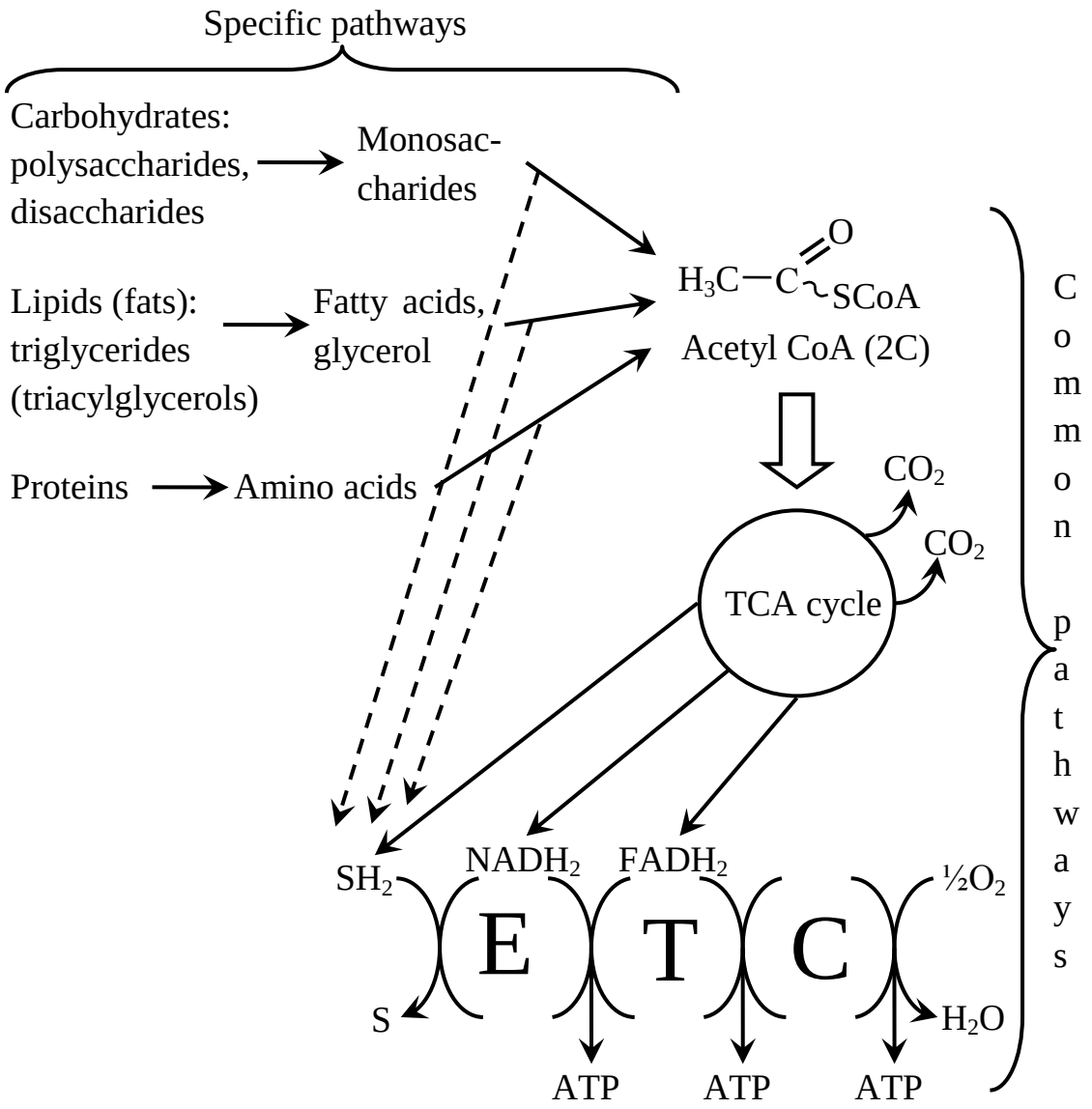


Fig. 9.1. Scheme of common and specific pathways of catabolism. TCA cycle – the tricarboxylic acid cycle; ETC – the electron transport chain. ***Specific pathways*** *of catabolism: carbohydrates, lipids, and proteins of the human body or from the food are degraded in the body to smaller molecules which are then converted to acetyl CoA.* ***Common pathways*** *of catabolism: acetyl CoA is utilized in the TCA cycle to form CO_2, reduced substrates (SH_2) and reduced coenzymes ($NADH_2$ and $FADH_2$). These reduced components are getting oxidized in the ETC to produce ATP.*

Experimental study of metabolism

Levels of study

1) the whole organism;
2) isolated organ;
3) organ slices;
4) cell culture;
5) homogenates of tissues (tissues may be ground to a homogenous state, i.e. may be undergone homogenization – destruction of cell membranes);
6) separated subcellular fractions;
7) purified enzymes.

Methods of study

1) colorimetry
2) spectrophotometry
3) centrifugation
4) chromatography
5) electrophoresis
6) determination of the enzymatic activity
7) isotope methods

The use of isotope tracers

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 to understand the metsbolic fate (distribution and conversions) of the labeled substance in the body, investigate the membrane permeability for the substance, assess the half-life time of the substance.

CHAPTER 10

MEMBRANES

Structure and composition of membranes

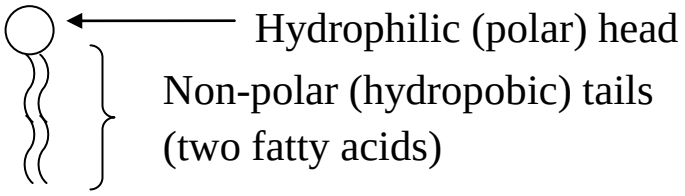
Major components of membranes are lipids, proteins and small amount of carbohydrates.

Carbohydrates in the membrane combine with lipids or proteins to form glycolipids and glycoproteins, respectively.

Membrane lipids

1) **Phospholipids**. This is the major class of the membrane lipids. Representatives: phosphatidylcholine, phosphatidyl-ethanolamine, phosphatidylserine, phosphatidylinositol, acetalphosphatides, cardiolipins.

Phosphilipids have polar head and non-polar tail (composed of two fatty acids):



2) **Sphingophospholipids** (sphingomyelin).

3) **Cholesterol.**

4) **Glycolipids** (cerebrosides, gangliosides and sulphatides). They have carbohydrate chains oriented towards the extracellular side of the membrane.

In membranes, all the lipids have hydrophilic and hydrophobic parts and 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 a constituent of membranes.

Membrane proteins

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

2) **Peripheral** proteins are situated on the surfaces of the bilayer.

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

Types of membrane proteins

1) **Structural** proteins (components of the membrane);

2) **Transport** proteins (they transfer substrates and ions across the membrane);

3) **Enzymes** (catalyze biochemical reactions);

4) **Receptors**;

5) **Tissue-specific antigens**.

Structure of membranes

The model of a membrane is called the fluid mosaic model. This model is compared with icebergs floating in a sea (the icebergs are proteins, the sea is phospholipids).

Properties of membranes

1) **Self-assembly**.

2) **Selective permeability**. Membranes are impermeable to many molecules. Certain molecules can freely pass through the membrane. For some other molecules there are specific channels in membranes.

3) **Asymmetry**.

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

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

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. The increase of fluidity leads to the increase of permeability.

Functions of membranes

1) **Separation**. Membranes separate:
   - the cell content – from the external environment,
   - one cell –from another,
   - different parts of the cell.

2) **Barrier** function. Membranes maintain different concentration of substances on the both sides of the membrane.

3) The plasma membrane provides **cell-to-cell interactions**.

4) **Receptor** function. Receptor can recognize and bind substances, e.g. hormones, lipoproteins.

5) **Catalytic** function. Some membrane proteins are enzymes.

6) **Transport** of molecules. Membranes contain channels and pumps thus providing selective transport of ions and substrates.

Transport mechanisms

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

- **Uniport** – one type of molecules moves across the membrane. E.g. calcium pump.
- **Symport** – two types of molecules move simultaneously at the same direction across the membrane. E.g. Na^+-glucose transporters and Na^+-amino acid transporters.
- **Antiport** – two types of molecules move in opposite directions across the memebrane, e.g. Na^+ moves out of the cell, and K^+ into the cell, i.e. sodium pump.

Types of transport mechanisms

1) Passive transport: simple diffusion and facilitated diffusion;
2) Active transport: primary and secondary active transport;
3) Transport of macromolecules across the plasma membrane: exocytosis and endocytosis.

Passive transport (diffusion)

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

There are two types of passive transport:

1) **Simple diffusion** – it does not require transfer proteins (e.g. transport of H_2O, CO_2, O_2).

2) **Facilitated diffusion** – requires transfer proteins which are called transporters, permeases, translocases, or carrier proteins. These carrier proteins are regulated by hormones. E.g. insulin activates the glucose transporter to transfer the carbohydrate into skeletal muscle cells and adipocytes. Glucocorticoids increase transport of amino acids into the hepatocytes. Growth hormone increases transport of amino acids into all cells.

Active transport

In this type of transport, molecules are transported across the membrane against their concentration gradient. This process requires energy. There are two subtypes of active transport: primary active transport and secondary active transport.

- **Primary active transport** uses ATP as source of energy. E.g. Na^+,K^+-ATPase, or sodium pump, Ca^{++}-ATPase, or calcium pump, H^+-ATPase.
- **Secondary active transport** uses energy of membrane potential produced by primary active transport (e.g. amino acids and carbohydrates are transported into the cell in such a way).

Endocytosis

This is transfer of extracellular macromolecules into the cell. 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** – “drinking by the cell”. The cell takes up fluid and fluid contents.

• **Phagocytosis** – “eating by the cell”. It is the engulfment of large particles such as bacteria, viruses, products of the cells destruction. Phagocytosis occurs only in specialized cells, such as macrophages and granulocytes.

Exocytosis

This is the release of macromolecules from the cell to the exterior. The secretory vesicles with the substance move towards the plasma membrane and fuse with it. E.g. in such a way, enzymes (trypsinogen), hormones (insulin) and neurotranmitters (acetyl choline) are released from the appropriate cells.

CHAPTER 11

ENERGY METABOLISM

Bioenergetics of the cell. Free energy

Any molecule or chemical process can contain definite types of energy:

• Enthalpy (ΔH) is the **total energy** (or heat content) which may be available from any system, or molecule, or chemical reaction.

• Free energy (ΔG) is portion of energy which can be used **to perform work** at the constant temperature and pressure (the Gibbs' free energy).

• Bound energy (TΔS) is the portion of energy which **cannot be converted to work.**

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

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.

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.

- Phosphate-containing macroergic compounds: creatine phosphate, 1,3-bisphosphoglycerate, phosphoenolpyruvate, carbamoyl phosphate, ATP.
- Sulfur-containing macroergic compounds (thioesters): acetyl CoA, acyl CoA, succinyl CoA

Biological role of macroergic compounds

ATP is universal energy currency because only this compound can immediately give its energy (accumulated in the macroergic bond) for the performing any type of work in the living cell. All the other macroergic compounds take part in reactions of substrate-level phosphorylation. Creatine phosphate is depot of energy in muscles.

ATP: ways of its formation and use

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

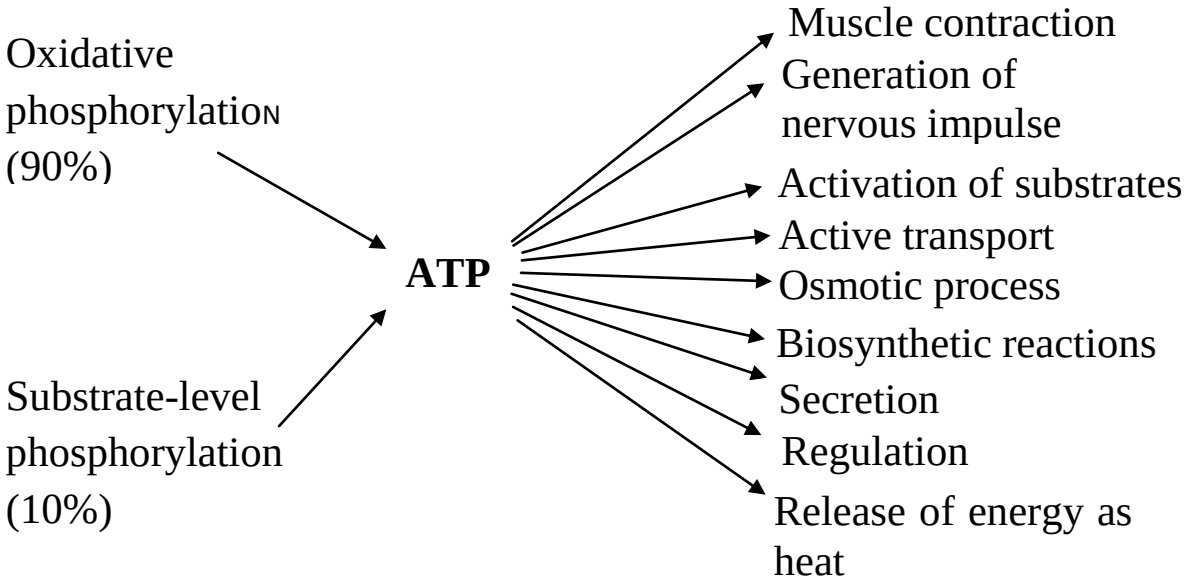


Fig. 11.1. Ways of the ATP formation and its use.

Oxidative phosphorylation is synthesis of ATP from ADP and P_i 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 P_i 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.

ATP plays central role in the energy metabolism in the body. ATP is used in many energy-requiring processes (various types of work and processes).

Electron transport chain

The electron transport chain (the ETC) is located in the inner mitochondrial membrane.

The ETC consists of several components (carriers) which follow one another in the definite sequence (Fig. 11.2). The components of the ETC transfer protons and electrons (or only electrons) from reduced substrates (SH_2) or from reduced coenzymes (such as $NADH_2$ or $FADH_2$) to oxygen (O_2) with the resultant formation of water. Thus, in the ETC, a number of oxidative-reduction reactions take place. (Oxidation is the loss of two electrons or two hydrogen atoms; reduction is the gain of them). Due to consecutive oxidation-reduction reactions in the ETC, 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.

There are two types of the ETC: the long chain and the short chain.

The **long chain contains**: NAD-dehydrogenase, FMN-dehydrogenase, coenzyme Q, and cytochromes (b, c_1, c, aa_3). In the long chain **three molecules of ATP** are produced (or, in oxidation of $NADH_2$ in the ETC, 3 ATP are produced).

The **short chain** contains: FAD-dehydrogenase, coenzyme Q, and cytochromes (b, c_1, c, aa_3). In the short chain **two molecules of ATP** are produced (or, in oxidation of $FADH_2$ in the ETC, 2 ATP are produced).

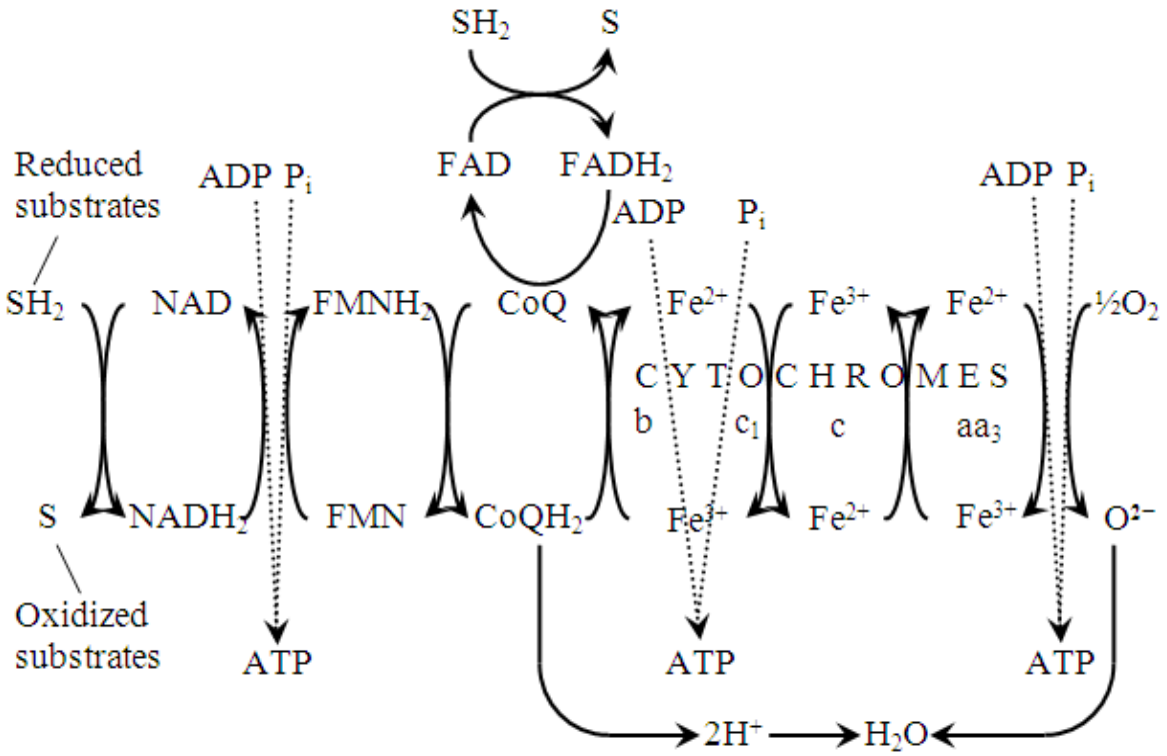


Fig. 11.2. Scheme of the electron transport chain (the ETC).

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-dehydrogenases:

$$SH_2 + NAD \rightleftharpoons S + NADH_2.$$

The substrates for NAD-dehydrogenases: malate, isocitrate, pyruvate, α-ketoglutarate, and glutamate.

In the long chain, NAD-dehydrogenases transfer hydrogen from a substrate to FMN.

NADP-dehydrogenases contain extra phosphate residue bound to the ATP ribose at position 2′; these enzymes mainly located in the **cytoplasm,** participate in **biosynthetic reactions** (e.g. in the 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 is located in the long ETC. The reaction catalyzed by FMN-dehydrogenases:

$$NADH_2 + FMN \rightleftharpoons NAD + FMNH_2.$$

FAD is located in the short ETC. The reaction catalyzed by FAD-dehydrogenases:

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

The substrates for FAD-dehydrogenases may be succinate and acyl CoA (the latter is active form of a fatty acid).

Both FAD- and FMN-dehydrogenases transfer hydrogen to coenzyme Q.

Coenzyme Q

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

Coenzyme Q accepts two hydrogen atoms from $FMNH_2$ (in the long chain) or from $FADH_2$ (in the short chain) 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 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. Beside iron, cytochrome aa_3 contains copper, which is reversibly reduced and oxidized between Cu^{2+} and Cu^{+}:

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

Oxidative phosphorylation

Oxidative phosphorylation is a process of generation of ATP from ADP and P_i as a result of energy production in the ETC. The components of the ETC form three large protein complexes in the inner mitochondrial membrane. Each complex uses energy from the transfer of electrons to pump protons from mitochondrial matrix to the outer side of the inner membrane.

The mechanism of oxidative phosphorylation is described by chemiosmotic theory proposed by P. Mitchell (Nobel laureate). According to this theory, **mitochondrial transference of electron is coupled to the ATP synthesis through a proton gradient**. This means that the energy derived from the transfer of each pair of electrons through the ETC is used to release three pairs of protons across the inner mitochondrial membrane from the matrix into the intermembrane space (Fig. 11.3).

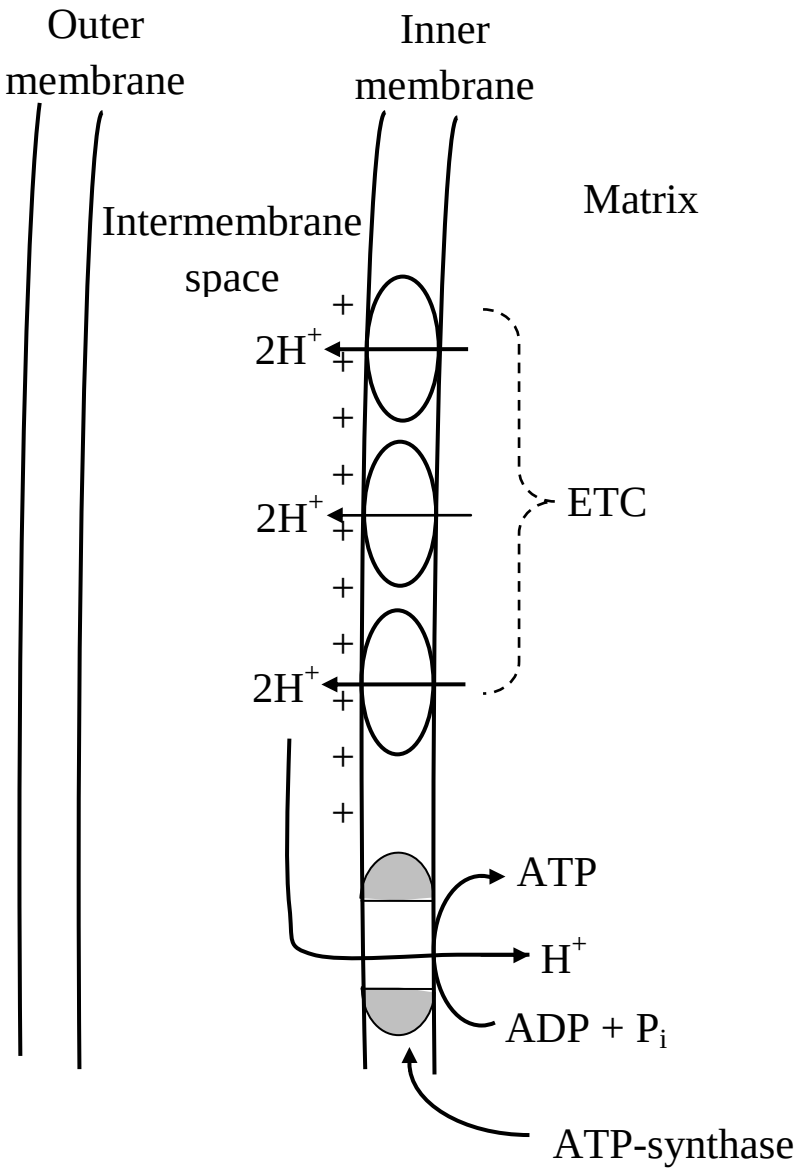


Fig. 11.3. Mechanism of oxidative phosphorylation of ADP (theory of P. Mitchell).

Consequently, the outer surface of the inner mitochondrial membrane acquires positive charge, and the inner surface of the inner membrane – negative charge. As a result, an **electrochemical potential** is generated on the inner mitochondrial membrane. This membrane is impermeable to protons. Protons can move back into the matrix only through the **ATP-synthase complex** located here in the inner membrane.

The ATP-synthase complex contains proteins which form a channel in the inner mitochondrial membrane. Through this channel, protons can flow. The transfer of protons from the zone of higher H^+

concentration into the zone of lower H^+ concentration is accompanied by the release of free energy. This free energy 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**.

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

The P/O ratio. The P/O ratio is used for the characteristics of energy production in the 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 consumed.

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 $NADH_2$ 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: ADP, P_i, oxygen, and reduced substrates (SH_2).

Inhibitors inhibit the ETC, and decrease synthesis of ATP. The inhibitors of the ETC: sleeping-draughts (barbiturates, amytal, amobarbital, chloropromasin, etc.); antibiotics (antimycin); piericidin; such poisons as cyanine and carbon monoxide (CO).

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 lost as heat.

Uncoupler agents uncouple the ETC and oxidative phosphorylation. As a result, the ETC keeps functioning (substrates are oxidizing, oxygen is consuming, and the energy is generating) but ATP production does not occur, and all the energy generated 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 production). The examples of uncoupler agents: 2,4-dinitrophenol, hormones thyroxine and progesterone, some antibiotics, calcium ions, dicoumarol (anticoagulant agent), fatty acids, components of the brown adipose tissue, constituents of microbial cells.

Disorders of energy metabolism. Hypoxia

The living cell has constantly the need in 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**.

Subtypes of endogenous hypoxia:

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 blood 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** is observed in **anemia** and is associated with the **decreased amount of red blood cells** or **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, carboxy hemoglobin 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 terminal enzyme of the ETC (cytochrome oxidase), which in turn inhibits consumption of oxygen by the cells.

The tricarboxylic acid cycle (the TCA cycle)

The TCA cycle is also called the citric acid cycle or the Krebs' cycle. It is located in the mitochondrial matrix.

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 is 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 $NADH_2$. These are the reactions catalyzed by isocitrate dehydrogenase, α-ketoglutarate dehydrogenase,

and malate dehydrogenase. Each of these reactions produces $NADH_2$. Then $NADH_2$ is oxidized in the ETC and forms 3 ATP. Totally, three $NADH_2$ produces 9 ATP.

b) One reaction produces $FADH_2$. This reaction is catalyzed by succinate dehydrogenase. $FADH_2$ is oxidized 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.

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

Regulation of the TCA cycle

The main sites for regulation of the TCA cycle are citrate synthase, isocitrate dehydrogenase, and α-ketoglutarate dehydrogenase. All these reactions are inhibited by $NADH_2$ (i.e. by the product of the TCA cycle), and ATP. Thus, the TCA cycle is ihibited when energy is abundant. When energy stores (ATP and $NADH_2$) 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 is the removal of electrons (2ē) or hydrogen atoms (2H) from a molecule.
- Reduction is the gain of 2ē or 2H by a molecule.

Oxidation of one molecule is accompanied by reduction of the other molecule.

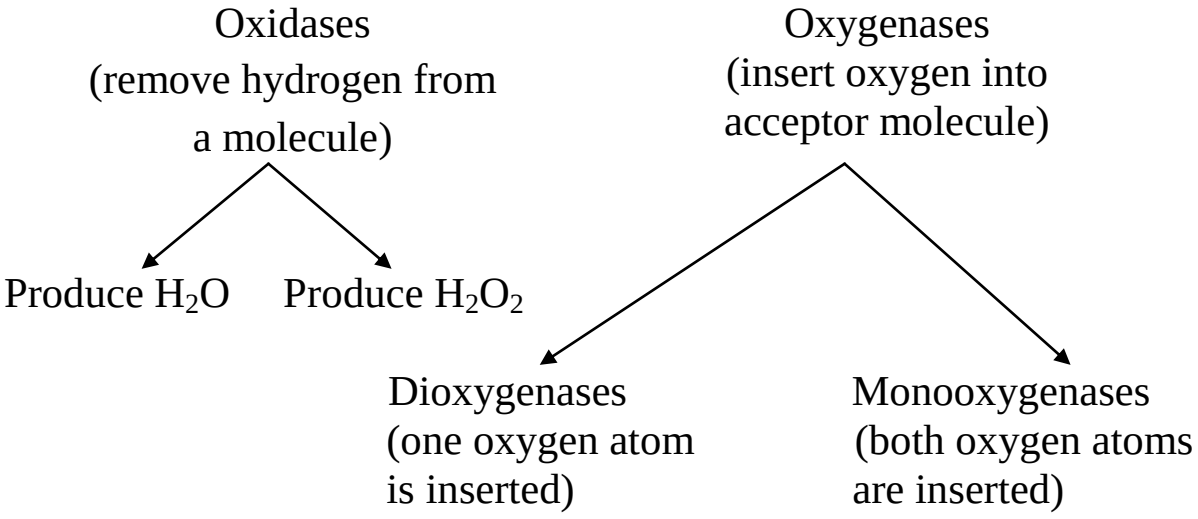
Oxidation of chemical substances in the body is performd by two ways:

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):

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

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

$AH_2 + O_2 + FAD$ (or FMN) $\rightarrow A + FADH_2$ (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 reaction is catalyzed by **monoxygenase,** or **hydroxylase,** and requires donor of hydrogen (usually $NADPH_2$):

$$A\text{-}H + O_2 + NADPH_2 \rightarrow A\text{-}\mathbf{O}H + NADP+ H_2O.$$

This type of oxidation takes place 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) $NADPH_2$ – donor of $2\bar{e}$ and $2H^+$;
2) Flavoprotein (FAD-containing enzyme) which is reffered to as **$NADPH_2$-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 $NADPH_2$ and divides them into two parts: two protons are released to form subsequently water, and $2\bar{e}$ are transferred to cyt P_{450}.

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 $NADPH_2$ 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.

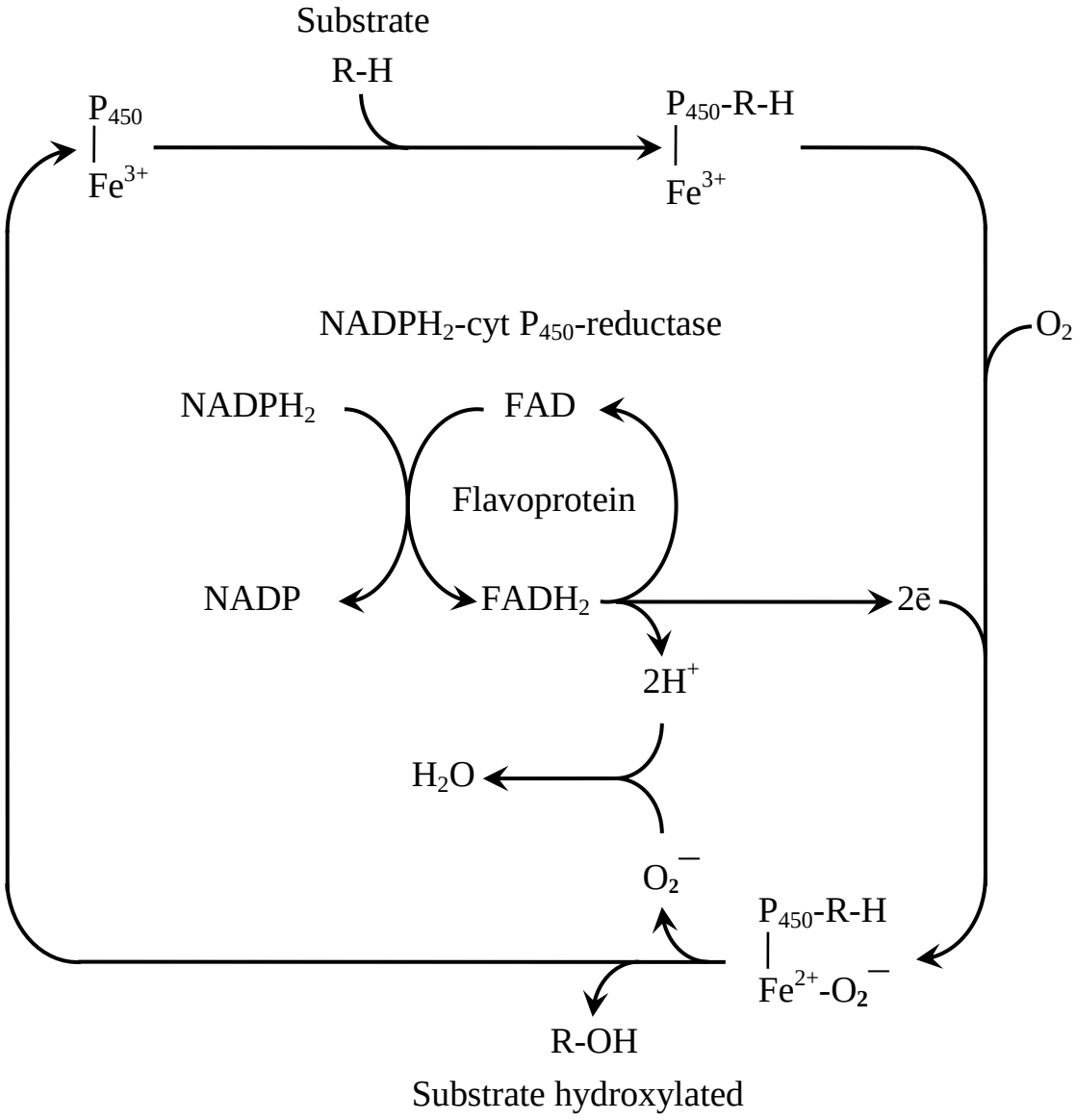


Fig. 12.1. Scheme of microsomal oxidation

Biological role of microsomal oxidation

1. **Detoxification of xenobiotics** (drugs, poisons, toxins, food additives, preservatives, adulterants, etc.). Due to microsomal oxidation, xenobiotics become more polar, water soluble and readily excreted from the body.

2. **Synthesis of compounds** (steroid hormones, bile acids, etc.).

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.

Oxygen radicals are formed when molecular oxygen undergoes series of one-electron reduction. Reactive oxygen species are as follows:

Superoxide anion, $^{\bullet}O_2^-$;

Peroxide radical, $H^{\bullet}O_2^-$;

Hydroxyl radical $^{\bullet}OH^-$.

Among oxygen radicals, **hydrogen peroxide H_2O_2** is also mentioned. Although hydrogen peroxide is not free radical (does not have superscript dot) this molecule is included into the group of reactive oxygen species because of its extreme reactivity.

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:

1) **Proteins** – oxidation of HS-groups of proteins leads to the loss of protein functions and inactivation of enzymes;

2) **Heteropolysaccharides** – ROS cause their degradation;

3) **DNA** – ROS lead to degradation of DNA strands, mutations which cause carcinogenesis and cell death;

4) **Lipids** – ROS initiate the lipid peroxidation which in turn leads to destruction of cell membranes;

5) **Hemoglobin** – ROS cause conversion of hemoglobin 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. Hydroperoxides are unstable and undergo degradation to form free radicals which involve other molecules of PUFAs into the peroxidation process (the chain reaction mechanism).

Lipid peroxidation:

- destroys membrane lipids, which leads to the destruction of membrane and death of a cell;
- participates in the development of cancer, atherosclerosis and ageing;
- impedes cell division and thus decreases healing of damaged tissues.

Antioxidant systems

Antioxidants protect the organism against harmful effects of ROS. Antioxidant systems include:

1) Enzyme systems:

- **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.

- **Catalase** – catalyzes the reaction: $2H_2O_2 \rightarrow 2H_2O + O_2$.
- **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 **$NADPH_2$**. 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. 12.2.

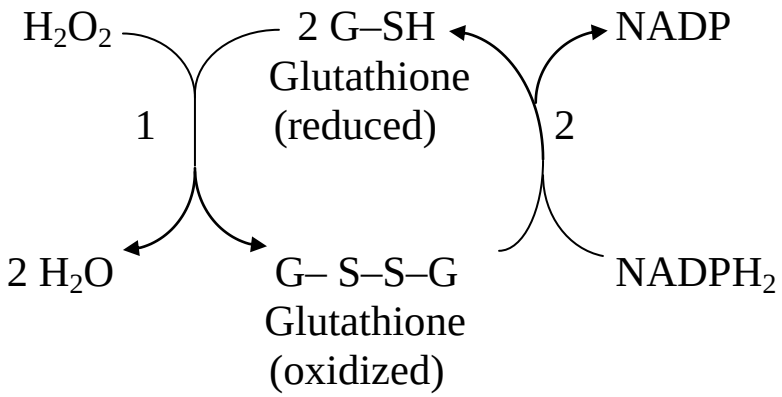


Fig. 12.2. Glutathione peroxidase system.
1 – glutathione peroxidase; 2 – glutathione reductase.

2) Water-soluble antioxidants: vitamin C (ascorbic acid), glutathione, uric acid, ceruloplasmin, ferritin, transferrin.

3) Fat-soluble antioxidants: vitamin A, β-carotene, vitamin E (α-tocopherol), ubiquinone, vitamin K (naphthoquinones).

CHAPTER 13

GENERAL CHARACTERISTICS OF HORMONES. HORMONES OF THYROID GLAND, PARATHYROID GLANDS, PANCREAS AND ADRENAL MUDULLA

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 (constancy of inner medium) 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);

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

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, hydrocortisone);

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

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

4) hormones stimulating growth (growth hormone – GH, 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 – ACTH, **t**hyroid stimulating hormone – TSH, follicle-stimulating hormone – FSH, prolactin – PRL, or lactotropic hormone – LTH, luteinizing hormone – LH).

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

8) hormones effecting the highest nervous activity (HNA), i.e. memory, attention, mentation, behaviour, mood, e.g. 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 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 represents a conjugated protein (glycoprotein), consisted of two parts – the carbohydrate and the protein ones. Hormone is bound precisely to 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 is always bound to its receptor unerringly, precisely, specifically, notwithstanding the small concentration of the hormone in the blood.

Tissues which contain receptors to the definite hormone are called target tissues. Receptors to thyroxine, glucocorticoids, or insulin are present in many tissues. There are also hormones to which receptors are present only in a few tissues (e.g. oxitocin).

MECHANISM OF ACTION OF POLYPEPTIDE HORMONES AND 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: cAMP, cGMP, inositol triphosphate, diacylglicerol, and calcium ions (Table 13.1). Each of the second messenger stimulates specific protein kinase which phosphorylate the cell proteins (enzymes) thus altering the activity of the 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 main 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 generating or degrading cAMP.

Cyclic AMP, cAMP

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. The hormone binds to the **receptor** to form the hormone-receptor complex. **G-protein** is so called because of its ability to bind to 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.

The binding of the hormone with its receptor causes consequent

changes in the three-dimensional structure of all the components of 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. Cyclic AMP stimulates **cAMP-dependent protein kinase** (**protein kinase A)**.

Protein kinase consists of four subunits, two of them are regulatory and two are catalytic ones. 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) altering their activity or functions.

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

MECHANISM OF ACTION OF STEROID AND THIROID HORMONES

Steroid and thyroid hormones enter the cell and bind with intracellular receptors. 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.

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

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 produce T_3 and T_4.

Thyroid stimulating hormone (TSH) stimulates ultimately the

release of free T_3 and T_4 into the blood.

In the blood, thyroid hormones bind with transporting proteins and reach the target tissues. The T_4 concentration 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 excepting 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 of thyroid hormones exert anabolic effect, and in high (excessive) concentrations they cause catabolic effect.

Action of normal (physiological) concentrations of thyroid hormones

The major effects of thyroid hormones 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. It is known that 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 is the age at which the thyroid hormone deficiency appeared 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 (opposite directed) are activated simultaneously, the equilibrium is preserved between them. The outer sign of the equilibrium is the heat formation for maintenance of the normal body temperature. Thus, **thyroid hormones maintain the energy equilibrium in the organism.**

Under normal conditions, due to participation of thyroid hormones, processes of excitation and inhibition in the brain are coordinated. The work for the maintenance of electrochemical gradient of sodium and potassium ion concentrations on both sides of the cell membrane is the basis for the neuron functioning. That is why the preservation of the energy equilibrium in the cell is of great importance for the normal functioning of nervous tissue.

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 high (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 electron transport chain (the ETC) is situated. Normally, major portion of energy produced in the ETC is accumulated in the phosphate bonds of ATP, and the other portion of energy is dissipated as heat for the maintenance of the normal body temperature. The process of ATP generation from ADP and P_i as a result of energy production in the electron transport chain is called oxidative phosphorylation. 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 of the decreased ATP production required for muscle contraction). 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 (tremulousness), 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; therefore the common symptoms in hypothyroidism are mucoid-like (mucous-like) swelling of tissues. 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, hypokinesis (slow to action), fatigue, lethargy (somnolence), apathy, decreased memory, slowed mentation, psychical inertness, 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. 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 appears as a result of insufficient dietary iodine supply. Most commonly, this disease occurs 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 compensatory enlargement (hypertrophy) of the thyroidal tissue at the expense of prevalent growth of the connective tissue; but 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. As a result, Ca^{2+} and phosphate concentrations are increased in the blood.

- In the **kidney** PTH acts on renal tubules to increase both reabsorption of Ca^{2+} and excretion of phosphate.
- In **intestinal epithelial cells** PTH increases Ca^{2+} and phosphate absorption.

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).

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;
- 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 synthesized by β-cells of the pancreas and deposited in secretory granules bound with zinc. The pancrease 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. Glucose may enter these cells and metabolize in them only in the presence of insulin.

2) Absolutely independent on (or 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 (essential to life) functions of the organism are fulfilled by these tissues:

- the brain – central regulation;
- medulla of kidney – secretion;
- erythrocytes – oxygen and carbon dioxide 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 (sensitive to) insulin tissues – these are all the other tissues.

The action of insulin

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

- Insulin increases membrane permeability for glucose to enter the cell (insulin increases transport of glucose into the cell).
- Insulin activates glucose utilization (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 type II (insulin-independent) diabetes mellitus, the adipose and muscle tissues are unable to take up glucose in the presence of normal amounts 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 is 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, kidney and the cardiac (but not skeletal) muscle.

The maximal amounts of glucagon are released from the islets during starvation. This is the main hormone which maintains the blood glucose levels. 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 since 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, glucose in the blood is entirely of the liver origin.

The 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 GLANDS

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.

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 reacts on epinephrine in no time (very fast).

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

4) The adrenal medulla secretes both epinephrine and norepinephrine into the blood. Epinephrine is not produced elsewhere in the organism beyond the adrenal medulla. Unlike epinephrine, norepinephrine is synthesized in terminals of sympathetic nervous system, functioning as a neurotransmitter.

Normally only 1-4% of epinephrine is execreted into the urine. This amount is too little to be detected by the routine methods;

therefore adrenalin 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** (VMA) 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 glucose blood 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 increase 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. The glucose and the fatty acid blood levels are increased. In the urine, epinephrine and glucose are observed (they are normally absent in the urine), and the excessive amount of VMA is also present.

CHAPTER 14

STEROID HORMONES. HORMONES OF HYPOPHYSIS. PROSTAGLANDINS

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 product of their inactivation called 17-ketosteroids (17-KS) is excreted into the urine and is used in diagnostics.

Glucocorticoids

Representatives of glucocorticoids (GCs): **cortisol** (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 the transport of substrates into the cell, and in the other target-tissues they, vice versa, activate catabolism and decrease the 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 the other target-tissues, GCs decrease the 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 fats (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 (enzymes) 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 in minimal 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).

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 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). The sodium reabsorption from the urine stimulated by aldosterone is accompanied by excretion of potassium into the urine. In 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 (ECG), heart failure, and heavy weakness.

Sweat glands are another target-tissue for aldosterone. The heat (high outer temperature) stimulates aldosterone production due to which 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.

Hypercorticoidism (hypercorticism). It is represented by 3 types of pathology:

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 is 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.

Hypocorticoidism (hypocorticism). Hypofunction of the adrenal cortex is called Addison's (or bronze) disease. This is a condition caused by lack of both mineralocorticoids and glucocorticoids. The main 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), enhanced water and sodium excretion and, related to the latter, subconscious preference of salt meals, and the lowered blood pressure (caused by lack of mineralocorticoids).

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.

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 (pituitary gland). Estrogens are responsible for the formation of sexual behaviour, instinct, and psychical status of a female.

Skeleton (bones and cartilages) 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 the state.

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 (pituitary gland). 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 (bones and cartilages) 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 boldness.

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 revealed to be harmful for the human organism: they didn't metabolize in human's tissues and didn't degrade during cooking (thermal processing of meals); therefore if the meat contained anabolic steroids had been consumed with meals this caused in some time the heavy damage of the liver (the liver cancer). Lately most countries refused from 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 the 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.

THE SYSTEM OF HYPOTHALAMUS-HYPOPHYSIS IN REGULATION OF ENDOCRINE GLANDS

Synthesis of hormones and their secretion into the blood are regulated by the 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 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)

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 a child sleeps little he grows 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 (ACTH)

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.

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.

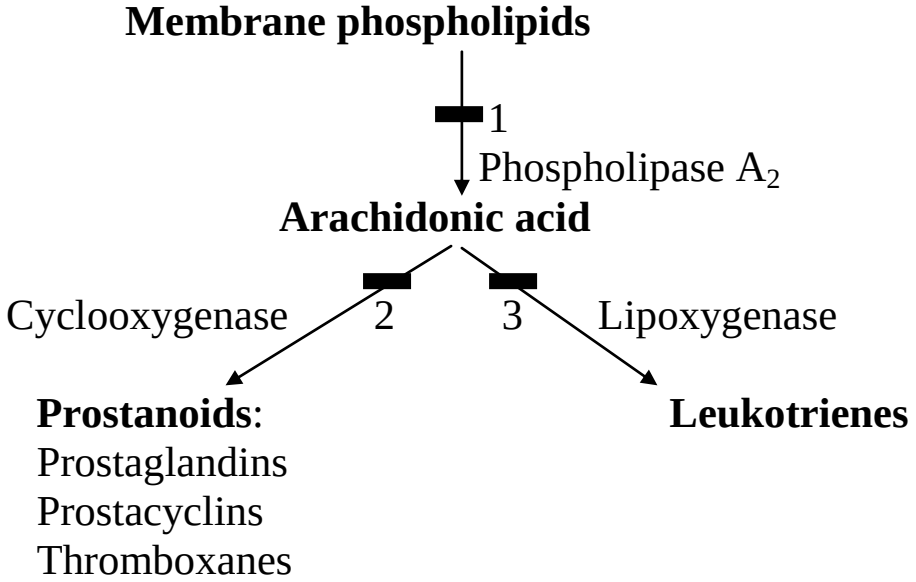


Fig. 14.1. Synthesis of eicosanoids. Inhibition of enzyme activity by: 1 – glucocorticoids; 2 – aspirin, indomethacin and other non-steroidal anti-inflammatory agents; 3 – vitamin E, vitamin P.

Prostaglandins are synthesized in all cells excepting erythrocytes, and 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.

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.

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 syptoms of excessive or insufficient nutrient intake?

Food provides the following functions:

- plastic role – 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 pentose). 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 (animal) 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 to 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 exchange toxic substances.

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 of the cell, they provide 60-70% of daily energy consumption. Carbohydrate metabolism is characterised 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 peristaltics and removes toxic final (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 acid.The daily requirement of lipids is 100 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, precursors of steroid hormone. Among the higher fatty acids so called essential higher fatty acids are present, which include linoleic, linolenic and arachidonic fatty acids. They are collectively nemed 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

Physico-chemical properties (in particular, solubility) of 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".

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).

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 Bc, folic acid (antianemic).

8. Vitamin H, biotin (anti-seborrhoeic).

9. Vitamin C, ascorbic acid (antiskorbic).

10. Vitamin P, rutin.

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

Name	***Daily requirement, mg***	***Coenzyme form***	***Biological functions***	***Characteristic signs of avitaminosis***
PP (niacin)	15-25	NAD, NADP	Acceptorsandhydrogen-carriers	Symmetricaldermatitis onexposed skin, dementia and diarrhea
H (biotin)	0.01-0.02	Biotin	Activation ofCO_2reactioncarboxylation(e.g.,pyruvateand acetyl-CoA)	Dermatitisaccompaniedenhancedactivityof the sebaceous glands
B_C (folic acid)	0.05-0.4	THFA	Transportof one-carbongroups	Anemia, leukopenia
B_{12} (Cyanocobalaminun)	0,001-0,002	Desoxyadenosilandmethyl-cobalamin	Transport methyl groups	Megaloblastic anemia
C (ascorbic acid)	50-75	-	Hydroxylation ofproline, lysine(collagen), an antioxidant	Bleeding gums,loosening ofteeth,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 15.2.

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-coenzyme (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 with 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);
- congenital defects in the enzymes involved in the conversion into vitamin coenzymes;
- effects of structural analogues of vitamins (antivitamin).

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 analysis 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, 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	Incorporatedinto the active siteof enzymesfolatzavisimyhand blocksthe 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 prothrombinprokonvertinaand other clotting factors in the liver (have anticoagulant effect).	For prevention and treatment of thrombosis (angina, thrombophlebitis, etc.).

Vitamin	***Antivitamin***	***Mechanism of action***	***Applicationof antivitamin***
Vitamin PP	Isonicotinicacid hydrazide(INH) and its derivatives (tubazid, ftivazid, metozid)	Antivitamins included in thestructure ofNAD and NADP, forming false coenzymes, which are notable to participate in redox reactions and other biochemical systems of Mycobacterium tuberculosis are 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 is a group of organic compounds based on the general formula $Cx(H_2O)y$. 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.

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 Kakes, 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. The main role of fiber is to regulate gut transit and motility.

Biological role of fiber

- 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.

Table 17.2

Digestion of carbohydrates

Section of GIT	***Enzymes***	***Localisation of enzymes***	***Products of hydrolysis***
Oral cavity	Amylase	Saliva	Dextrins
Duode-num	Amylase	Pancreatic juice	Maltose, maltotriose, glucose, small branched dextrins
Small intesine	maltase sucrase-isomaltase complex lactase	Brush border of the intestinal mucosal cells	Monosaccharides

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 trisaccharidemaltotriose, 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.

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 carbohydrate. 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 along 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. The glucose transporter family comprises five major species, named GLUT-1 to GLUT-5 (Table 17.3). Cells in insulin-sensitive tissues 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.

Table 17.3

Glucosetransporters

	Tissue Location	***Functions***
GLUT 1	Brain, kidney, colon, placenta, erythrocytes	Uptake of glucose
GLUT 2	Liver, pancreatic β cell, small intestine, kidney	Rapid uptake and release of glucose
GLUT 3	Brain, kidney, placenta	Uptake of glucose
GLUT 4	Heart and skeletal muscle, adipose tissue	Insulin-stimulated uptake of glucose
GLUT 5	Small intestine	Absorption of glucose

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 triose kinase 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 Gal-1-P uridyltransferase catalyzes an exchange between UDP-Glc and Gal-1-P to form UDP-Gal and Glc-1-P - that is, the Glc-1-P part of UDP-Glc is replaced with Gal-1-P, to give 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, uridyltransferase, or 4-epimerase. A deficiency in **uridyltransferase** is the best known cause.

The galactose concentration in the blood and in the eye is reduced by aldose reductasetogalactitol, which accumulates, causing cataract. In uridyltransferase deficiency, galactose 1-phosphate accumulates and depletes the liver of inorganic phosphate. Ultimately, liver failure and mental deterioration occurs. As the epimerase is present in adequate amounts, the galactosemic individual can still form UDP-Galfrom 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 glucosein mammary glands during lactation. The reversible convertion of UDP-Glucose to UDP-Galactose is catalyzed by UDPGal 4-epimerase.

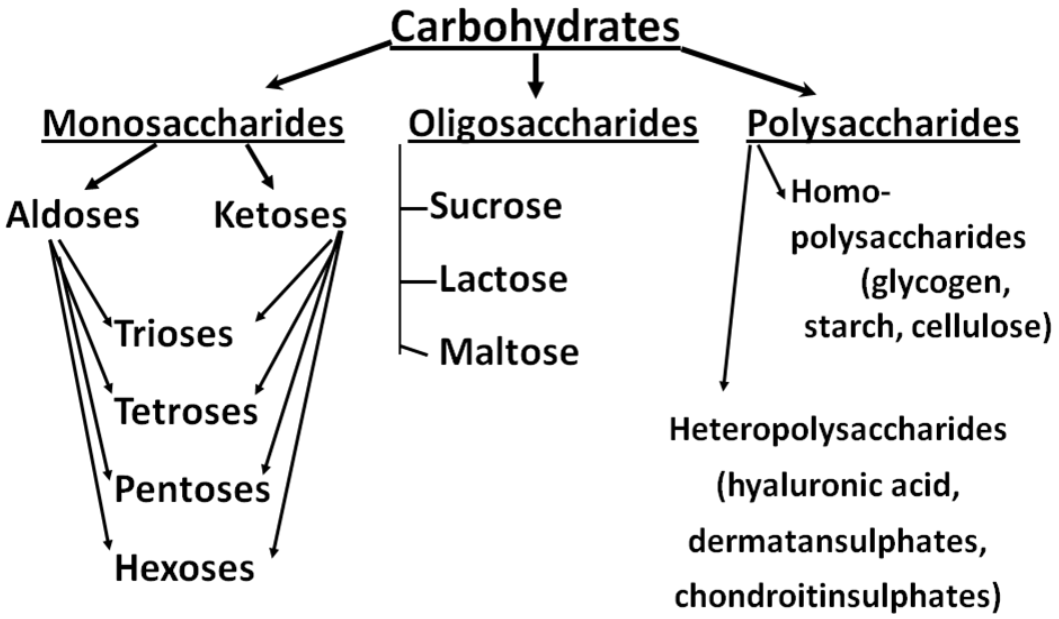
UDP-Glucose $\rightleftharpoons$ UDP-Galactose

Then UDPGal condenses with glucose to yield lactose, catalyzed by lactose syntase.

UDP-Galactose + Glucose $\longrightarrow$ Lactose + UDP

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.

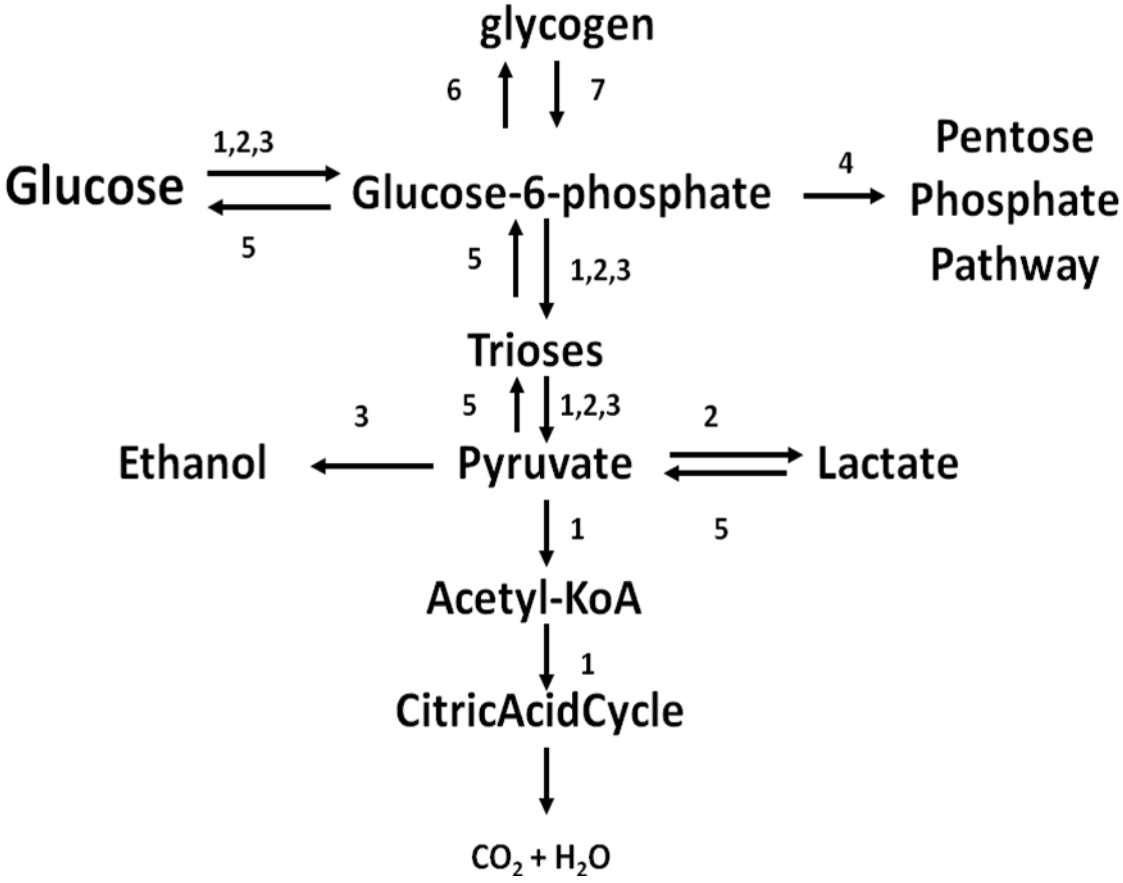


Fig. 18.1. The general scheme of pathways of glucose metabolism
1-aerobic glycolysis; 2-anaerobic glycolysis; 3-alcoholic fermentation; 4-pentose phosphate pathway; 5-gluconeogenesis; 6-glycogen synthesis; 7-glycogen degradation.

Glucose enters metabolism by phosphorylationto glucose 6-phosphate, catalyzed by **hexokinase**. Hexokinase is inhibited allosterically by its product, glucose 6-phosphate. Hexokinase has a high affinity (low Km) for glucose, and in the liver and pancreatic β islet cells is saturated under all normal conditions and so acts at a constant rate to provide glucose 6-phosphate to meet the cell's need. Liver and pancreatic β islet cells also contain anisoenzyme of hexokinase, **glucokinase**, which has a Km much higher than the normal intracellular concentration of glucose. The function of glucokinase in the liver is to remove glucose from the blood following a meal, providing glucose 6-phosphate in excess of requirements, which will be used for glycogensynthesis and lipogenesis.

Glucokinase is inducible by continued consumption of a high-carbohydrate diet. Unlike hexokinase, glucokinase is not inhibited by glucose 6-phosphate, so that the concentration of glucose 6-phosphate increases rapidly in the liver following a carbohydrate-rich meal, forcing glucose into all of the major pathways of glucose metabolism: glycolysis, the pentose phosphate pathway, and glycogenesis.

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.

Aerobic glycolysis 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.

Anaerobic glycolysis is the pathway of glucose degradation under anaerobic conditions to lactate as end product.

Glycolysis proceeds through a series of phosphorylated intermediates. During this process, 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. 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.

Pentose phosphate pathway

The pentose phosphate pathway is a cytosolic pathway present in all cells, so named because it is the primary pathway for formation of pentose phosphates for synthesis of nucleotides.

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

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.

Alcoholic 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.

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.

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 protein;
- glycerol released from triglycerides during lipolysis in adipose tissue.

Gluconeogenesis is conceptually the opposite of anaerobic glycolysis, 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 glucoseinto 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.

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 glucoseconcentration. 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 in part 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 linkages, chains of α1→4-linked glucoseresidues 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 four steps:

- 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 glucoseto 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.

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 glucosefrom 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.

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.

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 glucoseutilization 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 cyclic-3',5'-AMP (cAMP), a second messenger for action of glucagon (and other hormones). Cyclic AMP binds to the cytoplasmic enzyme protein kinase A, causing dissociation of regulatory subunits from the catalytic subunits of the enzyme, relieving inhibition. Cyclic AMP 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, theactivation 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 Caction, 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. $Ca2^{+}$ 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: first, insulin reverses the actions of glucagon in phosphorylation of proteins, turning off glycogen phosphorylase and activating glycogen synthase, promoting glucose storage; second, 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. More then 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
            - Plasmalagel
         - 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 *physicochemical 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. Uncombined 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. linoleic 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;

- Hormone (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 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.

Phospholipids are hydrolyced by pancreatic phospholipase A_1, A_2, C, and D. 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.

Lipid absorption occurs in the proximal part of the small intestine. 3-10% of fat food is absorbed without hydrolysis in the form of triacylglycerols. The main part of lipids is absorbed only in the form of degradation products. Suction hydrophilic digestion products (glycerol fatty acid with carbon number less than 12, phosphoric acid, choline, serine, ethanolamine, etc.) occurs independently and hydrophobic (cholesterol, long chain fatty acid di-and monoglitserols) are absorbed into the micelle composition. 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 involvs 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. 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 *apoproteins (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, LPID, 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***	***LPID***	***LDL***	***HDL***
Composition,%					
Protein	2	10	11	22	50
Phospholipids	3	18	23	21	27
Ch	2	7	8	8	4
Ether of Ch	3	10	30	42	16
TAG	85	55	26	7	3
Functions	Transport of exogenouslipid	Transport of endogenouslipid	Precursor of LDL	Transport of Ch into tissues	Transport of Ch from tissues, donor of Apo A and C-II
Place of synthesis	Intestines	Liver	Liver	Blood	Blood
Diameter,nm	> 120	30-100		21-100	7-15
Main apoproteins	B-48 C-II E	B-100 C-II E	B-100 E	B-100	A-I C-II E

When exogenous TAG resynthesize in enterocytes, they with phospholipids, cholesterol and proteins form ChM, 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 the 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 PhL. 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 – apoC-II and PhL. ApoC-II activates the enzyme and PhL 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 in the form of TAG (adipose tissue) or used as an energy source (muscles). Glycerol is transported in the blood 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 XM (remnant). They contain PhL,Ch, fat-soluble vitamins, apoB-48 and apoE.

Residual ChM enter hepatocytes that have receptors that interact with these apoproteins. Under the action 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 PhL form VLDL, which includes apoB100 and apoC. VLDL is the main form of transport for endogenous TAG. Once in the blood, VLDL gets apoC-II and apoE from HDL and converts into LPL. In this process, VLDL is first converted to LPID, and then LDL. Cholesterol becomes the main lipids 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 by blood, which can be used again for the synthesis of TAG.

All changes in PhL 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.

Hypolipoproteinaemias include the following conditions:

1. *Abetalipoproteinemia* occurs as a rare inherited disorder – a defect of apoprotein B gene, when protein synthesis of apoB-100 in the liver and apoB-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. *Family hypobetalipoproteinemiya*: concentration of PhL, containing apoB, is only 10-15% of normal levels, but the body is capable of forming ChM.

3. *Family insufficiency of alfa-LP* (Tangier disease): in plasma HDL is almost not detectable, and large amounts of cholesterol esters are accumulated in the tissues. The absence of apoC-II (activator of LPL) in patients leads to a characteristic increase in TAG concentration in plasma.

There are following types of hyperlipoproteinemia:

Type I – 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 – family 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 – dis-beta-lipoproteinemiya (broadband betalipoprote-nemiya) 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 reveals by xanthoma and atherosclerotic lesions of peripheral and coronary vessels. Treatment: diet therapy aimed at weight reduction.

Type IV – 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.

Gepatogenous 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.

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.

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.

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.

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 adenylate cyclase system activates hormonsensitive lipase that activates lipolysis and releases fatty acids and glycerol in the blood. In physical activity the secretion of adrenaline increases, which activates lipolysis through the adenylate cyclase 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.

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.

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.

Exchange 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 also glycerol. 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 activate 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. 21.1) 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 carnitinacyltransferasetranslocase on the inner surface of the inner mitochondrial membrane, wherein the enzyme carnitinacyltransferase-II catalyses acyltransfer on intramitochondrial CoA. Thereafter, acyl-CoA becomes included in β-oxidation reaction. Free carnitine returns to the intermembrane space of the same translocase.

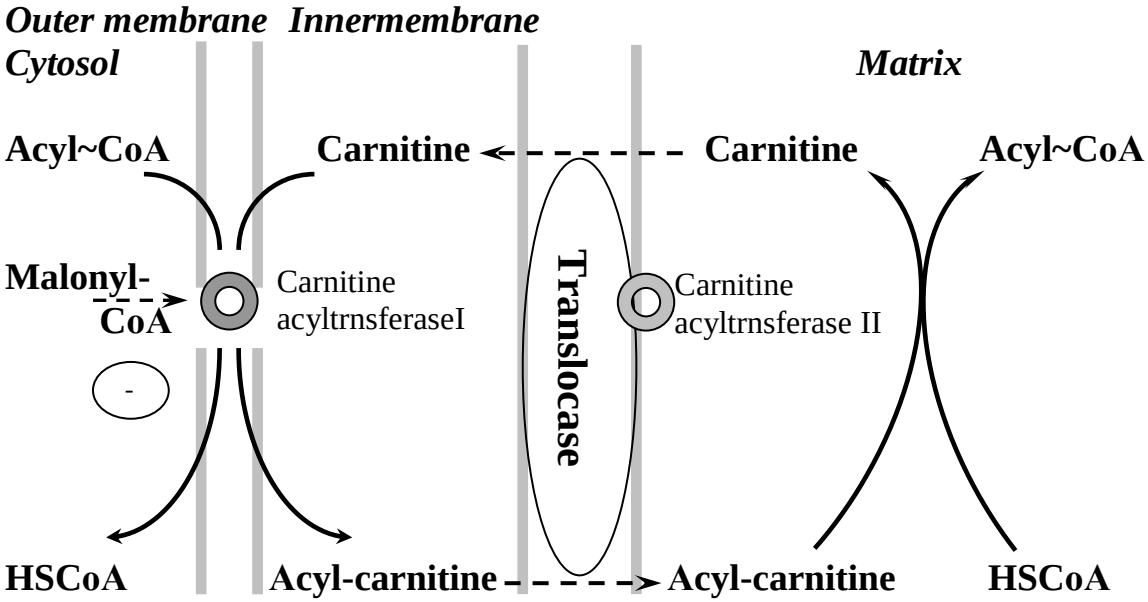


Figure 21.1. Transport of long chain fatty acids across the mitochondrial membrane.

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 by providing ATP synthesis in the respiratory chain.

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.

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.

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 carnitinacyltransferase-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. 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.

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.

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) activates 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.

Acetyl-CoA accumulates in the mitochondria of the liver and is used for the synthesis of ***acetoacetate***. 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.

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/literor up to 0.2 mmol/l) is 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 the main source of energy for the body are fatty acids - in the prolonged muscular work, starvation, diabetes melitus.

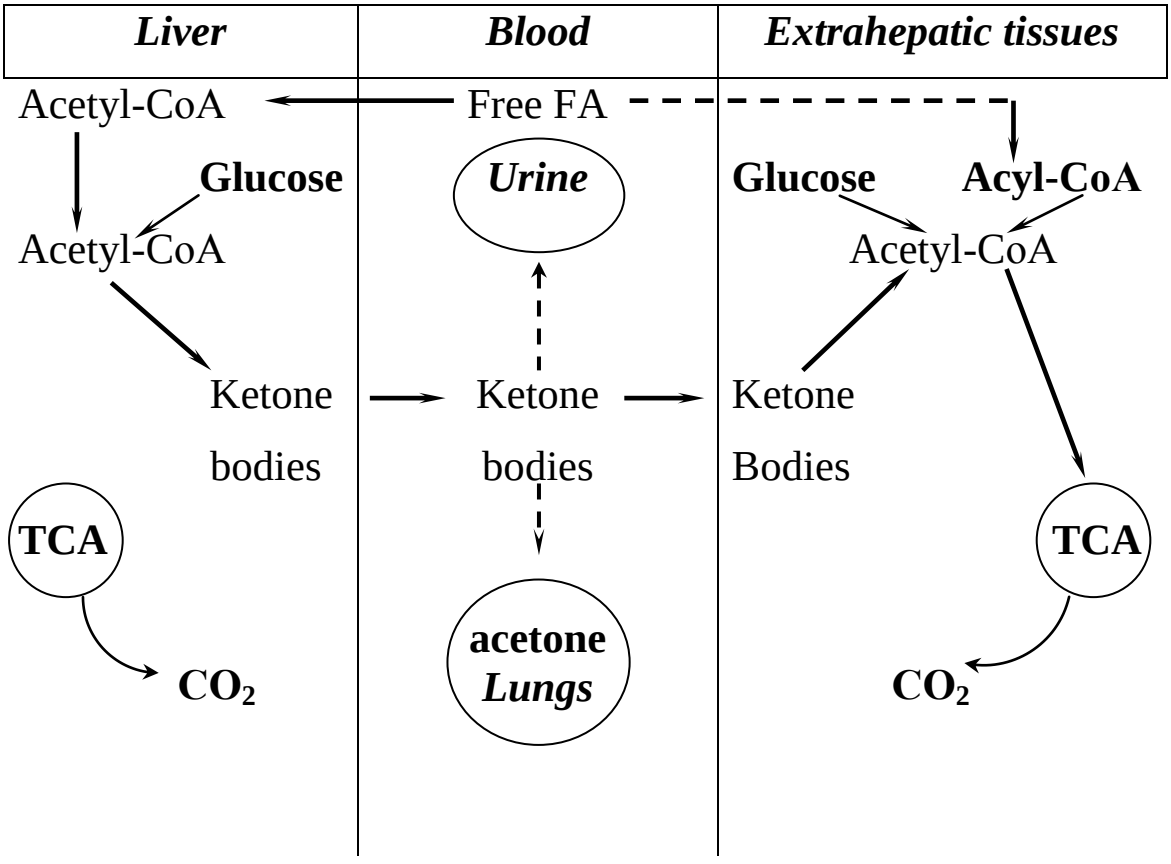


Figure 21.2. Formation, utilization and excretion of ketone bodies (the main path shown by continuous arrows)

Increasy 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 melitus.

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 malatedehidrogenase 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, enoylreductase and thioesterase) and acyl caring protein (ACP).

ACP is not an enzyme, its function is only associated with the transfer of acyl radicals. In the synthesis of 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 ketoacylsynthase.

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 hydrolyticaction 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 reaction in which the hydrogen donor is NADPH.

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 adenylate cyclase 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 citratlyase.

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 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 is a proteolipid, glycolipids and phospholipids. Unlike the simple lipid used as the energetic material, the plastic complex lipids perform 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 interaction, have a 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.

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 citidyndiphosphatediacylglicerol 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 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 this body 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. 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.

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 21.1).

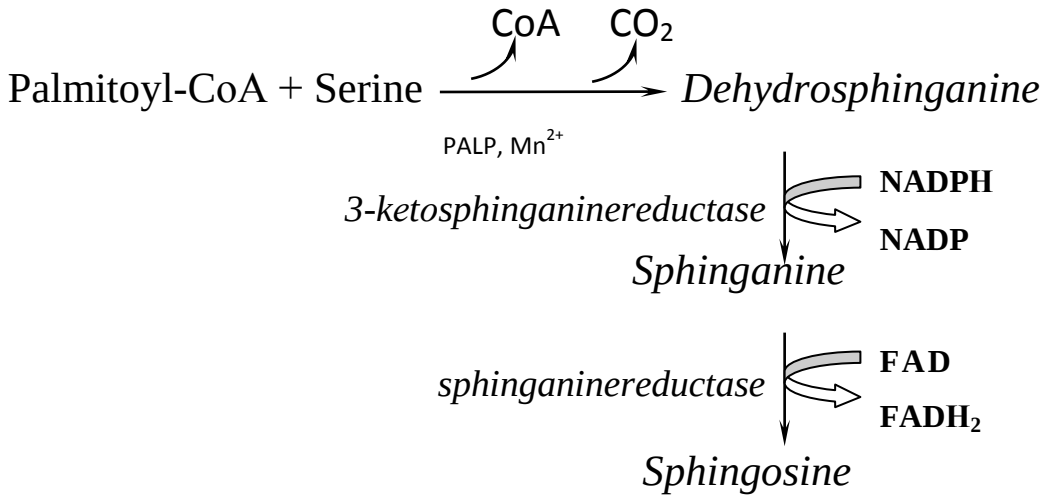


Figure 22.1. Scheme of the sphingosine synthesis

Sphingosine is subjected to acylation (accession of fatty acid residue). In the result ceramide is synthesized which is the precursor of cerebrosides, gangliosides, sulfatides and sphingomyelin (Figure 21.2).

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 in the result of nervous tissue cells destruction due to glycolipids accumulation.

In the breakdown of sphingomyelins (Figure 21.2) 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 there are hepatosplenomegaly and mental retardation 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.

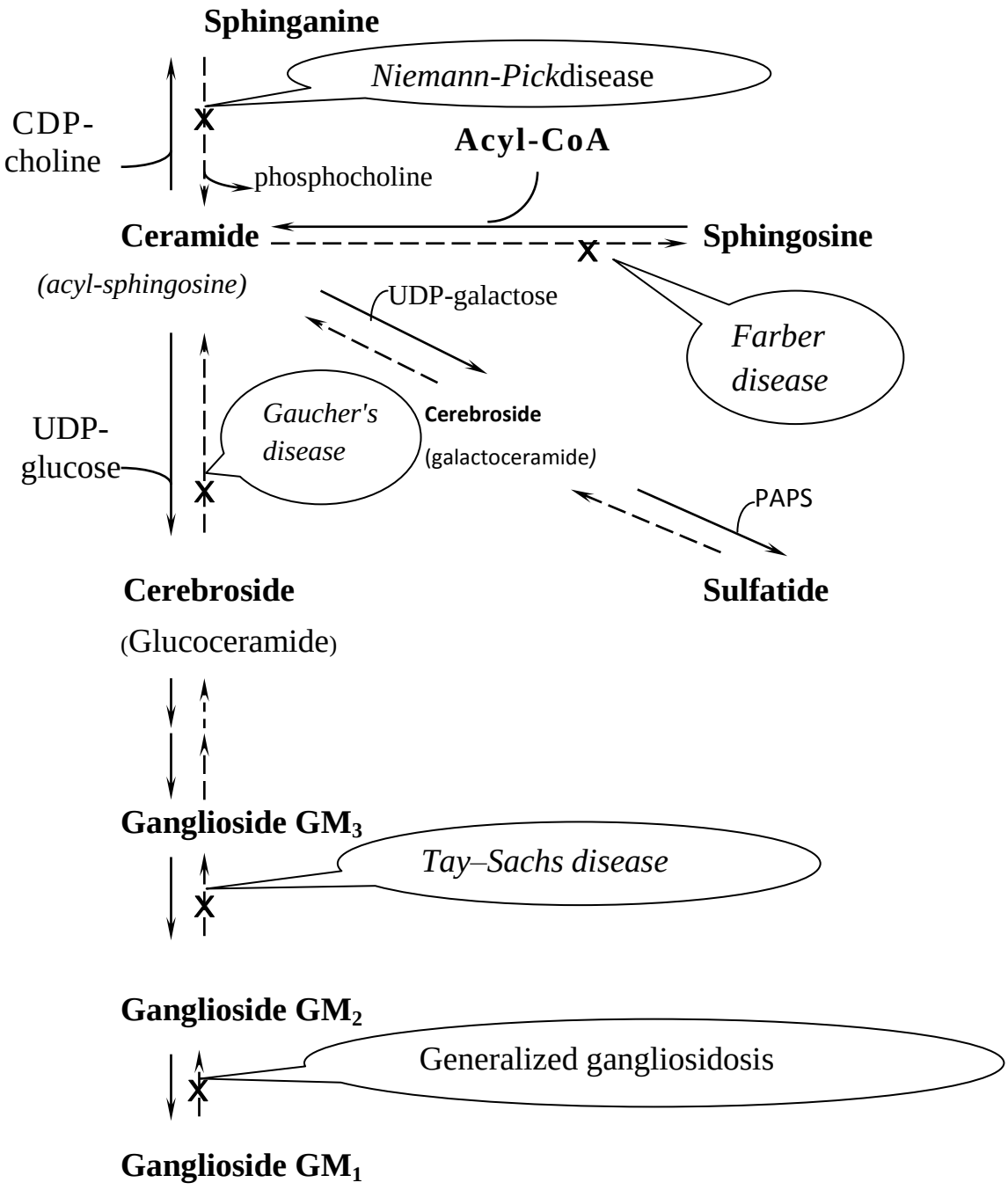


Figure 22.2. Biosynthesis (→) and degradation (- - →) of sphingolipids.

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 joint 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.

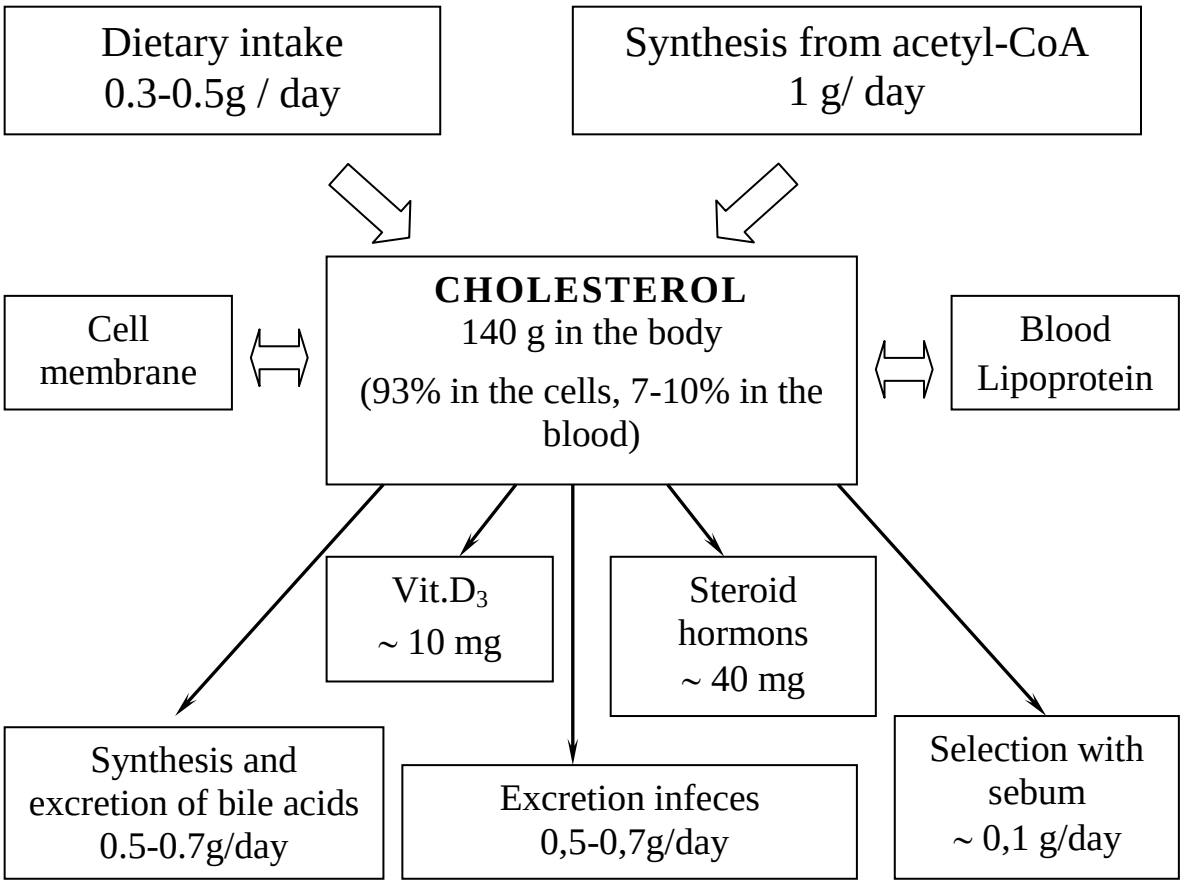


Figure 23.1. Formation and distribution of cholesterol in the body.

Cholesterol in human organism (total amount about 140 g) can be divided into three pools:

- pool A (30 g) quickly metabolised – 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.

In some tissues, the hydroxyl group is estherified to form cholesterol esters. Reaction is catalyzed by intracellular enzyme acylCoA: cholesterolacyltransferase. 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 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 the 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 22.2).

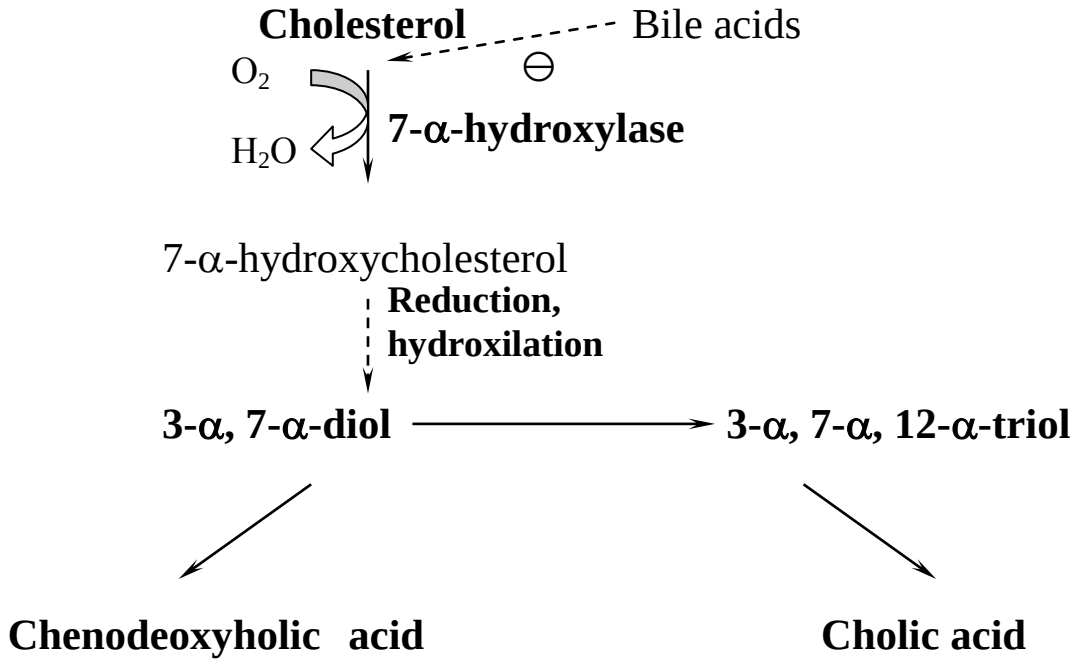


Figure 23.2. Scheme of the bile acid synthesis

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 undergoe 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 become attached, and the result is a 4 types of conjugates: taurocholic and taurochenodeoxycholic, glycocholic and glycochenodeoxycholic acid.

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 poor solubility and are slower absorbed in the intestine.

About 95% of bile acids secreted in the intestine are returned 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 faeces 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 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 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.

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.

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

METABOLISM OF AMINO ACIDS. DYNAMIC STATE OF BODY PROTEINS

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 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

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).

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

* - Half-essential amino acids

SOURCES OF AMINO ACIDS IN THE BODY AND WAYS OF THEIR USE

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).

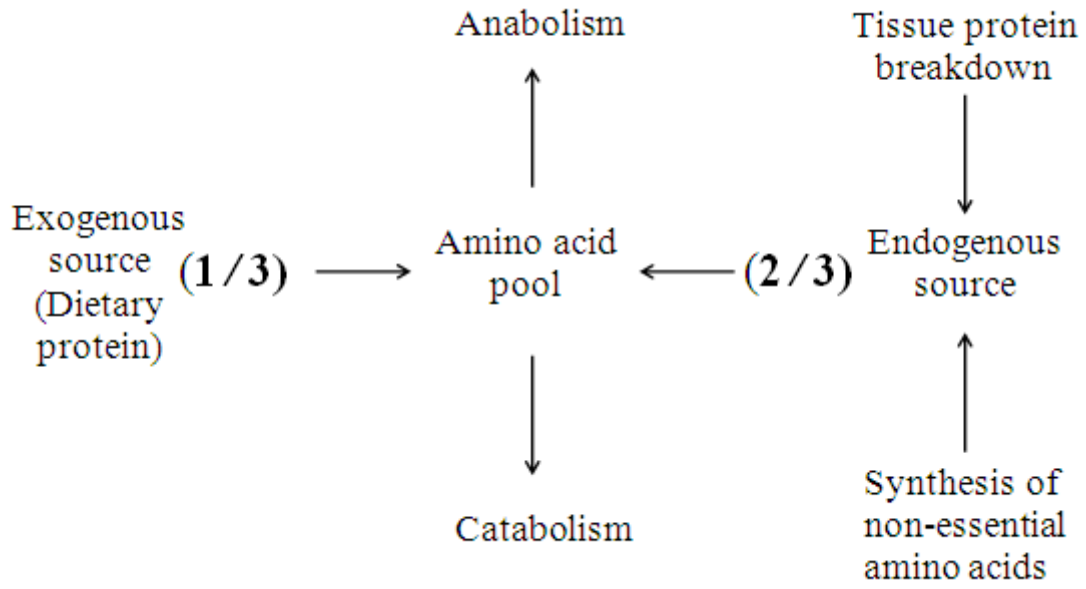


Fig. 24.1. Sources of amino acids in the body.

Ways of the amino acids use

Amino acids are used as building blocks for synthesis of **proteins** (enzymes, hormones, antibodies, etc.) 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.

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 scheme shown below represents the variety of routes to the use of amino acids in the body (Fig. 24.2 and Table 24.2).

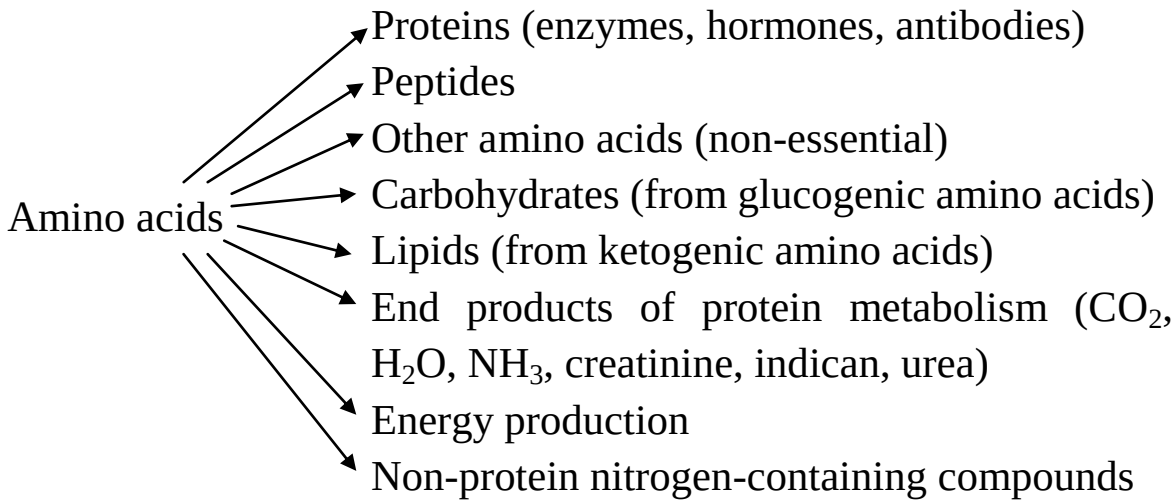


Fig. 24.2. Variety of routes to the use of amino acids in the body.

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 Fig. 24.3.

Proteolytic enzymes are secreted as inactive zymogens which are converted to their active forms in the intestinal lumen.

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

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 (HCl) also denatures proteins making them more easily digested.

The gastric juice contains three enzymes to digest proteins: pepsin, gastricsin and rennin.

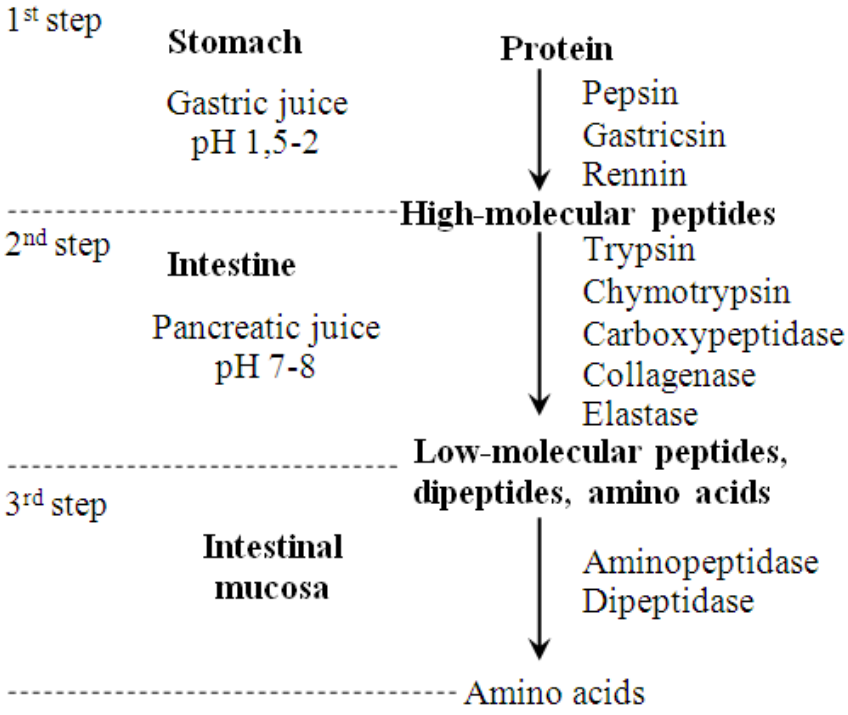


Fig. 24.3. Digestion of proteins in the gastrointestinal tract.

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 the 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 (small) 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.

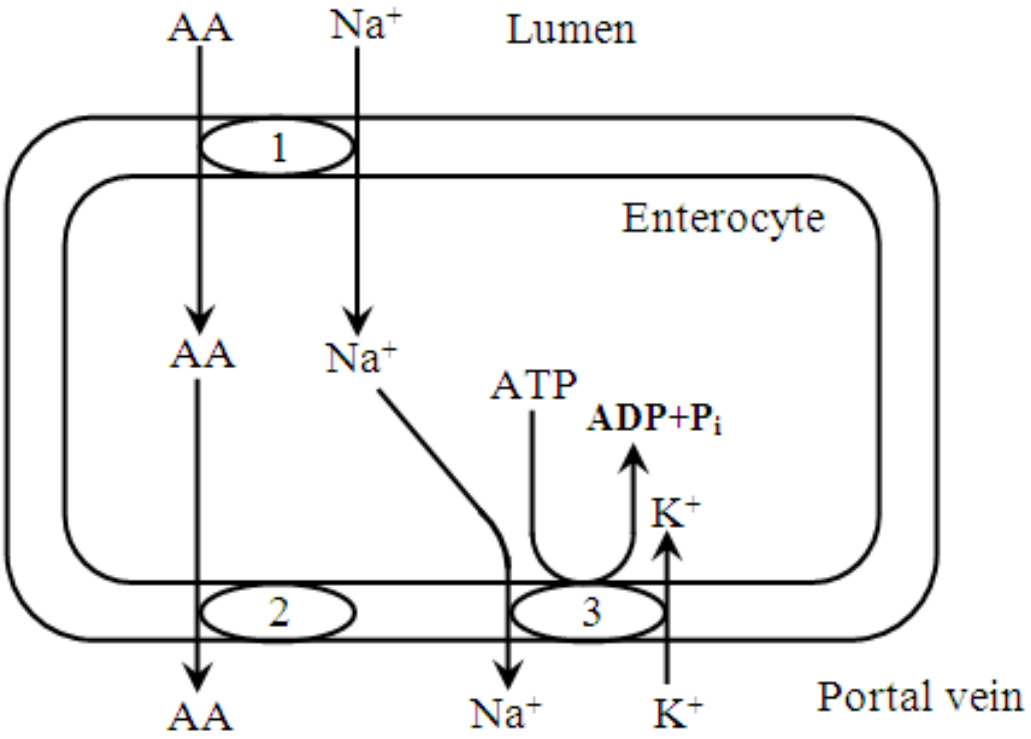


Fig. 24.4. Active transport of amino acids and facilitated diffusion. AA – amino acid; 1 – Na^+-dependent amino acid transporter (symporter); 2 – Na^+-independent amino acid transporter; 3 – Na^+,K^+-ATPase (sodium pump)

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 glutamic acid (glutamate), 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).

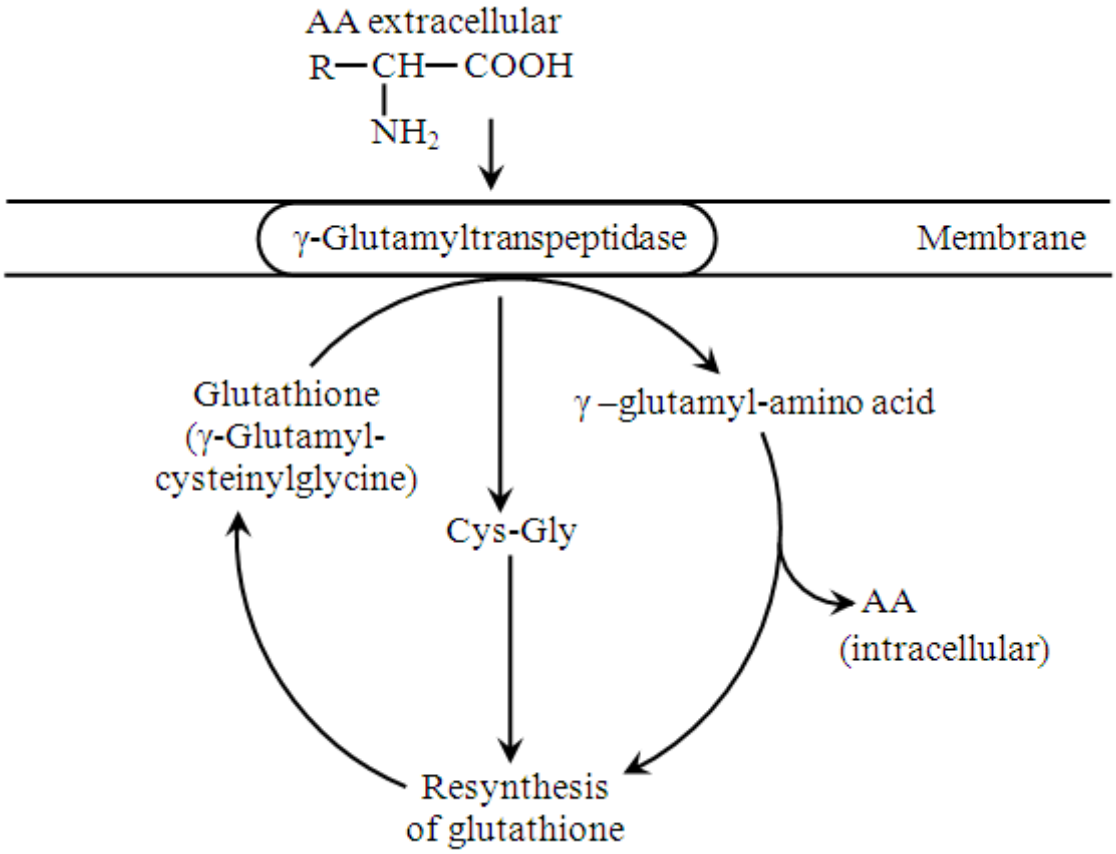
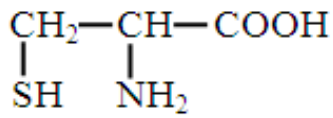


Fig. 24.5. Gamma-glutamyl cycle.

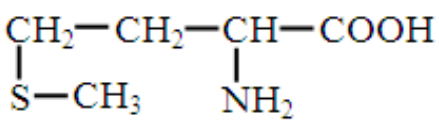
INTESTINAL PUTREFACTION OF PROTEINS (CONVERSION OF AMINO ACIDS BY INTESTINAL BACTERIA)

Intestinal bacteria have enzymes which catalyze conversion of amino acids to definite toxic products. This process is called **intestinal putrefaction of proteins**.

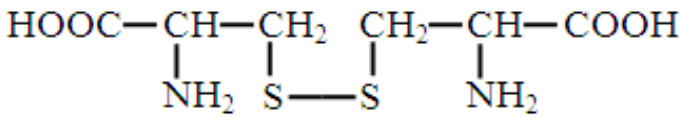
1) Putrefaction of sulfur-containing amino acids:



Cysteine



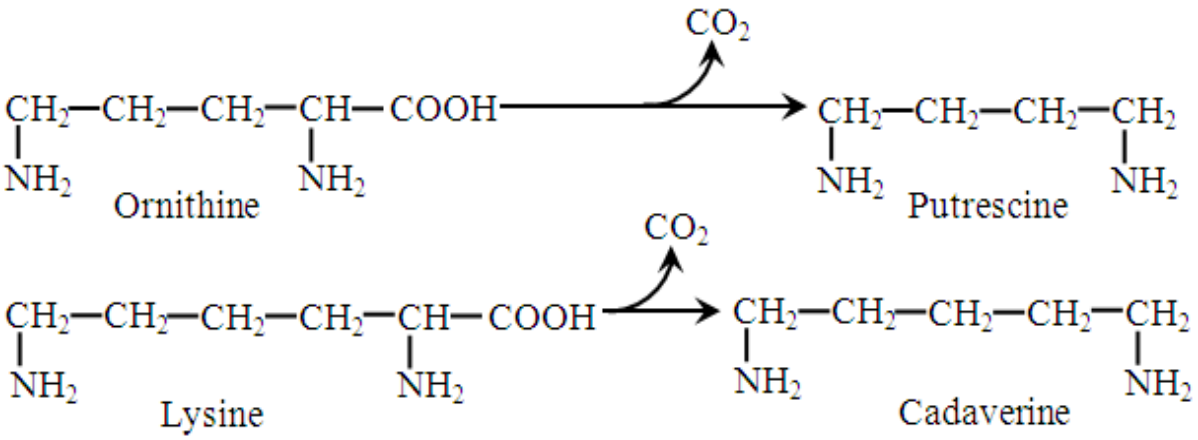
Methionine



Cystine

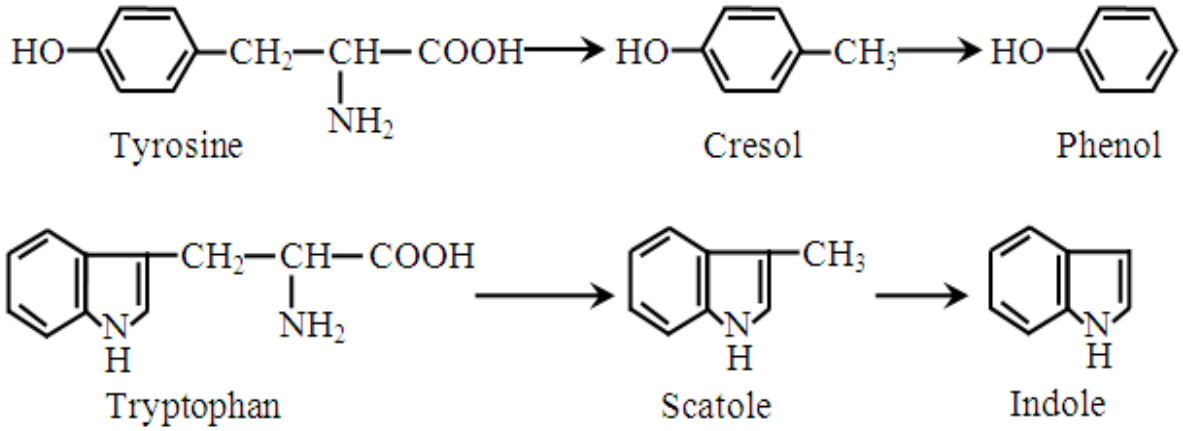
Putrefaction of these amino acids produces hydrogen sulfide (H_2S) and methylmercaptan (CH_3–SH), the products which are removed from the intestine with intestinal gas.

2) Putrefaction of diaminomonocarboxylic acids.

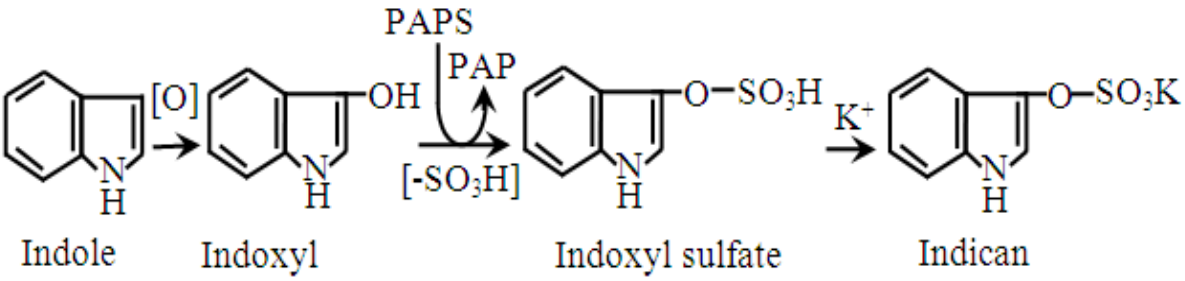


The toxic products (putrescine and cadaverine) are detoxified in enterocytes by diaminoxidases.

3) Putrefaction of aromatic amino acids:



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:



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);

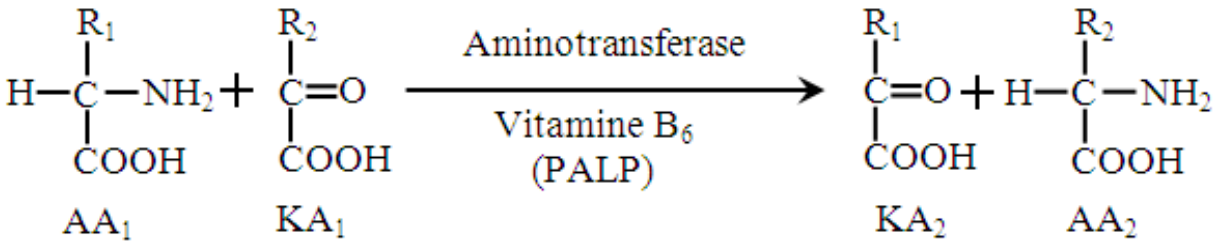
4) polymerization (synthesis of protein);

5) racemization (isomerization of D- and L-amino acids; reactions are typical of microorganisms only);

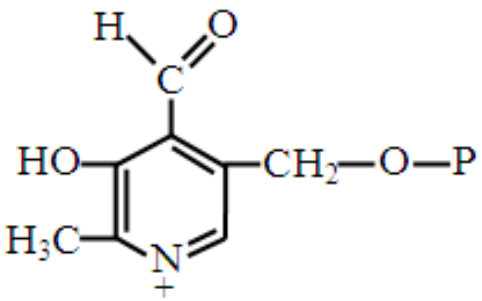
6) modification of a side chain radical.

TRANSAMINATION OF AMINO ACIDS

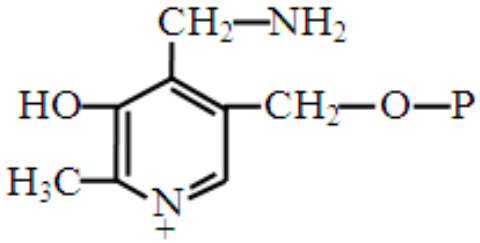
Transamination is the transfer of α-amino group from an amino acid (AA_1) to a keto acid (KA_1) with the resultant formation of another amino acid (AA_2) and another keto acid (KA_2), **without intermediary release of ammonia (NH_3)**. The general scheme of the reaction:



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:



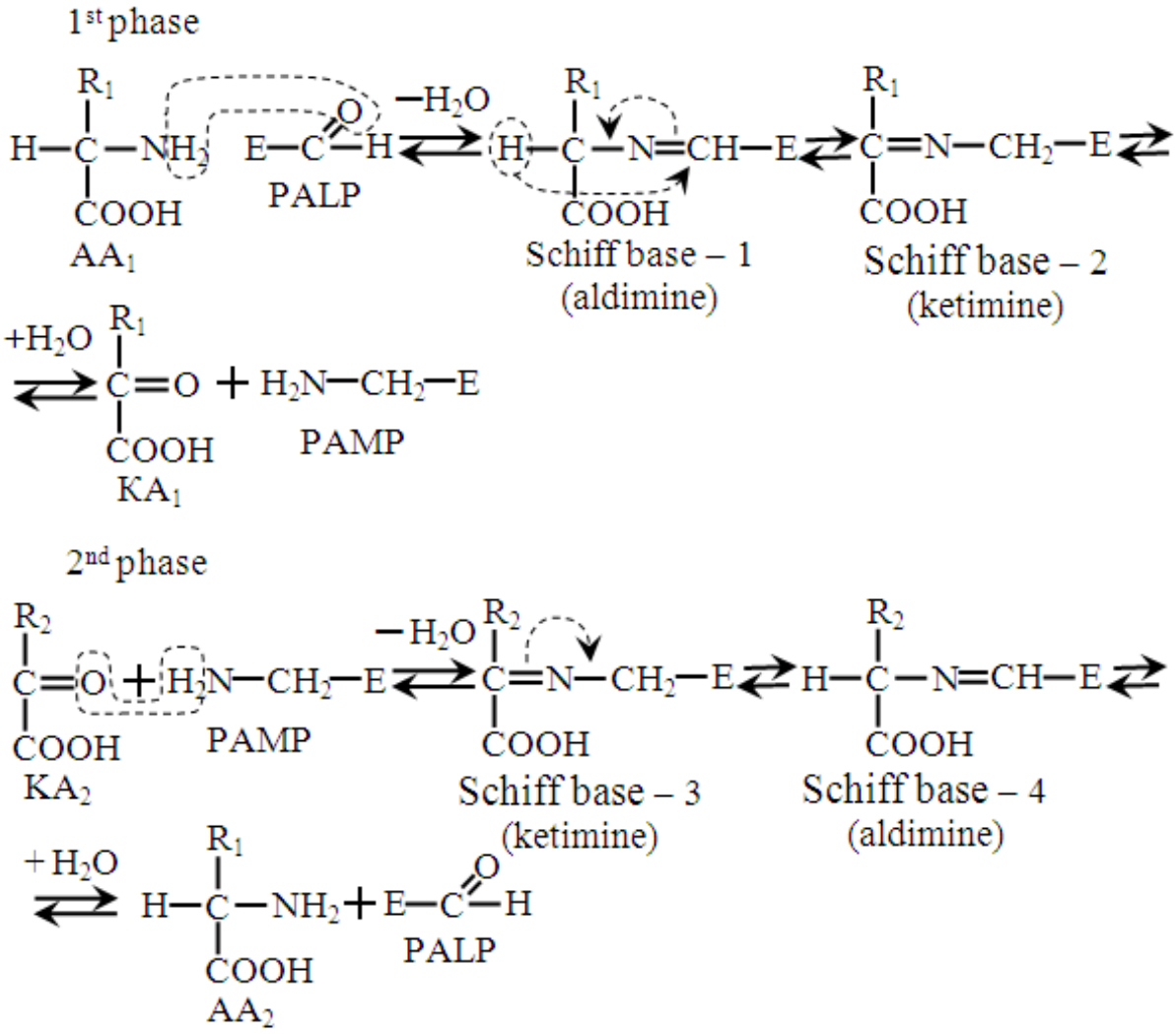
Pyridoxal phosphate
(PALP)



Pyridoxamine phosphate
(PAMP)

Biological role of transamination is synthesis of non-essential amino acids.

Mechanism of transamination



Of all, two transaminases are of clinical importance (Fig. 24.6):

1) **alanine aminotransferase** (AlAT or ALT); in the reaction, alanine is transaminated with α-ketoglutarate to form pyruvate and glutamate;

2) **aspartate aminotransferase** (AsAT or AST); in the reaction aspartate is transaminated with α-ketoglutarate to form oxaloacetate and glutamate.

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.

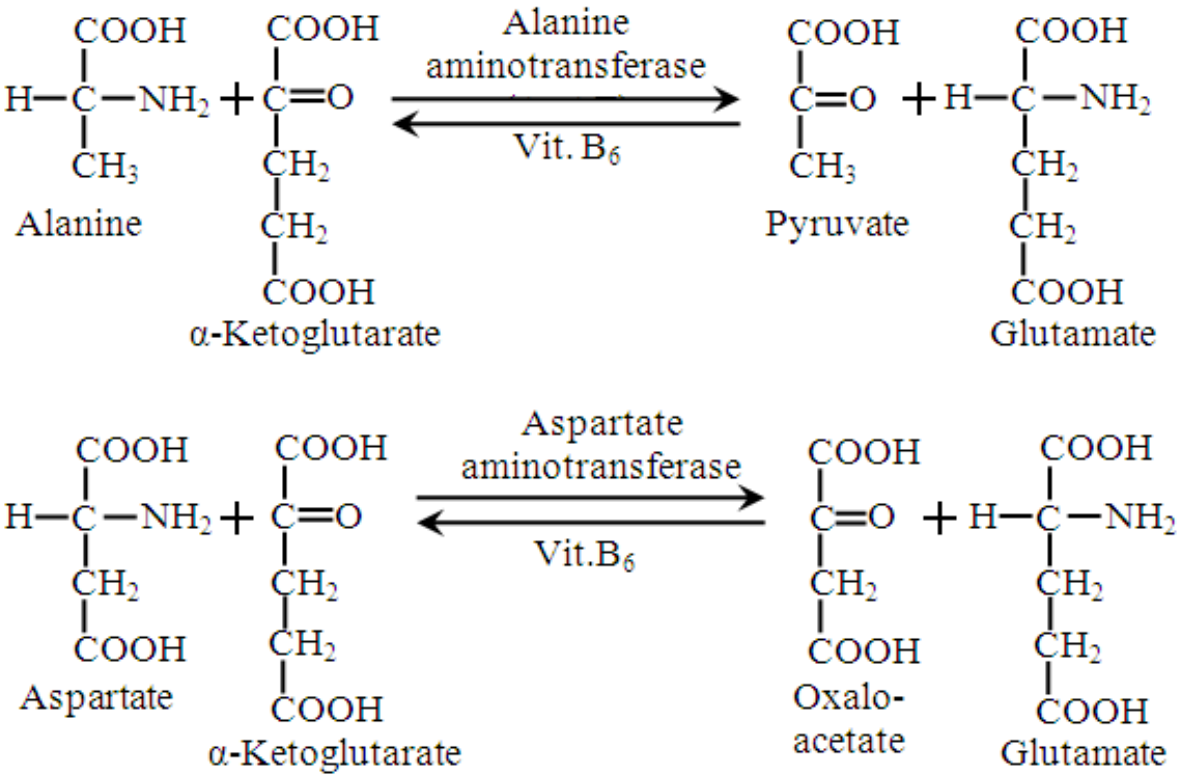


Fig. 24.6. Reactions catalyzed by alanine aminotransferase and aspartate aminotransferase.

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.

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. 24.7.).

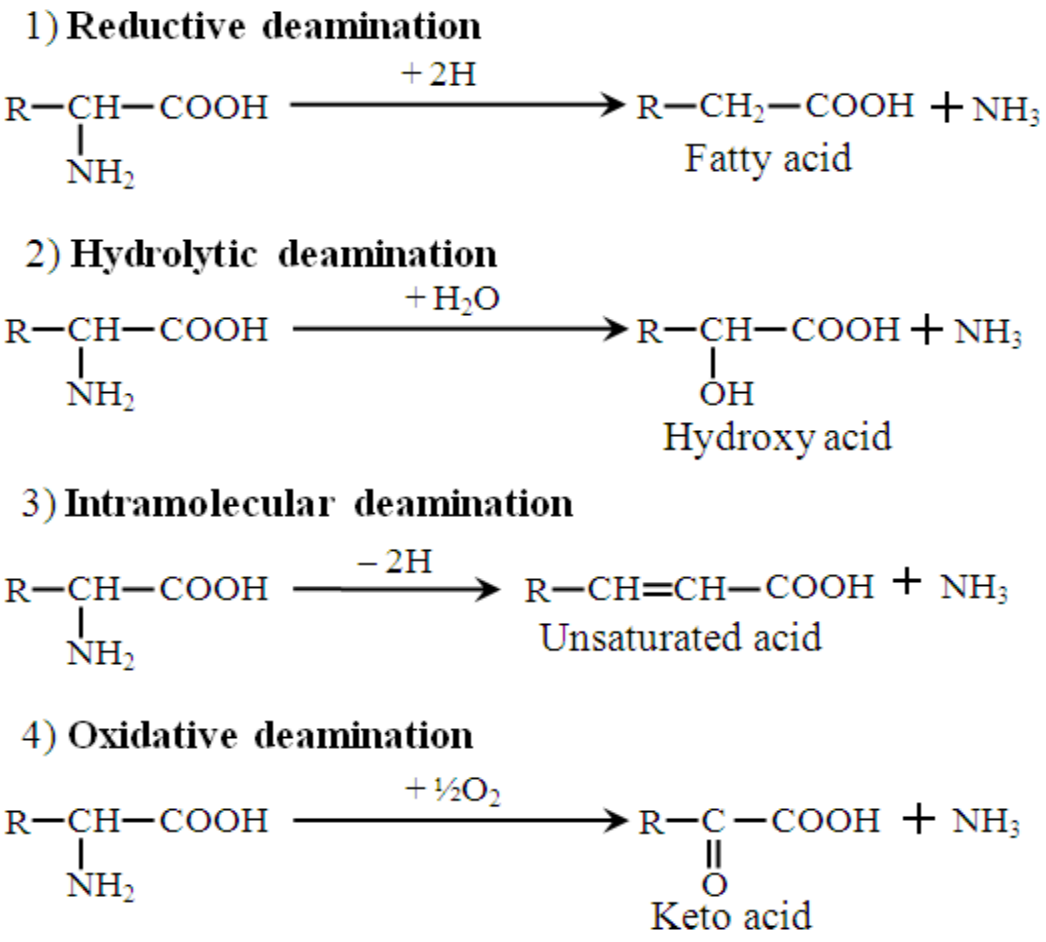
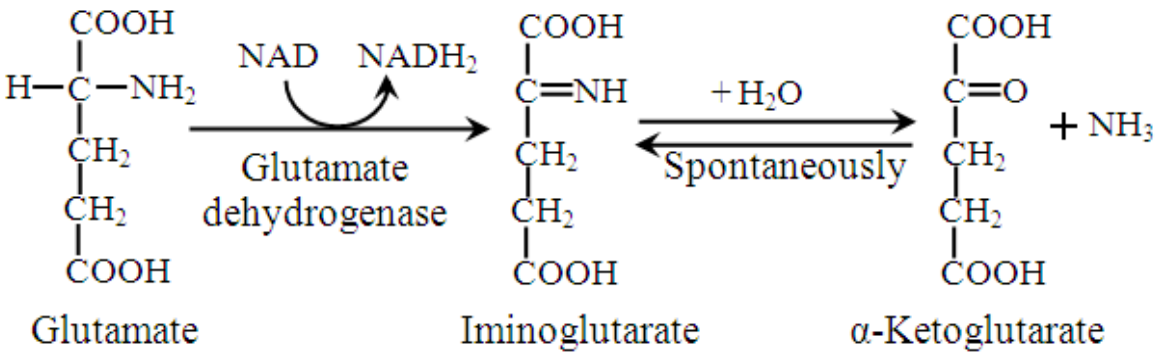


Fig. 24.7. Types of deamination.

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

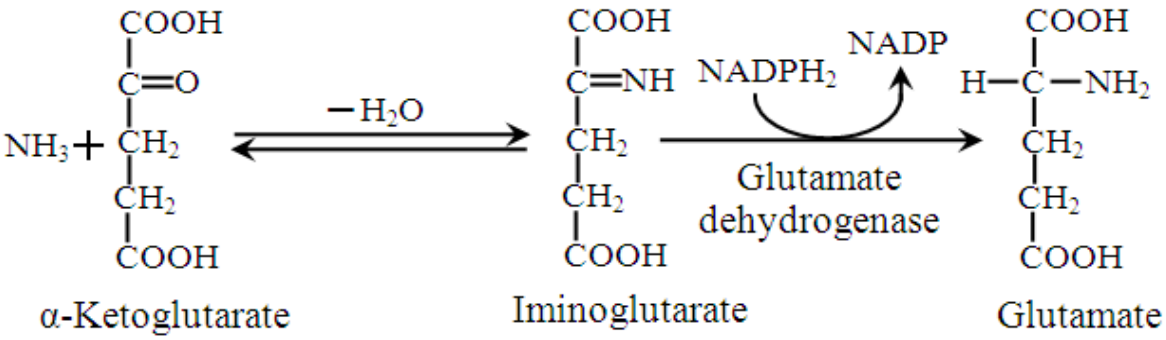
1. Deamination of excess molecules of glutamate, thus preparing them for further catabolism (degradation of carbon skeleton).

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 $NADPH_2$ 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.

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 (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. 24.8.

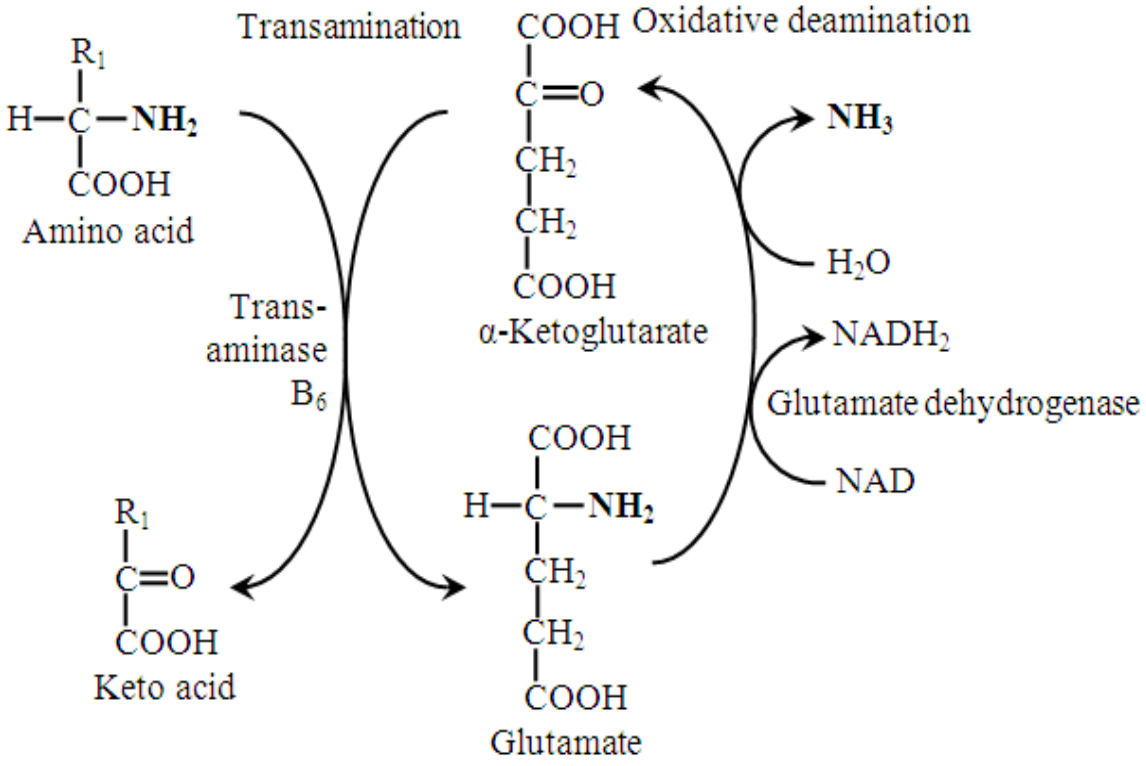


Fig. 24.8. Process of transdeamination. An amino acid first reacts with α-ketoglutarate in the transamination reaction to produce glutamate and corresponding keto acid. Glutamate thus formed is then subjected to the direct oxidative deamination by glutamate dehydrogenase.

CHAPTER 25

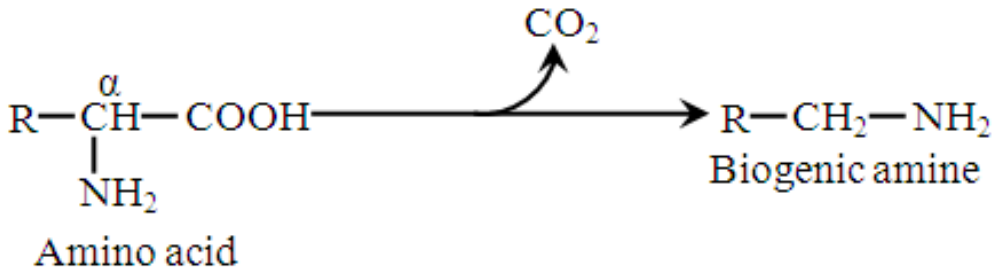
FORMATION AND DETOXIFICATION OF AMMONIA IN THE ORGANISM

DECARBOXYLATION OF AMINO ACIDS. TYPES OF DECARBOXYLATION, BIOLOGICAL ROLE

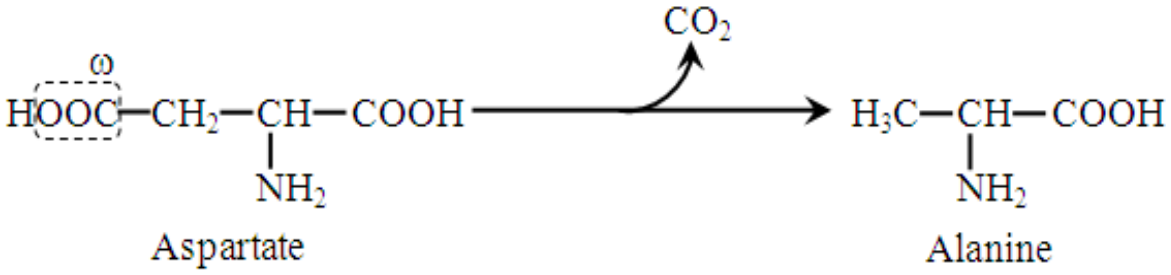
The removal of the carboxyl group from an amino acid with the release of CO_2 is called **decarboxylation**. The reaction is catalyzed by decarboxylases which coenzyme is pyridoxal phosphate (PALP), the derivative of vitamin B_6.

There are four types of the amino acid decarboxylation.

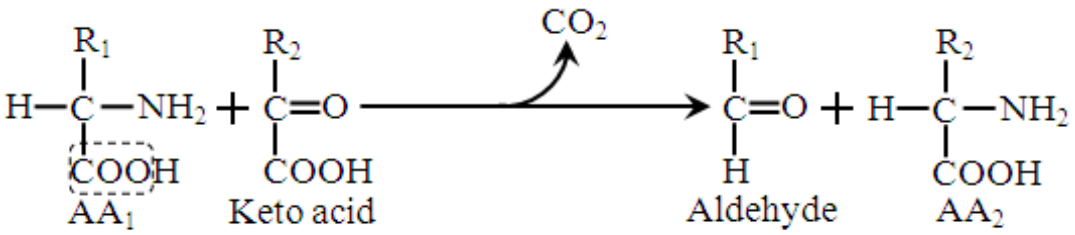
1) **α-Decarboxylation**. This type of decarboxylation is typical of animal tissues. Carboxyl group next to α-carbon of the amino acid molecule is removed. The reaction produces CO_2 and biogenic amines:



2) **ω-Decarboxylation**. This type of decarboxylation is typical of microorganism. By this pathway, a new amino acid may be produced.

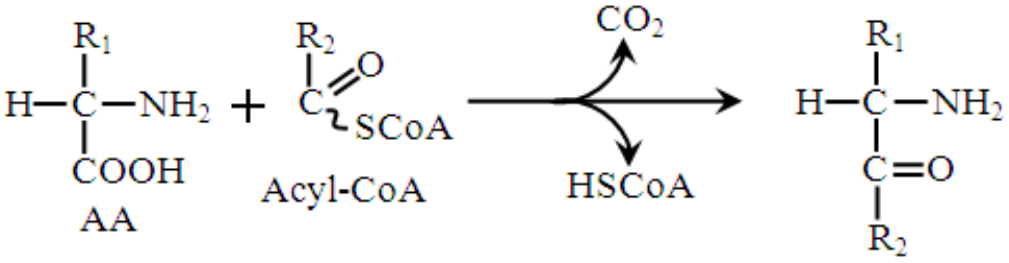


3) **Decarboxylation involving transamination reaction**.



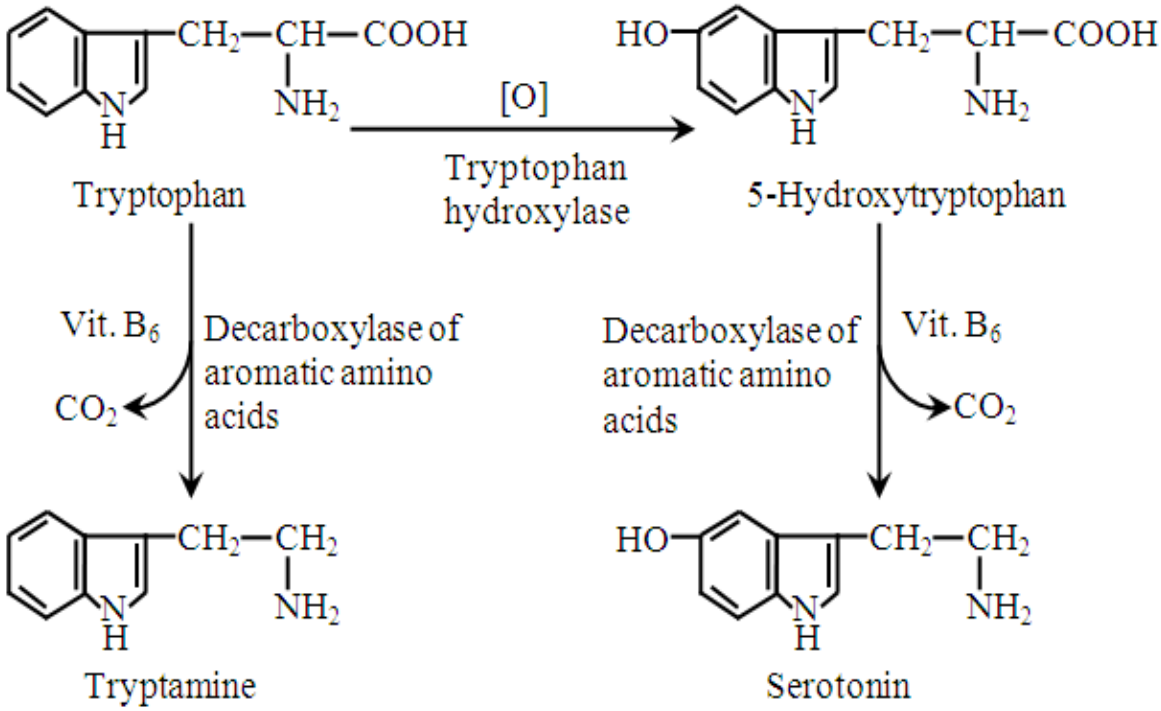
An aldehyde and a new amino acid, corresponding to the initial keto acid, are produced by this reaction.

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 exhibits **vasoconstrictive** action.

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). Fig. 25.1.

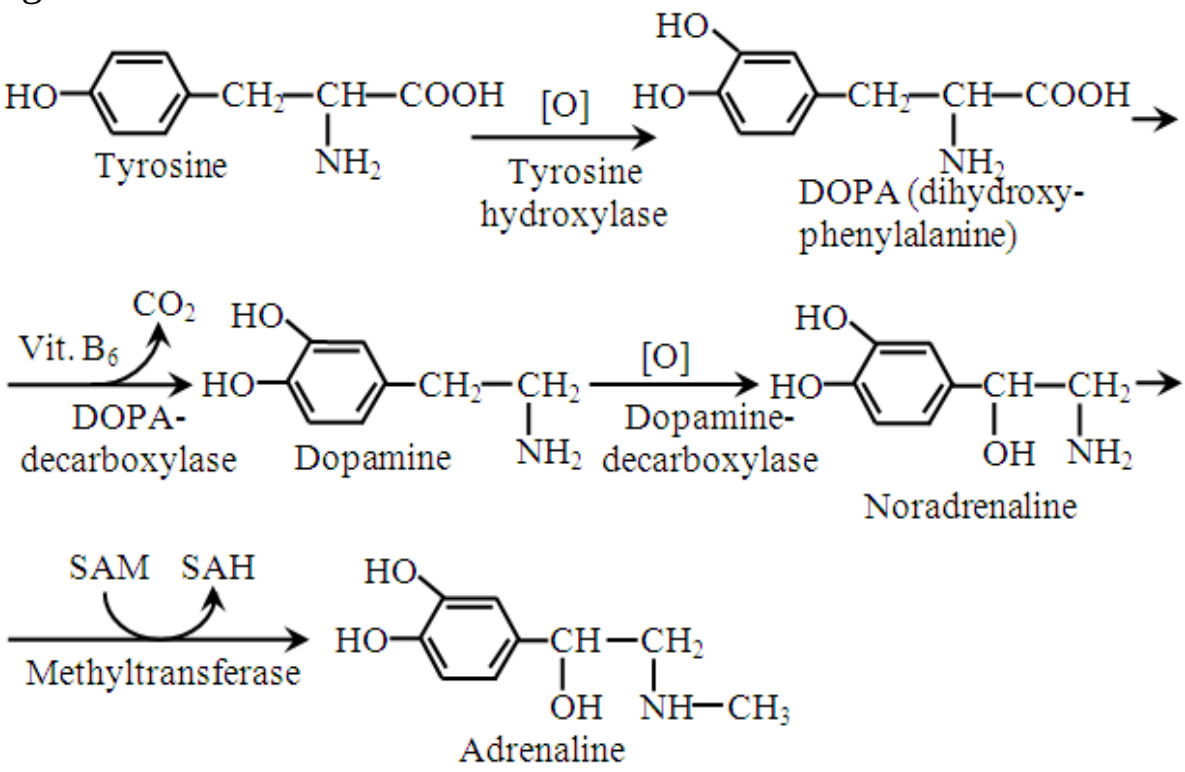


Fig. 25.1. Synthesis of dopamine, adrenaline and noradrenaline.

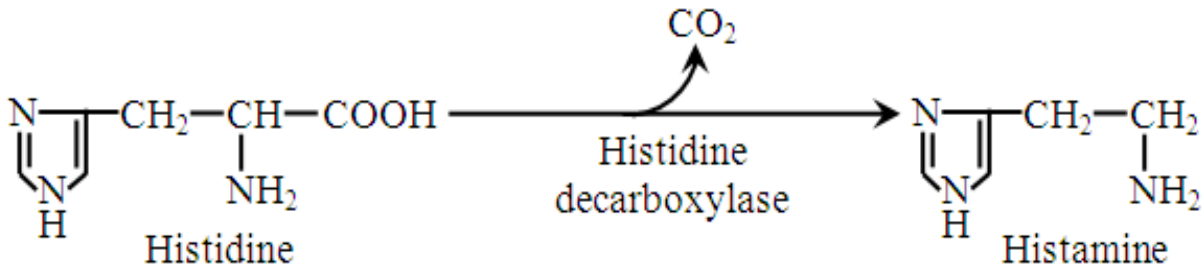
Dopamine is a **neurotransmitter**. It controls **voluntary movement**. Lack of dopamine causes **Parkinson's disease** which is characterized by **tremor** and difficulties in **initiating movement,** impairments in **emotional responses and memory**.

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**.

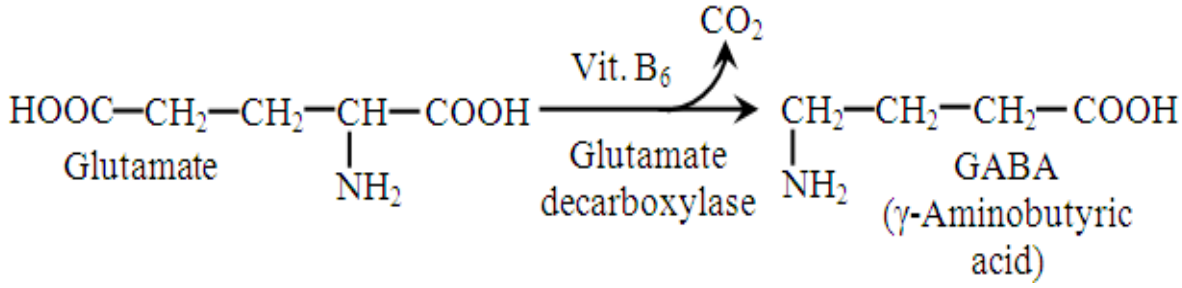
Epinephrine is a **hormone**. It is produced by adrenal medulla and is secreted in response to fright, flight, and exercise. Adrenaline **increases the blood pressure,** stimulates the **rate and force of myocardial contraction**, causes relaxation of smooth muscles of bronchi (**bronchodilatation**), increases **glycogenolysis** and **lipolysis**.

Biogenic amine – derivative of histidine



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**.

Biogenic amine – derivative of glutamate



GABA exerts the **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. 25.2), resulting in the formation of aldehyde and the release of ammonia.

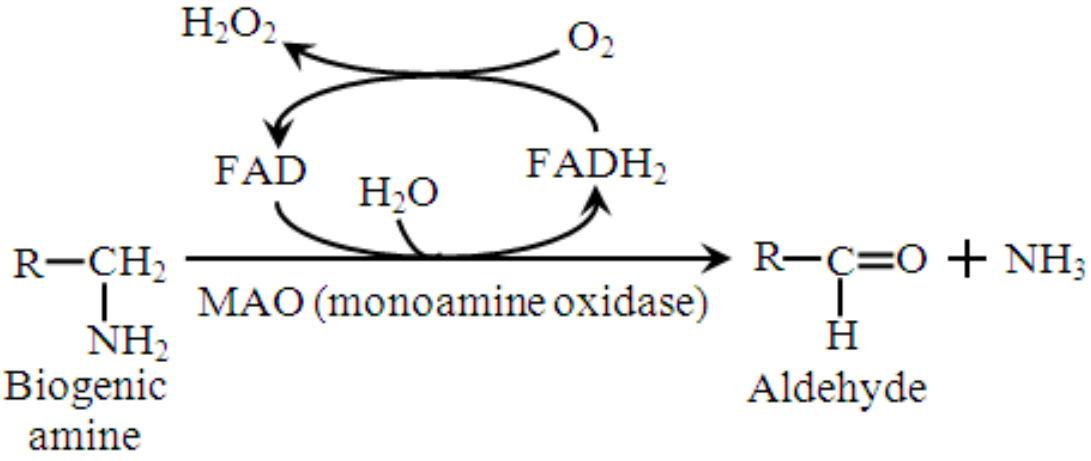


Fig. 25.2. Oxidation of biogenic amines.

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.

There are several ways for its detoxification:

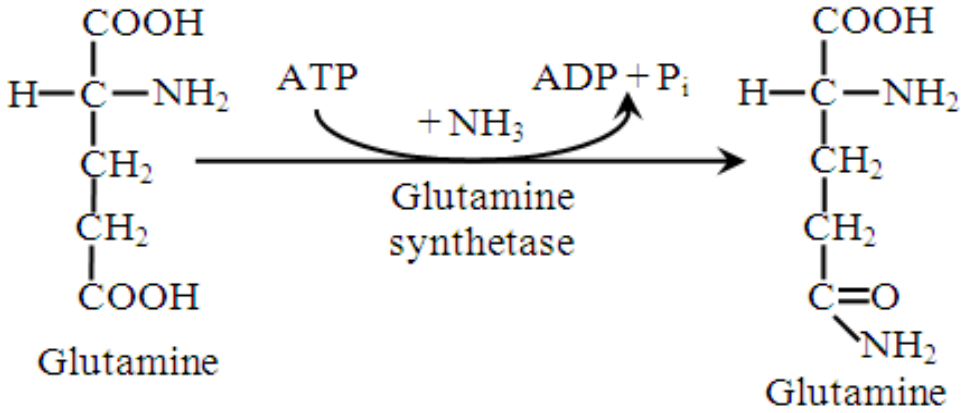
1) **Synthesis of carbamoyl phosphate** and its further conversion to urea which is excreted into the urine.

2) **Synthesis of amides**: glutamine and asparagine.

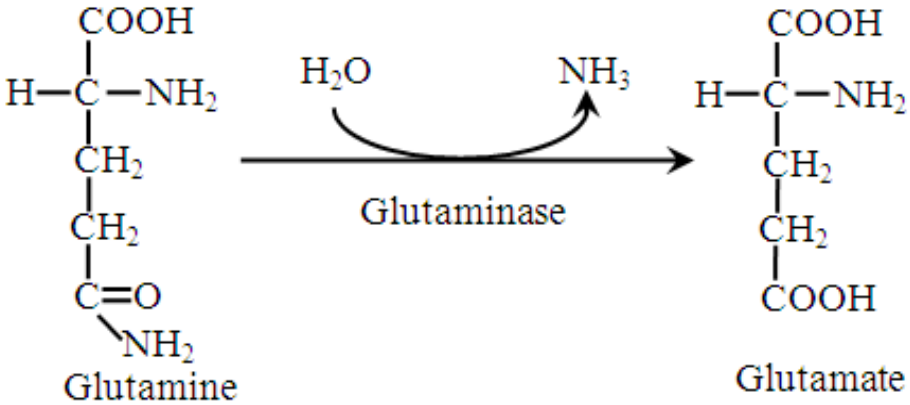
3) **Reductive amination** of α-ketoglutarate.

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 (glutamate) to form glutamine:

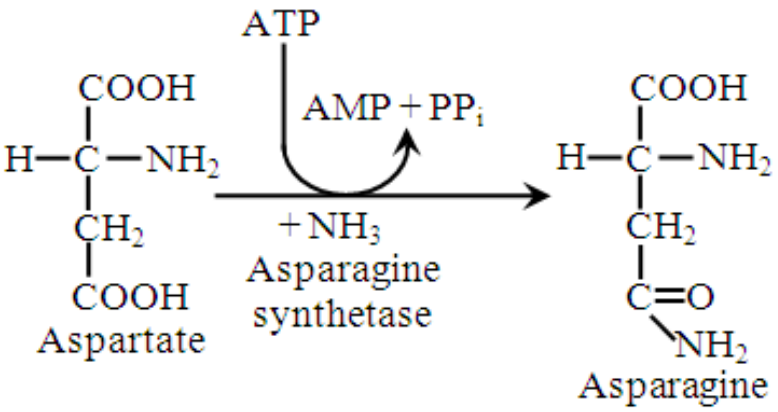


The glutamine is then transported to the liver, where the reaction is reversed by glutaminase:

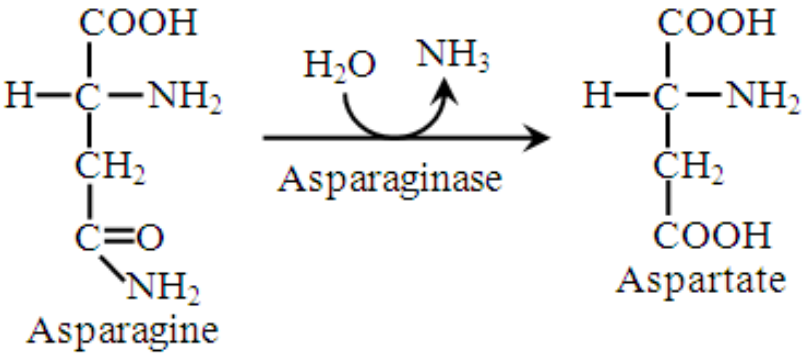


The ammonia, thus generated, is immediately detoxified in the liver by synthesis of urea.

Aspartic acid (aspartate) 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:

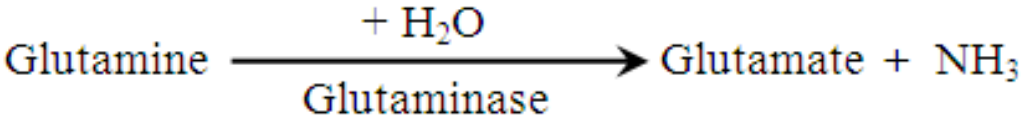


Glutamine and asparagine are the major transport forms of ammonia from the brain to the liver.

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:



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 (protein) metabolism.

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. 25.3.

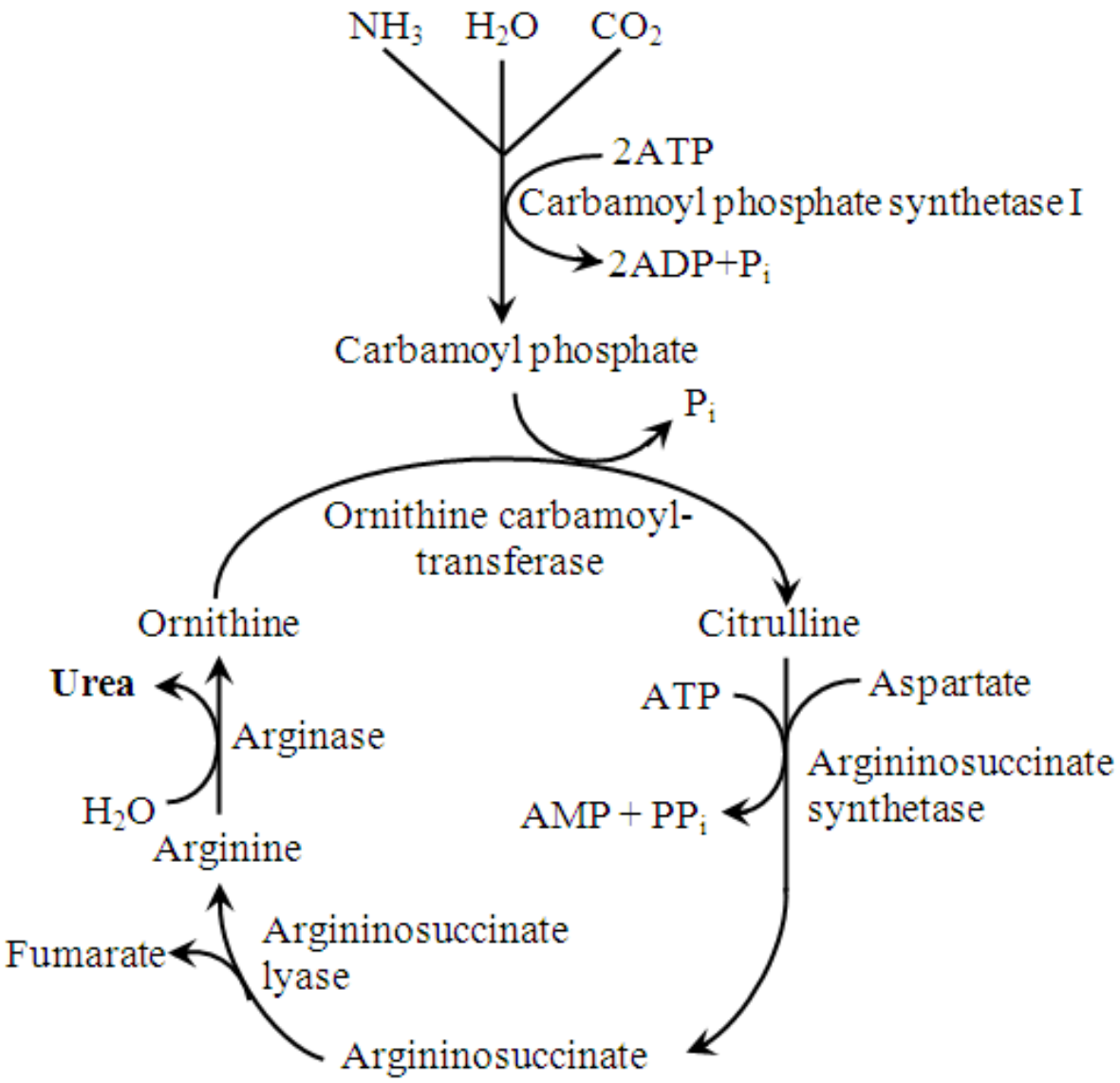
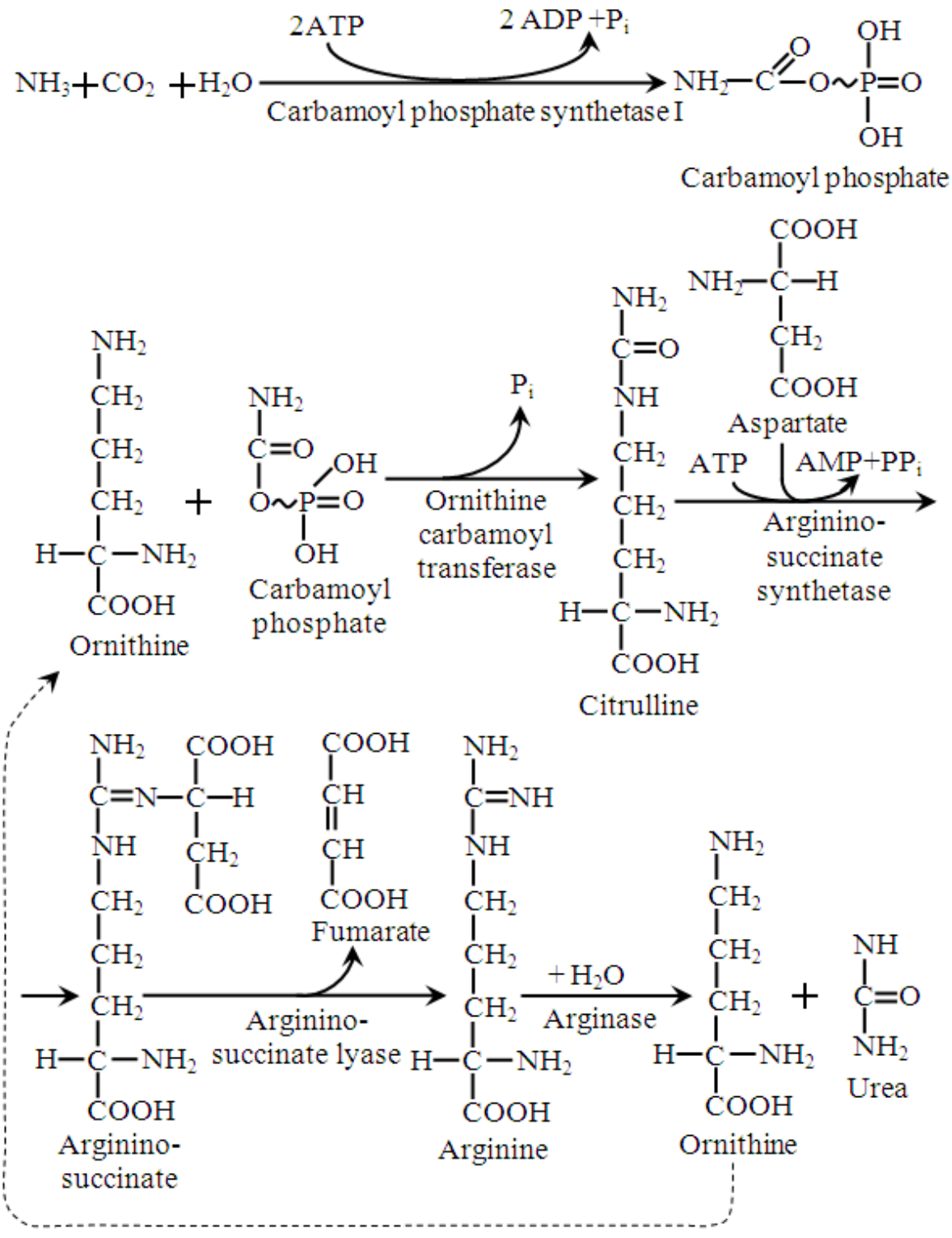


Fig. 25.3. Scheme of urea synthesis.

Normal concentrations of urea in the blood 2.5-8.33 mmol/L; its excretion into the urine 333-583 mmol/day.

Reactions 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 carbamoyltransferase
Citrullinemia	Argininosuccinate synthetase
Argininosuccinate aciduria	Argininosuccinate lyase
Hyperargininemia	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.

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**. 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.

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). Schematically, glucogenic and ketogenic amino acids are shown in Fig. 25.4.

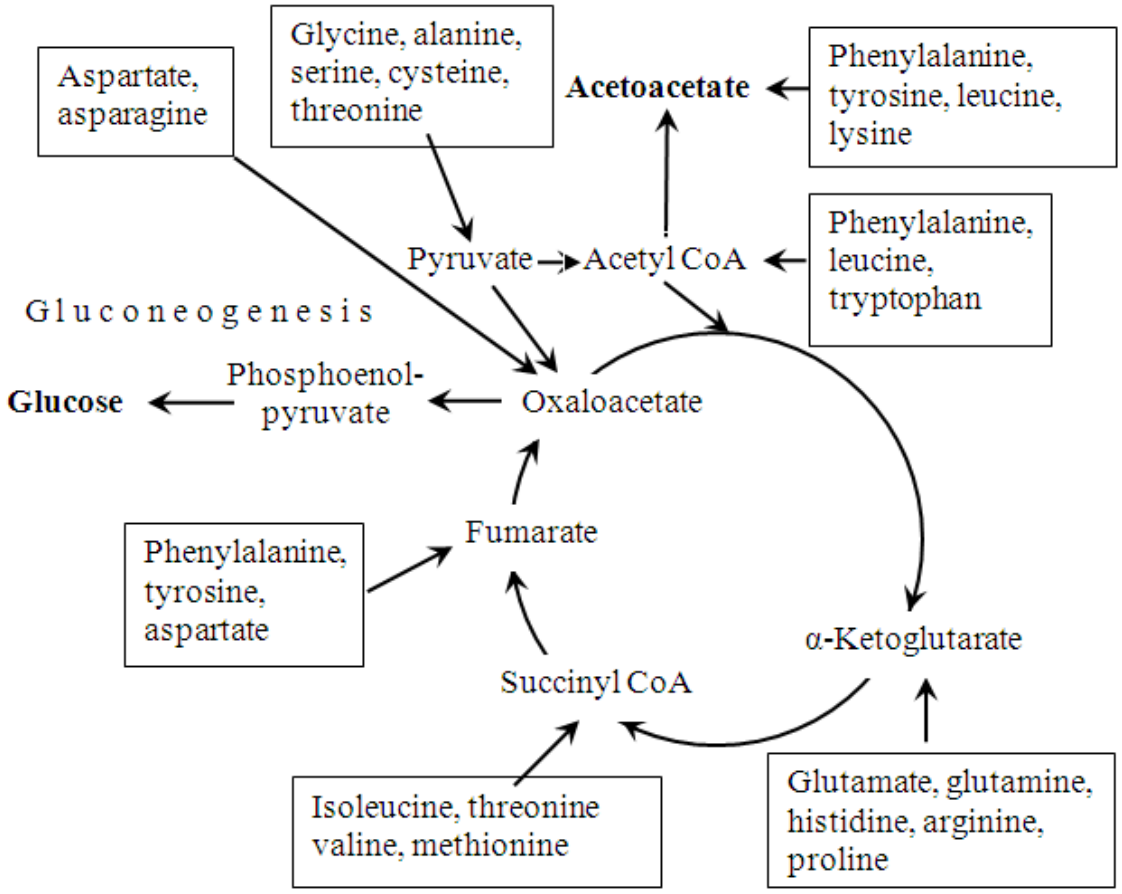


Fig. 25.4. Glucogenic and ketogenic amino acids.

CHAPTER 26

METABOLISM OF METHIONINE, PHENYLALANINE AND TYROSINE

METABOLISM OF METHIONINE. ROLE OF METHIONINE IN TRANSMETHYLATION REACTIONS

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.

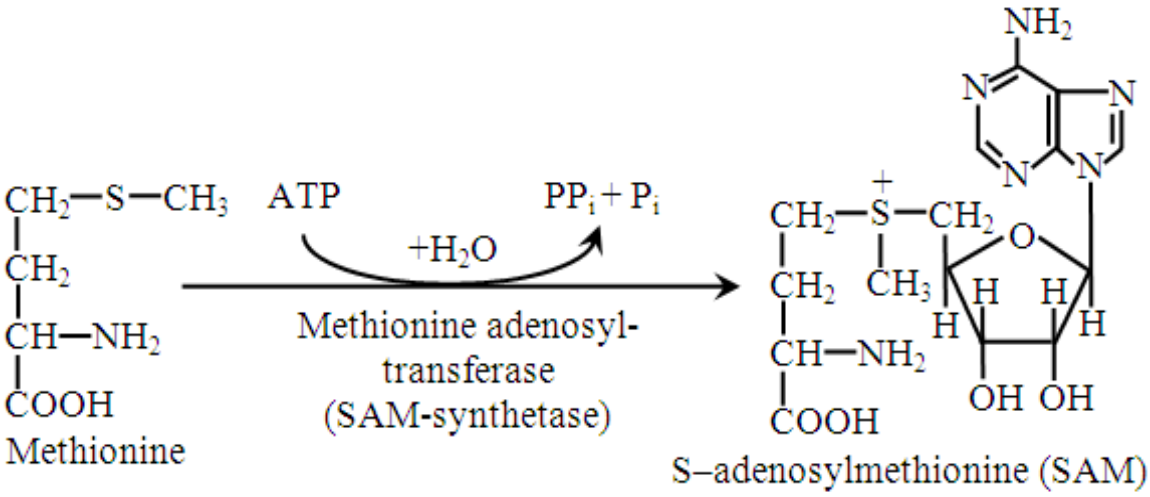
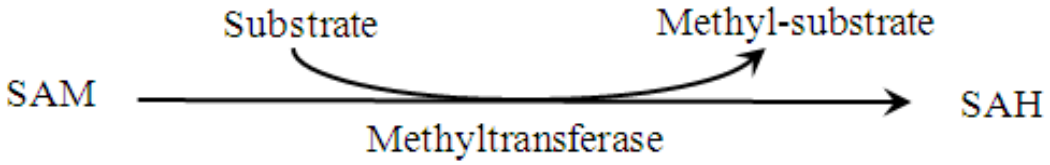


Fig. 26.1. Reaction of fomation of S-adenosylmethionine.

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 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

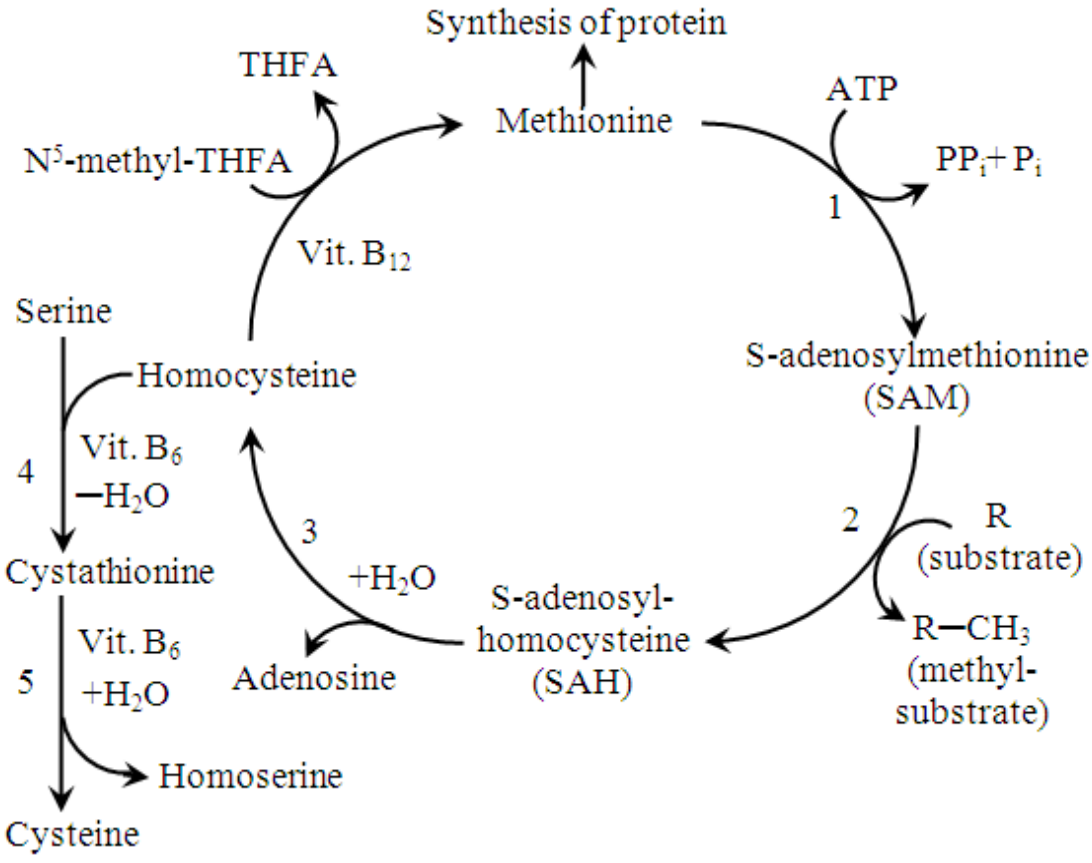


Fig. 26.2. The total scheme of methionine metabolism.
Enzymes: 1 – methionine adenosyltransferase; 2 – methyltransferase;
3 – SAH-hydrolase; 4 – cystathionine synthetase;
5 – cystathionine lyase.

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.

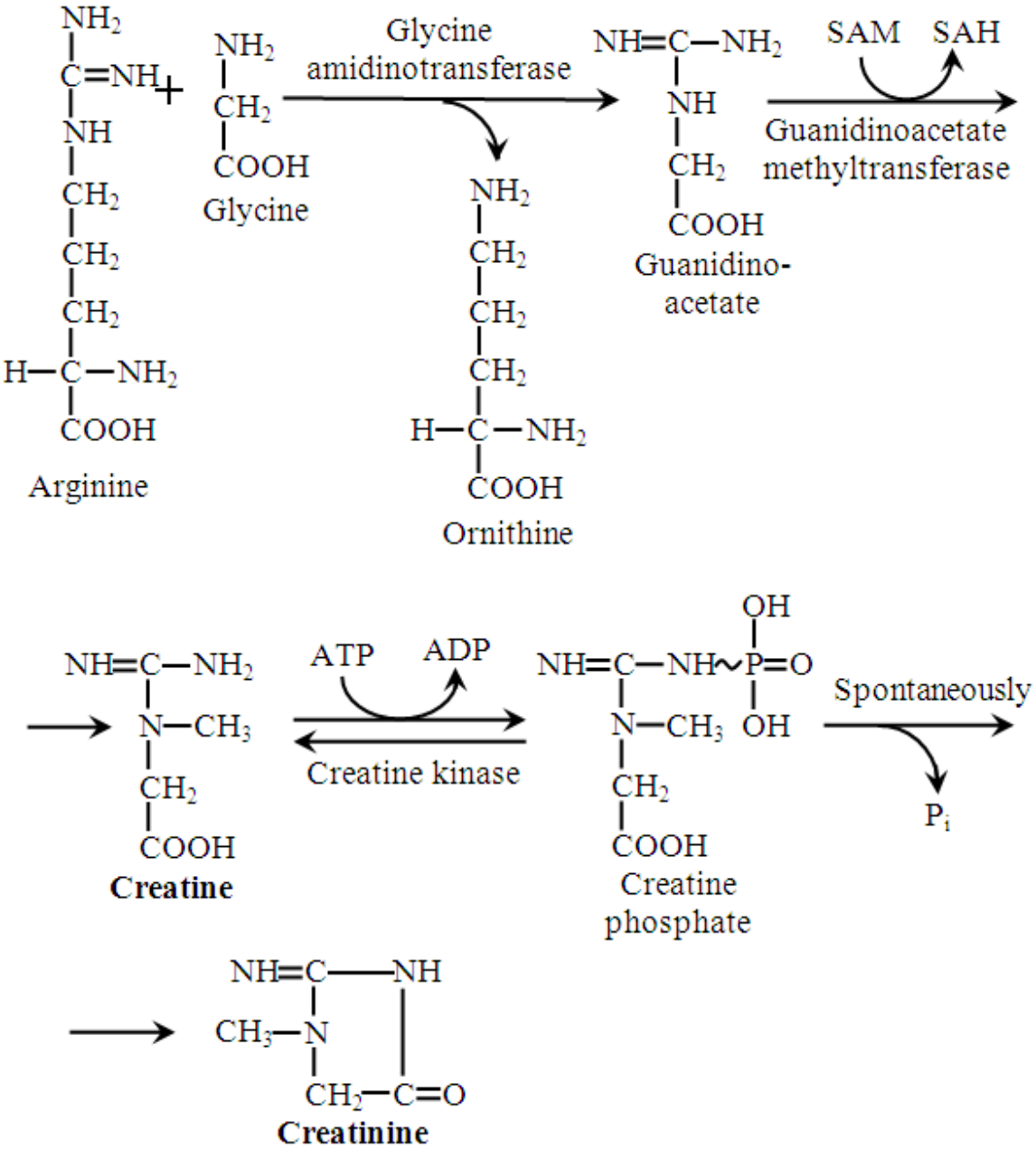


Fig. 26.3. Synthesis of creatine and creatinine.

Unlike creatinine, **creatine is practically absent from the urine in adult humans**. 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 into the urine). Determination of the creatine kinase activity in the blood serum is important for laboratory diagnosis of myocardial infarction.

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 either undergo catabolism or convert to catecholamines, melanin and thyroid hormones.

Catabolism of phenylalanine and tyrosine

The major pathway for phenylalanine metabolism begins with hydroxylation of this amino acid to form tyrosine (Fig. 26.4.).

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. 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.

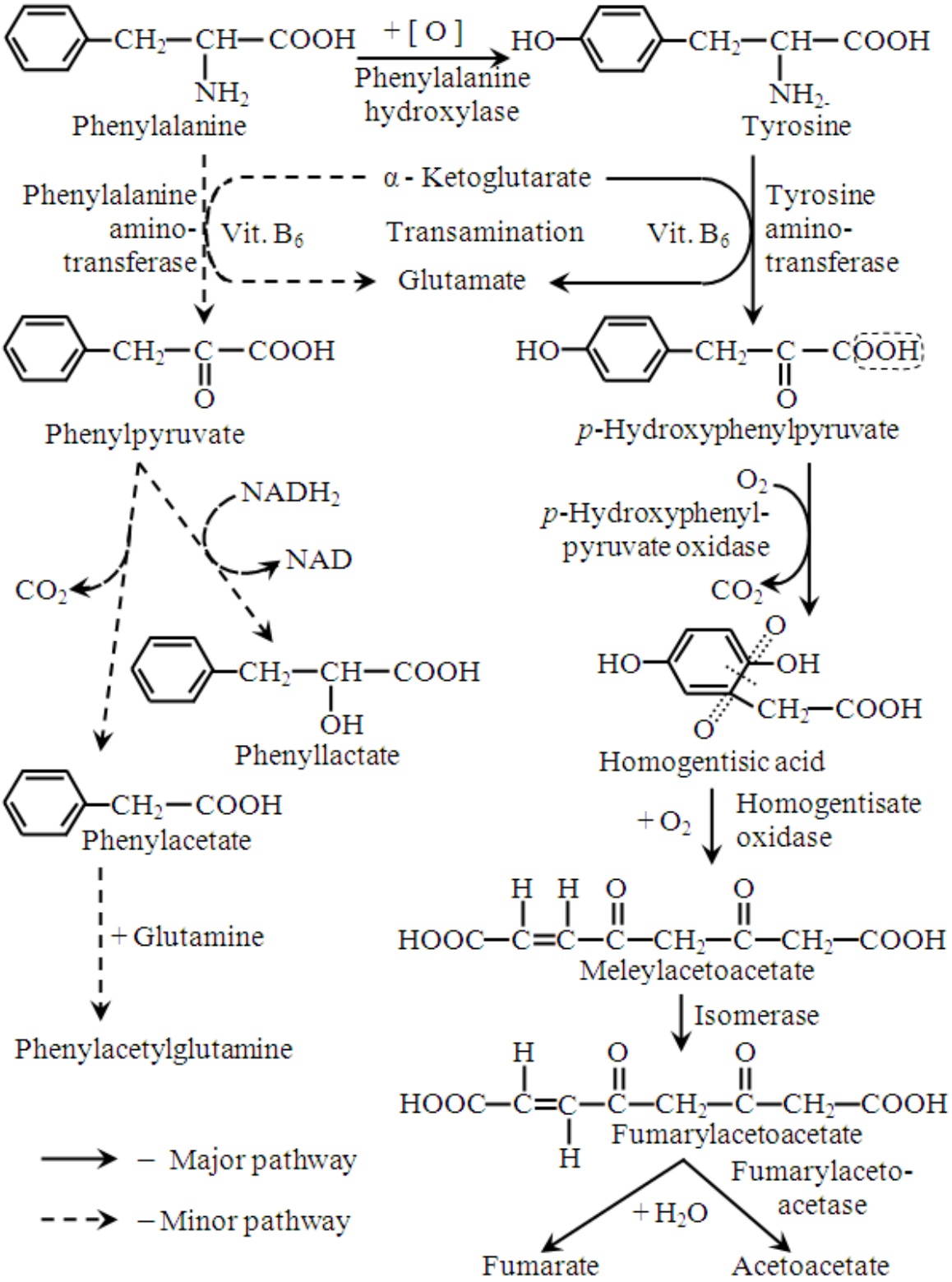


Fig. 26.4. Catabolism of phenlalanine and tyrosine.

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. 26.5).

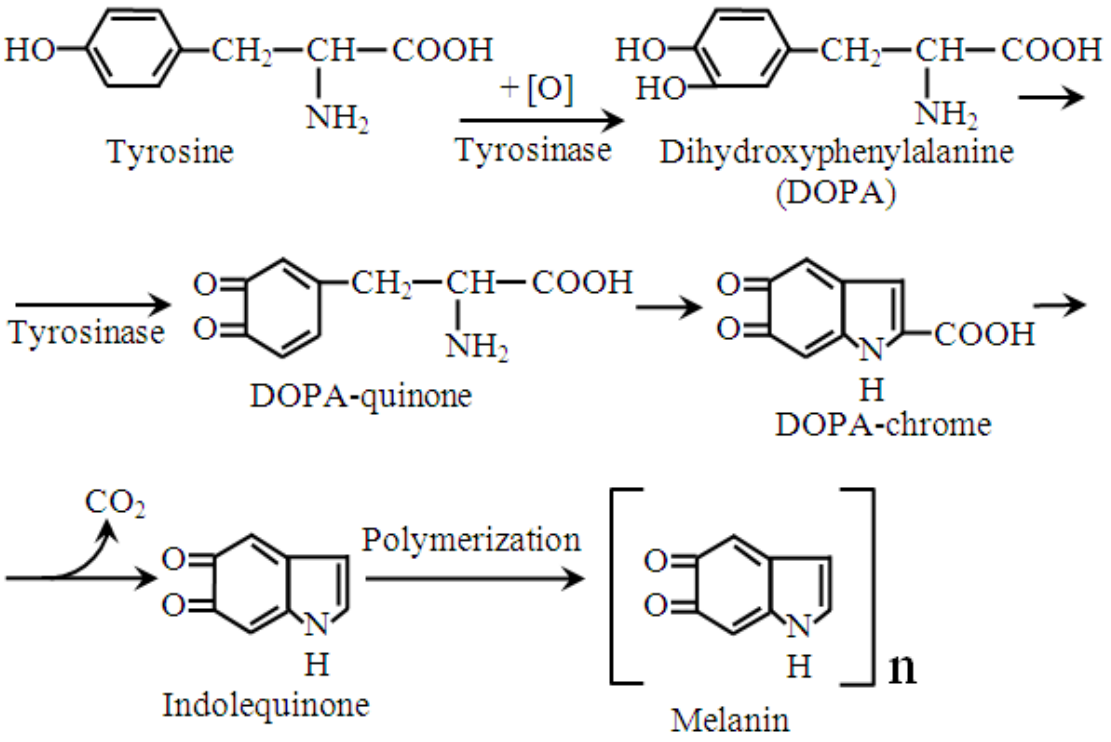


Fig. 26.5. Synthesis of melanin.

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.

General scheme of the phenylalanine and tyrosine metabolism as well as some genetic defects of the amino acids are shown in Fig. 26.7.

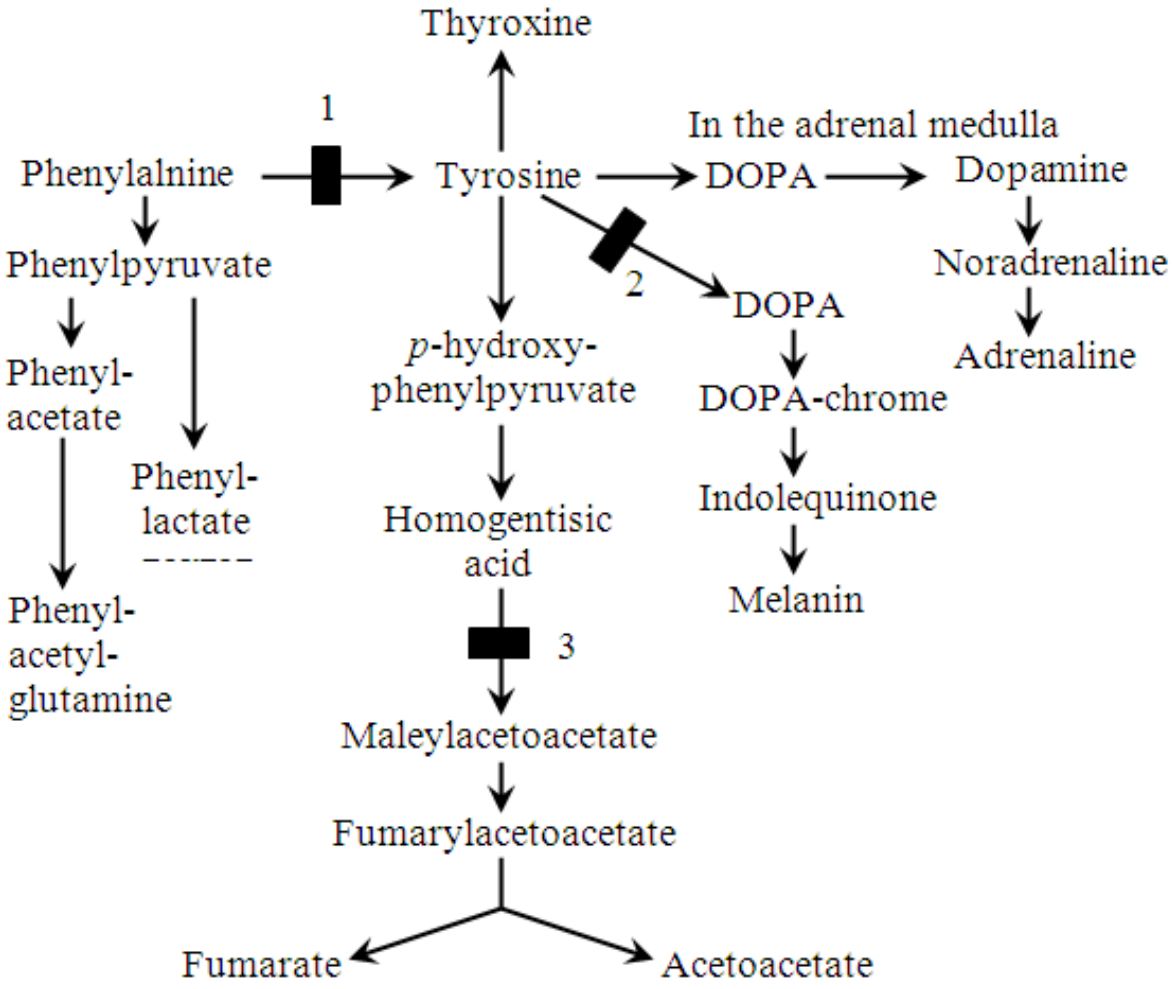


Fig. 26.7. Summary of the phenylalanine and tyrosine metabolism. The numbers indicate genetic block in diseases: 1 – phenylketonuria; 2 – albinism; 3 – alkaptonuria.

CHAPTER 27

METABOLISM OF NUCLEOTIDES

A nucleotide consists of three component parts: nitrogenous base (purine or pyrimidine), pentose (ribose or deoxyribose), and phosphate group. 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).

Nitrogenous base + (d)Ribose = (d) **Nucleoside**;

Nitrogenous base + (d)Ribose + P_i = (d) **Nucleotide**.

The names of nucleosides and nucleotides are given in Table 27.1.

Table 27.1.

Names of nitrogenous bases, nucleosides, and nucleotides

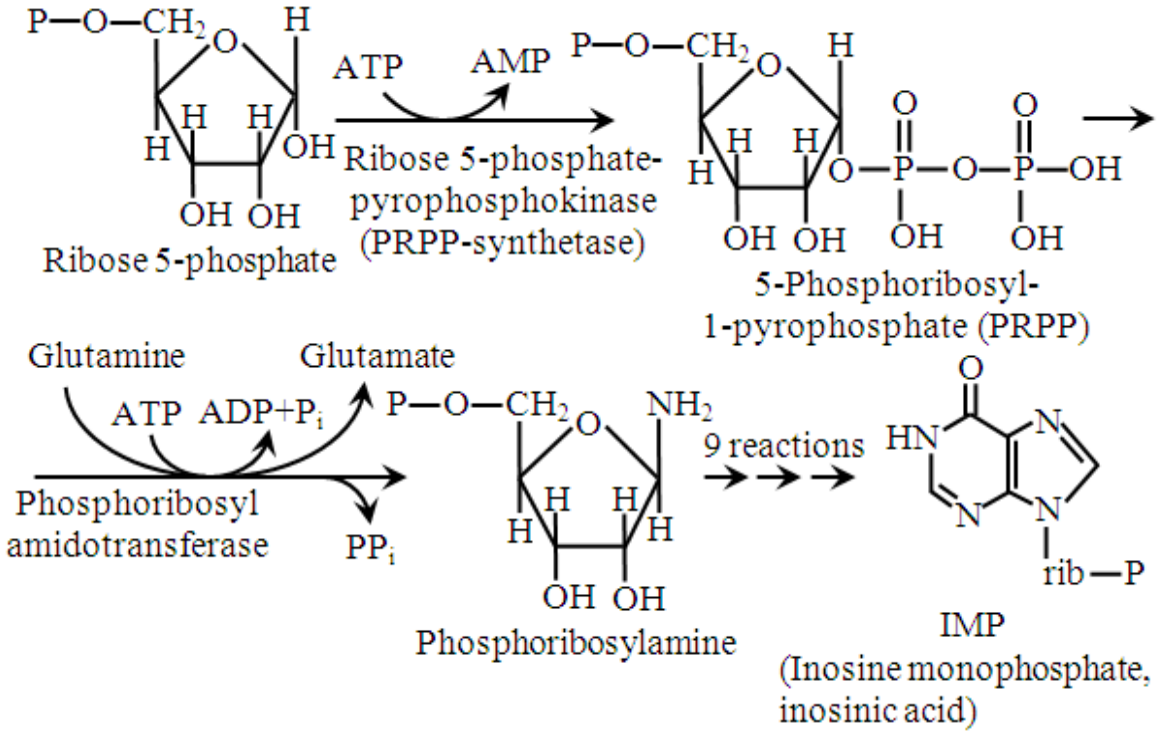
Nitrogenous base	***Nucleoside***	***Nucleotide***
Purines:		
Adenine	(d)Adenosine	(d)Adenosine monophosphate, (d)AMP, (d)adenylic acid
Guanine	(d)Guanosine	(d)Guanosine monophosphate, (d)GMP, (d)guanylic acid
Pyrimidines:		
Cytosine	(d)Cytidine	(d)Cytidine monophosphate, (d)CMP, (d)cytidylic acid
Thymine*	Thymidine	Thymidine monophosphate, TMP, thymidylic acid
Uracil*	Uridine	Uridine monophosphate, UMP, uridylic acid

* Thymine is mainly bound with deoxyribose, uracil – with ribose.

BIOSYNTHESIS OF PURINE NUCLEOTIDES

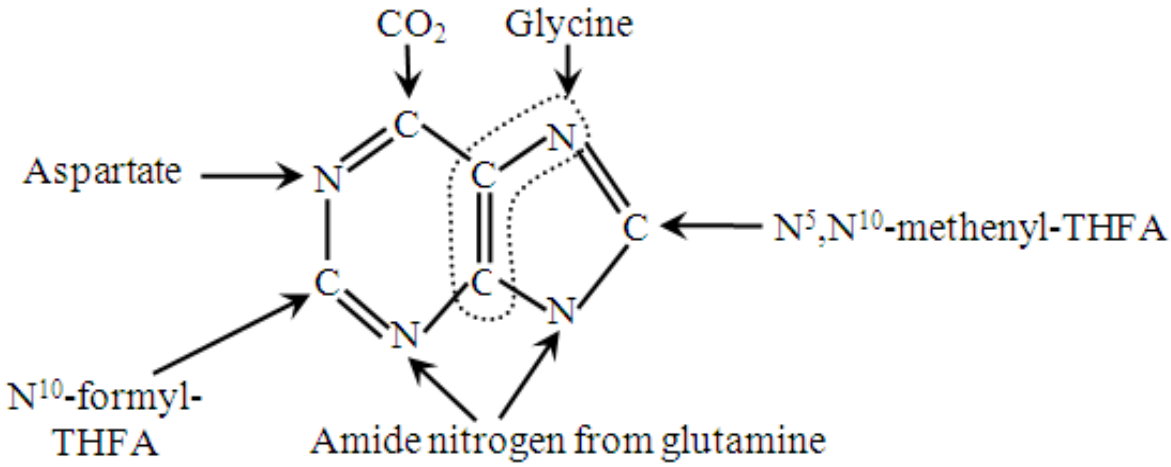
Biosynthesis of phosphoribosylamine

Biosynthesis of purine nucleotides takes place in the cytoplasm and starts from ribose 5-phosphate, a product of the pentose phosphate pathway. The initial reactions are as follows:



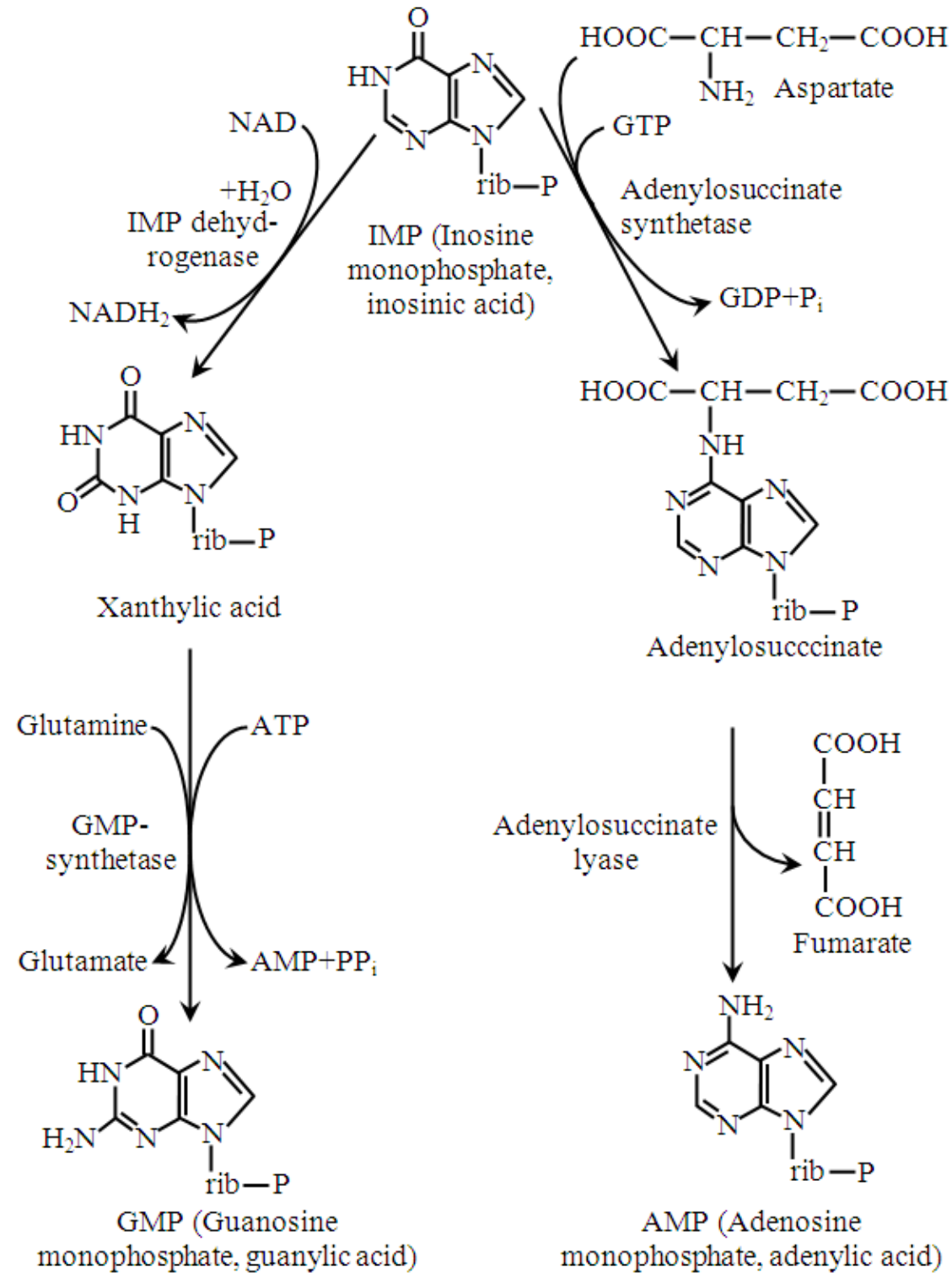
Origin of atoms in the purine ring

The contribution of different atoms from different sources for the formation of the purine ring may be represented as follows:



Inosinic acid as a precursor for synthesis of adenylic and guanylic acids

Inosinic acid (IMP) is a precursor for synthesis of adenylic acid (AMP) and guanylic acid (GMP):



Regulation of purine synthesis

The reaction catalyzed by **phosphoribosyl amidotransferase**. It is inhibited by AMP and GMP (the end products). 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. The activity of **ribose 5-phosphate pyrophosphokinase (PRPP synthetase)** is also inhibited by purine nucleotides. The regulatory effects of purine nucleotides on their synthesis are given in Fig. 27.1.

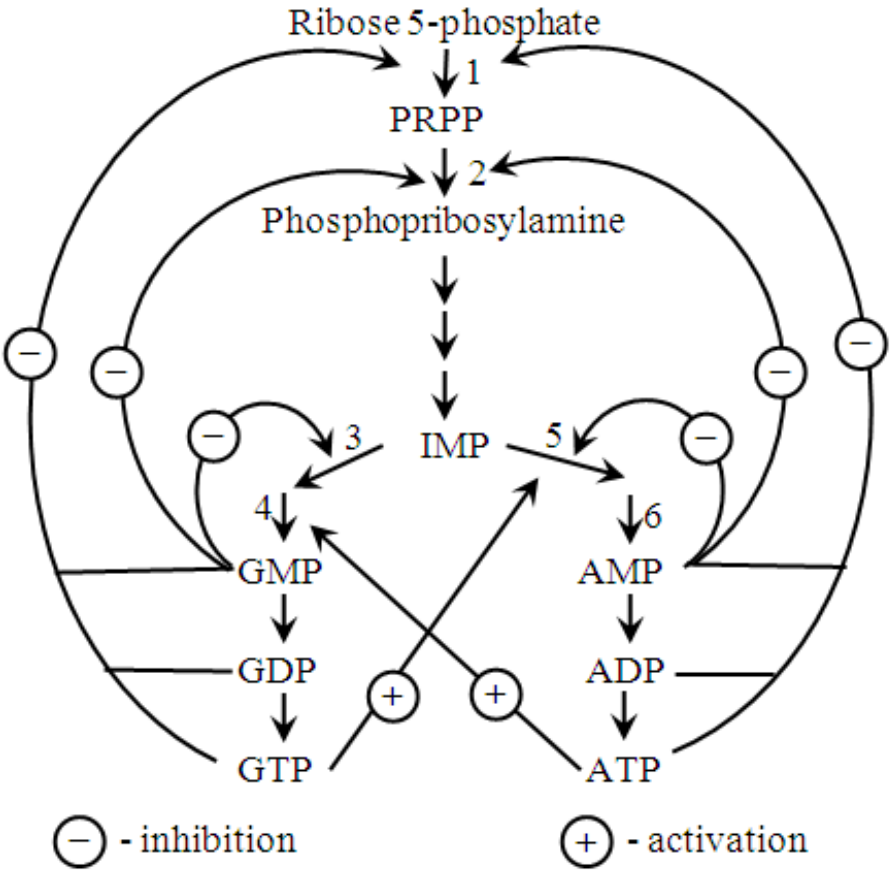


Fig. 27.1. Regulation of purine synthesis.
1 – ribose 5-phosphate pyrophosphokinase; 2 – phosphoribosyl amidotransferase; 3 – IMP dehydrogenase; 4 – GMP synthetase; 5 – adenylosuccinate synthetase; 6 – adenylosuccinate lyase.

BIOSYNTHESIS OF PYRIMIDINE NUCLEOTIDES

Biosynthesis of pyrimidine nucleotides (Fig. 27.2) 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.

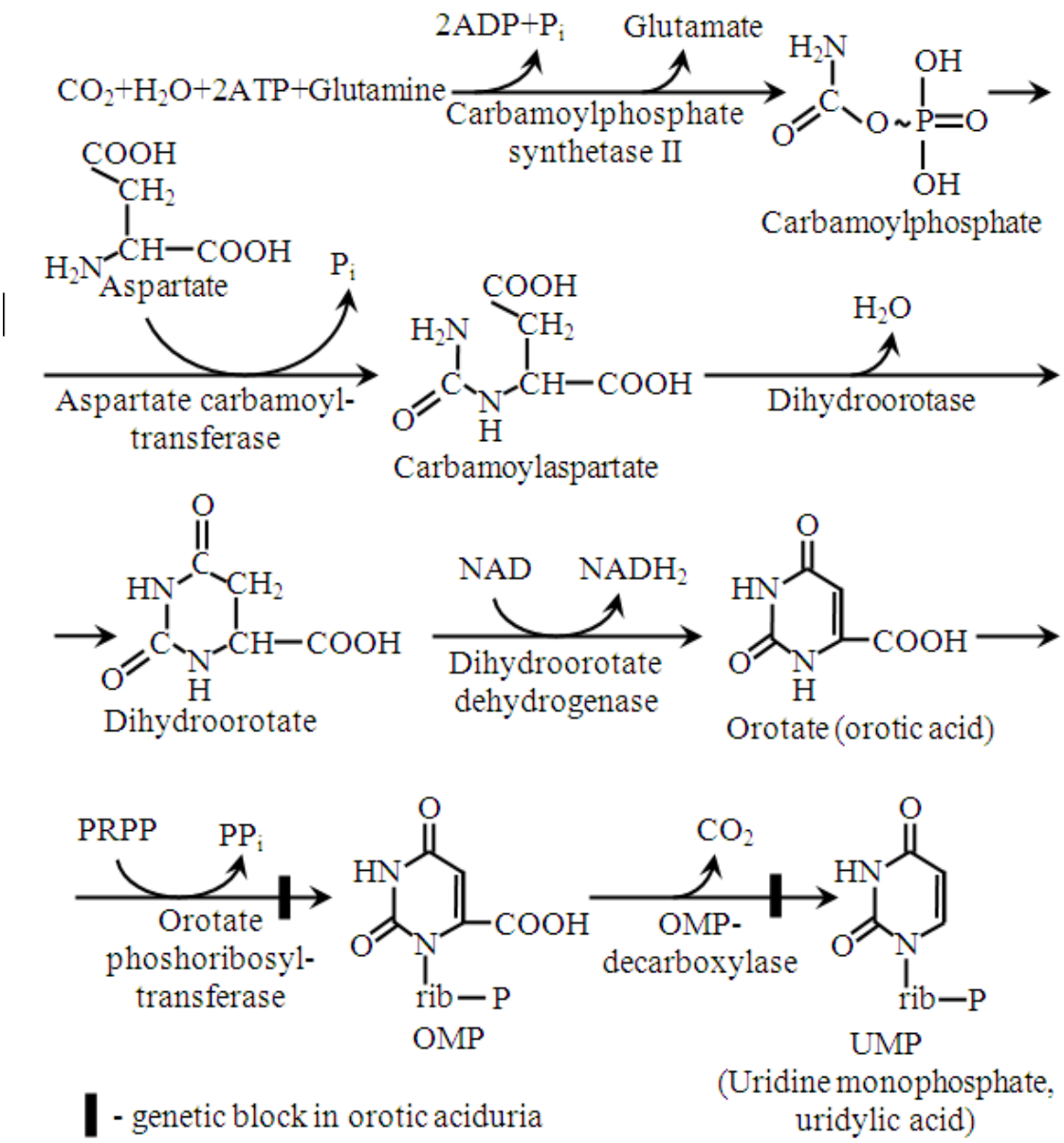
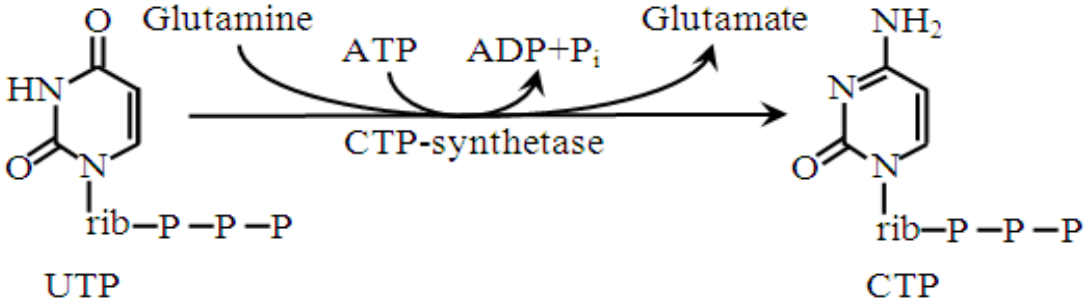


Fig. 27.2. Synthesis of pyrimidine nucleotides.

Of three pyrimidine nucleotides, UMP is initially synthesized. The other uridylic nucleotides (UDP, UMP) are formed due to phosphorylation by ATP: UMP +ATP↔UDP+ADP; UDP+ATP↔ ↔UTP+ADP. **CTP is synthesized from UTP:**



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. The regulatory effects of pyrimidine nucleotides on their synthesis are given in Fig. 27.3.

SYNTHESIS OF DEOXYRIBONUCLEOTIDES

As DNA consists 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. 27.4). 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.

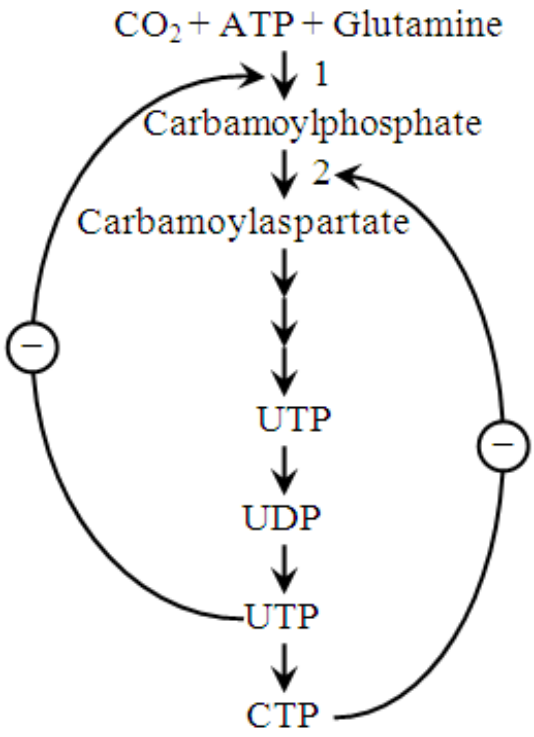


Fig. 27.3. Regulation of pyrimidine synthesis.
1 – carbamoylphosphate synthetase II; 2 – aspartate carbamoyltransferase.

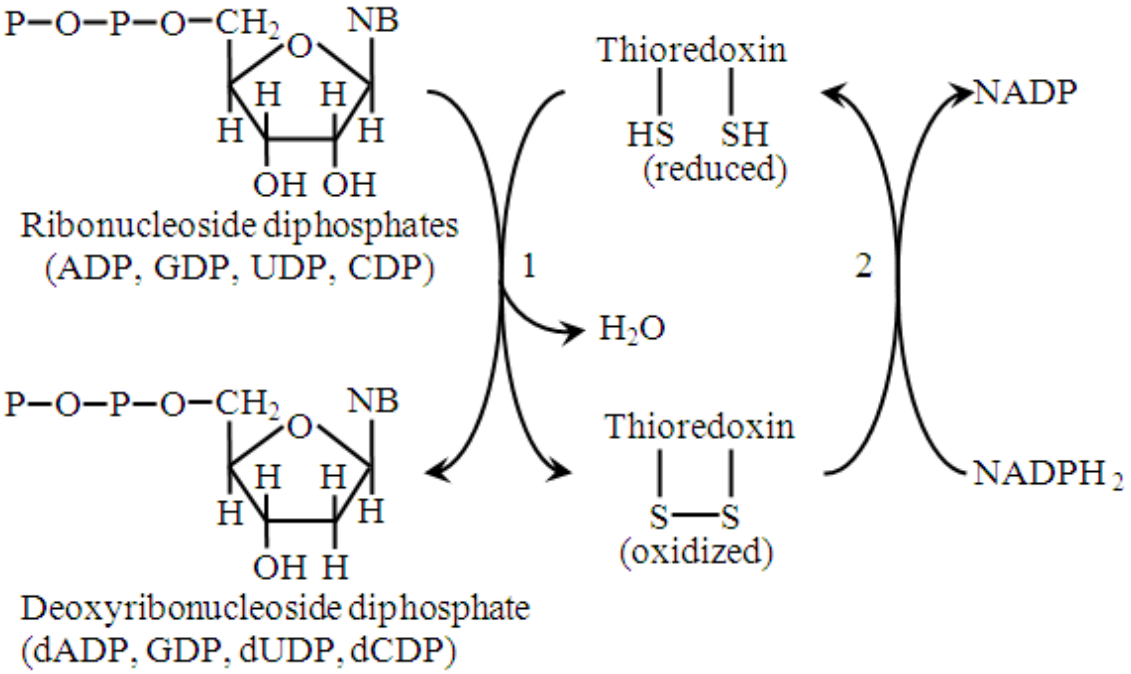


Fig. 27.4. Synthesis of deoxyribonucleotides. Enzymes: 1 – ribonucleoside diphosphate reductase; 2 – thioredoxin reductase. NB – nitrogenous bases.

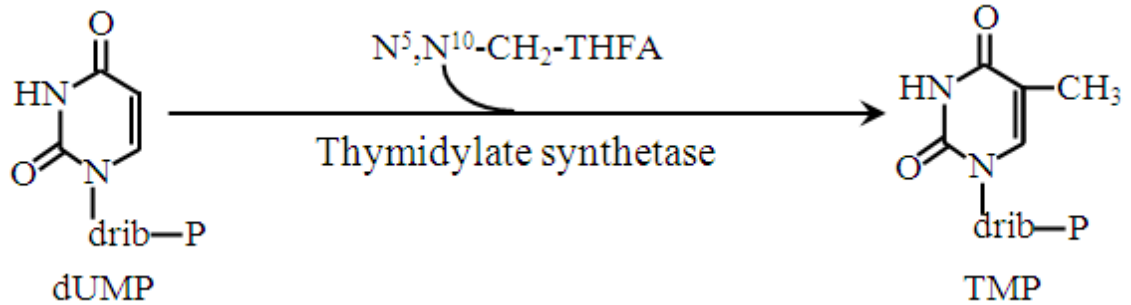
Thioredoxin is getting oxidized and undergoes subsequent reduction by **thioredoxin reductase** in the presence of $NADPH_2$.

SYNTHESIS OF THYMIDYLIC ACID

For synthesis of thymidylic acid (TMP), the molecule of dUMP is required. First, dUDP previously formed undergoes hydrolysis to dUMP:

$$dUDP + H_2O \longrightarrow dUMP + H_3PO_4.$$

The donor of methyl group for synthesis of thymidylic acid is N^5,N^{10}-methylene-THFA:



Disorders of pyrimidine synthesis. Orotaciduria (orotic aciduria)

The disease results from deficiency of **orotate phosphoribosyl transferase** and **OMP-decarboxylase**, i.e. the enzymes that catalyze conversion of orotate to UMP. The orotic acid accumulated in the organism is excreted into the urine. On standing, orotic acid forms specific **needle-like crystals** in the urine, but may also precipitate in the urinary tract causing its obstruction.

Due to the genetic defect, pyrimidines are not synthesized, which 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 **uridine** allows to bypass the metabolic block and provides the body with pyrimidines.

DIGESTION OF NUCLEIC ACIDS

Dietary nucleic acids are ingested as nucleoproteins. In the stomach, nucleoproteins are degraded by gastric enzymes and HCl to form polypeptides and nucleic acids (Fig.27.5.). 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.

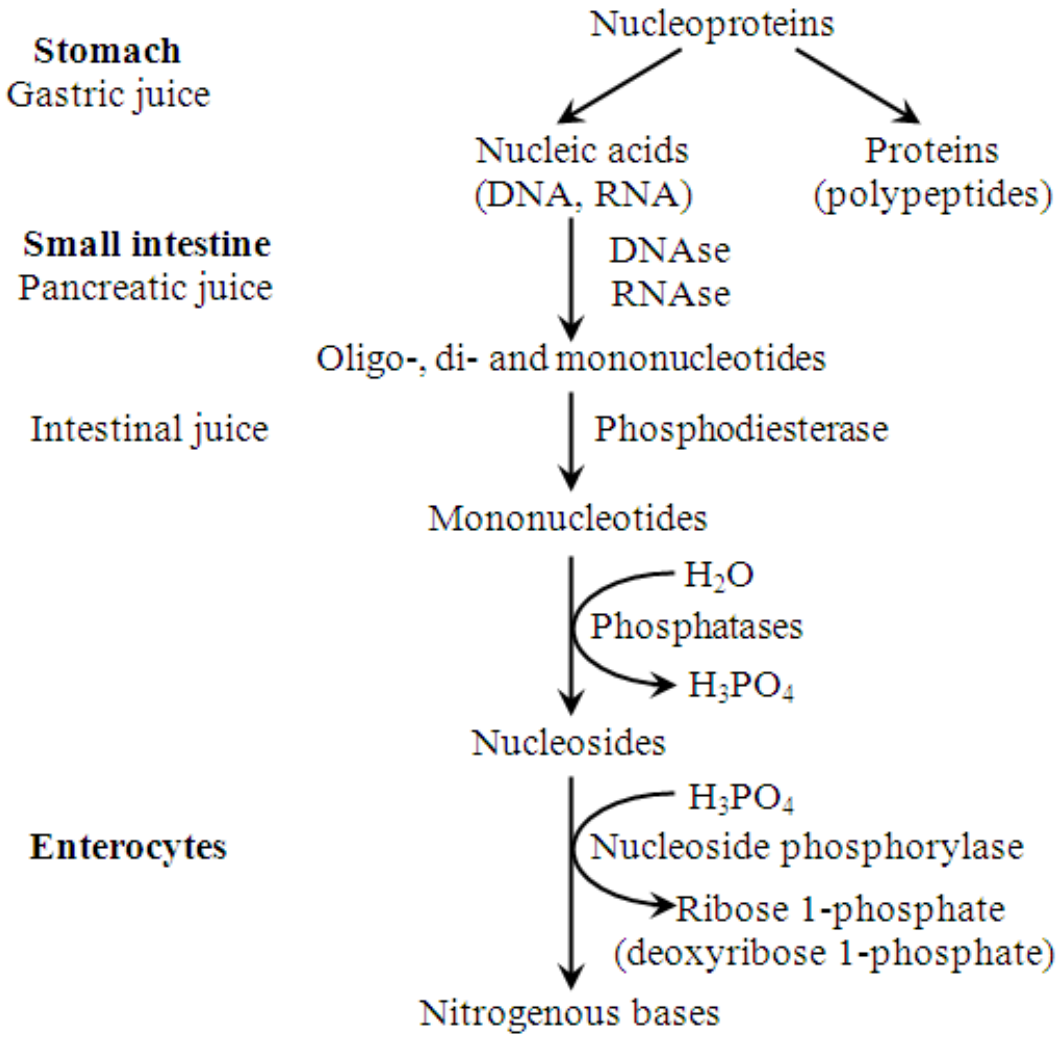


Fig. 27.5. Digestion of nucleic acids in the gastrointestinal tract.

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).

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**):

$$RNA + H_3PO_4 \longrightarrow RNA_{(n-1)} + RDP.$$

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)**. Each of purine nucleotides (AMP and GMP) can be converted into their corresponding nucleosides by **nucleotidase**. Reactions of purine nucleoside degradation is shown in Fig. 27.6.

Disorders of purine metabolism

Xanthinuria,or **xanthine oxidase deficiency**. The disease is due to genetic defect of xanthine oxidase and is characterized by decreased production of uric acid (**hypouricemia**) and increased excretion of hypoxanthine and xanthine. In alkali urine, xanthinuria may result is **xanthine lithiasis** (formation of **xanthine urinary stones**).

Gout. It is a disease caused by the increased accumulation of uric acid in the blood (hyperuricemia) and tissues, and **deposition of sodium urate crystals** in and around **joints**.

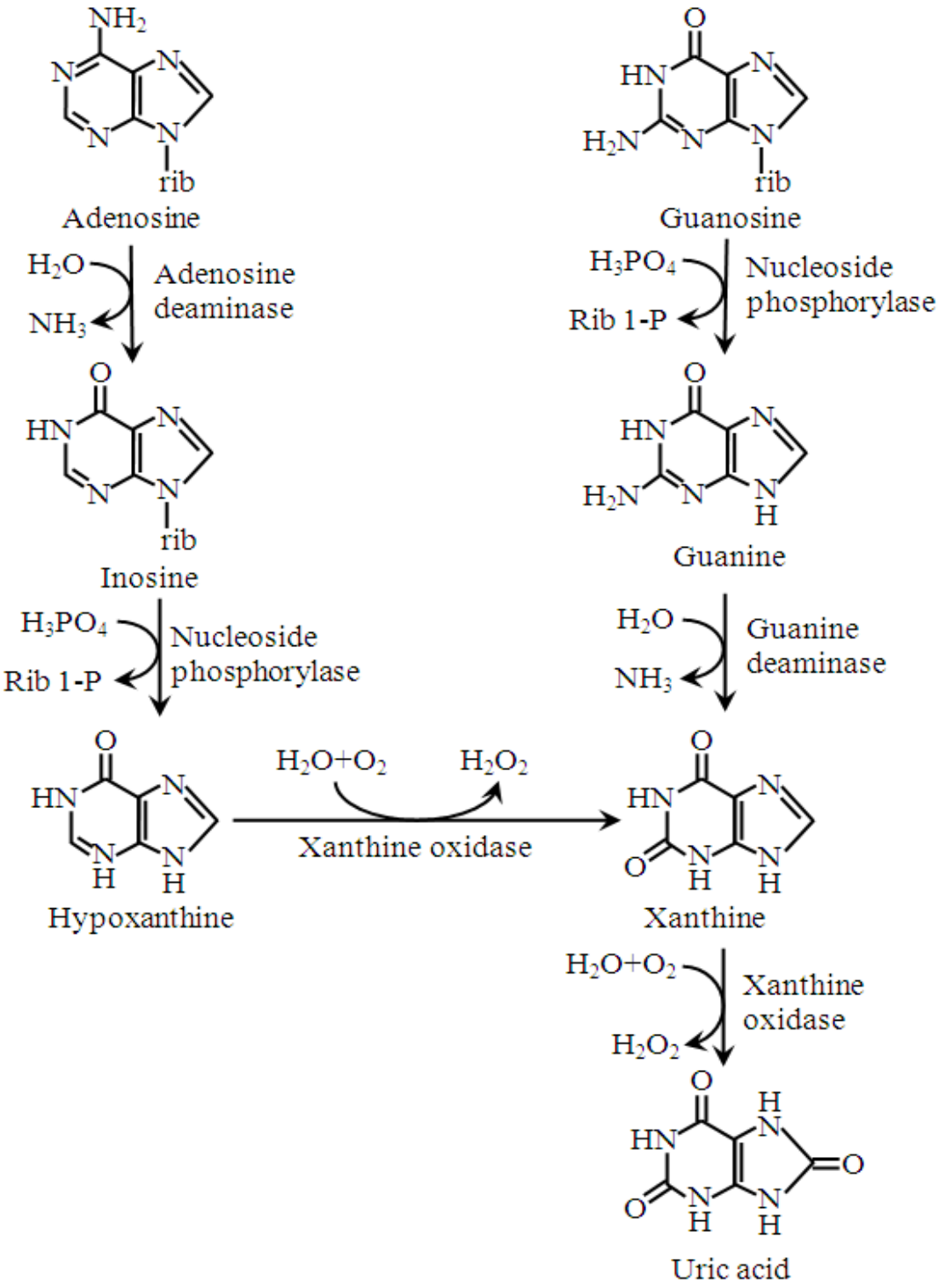


Fig. 27.6. Degradation of purine nucleosides.

Normal uric acid levels in the blood plasma are 0.12-0.24 mmol/L, excretion into the urine is 2.36-5.9 mmol/day.

Uric acid and its salts (urate salts) 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.

Incresed 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 of gout. 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.

DEGRADATION OF PYRIMIDINE NUCLEOTIDES

The pyrimidine nucleotides (CMP, UMP, and TMP) are dephosphorylated by nucleotidase to form nucleosides (cytidine, uridine, and thymidine) which undergo further degradation.

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.

The reactions of degradation of pyrimidine nucleosides are shown in (Fig. 27.7).

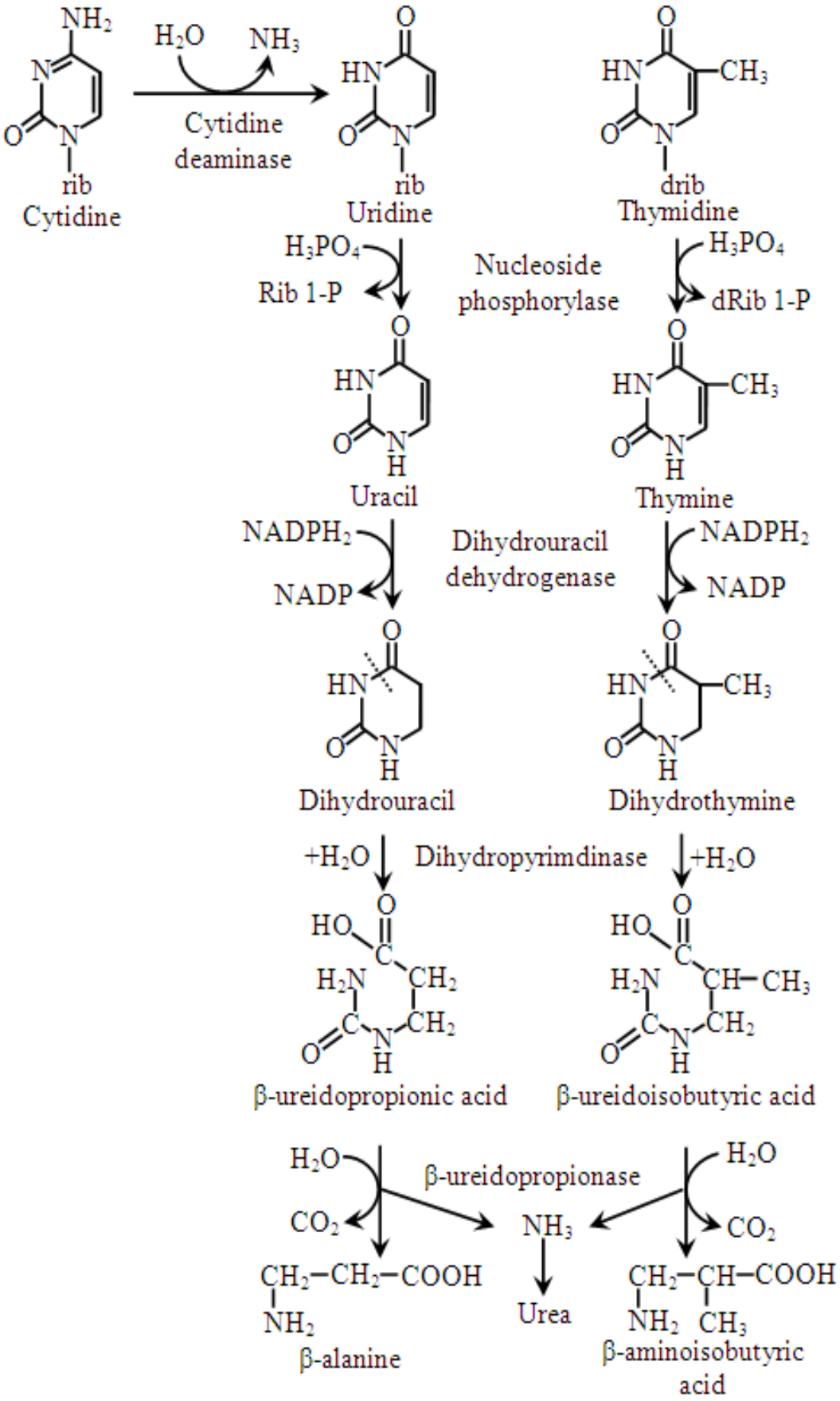


Fig. 27.7. Degradation of pyrimidine nucleosides.

CHAPTER 28

REGULATION AND CORRELATION 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.

Cellular metabolism is based on the principle of maximum economy. Cell consumes at any given moment such an amount of nutrients, which allows to satisfy its need in energy. This high organization and coordination of metabolism is achieved by regulatory mechanisms. These mechanisms are quite varied.

There are 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.
2. Regulation involving cyclic nucleotides and other secondary mediators.
3. Adjusting the amount of enzymes.
4. Adjusting the enzyme activity.
5. Hormonal regulation.

Regulation at the level of membranes can occur through several mechanisms:

- 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.

- The ability of membranes to fix hormones with the help of receptors.

- Enzymatic activity of membranes.

The cyclic nucleotides and other secondary mediators take part in realization of the whole row of hormones action.

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) play the opposite role – activate catabolism way, ensuring their conversion to ATP and inhibit 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 to 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 compensatoryly. As a result of glycolysis activation the rate of accumulation of acetyl-CoAm, being a substrate of the tricarboxylic acid cycle, increases. Activation of the tricarboxylic acid cycle leads to increased amounts of NADH + H^+, which is involved in the chain of tissue respiration, activity of functioning of which also increases. This leads to re-synthesis of ATP and replenishment of its pool, decreased as a result of muscle contraction.

Correlation 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, mononucleotides are closely interrelated.

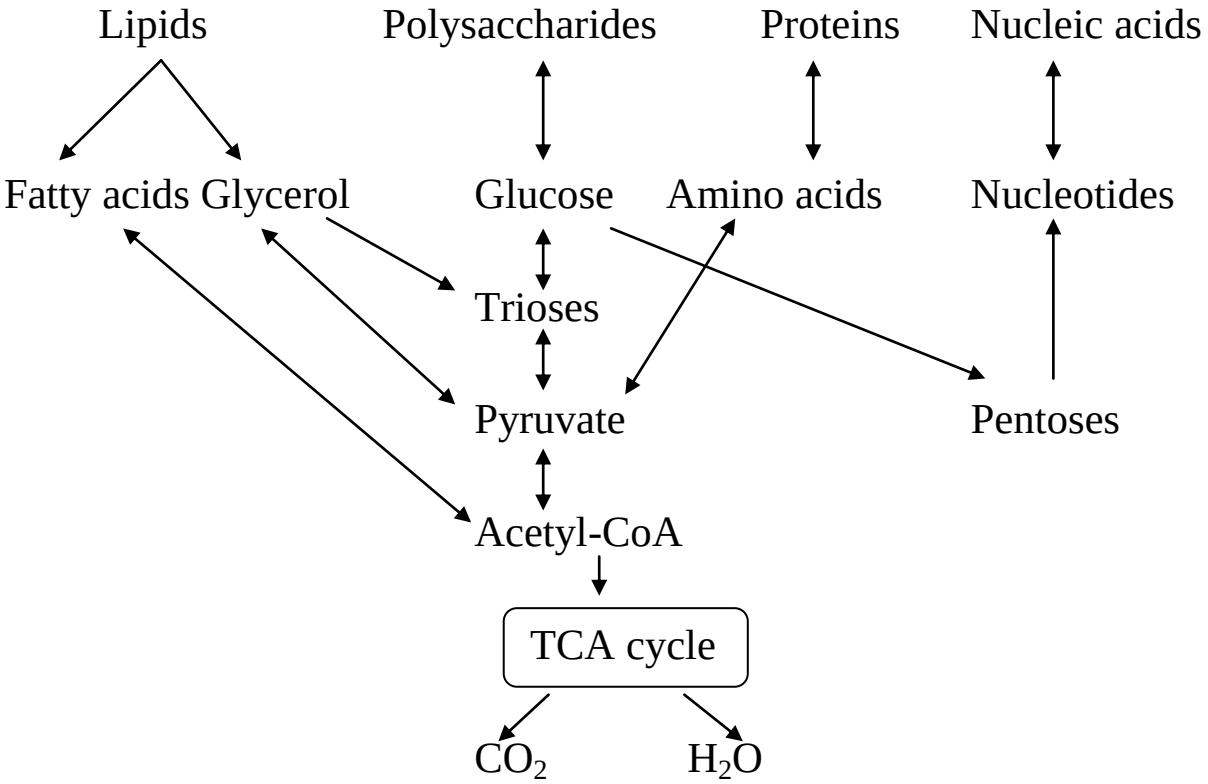


Figure 28.1. Metabolism correlation

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 pyruvate, glycerophosphate, acetyl-CoA, some metabolites of the citric acid cycle (Figure 28.1).

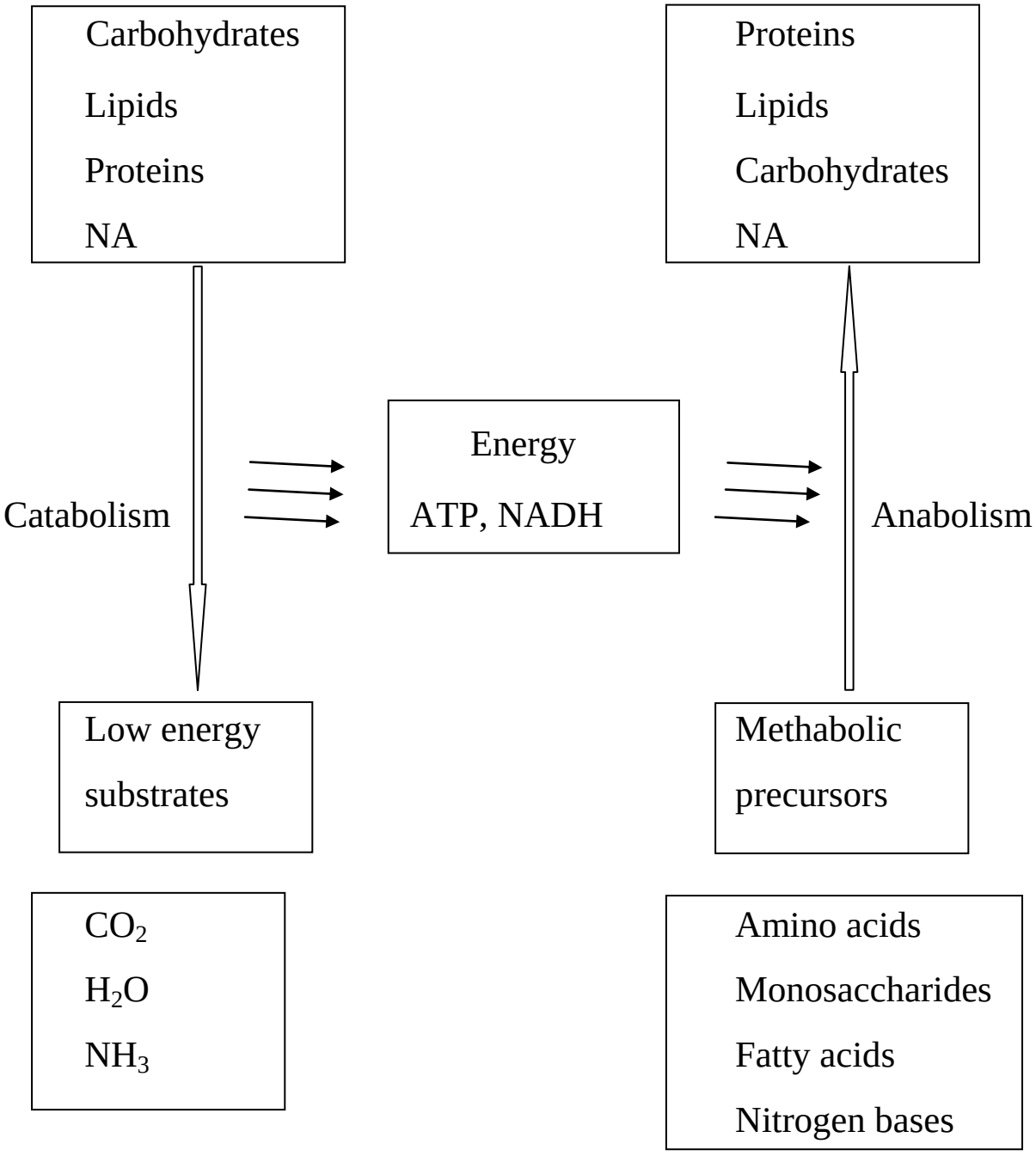


Figure 28.2. Energy relationship between catabolic and anabolic pathways.

CHAPTER 29

BIOCHEMISTRY OF THE LIVER

Weighing 1.5 kg, the liver is one of the largest organs in the human body. The liver has a central role in human metabolism; is extensively involved in the synthesis and catabolism of carbohydrate, lipids and proteins; synthesizes an array of acute phase proteins in response to inflammation and infection, and laboratory measurements of such proteins are clinically useful in monitoring disease progress; is specifically involved in the metabolism of bilirubin derived from the catabolism of heme; when affected by a variety of disease processes often causes the patient to present with jaundice due to hyperbilirubinemia; has a central role in the detoxification of drugs; its biochemical function is commonly and routinely assessed in clinical practice using a panel of blood tests, called liver function tests, abnormalities of which can point to disease affecting the hepatocelluar or biliary systems.

Functions of the liver

The most important functions of the liver are:

1. Uptake of nutrients supplied by the intestines via the portal vein.
2. Biosynthesis of endogenous compounds and storage, conversion, and degradation of them into excretable molecules.
3. Supply of the body with metabolites andnutrients.
4. Detoxification of toxic compounds by biotransformation.
5. Excretion of substances with the bile.
6. Storage. The liver not only stores energy reserves and nutrients for the body, but also certain mineral substances, trace elements, and vitamins, including iron, retinol, and vitamins A, D, K, folic acid, and B_{12}.

The liver has a central role in metabolism, because of both its anatomical placement and its many biochemical functions. It receives venous blood from the intestine, and thus all the products of digestion, in addition to ingested drugs and other xenobiotics, perfuse the liver before entering the systemic circulation.

Participation of the liver in carbohydrate metabolism

The liver has a central role in glucose metabolism, specifically in maintaining the circulating concentration of glucose. This function depends upon the ability of the liver both to store a supply of glucosein a polymerized form as glycogen, and to synthesize glucose from non-carbohydrate sources in the process of gluconeogenesis. Importantly the liver also possesses glucose-6-phosphatase which permits the dephosphorylation of glucose-6-phosphate, produced as a result of glycogen breakdown (glycogenolysis) or gluconeogenesis, which is a necessary prerequisite to the release of glucose to the blood.

On average, the adult liver stores about 80 g of glycogen, and in the fasting state releases 9 g of glucose each hour to the blood to maintain the peripheral glucose concentration. The contribution from gluconeogenesis increases progressively with fasting as glycogen stores become further depleted at the rate of 11% per hour. The carbon substrates for gluconeogenesis are derived from both lactate released by glycolysis in the peripheral tissues and from hepatic deamination of amino acids from the proteolysis of skeletal muscle. Energy for gluconeogenesis comes from the β-oxidation of fatty acids. The end product of this process, acetyl-CoA, also stimulates the activity of the first committed enzyme of gluconeogenesis, pyruvate carboxylase

On feeding, hepatic glucose production from gluconeogenesis is suppressed by insulin, which is carried directly from the pancreas to the liver by the portal vein. Glucose entering the circulation after feeding enters cells by specific transporters of differing kinetic properties. The activity of the GLUT2 transporter, present in the liver, is independent of insulin. The tissue takes up glucose at a rate which is proportional to the plasma glucose concentration, and in the liver glucose is converted to glycogen.

The role of the liver in lipid metabolism

The liver synthesizes fatty acids from acetate units. The fatty acids formed are then used to synthesize fats and phospholipids, which are released into the blood in the form of lipoproteins. The liver’s special ability to convert fatty acids into ketonebodies and to release these again is alsoimportant.The liver is the major site of both synthesis and catabolism of cholesterol, which is transported to other

tissues as a component of lipoproteins. Excess cholesterol is converted into bile acids in the liver or directly excreted with the bile.

Bile acids are key elements in fat metabolism. Bile acids have a detergent-like effect, solubilizing biliary lipids and emulsifying dietary fat in the gut to facilitate its digestion. They are synthesized by hepatocytes.

The role of the liver in amino acid and protein metabolism

The liver controls the plasma levels of the aminoacids. Excess amino acids are broken down.With the help of the urea cycle, the nitrogen from the amino acids is converted into urea and excreted via the kidneys.The carbon skeleton of the amino acids entersthe intermediary metabolism and serves for glucose synthesis or energy production. In addition, most of the plasma proteins are synthesized or broken down in the liver. Hepatic protein synthesis is important, as the majority of plasma proteins are synthesized in the liver, and hepatocellular disease may alter protein synthesis both quantitatively and qualitatively. Albumin is the most abundant protein in blood, and is synthesized exclusively by the liver. 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 liver also synthesizes most of the plasma α and β-globulins and a number of “acute phase proteins", which have been defined as those hepatically-derived proteins whose plasma concentrations change by more than 25% within 1 week of the inception of the inflammatory or infective insult.

Hepatic protein turnover is highly regulated. Mammalian cells possess several proteolytic systems. Plasma proteins and membrane receptors are endocytosed, to be hydrolyzed by acid proteases within intracellular organelles known as lysosomes; intracellular proteins are degraded within structures known as proteasomes.

Catabolism of amino acids generates ammonia (NH_3) and ammonium ions (NH_4^+). Ammonia is toxic, particularly to the central nervous system. Most ammonia is detoxified at its site of formation, by amidation of glutamate to glutamine. The remaining nitrogen enters the portal vein either as ammonia or as alanine, both of which are used by the liver for the synthesis of urea.The urea cycle is the major route by which waste nitrogen is excreted.

Biotransformation

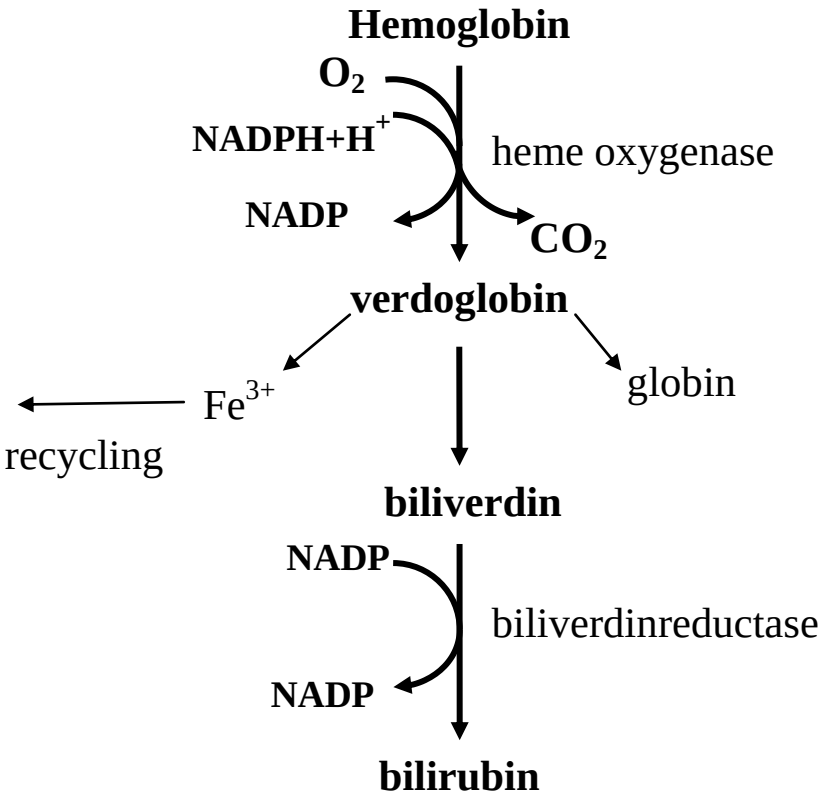
Steroid hormones and bilirubin, as well as drugs, ethanol, and otherxenobiotics are taken up by the liver and inactivated and converted into highly polarmetabolites by conversion reactions.

Most drugs are metabolized by the liver. Among other effects, this hepatic metabolism usually increases the hydrophilicity of drugs, and therefore their ability to be excreted. Generally, the metabolites that are produced are less pharmacologically active than the substrate drug; however, some inactive pro-drugs are converted to their active forms as a result of processing in the liver. Metabolism proceeds in two phases:

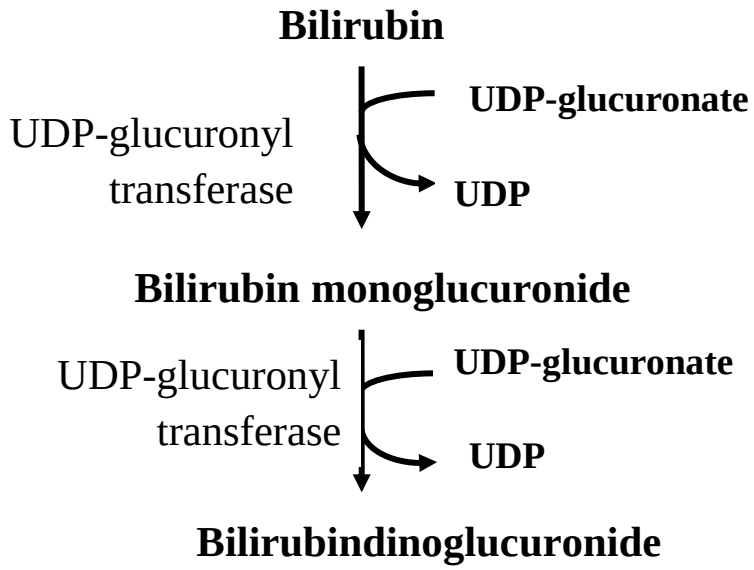
- **phase I**: the polarity of the drug is increased by oxidation or hydroxylation catalyzed by a family of microsomal cytochrome P450 oxidases;
- **phase II**: cytoplasmic enzymes conjugate the functional groups introduced in the first phase reactions, most often by glucuronidation.

Bilirubin metabolism

Bilirubin is a catabolic product of heme. About 75% is derived from the hemoglobin of senescent red blood cells, which are phagocytosed by mononuclear cells of the spleen, bone marrow, and liver; in normal adults, this contributes a daily load of 250-350 mg of bilirubin. The ring structure of heme is oxidatively cleaved to **biliverdin** by heme oxygenase, a P_{450} cytochrome. Biliverdin is, in turn, enzymatically reduced to **bilirubin**. Bilirubin is metabolized by the hepatocytes and excreted by the biliary system. Whereas biliverdin is water soluble, bilirubin, paradoxically, is not, and so must be further metabolized before excretion. This occurs in the liver, to which bilirubin is transported as a complex with plasma albumin; the hepatic uptake of bilirubin is mediated by a carrier. The hydrophilicity of bilirubin is increased by esterification of one or both of its carboxylic acid side chains with glucuronic acid.



Conjugated bilirubin is then secreted by the hepatocyte into the biliary canaliculi. Conjugated bilirubin in the gut is catabolized by bacteria to form **stercobilinogen**, also known as fecal urobilinogen, which is a colorless compound. On oxidation, however, stercobilinogen forms **stercobilin** (otherwise known as fecal urobilin), which is colored; most stercobilin is excreted in the feces, and is responsible for the color of feces. Some stercobilin may be reabsorbed from the gut and, being water soluble, can then be re-excreted by either the liver or the kidneys.



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 occurs when there is an imbalance between production and excretion. The causes of jaundice are conventionally classified as:

- **prehepatic:** increased production of bilirubin,
- **intrahepatic:** impaired hepatic uptake, conjugation, or secretion of bilirubin,
- **posthepatic:** obstruction to biliary drainage.

Prehepatic jaundice results from excess production of bilirubin after hemolysis, or a genetic abnormality in the hepatic uptake of unconjugated bilirubin. Hemolysis is commonly the result of immune disease, structurally abnormal red cells, or breakdown of extravasated blood. Intravascular hemolysis releases hemoglobin into the plasma, where it is either oxidized to methemoglobin or complexed with haptoglobin. More commonly, red cells are hemolyzedextravascularly, within phagocytes, and hemoglobin is converted to bilirubin; such bilirubin is unconjugated. Unconjugated and conjugated bilirubin can be chemically distinguished.

Intrahepatic jaundice reflects a generalized hepatocyte

dysfunction. Hyperbilirubinemia is usually accompanied by other abnormalities in biochemical markers of hepatocellular function

Posthepatic jaundice is caused by obstruction of the biliary tree. The plasma bilrubin is conjugated, and other biliary metabolites, such as bile acids, accumulate in the plasma. The clinical features are pale-colored stools, caused by the absence of fecal bilirubin and urobilin, and dark urine as a result of the presence of water-soluble conjugated bilirubin. In complete obstruction, urobilinogen/urobilin is absent from urine, as there can be no intestinal conversion of bilirubin to urobilinogen/urobilin, and hence no renal excretion of reabsorbed urobilinogen/urobilin.

Neonatal jaundice

About 50% of normal babies become jaundiced 48 hours after birth. This physiologic jaundice is caused by temporary inefficiency in bilirubin conjugation, and resolves in the first 10 days. The hyperbilirubinemia is unconjugated in nature; if severe, it may require phototherapy (ultraviolet light to photoisomerize bilirubin into a nontoxic form) or exchange blood transfusion to prevent damage to the brain. Jaundice in the first 24 hours of life is abnormal and requires investigation to exclude hemolysis. Jaundice that presents later - after 10 days - is always abnormal, and is likely to indicate an inborn error of metabolism or structural defects of the bile ducts.

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 uridine diphosphate (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 presents 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.

Biochemical tests of liver function

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).

A panel of biochemical measurements is routinely performed in the clinical laboratories on plasma or serum specimens. This group of tests is usually, and incorrectly, described as liver "function" tests, which commonly include the measurements of:

- bilirubin,
- albumin,
- transaminases: aspartate aminotransferase (AST) and alanine aminotransferase (ALT).
- alkaline phosphatase (ALP), sometimes in conjunction with:
- γ-glutamyl transferase (γGT).

Aspartate amino transferase (AST) and alanine transaminase (ALT) are involved in the interconversion of amino and ketoacids, and are therefore required for hepatic metabolism of nitrogen and carbohydrates. Both these transaminases are located in the mitochondria; ALT is also found in the cytoplasm. The serum activity of ALT is a better marker of liver disease than that of AST, which is also present in muscle and red blood cells. In more severe liver disease, the synthetic functions of the hepatocytes are likely to be affected, and so the patient would be expected to have a prolonged prothrombin time and low serum albumin concentration.

CHAPTER 30

WATER AND ELECTROLYTE BALANCE

Water constitutes about 60% of body weight (40-45 L). Deprivation of water causes death much more rapidly than deprivation of food. When water is completely withheld, death takes place in a few days after the body has lost only 10-20% of its water content. If water is accessible, but no food is taken, an individual may survive for several weeks in spite of the loss of most body fat and about 50% of the tissue protein.

WATER BALANCE

Normally, the intake of water equals its loss (Table 30.1).

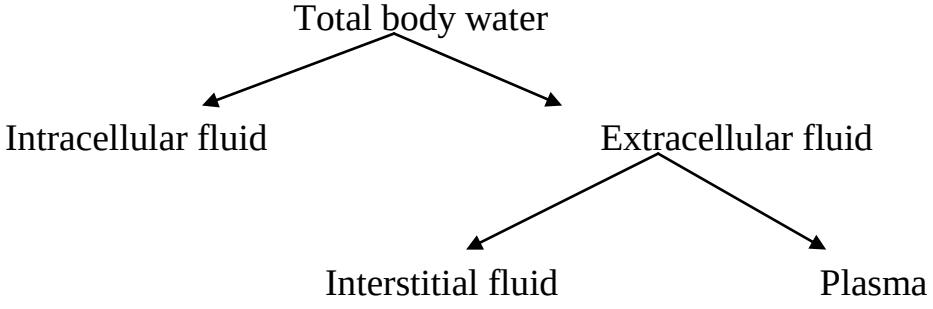
Table 30.1.

The intake and output of water in the organism

Intake per day		***Output per day***	
Water of food	500 ml	Urine	1200 ml
Drinking water	1200 ml	Expired air	500 ml
Metabolism	300 ml	Sweat	200 ml
		Feces	100 ml
Total	2000 ml	Total	2000 ml

BODY WATER COMPARTMENTS

About ⅔ of total body water is intracellular fluid (ICF), and ⅓ is outside cells, i.e. extracellular fluid (ECF). The ECF consists of interstitial fluid and plasma (intravascular fluid). Interstitial space is four times larger than the intravascular space.



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.

However, capillary wall is impermeable for proteins; therefore concentration of proteins is 4 times greater in plasma than that 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

All molecules dissolved in the body water create osmotic pressure.

Osmolality of a body fluid is the osmotic pressure exerted by the dissolved substances. 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.

Osmolality of the ECF is created mainly by Na^+. Besides, glucose and urea participate in plasma osmolality, but normally their contribution is small. Proteins (mainly albumin) create their own osmotic pressure referred to as **oncotic pressure**. Protein concentration is high in the ICF and in plasma, therefore the oncotic pressure is the highest in the ICF and in the intravascular fluid (plasma). If concentration of protein is getting decreased in plasma, this causes the decrease of oncotic pressure in the blood, and therefore water moves from plasma to interstitial space, leading to edema.

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.

Aldosterone is secreted by *zona glomerulosa* of adrenal cortex if concentration of Na^+ in the blood is decreased. Aldosterone is also referred to as **Na-retaining hormone**. It stimulates Na^+ reabsorption from the urine in the distal tubules and thus **increases concentration of Na^+ in the blood**.

Vasopressin (**antidiuretic hormone – ADH**). When osmolality of plasma rises, the **osmoreceptors** of **hypothalamus** are stimulated resulting in the increase of the ADH secretion. ADH is transported to the posterior lobe of hypophysis and secreted into the blood. ADH **increases the water reabsorption** by renal tubules.

Renin-angoitensin system. This system is stimulated by the decreased blood pressure and the decreased ECF volume. Both these reasons lead to the lowering renal plasma flow. The decreased renal plasma flow causes stimulation of **baroreceptors** in the **juxtaglomerular apparatus** and results in the release of **renin** by juxtaglomerular cells from kidney into the blood. In the blood, renin activates conversion of angiotensinogen into **angiotensin** I (AT-I). In the lungs, **angiotensin-converting enzyme (ACE)** converts AT-I into AT-II. In turn, AT-II increases blood pressure by causing constriction of arterioles. Drugs which inhibit ACE are used in the treatment of arterial hypertension. AT-II stimulates also production of aldosterone which increases reabsorption of Na and consequently water. Therefore, the blood pressure rises.

Atrial natriuretic peptides. They are synthesized in the atrium of the heart. Atrial natriuretic peptides cause vasodilatation, decrease blood pressure, decrease Na reabsorption in the kidney, and increase Na excretion into the urine.

MINERAL COMPONENTS OF TISSUES

Macrominerals (daily requirements of the organism is more than 100 mg): Na, K, Ca, Mg, Cl, P.

Microminerals or trace elements (daily requirement is less than 100 mg): Fe, I, Cu, Mn, Zn, Co, Mo, Cr, Se, F.

Sodium (Na)

Normal concentration of Na^+ in the blood is (130-150 mmol/L). Na^+ is the major cation of the ECF (i.e. of plasma and interstitial fluid).

Role of Na^+ in the organism. Na^+ maintains osmotic pressure of the ECF, acid-base balance, volume of the ECF (water balance),

participates in generation of membrane potential and nerve impulse.

Regulation of sodium metabolism. Aldosterone increases reabsorption of Na^+ from the urine, estrogens exhibit the same action, progesterone increases Na^+ excretion into the urine.

Potassium (K)

Normal concentration of K^+ in the blood is 3.6-5.4 mmol/L. K^+ is the major cation of the ICF.

Role in the organism is the same as for Na^+. Maintenance of normal K^+ concentration in the blood is important for heart contraction.

Regulation of potassium metabolism. Aldosterone increases excretion of K^+ into the urine.

Calcium (Ca)

Normal concentration of Ca^{2+} in the blood is 2-2.75 mmol/L.

Role in the organism. Ca^{2+} is a structural component of bones. Ca^{2+} as second messenger transfers the action of hormones into the cell. Also Ca^{2+} participates in muscle contraction, blood coagulation, transmission of nerve impulses, decreases permeability of capillaries (therefore is used for treatment of allergy), decreases neuromuscular excitability.

Regulation of calcium metabolism. Parathyroid hormone increases concentration of Ca^{2+} and decreases concentration of P in the blood. Calcitonin decreases concentration of both Ca^{2+} and P in the blood. Estrogens and androgens increase ossification (Ca^{2+} deposition) of epiphyses (growth zones of the bones). Vitamin D_3 (its active forms) also participates in regulation of Ca^{2+} and P metabolism.

Phosphorus (P)

Role in the organism. This is the second major intracellular anion. Plasma phosphates ($H_2PO_4^-$ and HPO_4^{2-}) are components of phosphate buffer system. Phosphate is a structural component of bones, component of many important substances: DNA, RNA, phospholipids, macroergic compounds, coenzymes (NAD, FAD, pyridoxal phosphate), active forms of carbohydrates (glucose 6-P, etc.).

Magnesium (Mg)

Role in the organism. This is the second major intracellular cation. Along with Ca^{2+}, Mg^{2+} is a component of bones, activator of enzymes

requiring ATP (kinases), decreases neuromuscular excitability.

Iron (Fe)

The major **iron-containing proteins and enzymes** are represented by hemoglobin, myoglobin, cytochromes, cytochrome oxidase, catalase, peroxidase, transferrin, ferritin, and hemosiderin. Irom deficiency may lead to anemia.

Iodine (I_2)

Is a component of thyroid hormones.

Copper (Cu)

Is a component of cytochrome oxidase (the enzyme of the ETC), and ceruloplasmin (a protein which transports Cu in plasma).

Cobalt (Co)

Is a component of vitamin B_{12} which is required for heme synthesis (vitamin B_{12} deficiency causes anemia).

Molybdenum (Mo)

Is a coenzyme of xanthine oxidase which participates in catabolism of purines.

Zinc (Zn)

Is important for normal functioning of pancreas and testis.

Fluorine (F)

Prevents caries.

Cromium (Cr)

This is a glucose tolerance factor: it improves binding of insulin with its receptor, i.e. increases sensitivity of cells to insulin.

Selenium (Se)

Is an antioxidant: it is a component of glutathione peroxidase which catalyzes breakdown of H_2O_2 and organic peroxides. Also Se is a cancer protecting agent.

CHAPTER 31

BIOCHEMISTRY OF THE KIDNEY. ANALYSIS OF THE URINE

BIOCHEMICAL FUNCTIONS OF THE KIDNEY

The primary designation of the kidney is to provide for a constancy of the internal medium of the organism. 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) **regulation of water and electrolyte balance** (regulation of volume and osmolality of the ECF);

3) **maintenance of the acid-base balance** (**pH**);

4) **endocrine function**: the kidney produces **renin** (in response to the decrease of the arterial blood pressure), **1,25 $(OH)_2$ cholecalcipherol** (active form of vitamin D_3 which regulates calcium homeostasis), and **erythropoietin** (which stimulates erythropoiesis, i.e. production of erythrocytes).

FORMATION OF THE URINE

Formation of the urine includes three major processes which occur in the nephron: **filtration** of plasma in glomeruli, tubular **reabsorption**, and tubular **secretion**.

Glomerular filtration is a passive process. When the blood flows through glomerular capillaries, plasma is filtered into the Bowman's space to produce **ultrafiltrate** of the blood, i.e. **the primary urine**.

Proteins and blood cells are incapable of penetrating into the capsular lumen of the glomerulus through the capillary wall and are retained in the blood. In certain pathologic states, the permeability of the glomerular filter (filtration barrier) becomes increased, which leads to the change of the ultrafiltrate composition. The earliest manifestation in the abnormality of the glomeruli is the appearance of albumin in the urine.

Tubular reabsorption. The overall length of renal tubules is about 120 km. When the primary urine passes along the tubules, most of the urine components are reabsorbed to yield **the secondary, or final, urine**; i.e. 99% of the total fluid filtered in the glomeruli is reabsorbed, and only 1% is converted into the urine which is excreted from the body.

In the course of total tubular reabsorption, about 99% of **sodium**, **chloride**, **bicarbonate** and **amino acids** is reabsorbed; 93% of **potassium** ions, 45% of urea, 100% of **glucose**.

Tubular secretion. In the distal convoluted tubules, H^+, K^+ ions and uric acid are secreted into the urine.

The participation of specific renal tubules in reabsorption

In the **proximal convoluted tubules**, the major part of the ultrafiltrate components is reabsorbed: **water** (80%), **sodium**, chloride, bicarbonate (70-85% each), urea (40-45%), **potassium** (70-100%); **glucose** and **amino acids** are completely reabsorbed (100%).

In the **descending limb** of the loop of Henle, water undergoes further partial reabsorption. In the **ascending limb** of the loop of Henle sodium and chloride ions (but not water) are reabsorbed.

In the **distal convoluted tubules**, water and sodium ions are reabsorbed, whereas **potassium and hydrogen** ions are **secreted** into the tubular lumen. Also, in this part of a nephron, reabsorption of Ca^{++} and secretion of uric acid occurs.

The fate of individual urine components in the different segments of tubules

Reabsorption of sodium. In the **proximal convoluted tubules**, Na^+ is reabsorbed by several mechanisms: through specific **ion channels** in exchange with the **hydrogen** ion ($Na^+ \rightarrow H^+$ exchange system), and by mechanism of **co-transport** with **glucose, amino acids**, phosphate and other ions. Once within the tubular cell, Na^+ migrates towards the basement plasma membrane (to the "blood side" membrane) of tubular cells to be transported by the Na^+,K^+-ATPase (sodium pump) into the interstitial fluid. About 85% of Na^+ filtered is reabsorbed in the proximal tubule.

When Na^+ is reabsorbed, the equivalent amounts of chloride are passively transported from the tubular lumen into the cells, to maintain the electrical neutrality. When Na^+ is exchanged for hydrogen ions by $Na^+ \rightarrow H^+$ exchange system, the concomitant reabsorption of bicarbonate takes place. In the proximal convoluted tubules, reabsorption of Na^+ causes the passive movement of water from the tubule lumen to the extracellular space, i.e. **water molecules move along with the sodium ions**.

In the **distal convoluted tubule**, still more Na^+ is reabsorbed by active transport controlled by **aldosterone,** and this is coupled with the secretion of hydrogen and potassium ions.

Reabsorption of water. About 80% of filtered water is reabsorbed in the proximal convoluted tubules. Water moves along with Na^+, Cl^- and HCO_3^- ions to maintain osmolality. In the distal tubule (and the collecting duct), water is again reabsorbed, and this is under the regulation of **antiduiretic hormone** which makes the tubular wall permeable to water independently of the sodium ion reabsorption.

Potassium. About 70-100% of K^+ in the glomerular filtrate is **reabsorbed** by **proximal** convoluted tubules. **Secretion** of K^+ occurs in the **distal** tubules in exchange for Na^+ reabsorption, under the effect of **aldosterone**.

Calcium. About 90% of Ca^{++} is reabsorbed from the glomerular filtrate in the distal convoluted tubules. The Ca^{++} reabsorption is regulated by parathyroid hormone, calcitonin and vitamin D_3.

Urea. Urea is freely filtered by the glomeruli, but about 40-45% is reabsorbed actively by the tubules. Urea makes up 80% of total urinary soluble components.

Glucose. Glucose is one of the compounds which excretion in the urine is dependent on the blood level. At normal or low plasma levels, glucose is completely reabsorbed and is not excreted into the urine. But when the blood glucose level is elevated, the tubular capacity for the glucose reabsorption is getting saturated, so that the excess will be excreted into the urine. The plasma level of a compound above which the compound is excreted into the urine is called the **renal threshold** of the substance. For glucose, the renal threshold is 10 mmol/L. This means that glucose appears in the urine when blood glucose level exceeds 10 mmol/L.

METABOLISM OF 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. About 70% of the consumed oxygen is used to generate energy for the active sodium transport which in turn determines reabsorption of glucose and amino acids. Approximately 80% of ATP available in the renal tubular cells is used for the functioning of the sodium pump (Na^+,K^+-ATPase).

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.

ACID-BASE BALANCE AND pH

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^-):

$$CO_2 + H_2O \rightleftarrows H_2CO_3 \rightleftarrows H^+ + HCO_3^-.$$

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.

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 constancy of pH in the organism is maintained by cooperative interaction of **buffer** systems of the blood, as well as **respiratory** mechanisms (lungs), and **renal** excretion 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

As the cell metabolism predominantly generates acids, so the body buffers have to contain certain alkali reserve to neutralize the acids.

The major buffers **in the blood** are the **bicarbonate** buffer system and the **hemoglobin** buffer system. Quantitatively, about 65%

of buffering action in the blood is prowided by bicarbonate system; 30% by hemoglobin system. The main **intracellular** buffers are the **protein** buffer system and the **phosphate** buffer system.

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^- :

$$CO_2 + H_2O \rightleftarrows H_2CO_3 \rightleftarrows H^+ + HCO_3^-.$$

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 is 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.

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 ($HHbO_2/HbO_2^-$).

Respiratory mechanism for regulation of pH

Due to respiration, CO_2 is eliminated from the blood. Removal of CO_2 is essential for maintenance of the blood pH.

Carbon dioxide produced in the cell can freely diffuse across the cell membrane and vascular capillary membrane to enter the blood plasma. Then, approximately 70% of CO_2 is converted to bicarbonate in the RBC. Inside the erythrocytes, carbonic anhydrase catalyzes the reaction due to which CO_2 is finally converted to bicarbonate:

$$CO_2 + H_2O \xrightleftharpoons{\text{Carbonic anhydrase}} H_2CO_3 \xrightleftharpoons{\text{Spontaneously}} H^+ + HCO_3^-.$$

The hydrogen ion resulting from dissociation of carbonic acid binds with hemoglobin. Bicarbonate, produced in the RBC due to the above reaction, is released to the blood plasma. In alveolar air, the pO_2 is high. Hemoglobin binds with O_2 and releases H^+ which reacts with bicarbonate to form CO_2:

$$H^+ + HCO_3^- \rightleftharpoons H_2CO_3 \rightleftharpoons CO_2 + H_2O.$$

CO_2 enters plasma and, in the lung, diffuses into the expired air.

Kidney (renal) mechanism for regulation of pH

The major mechanisms due to which the kidney regulates pH of the blood plasma are:

1) **reabsorption of bicarbonate** (alkali reserve), and
2) **excretion of H^+.**

Reabsorption of bicarbonate. 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.

In the tubular lumen, there is bicarbonate which was filtrated by glomerulus. **Hydrogen** ion secreted into the lumen of the tubule **binds** with the **filtered bicarbonate** to form carbonic acid which dissociates to produce CO_2 and H_2O. The CO_2 diffuses back into cells of the tubules and again combines with water to form carbonic acid. The latter, in turn, dissociates to form H^+ and HCO_3^-; then H^+ is secreted into the lumen in exchange for Na^+, and HCO_3^- is reabsorbed in plasma along with Na^+. Thus, **this mechanism serves to increase the alkali reserve** and prevents the loss of bicarbonate through urine. **The normal urine is bicarbonate-free**. At this stage, **hydrogen ions** are **secreted** into the tubular lumen, **but no** net **excretion** of **hydrogen ions** occurs.

Hydrogen ion excretion. The excretion of hydrogen ions takes place in the **distal** convoluted tubules of the kidney. Hydrogen ions may be excreted by two mechanisms:

1) as monosubstituted phosphates, or
2) as ammonium ion (NH_4^+).

1. **Excretion of H^+ as monosubstituted phosphates**. In the **distal** tubule cells, as well as in the proximal tubules, the CO_2 is converted into carbonic acid which immediately dissociates into hydrogen ion and bicarbonate. Then, bicarbonate is reabsorbed to plasma and hydrogen ion is secreted into tubule lumen in exchange for Na^+.

The fate of hydrogen ion 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**: disubstituted phosphate Na_2HPO_4 (basic phosphate) and monosubstituted phosphate NaH_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. Hence, disubstituted phosphate, Na_2HPO_4, is converted to monosubstituted phosphate, NaH_2PO_4, to be excreted into the urine. This process helps to excrete (eliminate) acids from the body.

2. **Excretion of H^+ as ammonium ion (NH_4^+)**. The major way of H^+ excretion is perfprmed by mechanism of the renal secretion of ammonia. This process occurs in the **distal** tubules. Ammonia is generated in the tubule cell due to **deamination of glutamine** by glutaminase, and **oxidative deamination of glutamate** by **glutamate dehydrogenase**.

Ammonia diffuses freely through the luminal membrane into the tubule lumen and combines with H^+ to form NH_4^+. The glutaminase activity is increased in acidosis. Therefore in acidosis, much H^+ is excreted as NH_4^+. The positively charged NH_4^+ combines with negatively charged Cl^- to form NH_4Cl. As a result, **Na^+ and K^+ are reabsorbed (saved for the organism), and ammonium ion is excreted into the urine as ammonium salt**.

GENERAL CHARACTERISTICS OF URINE

To characterize urine sample in clinical laboratories, several properties are used: volume, specific gravity, colour, pH, and transparency.

1. **Volume** (**daily diuresis**).

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, or increased urine volume, may be as **physiological** state after large (excessive) fluid intake, or after the dietary intake of food stimulating diuresis (e.g. melon, water melon, cucumber, pumpkin). The **pathologic** polyuria occurs in **diabetes mellitus** (water moves along with or is "dragged" behind glucose molecules), **chronic renal diseases** (nephritis, pyelonephritis) when protein present in the urine retains water from being reabsorbed, and in **diabetes insipidus** (due to lack of antiduiretic hormone the volume of the urine excreted may amount to 15 litres per day or more).

Oliguria, or decreased daily urine volume, may be observed as physiological state in deficient liquid intake. The pathological oliguria may be caused by:

1) Disorders in general circulation due to

a) the decrease of the blood volume; this may be because of dehydration (loss of water through the skin or as a result of excessive sweating in fever, diarrhea, vomiting, and severe burns);

b) low blood pressure (glomerular filtration stops when the blood pressure is 60 mm of Hg or below).

2) The narrowing of afferent arteriole (by adrenaline in stress or neurogenic shock).

3) Acute nephritis.

4) Poisoning with nephrotropic compounds, such as salts of lead, mercury, or arsenic salts.

5) Urolithiasis (obstruction of urinary tract with urinary stones).

Complete or nearly complete stop of urinary excretion is called anuria; a prolonged **anuria** leads to **uremia**.

2. **Specific gravity** of the urine is most commonly 1.010-1.020.

Normally, the volume of the urine and its specific gravity reversely correspond to each other: the lower is daily diuresis the higher is specific gravity, or vice versa. In pathological states, this interrelation may be the same or changed. E.g. in diabetes insipidus, the excreted urine is of a very low density (1.001-1.004), which is due to disturbed reabsorption of water in the kidney tubules and is accompanied by polyuria. In oliguria caused by acute nephritis, the urine has high specific gravity.

On the contrary, polyuria with high specific gravity of the urine may be in diabetes mellitus (due to presence of glucose in the urine)

and in nephrosis (due to protein which is lost with urine in this state).

3. **Colour**. Normal urine is straw-yellow or amber-yellow in colour. This colour is for the most part (95%) due to pigment urochrome. In pathology, the urine may be of some other colour due to presence of substances normally not occurred in the urine.

The urine of **red** colour is observed due to presence of **blood pigments** in the urine, i.e. in **hematuria** and **hemoglobinuria**, as well as after the intake of some drugs, e.g. rifampicin, or after having red beetroot as a meal. **Brown** colour is typical of high concentrations of **direct bilirubin** (obstructive jaundice), **urobilinogen** (hepatocellular jaundice), or **stercobilingen** (hemolytic jaundice). **Green** or **blue** colour of the urine is observed after administration of **methylene blue** which may be used as an antidote in cyanide poisoning, or in treatment of methemoglobinemia. This colour of urine also takes place as a result of intensified protein putrefaction in the intestine. In the case, large amounts of **indoxylsulphuric acid** are excreted into the urine; this acid may undergo degradation to yield indigo (of blue colour). Urine may be **black** in the case of alkaptonuria (due to presence of pigment **alkaptone** which is formed in the urine because of polymerization of homogentisic acid excreted in this genetic disease).

4. **Reaction** (**pH**). Normally, under ordinary dietary conditions, the urine reaction (pH) is weakly acidic (5.3-6.5). The pH of the urine depends on the type of diet. In meat-rich diet, the urine reaction is acidic; in vegetable diet, the urine reaction is alkaline.

Distinctly **acidic urine** reaction is observed in diabetes mellitus, starvation, and fever due to presence of ketone bodies in the urine (acetoacetate and β-hydroxybutyrate are acids).

The **alkaline urine** occurs in cystitis and pyelitis: microorganisms produce the enzyme urease which can degrade urea to form ammonia (alkaline solution NH_4OH) within the bladder.

5. **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 (the presence of fat in the urine is called lipuria).

CHEMICAL COMPOSITION OF URINE

Over 150 compounds are present in the urine. The components of the human urine are divided into organic and inorganic compounds.

Major organic components of urine. They are divided into nitrogen-containing and nitrogen-free compounds.

Nitrogen-containing compounds are represented by urea, uric acid, creatine, creatinine, amino acids, bilirubin, indican. Most of them are products of nitrogen metabolism (metabolism of amino acids and nitrogenous bases); bilirubin is a product of heme breakdown.

Nitrogen of urea accounts for 80% of the urinary nitrogen. Nitrogen-free organic components of the urine include organic acids such as oxalic, lactic, citric, butyric, valeric, β-hydroxybutyric, acetoacetic acid, and some others.

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

The components referred to as “pathological components of the urine” are always present in the normal urine, but at very low (minimal, or trace) amounts which are not detectable by routine analytical techniques. In practice, it is admitted that these compounds are absent in the normal urine. The pathological components of the urine include: protein, blood, glucose, ketone bodies, and bile pigments.

Proteins

The presence of protein in the urine is called **proteinuria**. Proteinuria is divided into two groups: renal and extrarenal proteinuria.

Renal proteinuria. In renal proteinuria, proteins present in the urine originate from the blood plasma (glomerular proteinuria).

The glomeruli of the kidney are not permeable to substrates which molecular weight is more than 69,000, and so **plasma proteins are absent in the normal urine**. 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.

The smaller molecules of albumin pass through the damaged glomeruli more readily than the larger globulins. Therefore, when proteins appear in the urine, the albumin fraction is predominant. **Albuminuria is always pathological**. Large quantities of albumin (a few grams per day) are lost in the urine of patients with **nephrosis**. Smaller quantities are present in the urine in **nephritis**. Proteins can also 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**).

Extrarenal proteinuria. The extrarenal proteinuria is due to inflammation of lower urinary tract (cystitis, pyelitis, urethritis, prostatitis).

Proteinuria may be detected by either nitric acid or sulphasalicylic acid. The latter method is more sensitive than the method with HNO_3.

Blood

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 cytologic method (**microscopically**), i.e. by examining the urine sediment through the microscope when red blood cells become visible in case of hematuria.

Also, chemical technique (bezidine test) helps to detect blood

pigments 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 coloured molecule. Developing blue-green colour indicates the presence of blood or blood pigments.

Glucose

Glucose appears in the urine when blood levels exceed 10 mmol/L. The presence of glucose in the urine is called **glucosuria**. This is one of manifestations of diabetes mellitus. In certain genetic diseases, other carbohydrates appear in the urine, e.g. galactose (in galactosemia), fructose (in essential fructosuria).

Ketone bodies

The increased concentration of ketone bodies in the urine is called **ketonuria**. Ketonuria is observed in **diabetes mellitus, starvation,** or in deficiency of carbohydrates in the body (e.g. low-carbohydrate diet), or in the increased utilization of carbohydrates in the organism (**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 32

BIOCHEMISTRY OF THE BLOOD

Human blood constitutes about 8% of the body's weight. It consists of cells and cell fragments in an aqueous medium, the blood plasma. The proportion of cellular elements, known as hematocrit, in the total volume is approximately 45%. The blood is the most important transportmedium in the body. It serves to keep the homeostasis and it plays a decisive role in defending the body against pathogens.

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 7.36-7.4. 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.

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.

Formed elements of blood

The formed elements of blood are erythrocytes, leukocytes and thrombocytes (platelets). They are suspended in an aqueous solution (plasma) and have several specialized functions such as transport of oxygen, destruction of external agents, and clotting of blood. Plasma without fibrin is called **serum.** Most biochemical tests are done on serum. To obtain plasma, blood must be taken into a test tube containing an anticoagulant.

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 the 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.

The erythrocyte is unique among all cells in the body - it uses glucose and glycolysis as its sole source of energy.The ATP formed during glycolysis serves mainly to supply Na^+/K^+-ATPase, which maintains the erythrocytes' membrane potential.

In the red cell, 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. The pentose phosphate pathway, accounts for about 10% of glucose metabolism in the red cell. In erythrocyte this pathway has a special role in protection against oxidative stress. Erythrocytes also have systems that can inactivate reactive oxygen species (superoxide dismutase, catalase, glutathione). The pentose phosphate pathway supplies $NADPH+H^+$, which is needed to regenerateglutathione (GSH) from GSSG 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 reactive oxygen species with unsaturated fatty acids in the erythrocyte membrane.

The reduction of methemoglobin (Hb Fe^{3+}) to Hb (Hb Fe^{2+}) is carried out by GSH or ascorbate by a non-enzymatic pathway; however, there are also NAD(P)H dependent Met-Hb reductases.

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. In order to function correctly, leukocytes have the ability to migrate out of the bloodstream into surrounding tissues.

Major biochemical features of leukocytes

- active synthesis of proteins and nucleic acids
- active glycolysis
- active pentose phosphate pathway
- moderate oxidative phosphorylation
- rich indegradative enzymes

Thrombocytes (platelets) are cell fragments that arise in the bone marrow from large precursor cells, the megakaryocytes. They have a key role in the process of blood clotting.

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

monoxide and 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. Embryonicand 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

Sickle cell anemia is caused by an inherited structural abnormality in the β-globin polypeptide. The mutation is Glu6 β→Val.

Clinically, an individual with sickle cell disease 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.

Other hemoglobinopathies. More than 600 mutations in the genes encoding the α- and β-globin polypeptides have been documented. Hemoglobinopathies are classified according to the type of structural change and altered function and the resulting clinical characteristics.

Plasma proteins

Plasma contains many proteins broadly classified into albumin and globulins (predominantly immunoglobulins). Albumin functions as a major transport protein for several ligands – trace metals, hormones, bilirubin, and free fatty acids.

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 with different protective functions.

Changes in the concentration of plasma proteins give important clinical information. A characteristic pattern with decreased albumin, transthyretin and transferrin and increased α1-antitrypsin, fibrinogen and C-reactive protein indicates the acute phase response. Serum and urine protein electrophoresis is an important way of identifying the presence of monoclonal immunoglobulins.

A number of plasma proteins have the ability to bind certain ligands. These proteins can then act as a reservoir for the ligand and help control its distribution and availability by transporting it to tissues

throughout the body. Binding to a protein can also render a toxic substance less harmful to the tissues. Albumin, in addition to its functions as a protein reserve in nutritional depletion and as an osmotic regulator, is a major transport protein. Albumin, the predominant plasma protein having no known enzymatic or hormonal activity, accounts for approximately 50% of the protein found in human plasma, and is present normally at a concentration of 35-45 g/L.

Immunoglobulins are proteins 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. 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 along with a decrease in the plasma concentration of some others. 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. 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.

Iron metabolism

Iron (Fe) is quantitatively the most important trace element. The human body contains 4-5g of iron, which is almost exclusively present in protein-bound form. Approximately three-quarters of the total

amount are found in hemoproteins, mainly hemoglobin and myoglobin. About 1% of the iron is bound in iron-sulfur clusters, which function as cofactors in the respiratory chain, and in other redox chains. The remainder consists of iron in transport and storage proteins.

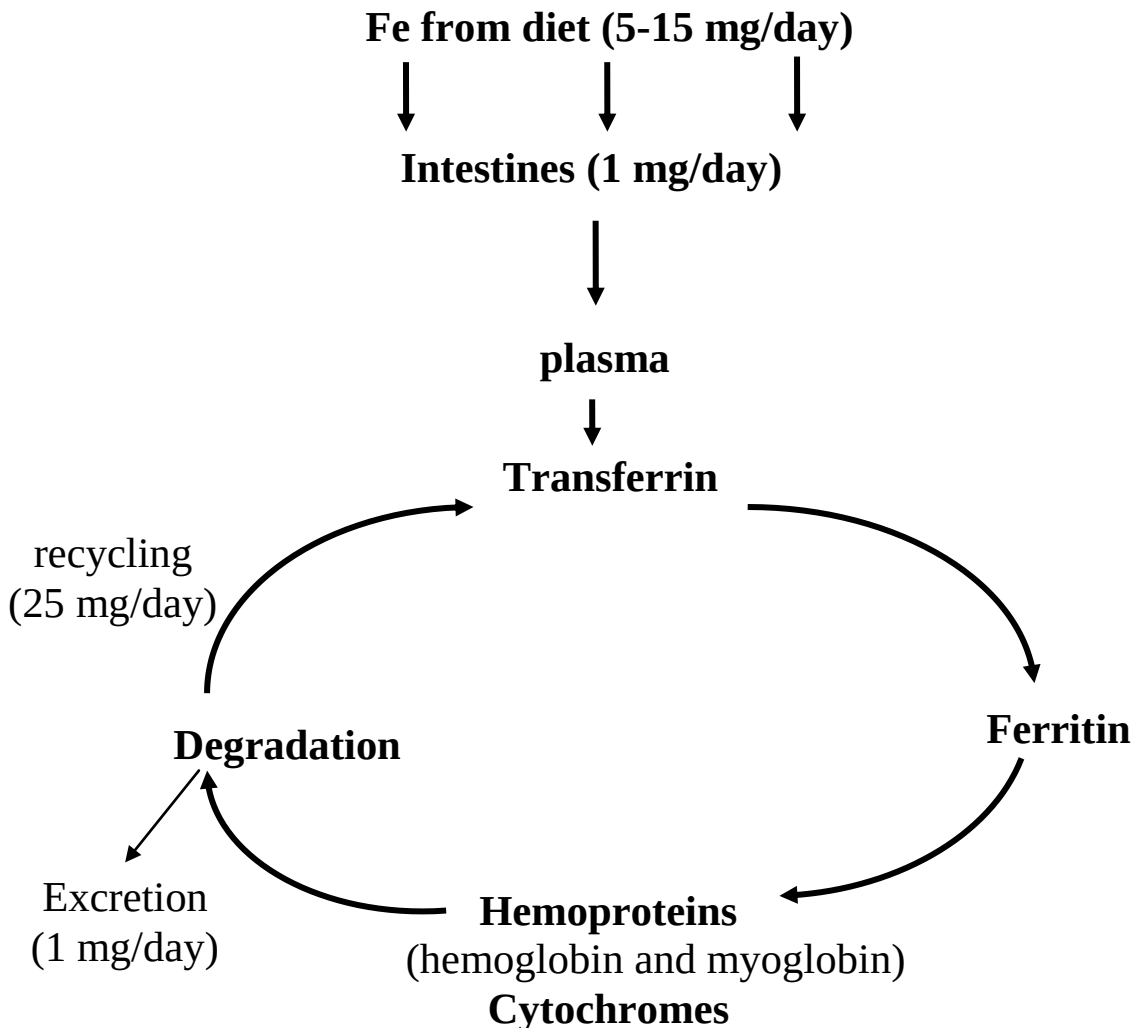
Iron can only be resorbed by the bowel in bivalent form (Fe^{2+}). Incoming iron in the Fe^{3+} state is reduced to Fe^{2+} by a ferrireductase present on the surface of enterocytes. Vitamin C in food is also preferrable.

Reduction of ferric iron to ferrous form. Within the plasma, iron with transferring is transported as ferric ions (Fe^{3+}) and is released from the protein into tissues after it has bound to specific cell receptors.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.

Most of the resorbed iron serves for the formation of red blood cells in the bone marrow. In the blood, 2.5–3.0 g of hemoglobin iron circulates as a component of the erythrocytes. After the heme degradation the iron returns to the plasma pool. The quantity of heme iron recycled per day is much larger than the amount resorbed by the intestines.

Excess iron is incorporated into **ferritin** and stored in this form in the liver and other organs. Each ferritin molecule is capable of storing several thousand iron ions. In addition to ferritin, there is another storage form, **hemosiderin**.

- **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. The concentration of ferritin in plasma is proportional to the amount of stored iron, and so measurement of plasma ferritin is one of the best indicators of iron deficiency;
- **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;



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

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 occur, resulting in cessation of blood loss from injured vessels within a few minutes (hemostasis). 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 cell fragments 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.

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. Prothrombin is activated in two ways: intrinsic and extrinsic.

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** III binds to various proteinases in the cascade and thereby inactivates them. **Heparin**, an anticoagulant, potentiates the effect of antithrombin III. **Thrombomodulin**, which is located on the vascular endothelia, also inactivates thrombin.

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 hereditary genetic 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 haemophiliacs 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 33

METABOLISM IN NERVOUS TISSUE

The human brain is the most complicated of all known living structures. Nervous system and, primarily, the brain plays 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. The brain is an instrument of human cognitive activity and the question of how the human brain works – remains one of the central problems in science.
Nerve tissue is composed of several cell types. Neuron – is a nerve cell with all its processes. 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 second type of cells in the nervous tissue is glia. 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 is more than 10 times exceeds 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).

Methods of separate biochemical analysis of neurons and glia:

1. Micromanipulation method (1950-1960. – Hiden and Endstrem in Sweden, Lowry in the U.S.).
2. Method of quantitative cytochemistry – Casperson, 30 years of the XX century.
3. Method of fractions enrichment – Rose, 1965.

Blood-brain barrier (BBB)

Most walls of brain capillaries (85-90%) are covered by outgrowths of astrocytes, and the rest of the surface itself is surrounded by bodies of glial cells. Contact between astrocytes and capillary walls so close that the outer surface of the membranes of these two elements seem to merge to form a double septum. Due to such a double baffle there appeares a barrier through which many soluble in the blood substances permeate with difficulty. Morphological basis of BBB is brain vascular endothelium, perivascular base membrane and the plasma membrane of glial cells.

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. Level of activity and metabolism of nervous tissue is a factor that regulates the function of the BBB. On the one hand, the BBB plays a role in protecting the brain against endogenous and exogenous toxins, circulating in the blood, and on the other hand, 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.

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.

Summarizing the data on the exchange of free amino acids in the brain, we can draw the following conclusions:

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- atsetilasparagin acids 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 separeted 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

In recent years the interest in the management of the most important brain functions with the help of peptides has significantly increased. There 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. Compared with other systems of

intercellular signaling peptide system appeared to be the most numerous (by now over 600 natural neuropeptides have been discovered) and multifunctional.

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 intercellular information transmitters. They frequently perform at the same time the function of neurotransmitters, neuromodulators and distant regulators. Neuropeptides (together with other regulatory compounds) form a functionally continuous system.

Each neuropeptide has a unique combination of biological activities. 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). There is a complex hierarchical system in which some neuropeptides induce or inhibit the output of other neuropeptides. Moreover, these neuropeptides-inductors possess, moreover, the capacity to cause a series of biochemical and physiological effects.

Energy metabolism in the neural tissue

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. The impossibility of replacing the main energy substrate, glucose, by other compounds extensively oxidizing in other tissues.

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.

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.

3. Dominant mode of glucose metabolism in nervous tissue is aerobic glycolysis.

4. Important role for brain metabolism hexokinase, as the main mechanism involving glucose in glycolysis.

5. Existence of a single functional complex of two glycolytic enzymes – hexokinase and phosphofructokinase, synchronously controlled by pool of adenine nucleotides.

Lipid metabolism in neural tissue

Lipid composition of the brain is unique not only by high concentrations of lipids, but also by the high content of the individual fractions. Almost all brain lipids are represented by three main fractions: glycerophospholipids, sphingolipids and cholesterol, which is always found in a free and not esterified state, typical for most other tissues.

Lipid metabolism in the nervous tissue has the following characteristics:

1. The brain has a high ability to synthesise fatty acids;

2. In the brain there is practically no β- oxidation of fatty acids;

3. Rate of lipogenesis in the brain varies in different periods of postnatal life;

4. Constant composition of lipids in the mature brain confirms low speed of their renewal;

5. Phosphatidylcholine and phosphatidylinositol are restored quickly in the brain tissue;

6. Rate of cholesterol synthesis in the brain is high in the period of CNS formation. With age, the activity of this process reduces;

7. Synthesis of cerebrosides and sulfatide occurs most actively during myelination.

In the mature brain 90% of cerebrosides are the myelin sheath, while gangliosides are the components of a typical neuron.

Role of mediators in the transmission of nerve impulses

Most synapses in the mammalian nervous system are chemical. The process of transmitting the signal is carried out by chemical synaptic neurotransmitter release from presynaptic nerve endings. Four groups of substances currently concern to neurotransmitters: monoamines, amino acids, purine nucleotides, peptides. In individual neurons, as a rule, several neurotransmitters of different chemical nature are synthesized. In addition there is an extensive class of neurotransmitter compounds – neuromodulators regulating the level of synaptic transmission.

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.

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. Short-term memory (duration from several milliseconds to several minutes).
2. Intermediate (from several seconds to several hours).
3. Long-term memory (years, decades and throughout life).

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 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 of a wide spectrum of neurochemical processes occurs 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 transcriptons. 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.

Spinal fluid
(cerebrospinal fluid, CSF)

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.

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

CHAPTER 34

BIOCHEMISTRY OF MUSCLE TISSUE

Mobility is a characteristic feature of all forms of life – chromosome segregation in mitotic apparatus of cells, air-screw motion of bacterial flagella, bird wings, the precise movements of the human hand, powerful work the leg muscles. All this is achieved by the work of muscles through their contraction and relaxation.

There are three types of muscle tissue:

- skeletal muscle;
- heart muscle;
- smooth muscle.

There is also a division into:

- smooth muscle;
- striated muscle.

Striated muscles include:

- skeletal;
- muscles of the tongue and upper third of the esophagus;
- external muscles of the eyeball, and others.

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.

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;
- actin;
- actomyosin;

as well as the so-called regulatory proteins:

- tropomyosin;
- troponin;
- α- and β-actin.

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

Biogeochemical 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 Pi, but does not provide release of the hydrolysis products. A stable complex "myosin – ADP – Pi" 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.

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.

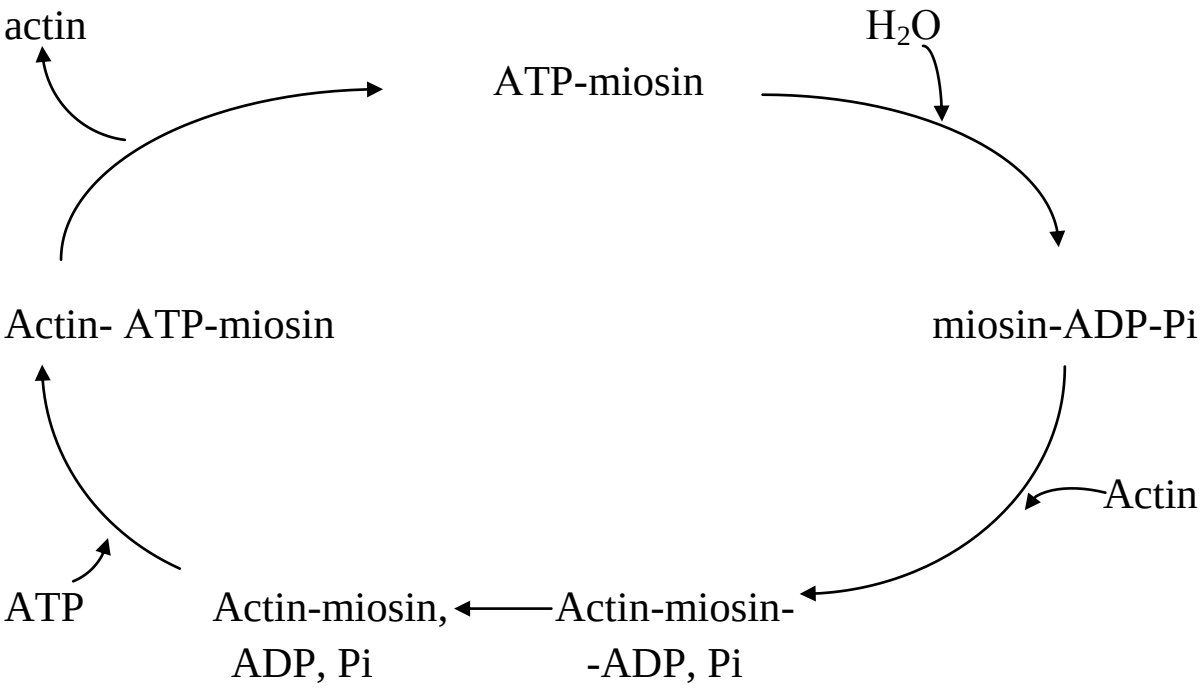


Fig. 34.1. Cycle of muscle contraction

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.

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 10^{-7} to 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.

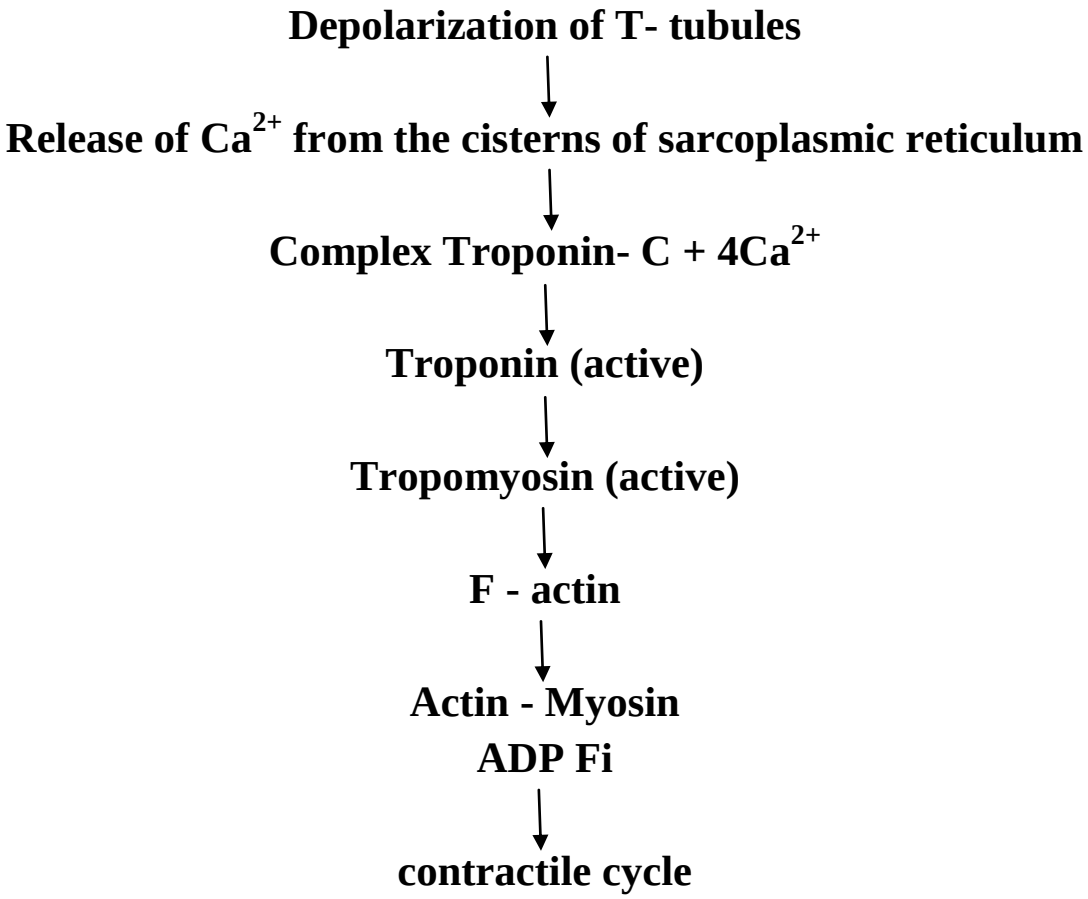


Fig. 34.2. Role of calcium ions in muscle contraction

Biochemistry of muscle fatigue

Fatigue is a body condition that occurs due to prolonged muscle load and is characterised 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.

CHAPTER 35

BIOCHEMISTRY OF CONNECTIVE TISSUE

Connective tissue comrises about half of the dry weight of the body. All varieties of connective tissue, despite their morphological differences, 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 main components of the extracellular matrix are structural proteins collagen and elastin, glycosaminoglycans, proteoglycans and non-collagenous structural proteins (fibronectin, laminin, tenascin, osteonectin, etc.) which form specific fibrous structures.

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).

Unusual mechanical properties of collagen are associated with their primary and spatial structures. Collagen molecules consist of three polypeptide chains, called α-chains. Over 20 α-chains are identificated, the majority of which 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 structure of collagen α-chains is unusual. Since every third amino acid in the polypeptide chain is presented by glycine, about 25% – by proline or 4 – hydroxyproline, about 10% – by alanine. In collagen such amino acids as cysteine and tryptophan are absent. The primary structure of collagen α-chain also contains the same unusual amino acid hydroxylysine.

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.

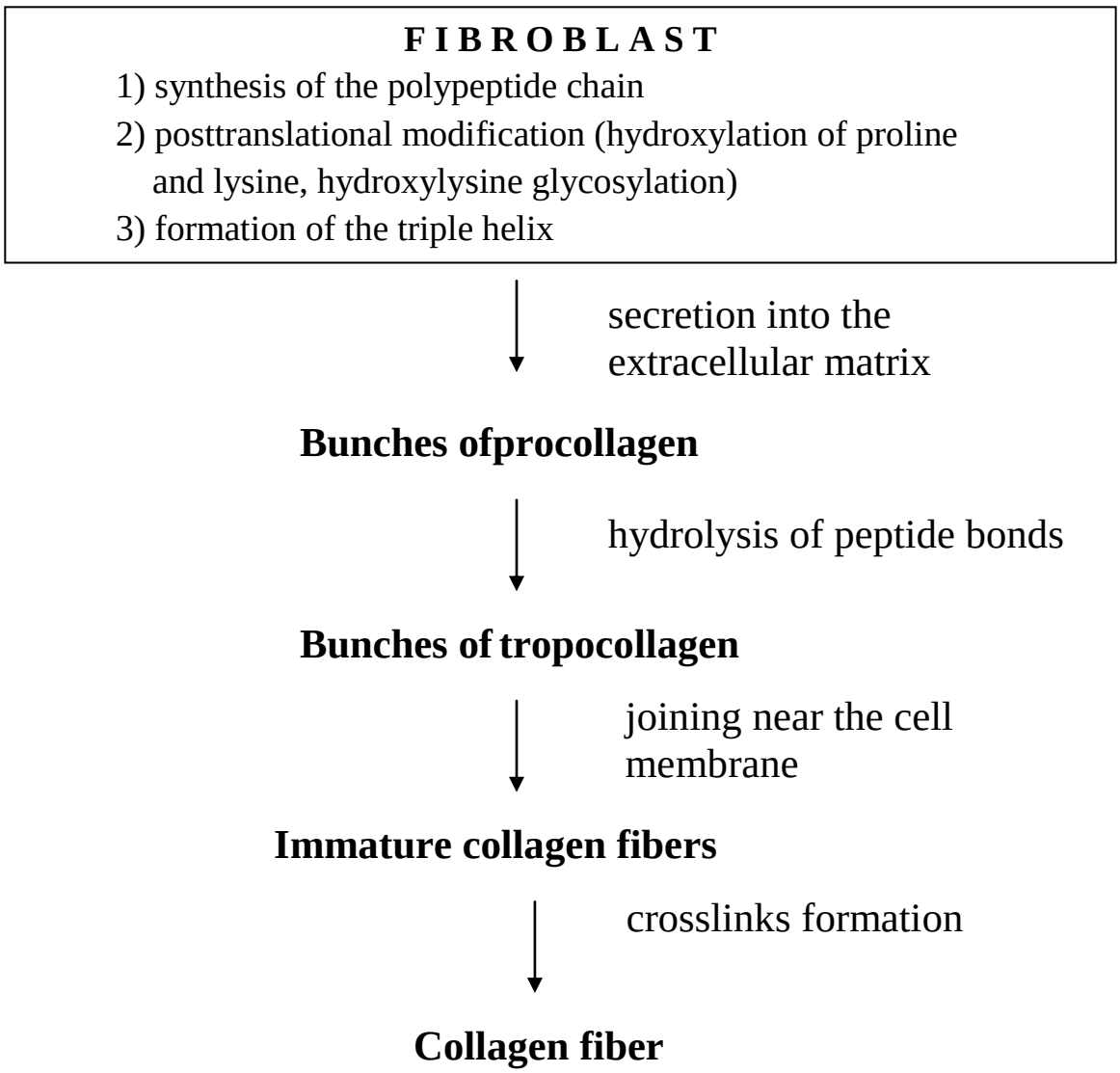


Fig. 34.1. Stages of formation of collagen fibers.

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.

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.

Elastin

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.

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 a little hydroxyproline, and hydroxylysine completely absent. The presence of large amounts of hydrophobic radicals prevents the creation of a stable globules resulting polypeptide chains do not form 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.

It should be noted that the elastin is synthesised as soluble monomer, which is called “tropoelastin”. After crosslinking elastin acquires its final shape, which is characterized by insolubility, high stability and a very low metabolic activity.

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.

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;
• as polyanions 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. 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.

3. Keratan sulfates 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-galactosamine-6-sulfate. They are part of the cornea, cartilage and intervertebral discs.

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. Disaccharide units are composed of D-glucuronate-2-sulfate and N-acetylglucosamine-6-sulfate.

6. Heparan sulphate is a part of the basal membrane proteoglycans. Disaccharide unit structure is the same as that of heparin, but has more N-acetyl groups.

In the extracellular matrix there are different types of proteoglycans. Among them, there are very large ones, for example aggrecan. 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.

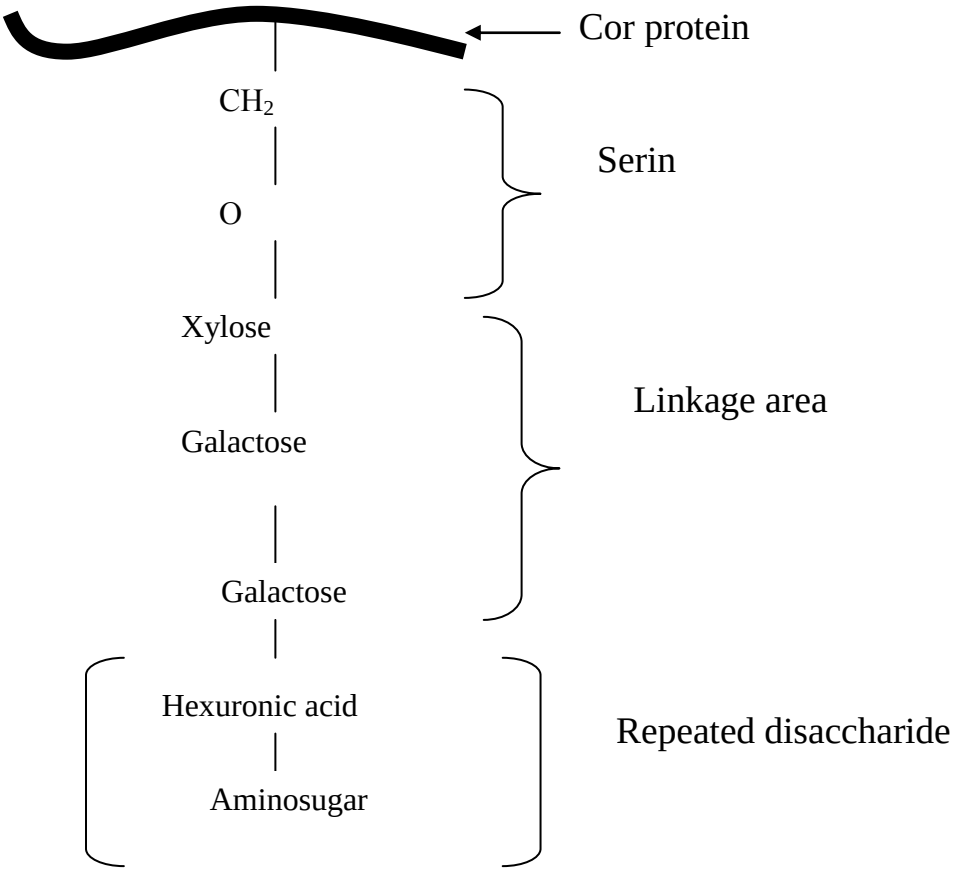


Fig. 34.2. The general scheme of the structure of glycoproteins.

Polysaccharide chains are synthesized by successive addition of monosaccharides. Appropriate nucleotide sugars are generally donors of monosaccharides. Synthesis reaction are 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 builts up. Sulfation of the carbohydrate portion is done by 3-phosphoadenosine-5-phosphosulfate (PAPS).

Glucocorticoids affect on 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.

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Под общей редакцией профессора В.В. Лелевича

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