МИНИСТЕРСТВО ЗДРАВООХРАНЕНИЯ РЕСПУБЛИКИ БЕЛАРУСЬ
БЕЛОРУССКИЙ ГОСУДАРСТВЕННЫЙ МЕДИЦИНСКИЙ УНИВЕРСИТЕТ
КАФЕДРА БИОЛОГИЧЕСКОЙ ХИМИИ

# БИОЛОГИЧЕСКАЯ ХИМИЯ. ВСПОМОГАТЕЛЬНЫЙ МАТЕРИАЛ К ЛЕКЦИЯМ

# BIOLOGICAL CHEMISTRY. LECTURE NOTES

Учебно-методическое пособие

*2-е издание, переработанное*

Минск БГМУ 2014

УДК 577.1(811.111)-054.6(075.8)
ББК 28.072 (81.2 Англ-923)
Б63

Рекомендовано Научно-методическим советом университета в качестве учебно-методического пособия 28.05.2014 г., протокол № 9

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**Биологическая** химия. Вспомогательный материал к лекциям = Biological chemistry. Б63 Lecture notes : учеб.-метод. пособие / А. Д. Таганович [и др.]. – 2-е изд., перераб. – Минск : БГМУ, 2014. – 144 с.

ISBN 978-985-567-033-0.

Издание содержит схемы, рисунки, пояснительный текст к лекциям по основным программным разделам курса биологической химии для студентов медицинского факультета иностранных учащихся, обучающихся на английском языке по специальностям «Лечебное дело» и «Стоматология». Предназначено для облегчения конспектирования лекций и улучшения усвоения материала. Рекомендуется для подготовки к лекциям, практическим занятиям и экзамену по биологической химии. Первое издание вышло в 2013 году.

Предназначено для иностранных студентов 1–2-го курсов, обучающихся на английском языке.

**УДК 577.1(811.111)-054.6(075.8)**
**ББК 28.072 (81.2 Англ-923)**

Учебное издание

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Подписано в печать 28.05.14. Формат 60×84/8. Бумага писчая «Снегурочка».
Ризография. Гарнитура «Times».
Усл. печ. л. 16,74. Уч.-изд. л. 11,35. Тираж 90 экз. Заказ 356.

Издатель и полиграфическое исполнение: учреждение образования
«Белорусский государственный медицинский университет».
Свидетельство о государственной регистрации издателя, изготовителя,
распространителя печатных изданий № 1/187 от 18.02.2014.
Ул. Ленинградская, 6, 220006, Минск.

**ISBN 978-985-567-033-0**

# CHAPTER 1
# PROTEINS: STRUCTURE AND FUNCTIONS

Proteins perform multiple critically important roles in living systems (table 1.1). Virtually, proteins provide molecular basis for vital activity.

*Table 1.1*

**Some proteins and their functions**

| Proteins | Function |
|---|---|
| *Collagen*<br>*Elastin* | Structure |
| *Enzymes* | Catalysis |
| *Myosin*<br>*Actin* | Contraction |
| *Hemoglobin*<br>*Transferrin*<br>*Aquaporin* | Transport |
| *Immunoglobulins* | Immune defence |
| *Albumin* | Provide colloid osmotic (oncotic) blood pressure |
| *Insulin*<br>*Prolactin*<br>*Growth factors*<br>*Cytokines* | Hormones and regulatory factors |
| *Receptors*<br>*G-proteins* | Recognition and signal transduction |
| *Prothrombin*<br>*Fibrinogen* | Blood clotting |

Structurally, **proteins** are complex macromolecules, polymers, consisting of amino acids linked via peptide bonds. As a rule, protein contains more than 80 amino acids. **Peptide** is the name assigned to short polymers of amino acids. Peptides are classified according to the number of amino acid units in the chain. Dipeptides have two amino acid residues, tripeptides have three, tetrapeptides four, and so on. The chains of less than 12 amino acid residues are usually referred to as oligopeptides, and when the chain exceeds dozen amino acids in length, the term polypeptide is used. However, the distinctions in this terminology are not precise. **Biologically important peptides** include neuropeptides (enkephalins), vasoactive peptides (angiotensins), hormones (oxytocin, glucagon, others), antioxidants (glutathione), toxins (α-amanitin), antibiotics (gramicidin).

## AMINO ACIDS

There are 20 proteinogenic amino acids, which are used for the synthesis of proteins on ribosomes. Most of amino acids contain a carboxyl group, an amino group, and a side chain (R group), all attached to the α-carbon. The general structure of α-amino acids is shown in figure 1.1. Proteinogenic amino acids are usually of the L-configuration. Rare exceptions are bacterial membrane proteins, which contain a few D-amino acids.

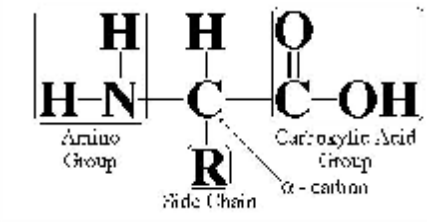



*Fig. 1.1.* Structure of an α-amino acid

The **classification** of amino acids is based on the chemical structure of their **side chains** (figure 1.2). An alternative way is to classify the common 20 amino acids according to the **polarity** of their side chains:

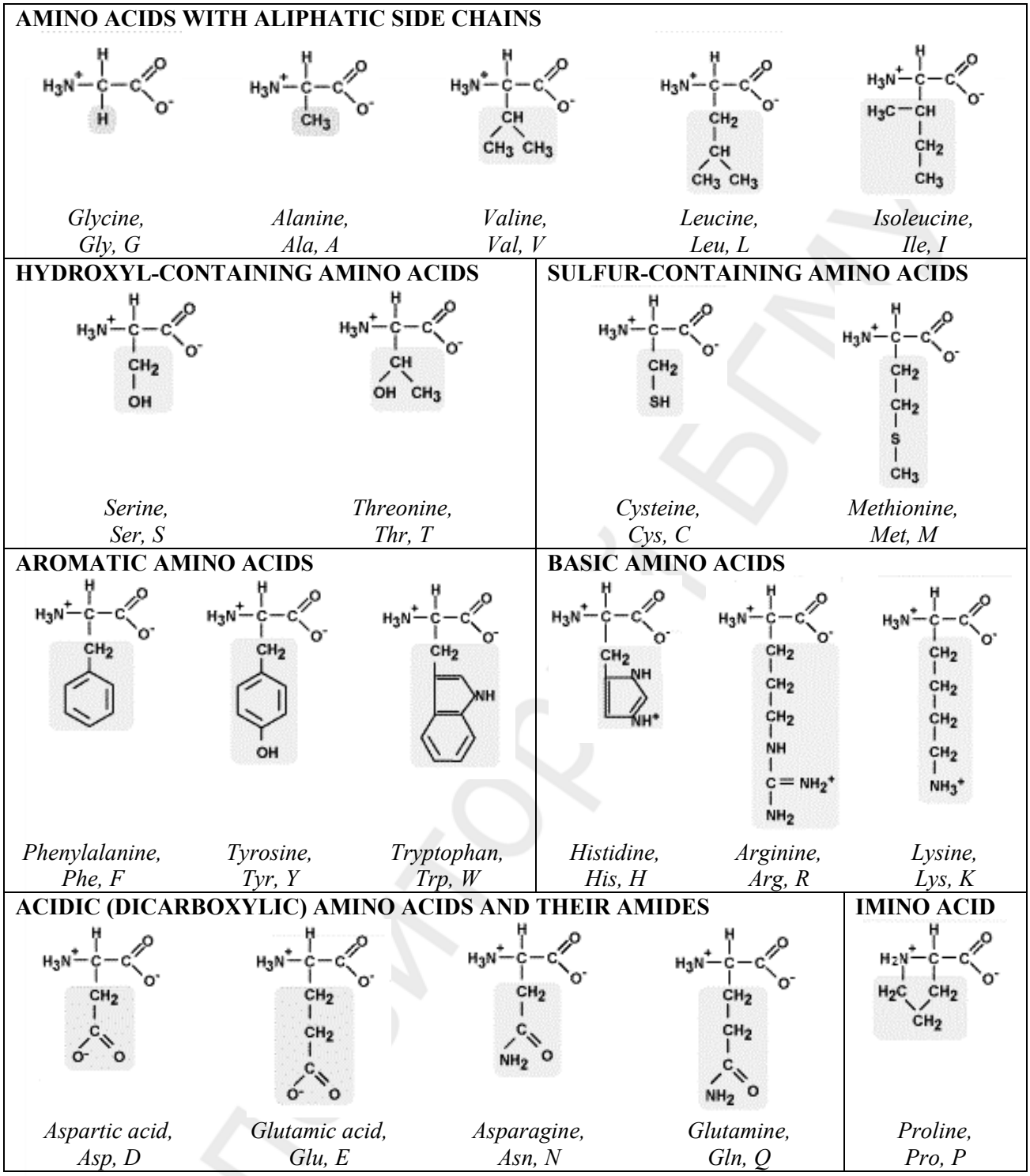



*Fig. 1.2.* Structure of amino acids

**Nonpolar (hydrophobic) amino acids** include all those with alkyl chain R groups (alanine, valine, leucine, and isoleucine), as well as proline, methionine, and two aromatic amino acids, phenylalanine and tryptophan.

**Polar (hydrophilic), uncharged amino acids** include glycine, and those amino acids which contain R groups that can form hydrogen bonds with water. The amide groups of asparagine and glutamine, the hydroxyl groups of tyrosine, threonine, and serine, and the sulfhydryl group of cysteine are all good hydrogen bond-forming moieties.

**Polar, acidic amino acids** include aspartic acid and glutamic acid. Their R groups contain a carboxyl group, which provides a net negative charge at pH 7.

**Polar, basic amino acids** — histidine, arginine, and lysine have side chains with net positive charges at neutral pH.

**Properties of amino acids**

**1. Stereochemistry**. The common amino acids (except glycine) have four different substituents (R, H, COOH, $NH_2$) covalently bound to an α-carbon. Such asymmetric carbon (also called a **chiral center**) ensures existence of L- and D-stereoisomers of amino acids. The solutions of L- and D-isomers rotate the plane of polarized light in opposite directions (i. e., exhibit **optical activity**).

**2. Acid-base properties.** Amino acids possess **amphoterism**, which is due to the presence of both acidic (COOH) and basic ($NH_2$) groups. Although the general structure of amino acids shown in the figure 1.1 is chemically correct, it ignores the conditions in vivo. In the solution at neutral pH the carboxylic group dissociates and looses a proton, while the amino group gains the proton. As a result, amino acid forms a **bipolar ion** (**zwitterion**, figure 1.3). The concentration of free protons in the surrounding medium (pH) strongly impacts the group ionization, thus influence on the net charge of the amino acid (figure 1.3). **At pH < 7** (acidic medium), carboxylic group will be protonated, so the net charge of the molecule becomes positive. **At pH > 7** (alkaline medium), the amino group will lose its proton, and the amino acid acquires the net negative charge.

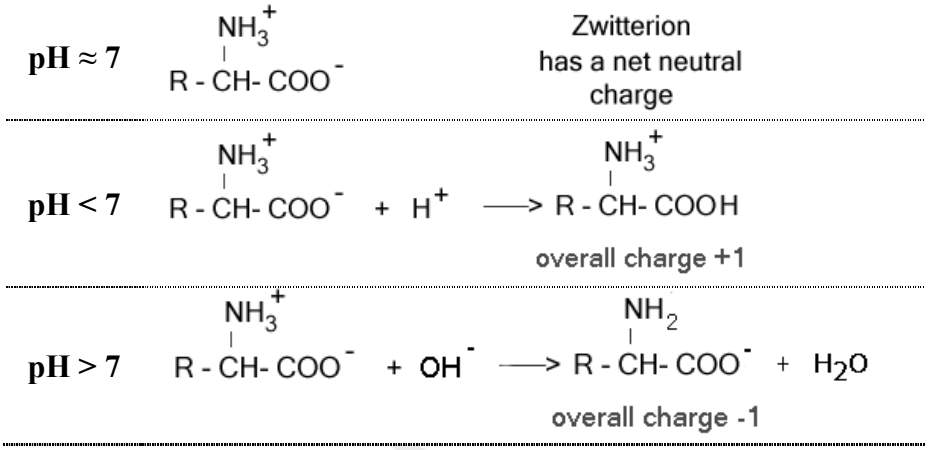



*Fig. 1.3.* The influence of pH on the overall charge of amino acids

The pH, at which an amino acid is in its zwitterion form and is electrically neutral, is called **isoelectric pH**.

**3.** Amino acids undergo a variety of **common chemical reactions** typical to $–NH_2$ and –COOH groups, including peptide bond formation.

**4. Specific properties** are determined by the chemical nature of the amino acid side chain (e. g., formation of disulfide bonds by cysteine, absorption of UV at 280 nm by aromatic amino acids).

## STRUCTURAL ORGANIZATION AND PROPERTIES OF PROTEINS

The architecture of protein molecules is quite complex. Nevertheless, this complexity conforms to the four levels of structural organization.

**Primary structure**. The primary structure of a protein corresponds to the linear **sequence of amino acids**, or the configuration[1] of the polypeptide chain.

The sequence of amino acid residues is encoded in DNA and determines the native three-dimensional conformation of the protein.

[1] The terms configuration and conformation are often confused. **Configuration** refers to the geometrical relationship between a given set of atoms; changes in configuration require breaking covalent bonds. **Conformation** refers to the spatial relationship of every atom in the molecule formed by rotation about single bonds; such rotations create many possible orientations for the protein chain. Changes in conformation occur without covalent bond rupture.

The primary protein structure is stabilized by **peptide bonds,** which are formed between α-carboxylic group of one amino acid and the α-amino group of another (figure 1.4).

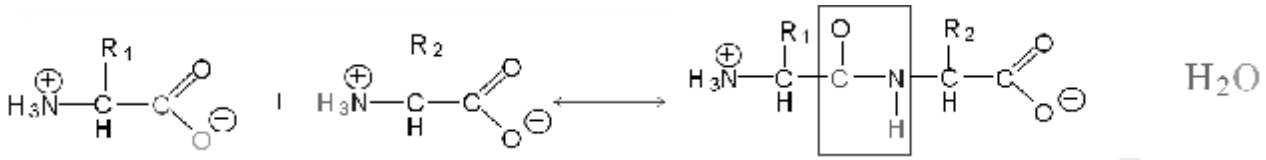



*Fig. 1.4.* Formation of a peptide bond

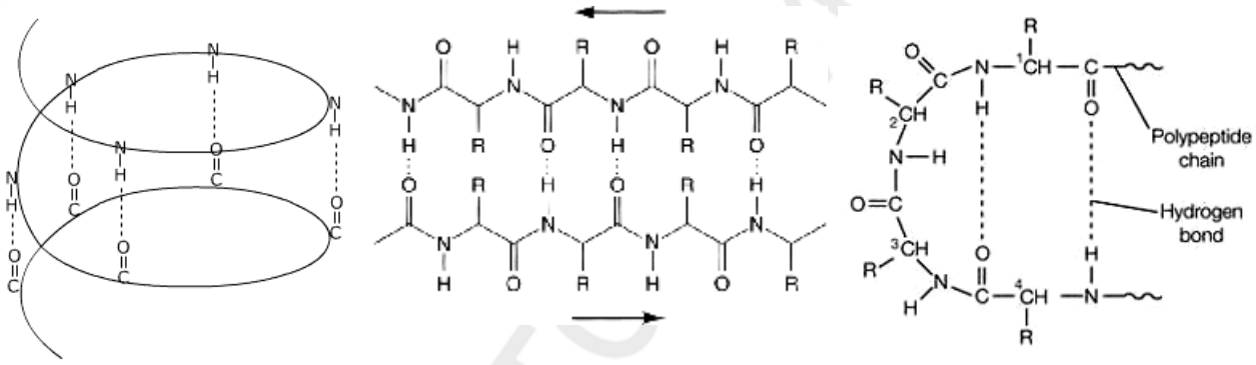
**Properties of the peptide bond:**

- Covalent, extremely stable.
- Partial double, planar, without possibility of rotation.
- *Trans*-position of O and H atoms provides maximal H-bonding.

**Disulfide bonds** between cysteine residues also may contribute to the stabilization of primary protein structure.

**Secondary structure**. Secondary protein structure includes various types of **local conformations**, which are stabilized by the **hydrogen bonding** between peptide units; the atoms of side chains are not involved.

Three basic types of secondary structure exist: α-helix, β-sheet, and β-turn (figure 1.5).




α-helix β-sheet (antiparallel) β-turn

*Fig. 1.5.* Secondary structure types

In **α-helix** H-bonds are oriented along the axis of the structure. Proline, and amino acids with charged or large side chains disrupt the regular structure of α-helix.

**β-Sheets** are formed from two extended fragments of a polypeptide chain; H bonds are oriented crosswise to the axis of the structure. The pleated sheets are parallel if the chains run in the same direction, and antiparallel if the chains run in the opposite directions.

**β-Turn** involves four amino acid residues, in which the first residue is H-bonded to the fourth, resulting in a tight 180º-degree turn. Proline and glycine are often present in β-turns.

Secondary level of protein structure organization is characterized by local ordered regions helices, sheets, turns), which are alternated with non-ordered regions (loops).

**Supersecondary structure.** Supersecondary structure represents a local ordered organization of secondary structures (helix-turn-helix, zinc fingers, βαβ, αααα). The sections of protein with supersecondary structure form distinct globular regions in the polypeptide chain — domains (functional and structural). Domains perform a particular task: anchor the protein to a membrane, or interact with regulatory molecules, or bind a substrate. However, the formation of a functionally active protein requires the completing of its tertiary (or quaternary) structure.

**Tertiary structure**. The term "tertiary structure" refers to the entire three-dimensional arrangement of a polypeptide chain. It is produced as a result of interaction between the side chains of amino acids, which may be located at a considerable distance from each other in the primary structure.

3-D structure of the protein is stabilized primarily by the noncovalent interactions: **hydrophobic**, **hydrogen**-bonding, **ionic** forces (figure 1.6). **Disulfide** bonds also may support the stability of the folded polypeptide chain. Principal among these are hydrophobic interactions that drive most hydrophobic amino acid R groups into the interior of the protein, shielding them from water, while hydrophilic residues are usually found on the surface.

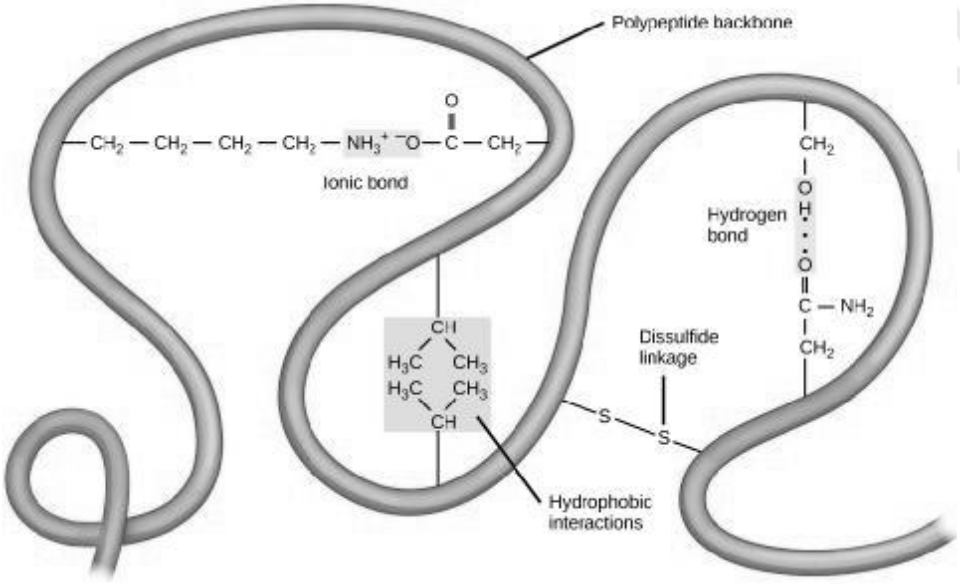



*Fig. 1.6.* Interactions between amino acid residues in a polypeptide chain

**Quaternary structure**. Quaternary structure defines the association of several (more than one) polypeptide chains, each having tertiary structure. Individual polypeptide chains are called **protomers** or **subunits**, and their association forms a new oligomeric protein with a new function. The subunits are joined together by the same types of noncovalent interactions that are used for stabilization of tertiary structure. Rarely disulfide bridges link protomers.

The proteins with quaternary structure gain some **advantages:** economy of genetic material, decrease of mistakes during protein synthesis, expansion of proteins functional capabilities.

For instance, difference in structure between **hemoglobin and myoglobin** determines their different biological roles. Both proteins are built on a common structural motif, and contain **heme**, a cyclic tetrapyrrole with one atom of ferrous iron ($Fe^{2+}$) in the center of the ring (figure 1.7). Due to the presence of heme, these proteins may bind oxygen. **Myoglobin** contains a single polypeptide chain folded about a prosthetic group (heme). **Hemoglobin** is a tetrameric protein, each polypeptide subunit closely resembles myoglobin. The multiple subunit structure of hemoglobin gives it important oxygen binding properties that are different from myoglobin's.

The $O_2$ binding curve of myoglobin in solution at neutral pH, shown in figure 1.8, illustrates that myoglobin has a high affinity for oxygen — an important characteristic for a protein that must extract oxygen from the small amounts present in blood. At the oxygen concentration existing in the capillaries, the myoglobin in adjacent tissues is nearly saturated. When cells become metabolically active, their internal $PO_2$ falls to levels where myoglobin will lose (deliver) its oxygen. Such properties of myoglobin make it useful for oxygen storage (e. g., in muscles), but not useful for oxygen transport, since transport protein must accept oxygen efficiently at the partial pressure found in lungs (approximately 100 mm Hg) and then deliver an appreciable fraction of it at the partial pressure found in tissues (about 30–40 mm Hg).

Hemoglobin, which has a sigmoidal binding curve (figure 1.8), transports oxygen very efficiently, allowing nearly full oxygen saturation of the protein in the lungs, and maximal release of oxygen in the capillaries. Sigmoidal curve indicates the existance of **cooperative effect** among subunits in hemoglobin molecules, i. e, filling the first subunit with oxygen increases the affinity of

the remaining sites for oxygen. Conversely, losing an oxygen from hemoglobin makes it easier for the protein to lose its remaining oxygen molecules.

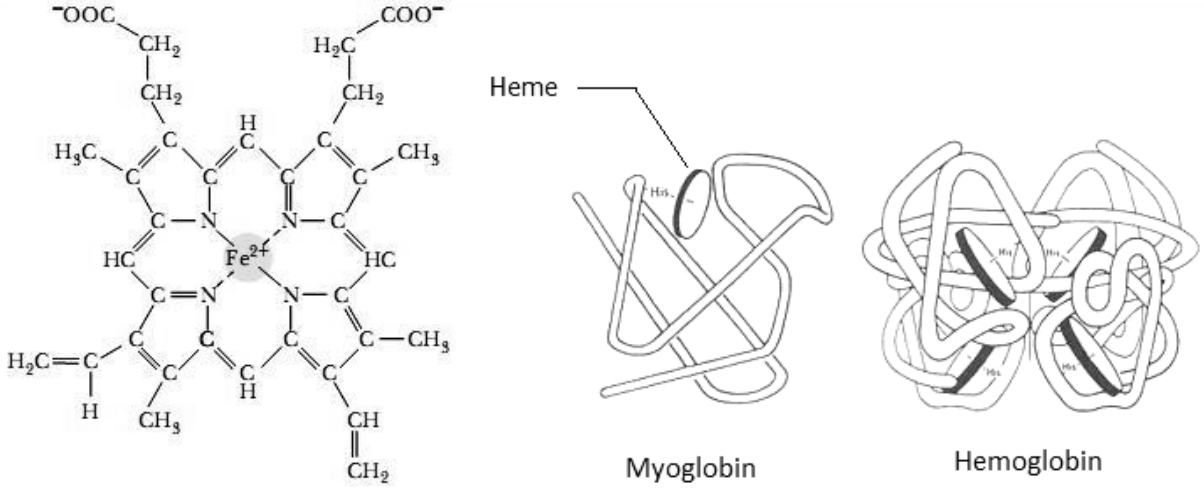



*Fig. 1.7.* Structure of heme (A), myoglobin and hemoglobin (B)

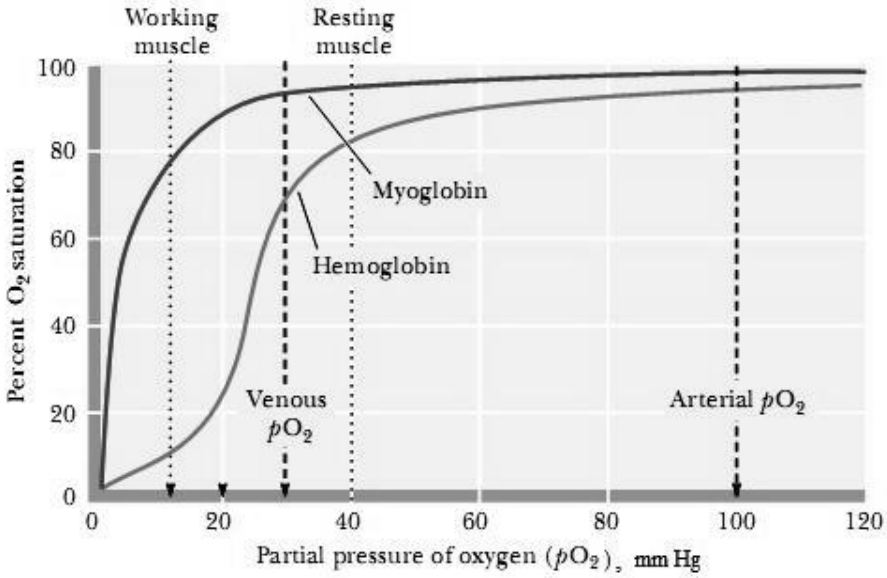



*Fig. 1.8.* Oxygen-binding curves for myoglobin and hemoglobin

**Denaturation** of proteins is the destruction of spatial protein structure ($4^{ry}$, $3^{ry}$, $2^{ry}$) followed by the loss of protein function. Many physical and chemical agents may cause unfolding of polypeptide chains through the disruption of relatively weak forces, which stabilize the higher orders of protein structure, without causing hydrolysis of peptide bonds (table 1.2).

*Table 1.2*

**Denaturing agents**

| Physical | Chemical |
|---|---|
| Heat | Urea |
| Cold | Organic solvents (acetone, alcohol) |
| Vibration | Detergents |
| UV | pH extremes (acids, alkali) |
| Ionizing radiation | Reducers |
| | Heavy metals |

If a denatured protein returns to its native state after the denaturing agent is removed, the process is called **renaturation**.

**General physical and chemical properties of proteins:**

- solubility, which depends on the following factors:
  - protein charge;
  - hydrate shell;
  - weight and shape of the molecule;
- viscosity;
- high oncotic and low osmotic pressure;
- optical activity;
- UV absorption;
- mobility in the electric field;
- low diffusion (virtual inability to pass through membranes).

## CLASSIFICATION OF PROTEINS

Proteins may be classified in several ways:

- according to their function (catalytical, structural, transport, etc.);
- according to their molecular shape (globular and fibrous).
- according to their constitution (simple and compound, or conjugated).

**Simple proteins** contain amino acids only. **Conjugated proteins** besides amino acids contain nonprotein component (**prosthetic group**). Examples and biological roles of some simple and conjugated proteins are given in the tables 1.3 and 1.4.

*Table 1.3*

**Simple proteins**

| Simple Protein | Functions |
|---|---|
| **Albumin**<br>55–65 % of plasma proteins, have relatively low molecular weight and strong negative charge | Provide plasma oncotic pressure<br>Transport hydrophobic substances in blood (fatty acids, steroid hormones, bilirubin, drugs, etc.) |
| **Histones**<br>Rich in Arg, Lys; bear positive charge and easily bind to nucleic acids | Take part in packing of DNA in the nucleus |

*Table 1.4*

**Conjugated proteins**

| Class of proteins | Nonprotein part | Type of bonding between apoprotein and nonprotein part | Functions and examples |
|---|---|---|---|
| Glycoproteins | Carbohydrate | Covalent<br>– O-glycosidic<br>– N-glycosidic | Structure (collagen)<br>Recognition (antigens, antibodies, receptors, hormones) |
| Lipoproteins | Lipids | Hydrophobic or Ionic | Transport of lipids in blood (chylomicrons, VLDL and others)<br>Structure (in membranes) |
| Metalloproteins | Mg, Ca, Mn, Fe, Co, Cu, Zn, etc. | Coordination | Transport and storage of metals (transferrin, ferritin)<br>Enzymes (carboxypeptidase A) |
| Phosphoproteins | Phosphate | Covalent | Calcium binding<br>Phosphorylation and dephosphorylation has effect on protein activity |
| Nucleoproteins | DNA, RNA | Ionic | Packing DNA in the nucleus<br>Control over transcription |
| Chromoproteins<br>Hemoproteins<br><br><br>Flavoproteins | Colored group<br>Heme<br><br><br>FMN or FAD | <br>Coordination<br><br><br>Covalent | <br>Transport and storage of oxygen (hemoglobin, myoglobin)<br>Enzymes (catalase)<br>Enzymes (succinate dehydrogenase) |

## SEPARATION AND PURIFICATION OF PROTEINS

Highly purified protein is essential for the detailed examination of its physical and biological properties. Cells contain thousands of different proteins, each in widely varying amounts. The isolation of a specific protein in quantities sufficient for analysis is an incredibly hard work that may require multiple successive purification techniques. Classic approaches to separation and purification of proteins exploit their certain differences in physical and chemical properties: solubility (isoelectric precipitation, salting out with ammonium sulfate), molecular size and weight (dialysis, size exclusion chromatography), surface charge (electrophoresis), ability to bind specific ligands (affinity chromatography).

### Polyacrylamide gel electrophoresis (PAGE)

PAGE in the presence of anionic detergent sodium dodecyl sulfate (SDS) nowadays is the most widely used method in protein research. Common electrophoresis separates charged biomolecules based on the rates at which they migrate in an applied electric field. For SDS-PAGE, acrylamide is polymerized and cross-linked to form a porous matrix (gel), which retards the movement of molecules according to their dimensions relative to the size of the pores in the matrix. SDS solves the task of making proteins equal in shape and charge. SDS is a detergent that disrupts protein folding and causes protein denaturation. The hydrophobic tail of SDS interacts strongly with polypeptide chains at a ratio of one molecule per two peptide bonds. Each dodecylsulfate contributes two negative charges. Collectively, these charges overwhelm any intrinsic charge that the protein might have.

Thus, in SDS-PAGE proteins migrate at a rate that reflects their molecular weight (the smaller – the faster). Individual proteins trapped in the gel are visualized by staining with dyes (figure 1.9).

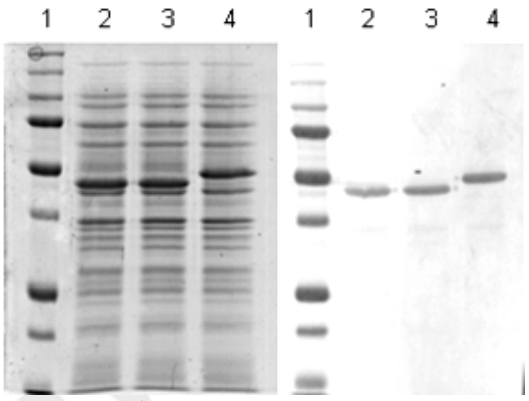



*Fig. 1.9.* Separation of proteins by SDS-PAGE (A) and identification of specific protein in the mixture by Western blot (B). Lane contents:
1 — protein standards of the known weight; 2–4 — proteins obtained from cell lysates; A — stained with Coomassie blue; B — treated with specific antibodies

### Western blot

Western blot allows to identify the required protein and to measure its relative content in the sample. Principal steps of Western blot analysis:

1. Extraction of proteins from biologic material.
2. Separation by SDS-PAGE.
3. Transfer of protein fractions to the nitrocellulose paper (blotting). The proteins retain the same pattern of separation they had on the gel.
4. Blocking with nonspecific protein (such as milk proteins) to bind to any remaining sticky places on the nitrocellulose.
5. Addition of primary antibodies which are able to bind to the required protein.
6. Addition of tagged secondary antibodies (molecular probe). These antibodies have an enzyme, a dye, or radioactive element attached to them.

7. Washing and protein detection. The location of the enzyme-labeled antibodies can be revealed by incubating the nitrocellulose membrane with a colorless substrate that the attached enzyme converts to a colored product that can be seen and photographed. The location of radioactive-labeled antibodies can be revealed by autoradiography.

## CLINICAL ASPECTS

**Medical implications of Western blot**

Immunoassays (including Western blot) are widely used for identification of antigens and antibodies present in the biological material. This is essential for diagnosis of AIDS, different types of hepatitis and other infectious diseases.

**Diseases of protein folding**

A number of human diseases are linked to abnormalities of protein folding. In **Alzheimer's disease** a misfolded β-amyloid peptide accumulates in human neural tissue, forming deposits known as neuritic plaques. Mutations which result in misfolding of the protein p53 cause **cancer**. P53 prevents cells with damaged DNA from dividing through induction of apoptosis. The misfolded protein is unstable and is quickly destroyed.

**Medical implications of protein denaturation**

The medically important aspects of protein denaturation are based on the fact that denatured proteins lose their properties. For example, the loss of protein antigenic properties, caused by a wide range of chemical and physical agents, is the basis for sterilization. The loss of blood protein solubility, caused by electro- or cryoprecipitation, is used for arrest of bleeding. Proteins form insoluble complexes with heavy metals and are used as antidote in poisonings by mercury, copper, lead salts, etc.

# CHAPTER 2
# ENZYMES

**Enzymes** are biological catalysts produced by living cells. The reactions in the body would not occur rapidly enough to sustain life if enzymes were not present. With the exception of catalytic RNA molecules, or ribozymes, **enzymes are proteins**. Like all catalysts, enzymes increase the rate of chemical reactions being neither consumed nor changed in a reaction.

In enzyme-catalyzed biochemical reactions one or more compounds (**substrates**) are converted into one or more other compounds (**products**). Enzymes do not affect the equilibrium concentrations of the substrates and products.

## GENERAL PROPERTIES OF ENZYMES:

- High molecular activity
- Working under mild conditions ($t^o \approx 37$ ºC; pH ÷ 6–8; p = 1 atm; aqueous medium)
- High sensitivity to chemicals and other surrounding changes
- Specificity
- Capability for regulation

Usually enzymes are highly specific both for the type of reaction catalyzed and for the substrates. In the case of **absolute specificity**, a single substrate can be recognized by an enzyme, e. g., lactose for lactase. Some enzymes can act on a set of closely related substrates that is they possess **group specificity**, e. g. alcohol dehydrogenase can oxidize different alcohols; esterase cleaves ester bonds in different substrates. Enzymes are also **stereospecific** catalysts and typically catalyze reactions only of one stereoisomer of a given compound — for example, D- but not L-sugars, L- but not D-amino acids.

Specificity depends on the structure of the enzyme **active site**. Active site of an enzyme is a region, where substrates bind, are converted to products, and are released. An active site is formed from amino acid residues brought together at the tertiary level of protein structure, and contains:

1. Assistant groups — provide proper position of substrate regarding to the active site.
2. Contact site — place for the substrate binding.
3. Catalytic site — responsible for carrying out chemical changes in a substrate molecule.
4. Auxiliary groups — remove the products of reaction.

All reactions have energy “barriers” to them. An activated or “transition state” for a molecule occurs when it has reached an energy that is sufficient to react. Therefore reactions usually go faster at higher temperatures. It was found that transition state for enzyme-substrate complex can be achieved at lower energy level than for substrate alone. Thus, an **enzyme works by lowering the free energy of activation**, $\Delta G^0$.

Two models were proposed to describe the interaction between an enzyme and a substrate and to explain enzymatic catalysis. The **lock-and-key model** originally proposed by Emil Fischer in 1894 supposes that an enzyme/substrate pair is like a lock and key. In 1958, Daniel Koshland proposed the **induced fit model** which supposes that distortion of the enzyme and the substrate is an important event in catalysis.

## ENZYME NOMENCLATURE AND CLASSIFICATION

The commonly used names for most enzymes describe the **substrate** or **type of reaction** catalyzed, followed by the suffix ***-ase***. For example, protein*ases* hydrolize proteins; dehydrogen*ases* remove hydrogen atoms. Systematic name and unique code number are given to each enzyme according to enzyme classification.

The **Enzyme Commission (EC)** of the International Union of Biochemistry and Molecular Biology (IUBMB) devised a system of enzyme classification, naming and numbering **based on the type of chemical reaction** catalyzed by an enzyme. All enzymes are divided into six major classes, with sub-classes and sub-subclasses to define their functions more precisely. The major classes are as follows:

1. ***Oxidoreductases*** catalyze oxidation-reduction reactions.
2. ***Transferases*** catalyze transfer of functional groups from one molecule to another.
3. ***Hydrolases*** catalyze hydrolytic cleavage.
4. ***Lyases*** catalyze removal of a group from or addition of a group to a double bond, or other nonhydrolytic cleavages.
5. ***Isomerases*** catalyze intramolecular rearrangement.
6. ***Ligases (synthetases)*** catalyze reactions in which two molecules are joined due to ATP (GTP) energy.

Each enzyme is given a number with four parts, such as **EC** 2.7.1.2. The first three numbers define major class, subclass, and sub-subclass, respectively. The last is a serial number in the sub-subclass, indicating the order in which each enzyme is added to the list, which is continually growing.

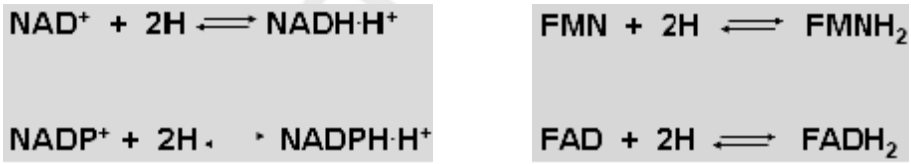
## COENZYMES

Many enzymes carry out their catalytic function relying solely on their protein structure. Such enzymes can be called simple or single-component. Many other enzymes require nonprotein "assistants". Such enzymes can be called compound or bi-component. Their protein part is termed apoenzyme; it is catalytically inactive. The catalytically active complex of protein and nonprotein parts is termed holoenzyme. Nonprotein parts may be designated as **cofactors** or **coenzymes** or **prosthetic groups.**

(Coenzymes usually bind to the enzyme in transient, dissociable manner (as $NAD^+$ does). Others may be firmly associated with their enzymes by covalent bonds (like FMN or FAD). Such tightly bound coenzymes are referred to as prosthetic groups.)

Coenzymes may be metal ions, vitamins and/or their active forms, different aromatic and aliphatic compounds. Usually coenzymes are **actively involved in the catalytic reaction** of the enzyme, often serving as intermediate carriers of functional groups in the conversion of substrates to products. The presence of coenzyme in most cases contributes to the **stabilization of the enzyme 3D-structure.** Cofactor may be involved in **substrate binding**.

The structures of $NAD^+$, $NADP^+$, FMN, and FAD, major coenzymes involved in biological oxidation, are given in figure 2.1. These molecules exist in two forms, oxidized and reduced, that are readily converted to each other depending on whether or not they are carrying electrons:




**Nicotinamide** coenzymes are derived from the **vitamin PP (niacin)**. **Flavin** coenzymes are derived from the **vitamin $B_2$ (riboflavin)**.

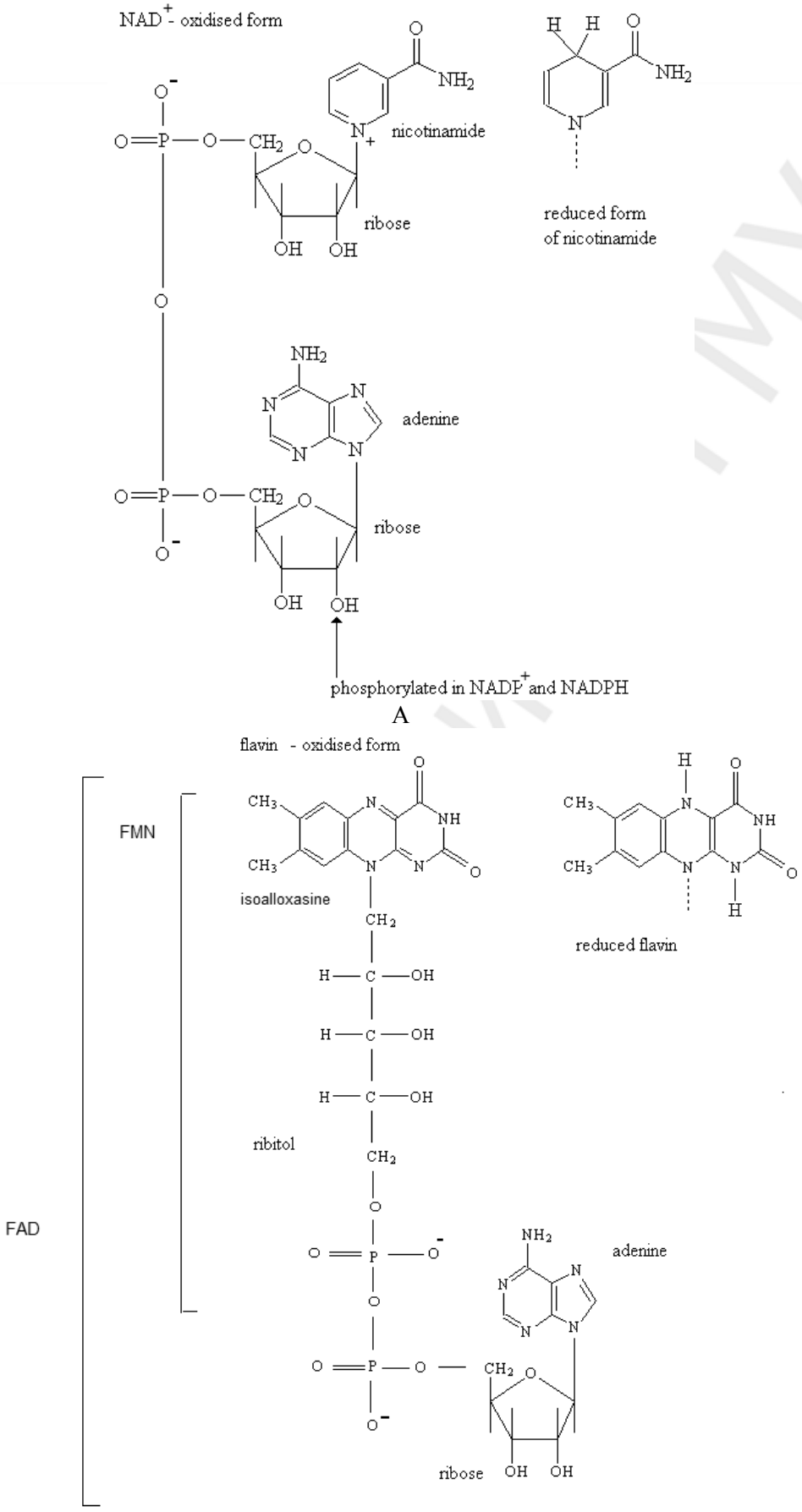



*Fig. 2.1.* Structures of: A — $NAD^+$ (Nicotinamide Adenine Dinucleotide), $NADP^+$ (Nicotinamide Adenine Dinucleotide Phosphate); B — FMN (Flavin MonoNucleotide), FAD (Flavin Adenin Dinucleotide)

## ENZYME KINETICS

Enzyme kinetics studies the influence of different factors on the rate of enzyme-catalyzed reactions. It was found out that the rate (or *velocity, V*) of enzyme catalyzed reaction, mainly, depends on

- Substrate concentration [S];
- Enzyme concentration [E];
- $t^o$;
- pH;
- Presence of inhibitors or activators.

**The dependence of the enzyme-catalyzed reaction velocity on the substrate concentration** was described by L. Michaelis and M. Menten (1913). The plot describing this dependence looks as follows (figure 2.2) for most of enzymes.

Increase in the substrate concentration leads to the increase in velocity of the reaction, but quickly the "steady state" is reached, when all the active sites of the enzyme are saturated with substrate, and the rate of ES complex breakdown is equal to the rate of its' formation. This steady state will persist until almost all of the substrate has been consumed.

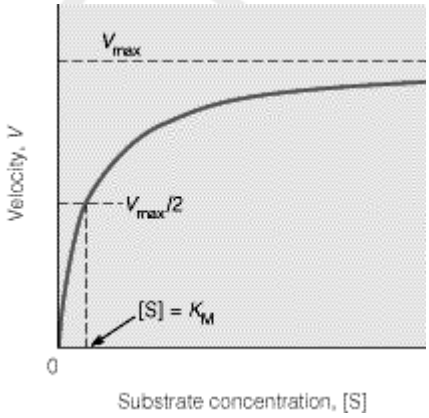



*Fig. 2.2.* Dependence of velocity of enzyme-catalyzed reaction on [S]

The dependence of V on [S] can be described by an equation, called **the Michaelis–Menten equation**:

$$v = \frac{V_{max}[S]}{K_m + [S]}$$, where $K_M$ is the **Michaelis constant**.

By mathematical rearrangement of the equation we find that $\mathbf{K_M = [S],}$ at which $\mathbf{V = V_{max}/2}$.

$\mathbf{K_M}$ is an important characteristic of the reaction. It **shows the affinity of enzyme for substrate(s)**: the higher $K_M$ — the higher [S] is required to achieve a given velocity — the lower affinity.

Since Michaelis-Menten kinetics is hyperbolic, it is difficult to determine Vmax accurately. The best way is to rearrange equation in such a way that it corresponds to a linear graph. The most common approach is to use a **double reciprocal plot**, also called **Lineweaver–Burk plot** (fig. 2.3). The intercept of the Lineweaver–Burk plot with the *Y* axis gives 1/Vmax; the intercept with the *X* axis gives — $1/K_M$.

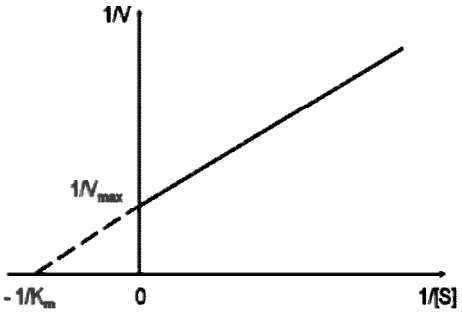



*Fig. 2.3.* Lineweaver–Burk plot

**The dependence of V on [E].** If the substrate concentration is constant, the velocity of the reaction is proportional to the enzyme concentration.

**The dependence of V on tº.** The plot describing the dependence of velocity of enzyme-catalyzed reaction on temperature is bell-shaped. The increase in temperature leads to the increase in kinetic energy of the reacting molecules and results in increasing velocity. The optimal temperature for most enzymes is about 40–50 ºC. Further heating will cause abrupt decrease of reaction rate resulting from denaturation of an enzyme.

**The dependence of V on pH.** Each enzyme-catalyzed reaction has an optimal pH at which appropriate charges are present on both the enzyme and the substrate, and the velocity is at a maximum. Changes in the pH may alter these charges so that the reaction proceeds at a slower rate. If the pH is too high or too low, the enzyme may also undergo denaturation.

## REGULATION OF ENZYME-CATALYZED PROCESSES

Capability for regulation is one of the important properties of enzymes. It allows to turn ON or OFF biochemical reactions in cells at appropriate time according to the cell needs.

The common mechanisms of regulation of enzyme-catalyzed processes, available *in vivo*, are

- *Substrate and/or product amount regulation*;
- *Enzyme amount regulation*;
  - Enzyme synthesis (regulation of gene expression);
  - Enzyme degradation;
- *Enzyme activity regulation*;
  - Inhibitors and activators;
  - Covalent modification of enzyme structure.

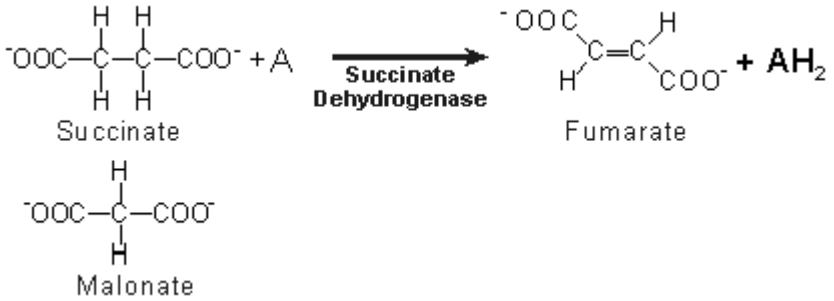
**INHIBITORS** slow down the rate of enzymatic reactions. Some substances (e. g. strong acids, alkali, heavy metals, detergents) can be called **non-specific inhibitors**, since they cause denaturation, thus, influence on all enzymes. **Specific inhibitors** act on a certain enzyme or group of enzymes.

**Irreversible inhibitors** usually bind tightly (through a covalent bond) to the enzyme and inactivate it forever. Some poisons and medicines act as irreversible inhibitors, e. g. aspirin (for cyclooxygenase), KCN (for cytochrome oxidase), penicillin (inhibit biosynthesis of peptidoglycans in bacterias' cell wall). **Reversible inhibitors** can be eliminated.

According to the mechanism of action inhibitors are divided into competitive and noncompetitive.

**Competitive inhibitors** 1) are similar in structure to substrate; 2) bind to the active site of an enzyme; 3) such inhibition may be reversed by increasing the substrate concentration. Thus, competitive inhibitors compete with the substrate for the active site of an enzyme. Vmax remains the same, but $K_M$ is increased.

The figure shows the structure of two molecules: succinate and malonate. Succinate is a substrate, and **malonate** is a competitive inhibitor for succinate dehydrogenase:




**Sulfanylamides** (antibacterial drugs) are similar to p-aminobenzoic acid (PABA), component of vitamin $B_9$ (folic acid). Since PABA is an essential factor for bacteria, their growth and reproduction becomes impossible in the presence of sulfanylamides — competitive inhibitors of folic acid biosynthesis.

**Noncompetitive inhibitors** 1) have no similarity to substrate; 2) bind to the enzyme at a site different from the active site; 3) and increasing [S] doesn't result in the enzyme reactivation.

**Allosteric regulation** can be considered as a kind of noncompetitive regulation, because allosteric enzymes bind effectors at sites other than the active site. An allosteric enzyme has two or more subunits (quaternary level of protein structure organization, figure 2.4). Subunits are designated as catalytic (carrying an active site for substrate binding) and regulatory (carrying an allosteric site for effectors). Subunits exhibit cooperative effect: binding of a regulatory molecule at the allosteric site has an impact on the conformation of both regulatory and catalytic subunits, and thus the enzyme activity changes. Allosteric effectors may have negative or positive effect (inhibitors or activators).

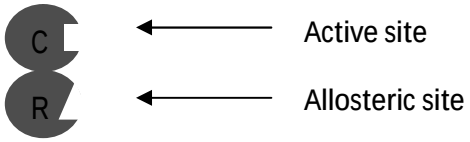
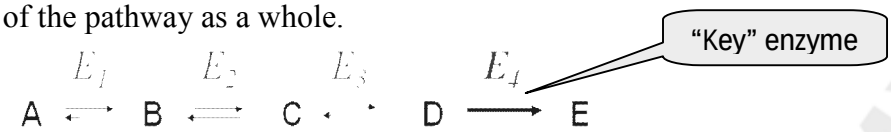



*Fig. 2.4.* Allosteric enzyme (schematic structure)

**Allosteric activators** shift the enzyme toward the high-affinity form, which binds substrate easily. **Allosteric inhibitors** cause a shift toward the low-affinity form, which binds substrate less readily.

**"Key" enzymes** of metabolic pathways are always allosteric. They catalyze the slowest (rate-limiting), often irreversible, reaction. Activation (inhibition) of "key" enzyme results in activation (inhibition) of the pathway as a whole.




The end products of metabolic pathways usually serve as allosteric inhibitors of key enzymes (in the example above — product E for enzyme *E4*). Such type of regulation is called **feedback inhibition**. Substrates A, B, C, D may serve as allosteric activators for enzyme *E4*. Such type of allosteric regulation is called **activation by a precursor**.

**Regulation of enzyme activity by covalent modification**

Regulation of enzyme activity may be performed by the covalent addition (or removal) of some groups. As a result, conformational reorganization of the enzyme occurs that increases or decreases its activity. The most common forms of covalent modification are partial proteolysis and phosphorylation-dephosphorylation.

**Partial (selective) proteolysis** is the way of irreversible activation of proteolytic enzymes (e. g. pepsin, trypsin, thrombin). Such enzymes usually are produced in the form of inactive zymogens (pepsinogen, trypsinogen, prothrombin). They become active only after being cleaved at a specific site in their polypeptide chain.

**Phosphorylation — dephosphorylation** is the way of reversible regulation of enzyme activity. Many enzymes may be activated or inhibited by phosphorylation. Phosphate groups are transfered from ATP to OH-groups of serine, threonine or tyrosine residues under the action of protein kinases. Protein phosphatases remove the phosphate groups, thus altering the activities of enzymes.

This way is under a hormonal control. Biological action of glucagon, epinephrine and some other hormones is mediated by activation of proteinkinases and subsequent phosphorylation of enzymes, whereas insulin causes activation of phosphatases. So, glucagon and epinephrine exhibit similar effects, which are opposite to those of insulin.

Other means of enzyme regulation may include addition of a protein to the active enzyme, covalent binding of substrate analogues to the active site of an enzyme, joining of enzymes into multi-enzyme complexes, compartmentation.

## MULTIPLE FORMS OF ENZYMES

There are two groups of multiple enzyme forms: true multiple forms and isoenzymes.

**True multiple forms** of enzymes are produced by covalent modification (see above).

**Isoenzymes** (or isozymes) are enzymes that catalyze the same reaction but differ in structure and properties. Tissues and cellular compartments contain characteristic isoenzymes, which are adapted to working under different surrounding conditions. For example, the enzymes of glycolysis differ in structure in the liver and muscles (organ isoenzymes); there are different forms of malate dehydrogenase in cytosol and mitochondria (cellular isoenzymes).

**Hybrid isoenzymes** are formed by assembling from different subunits. **Lactate dehydrogenase** consists of 4 subunits, each subunit may be either of 2 types — H (heart) and M (muscle). Five isoenzymes exist: $LDH_1$ (HHHH), $LDH_2$ (HHHM), $LDH_3$ (HHMM), $LDH_4$ (HMMM), $LDH_5$ (MMMM). **Creatine kinase** (creatine phosphokinase, CPK) consists of 2 subunits of 2 types — M (muscle) and B (brain). Three isoenzymes exist: MM, BB, MB.

$LDH_1$, $LDH_2$ and the MB fraction of CPK are most prevalent in heart muscle.

**Mutant isoenzymes** are formed as a result of gene mutation. In this way different isoenzymes of **glucose-6-phosphate dehydrogenase** in human population originated. Carriers of mutant enzyme form are resistant to malaria.

## MEDICAL ASPECTS OF ENZYMOLOGY

**Enzymes in pathology**. Genetic abnormalities resulting in enzyme deficiency (or defect) are causative agents of thousands of diseases, many of which are rare. For example, incidence of **phenylketonuria** (PKU) is about 1 in 10 000 births. In PKU the enzyme phenylalanine hydroxylase, converting phenylalanine to tyrosine, is defective. Phenylalanine and its' toxic metabolites (phenylpyruvate, phenyllactate) accumulate, and severe mental retardation develops. **Lactase deficiency** is more common. Lactose can't be digested, accumulates in the gut and is consumed by bacteria. Lactose intolerance manifests in bloating, abdominal cramps and watery diarrhea.

**Enzymes in diagnosis**. Activities of those enzymes, which are produced and working inside of cells/tissues, are low in the blood under normal conditions. Elevated serum enzyme level results from tissue damage (necrosis or inflammation), or tumor. A short list of tissue specific enzymes and their diagnostic use is given in the table 2.1.

*Table 2.1*

**Serum enzymes and their diagnostic value**

| Serum enzyme | Diagnostic use |
|---|---|
| *Alanine aminotransferase (ALT)*<br>*g-Glutamyl transferase (GGT)* | Hepatitis |
| *Aspartate aminotransferase (AST)*<br>*LDH (isozymes 1–2)*<br>*CPK (isozyme MB)* | Myocardial infarction |
| *Amylase*<br>*Lipase* | Pancreatitis |
| *Phosphatase, acid* | Metastatic carcinoma of the prostate |
| *Phosphatase, alkaline* | Bone disorders, obstructive liver disease |

**Enzymes in therapy.** There are some limitations for usage of enzymes as medicinal preparations. The most important of them are: rapid inactivation and/or degradation of enzymes; difficulties in accurate delivery of the enzyme to the site of its action (especially through the cell membrane); potential allergenic capacity. Nevertheless, certain enzymes have useful effects that may be used in therapy:

– Substitutive therapy — administration of digestive enzymes for compensation of their deficiency *(Mezym forte, Panzinorm forte).*

– Mucolytic effect of *trypsin, chymotrypsin* is used in treatment of bronchitis and pneumonia.

– Necrolytic effect of *proteases and nucleases* is used in treatment of wounds, abscesses, ulcers, caries, pulpitis, periodontitis, etc.

– *Hyaluronidasum, collagenase* help in tissue remodeling and struggle against joints contracture, burn and surgical scarring.

– *Streptokinase* is used in treatment of thromboses.

**Enzyme inhibitors** are widely used **in therapy**. Several examples are given in table 2.2.

*Table 2.2*

**Therapeutic application of enzyme inhibitors**

| Inhibitor | Target enzyme | Effect |
|---|---|---|
| *Contrical, Gordox* | Proteases | Prevent "autodigestion" of pancreas in pacreatitis, treatment of hemostasis disorders |
| *Enalapril, Captopril* | Angiotensin converting enzyme (ACE) | Antihypertensive |
| *Disulfiram* | Aldehyde dehydrogenase | Treatment of alcohol abuse |
| *Pyrazidol* | Monoamine oxidase (MAO) | Antidepressant |
| *Allopurinol* | Xanthine oxidase | Treatment of gout |
| *Aspirin* | Cyclooxygenase | Anti-inflammatory, anti-thrombotic |
| *Lovastatin, Simvastatin* | HMG-CoA reductase | Decrease blood cholesterol level |
| *5-Fluorouracil* | Thymidilate synthase | Antiproliferative |

# CHAPTER 3
# INTRODUCTION TO METABOLISM AND BIOENERGETICS

The word metabolism derives from the Greek word for "change". **Metabolism** represents the sum of the chemical changes that are taking place within cells from the moment of nutrients entrance into the body till formation and excretion of the end metabolic products. Nutrients, the "raw materials" provide the living organisms with energy and building blocks, which can be converted to the chemically complex essential cellular components (proteins, lipids, nucleic acids), specialized signal molecules or other products.

Metabolism consists of literally hundreds of enzymatic reactions organized into discrete pathways. These pathways are spatially arranged in the cells and proceed in a stepwise fashion, transforming substrates into end products through many specific chemical intermediates. The common intermediates produced in different pathways ensure a wide variety of interconnections between them, and if the metabolic processes do not represent chaos, it is only due to the existence of fine regulatory mechanisms, which allow turning ON and OFF specific pathways to meet the needs of a cell at the moment.

Metabolism consists largely of two contrasting processes: **catabolism** and **anabolism** (figure 3.1). Catabolic pathways are characteristically energy yielding, whereas anabolic pathways are energy requiring. Catabolism involves the oxidative degradation of complex nutrient molecules (carbohydrates, lipids, and proteins) obtained either from the environment or from cellular reserves. The breakdown of these molecules by catabolism leads to the formation of simpler molecules such as lactic acid, carbon dioxide, water, urea, or ammonia. Catabolic reactions are usually exergonic, and often the chemical energy released is captured in the form of ATP.

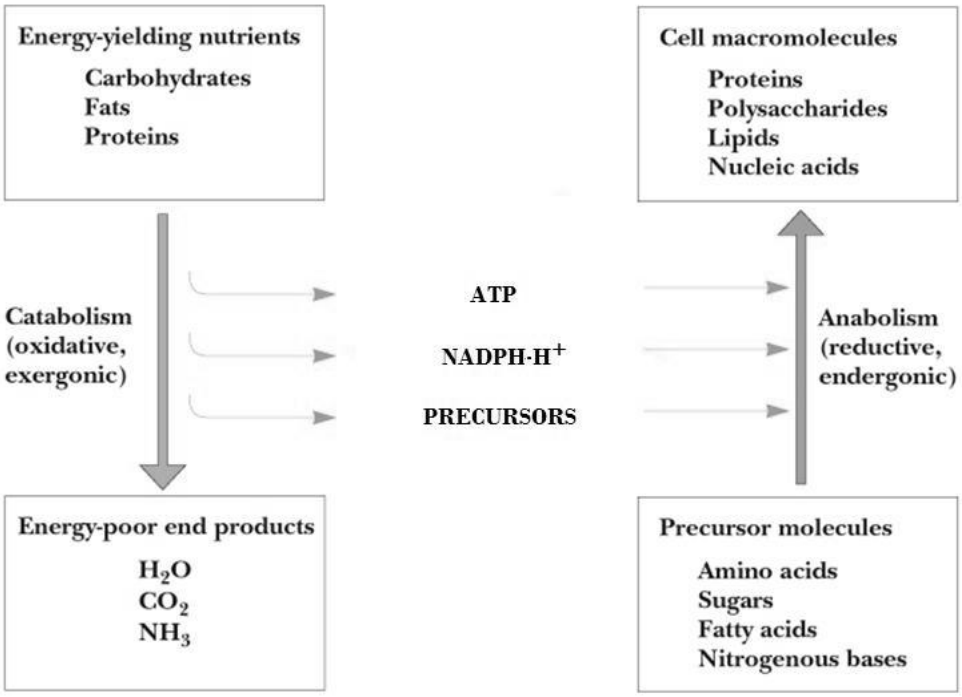



*Fig. 3.1.* General characteristics of catabolism and anabolism

Because catabolism is oxidative for the most part, part of the chemical energy may be conserved as energy-rich electrons transferred to the coenzymes $NAD^+$ and $NADP^+$. These two reduced coenzymes have very different metabolic roles: oxidation of NADH back to $NAD^+$ by means of respiratory chain serves to generate ATP; in contrast, NADPH is the source of the reducing power needed to drive reductive biosynthetic reactions.

Anabolism is a synthetic process in which the varied and complex biomolecules (proteins, nucleic acids, polysaccharides, and lipids) are assembled from simpler precursors. Such

biosynthesis involves the formation of new covalent bonds, and an input of chemical energy is necessary to drive such endergonic processes. The ATP generated by catabolism provides this energy. Despite their divergent roles, anabolism and catabolism are interrelated in that the products of one provide the substrates of the other. Many metabolic intermediates are shared between the two processes, and the precursors needed by anabolic pathways are found among the products of catabolism.

The whole energy that is released during catabolism of the principal energy-giving compounds (carbohydrates, lipids, and proteins) cannot be stored as ATP, the part will be gone with heat. The three stages of aerobic catabolism (figure 3.2) differ in possibilities to capture energy in the form of ATP. In **stage I,** the nutrient macromolecules are broken down into their respective building blocks (amino acids, glucose, glycerol and fatty acids).This breakdown occurs in the digestive tract, the energy is released as heat.

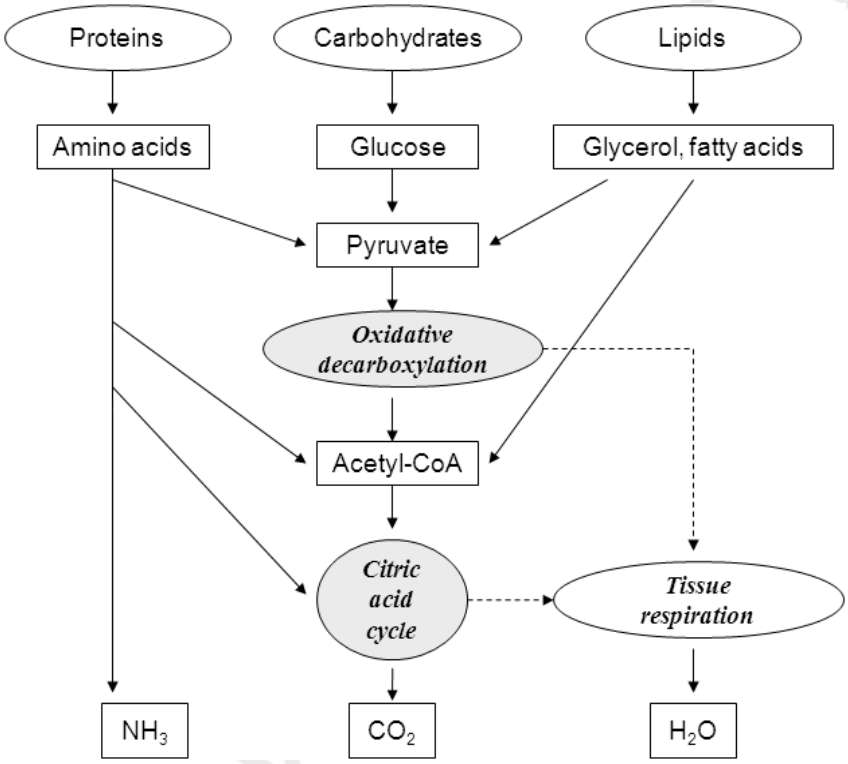



*Fig. 3.2.* The general diagram of catabolism

In **Stage II,** the building blocks generated in stage I are further degraded to produce relatively small common metabolic intermediates. The major of them are **pyruvate** (the three-carbon α-keto acid) and **acetyl-CoA** (the two-carbon residue). The stage II occurs inside of cells (in cytoplasm and mitochondria), the energy is partially released as heat, partially stored in the form of reduced coenzymes ($NADH+H^+$, $NADPH+H^+$), and partially used for ATP synthesis.

The combustion of the acetyl groups of acetyl-CoA in mitochondria by the citric acid cycle represents **stage III** of catabolism. The citric acid cycle is closely related to tissue respiration and oxidative phosphorylation. The end products of these pathways, $CO_2$ and $H_2O$, are the ultimate waste products of aerobic catabolism. The oxidation of acetyl-CoA during stage III generates most of the ATP produced by the cell.

### ATP AND ADENYLATE SYSTEM

**ATP** (**adenosine triphosphate**) plays a central role in energy exchange of the cell. Structurally ATP is a purine nucleotide consisting of the nitrous base **adenine**, the pentose sugar **ribose**, and **three phosphate groups** (figure 3.3). The important feature is that there are two "high-energy" phosphoanhydride bonds in ATP, each produce approximately 31 kJ/mol (7,3 kcal/mol) of energy on hydrolysis.

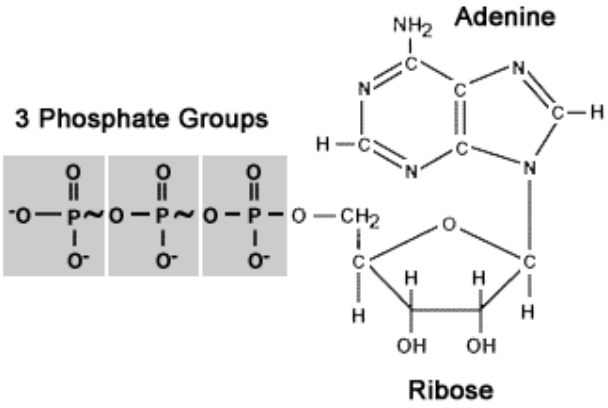



*Fig. 3.3.* The structure of ATP

ATP may be hydrolyzed to **ADP** and inorganic phosphate (**$P_i$**), or to **AMP** and pyrophosphate (**$PP_i$**). The free energy released on ATP hydrolysis is used in virtually all processes requiring energy input in the cell (e. g., to drive biosynthetic reactions, to activate molecules, for muscle contraction, etc.). ATP can be regenerated by the reaction of ADP phosphorylation. AMP cannot be directly converted to ATP, but only after conversion to ADP through *adenylate kinase* reaction:

$$ATP + AMP \leftrightarrow 2\ ADP$$

So, ATP, ADP, AMP may be interconverted through the, such called **adenylate system**, which includes ATP, ADP, AMP, phosphate, pyrophosphate, and cAMP.

**Synthesis of ATP** is referred to as **phosphorylation,** since occurs according to the equation:

$$ADP + P_i + Energy = ATP + H_2O$$

There are three mechanisms of ATP production:

1. **Photosynthetic phosphorylation** — ATP synthesis due to the sunlight energy.
2. **Substrate-level phosphorylation** — ATP synthesis due to the energy released on breakdown of the other high-energy compound (creatine phosphate, etc.).
3. **Oxidative phosphorylation** — ATP synthesis due to the energy released on substrate oxidation and subsequent electron transport via respiratory chain.

### THE CENTRAL METABOLIC PATHWAYS

As follows from the diagram in the fig. 3.2, **oxidative decarboxylation of pyruvate** and **citric acid cycle** can be regarded as **central metabolic pathways**, since these pathways are common for all metabolic fuels.

### OXIDATIVE DECARBOXYLATION OF PYRUVATE

In the pathway, pyruvate is converted to acetyl-CoA by the mitochondrial multi-enzyme system, known as ***pyruvate dehydrogenase complex*** (often called simply ***pyruvate dehydrogenase)***. This complex consists of three enzymes, designated $E_1$, $E_2$, and $E_3$:

$E_1$ — *pyruvate decarboxylase*, requires the active form of vitamin $B_1$, thiamine pyrophosphate (TPP), as coenzyme.

$E_2$ — *dihydrolipoyl transacetylase*, requires lipoic acid and the active form of pantothenic acid, CoA-SH, as coenzymes.

$E_3$ — *dihydrolipoyl dehydrogenase*, requires active forms of vitamins $B_2$ (FAD) and PP ($NAD^+$) as coenzymes.

The enzymes act in turn and catalyze the following reactions (figure 3.4). Initially, pyruvate is decarboxylated by *TPP-$E_1$,* and hydroxyethyl derivative remains bound to thiamine pyrophosphate (**hydroxyethyl**-*TPP-$E_1$*). Then, two-carbon residue (acetyl) is transferred from thiamine pyrophosphate to the oxidized *lipoyl-$E_2$*, and **acetyl**-*lipoyl-$E_2$* is formed. Acetyl-*lipoyl-$E_2$* reacts

with coenzyme A to form **acetyl-CoA** and reduced *lipoyl-$E_2$* (**dihydrolipoyl-$E_2$**). The process is completed when the reduced *lipoyl-$E_2$* is reoxidized by *FAD-$E_3$*. Finally the hydrogens are transferred from *$FADH_2$-$E_3$* to $NAD^+$, and $NADH+H^+$ is formed.

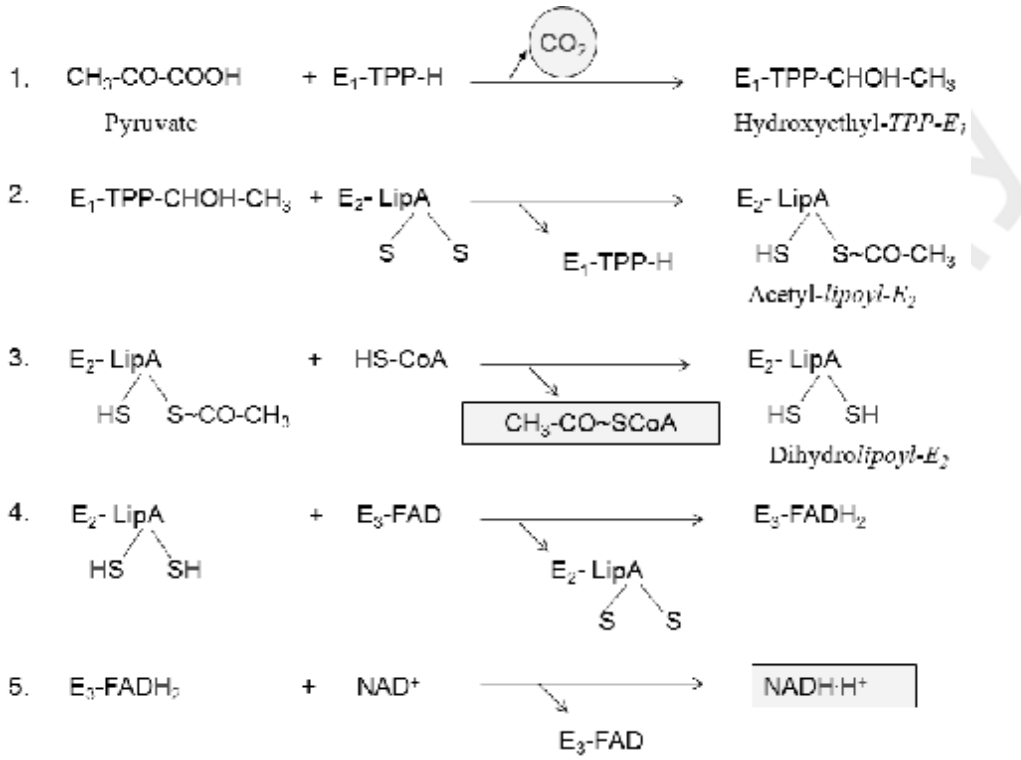
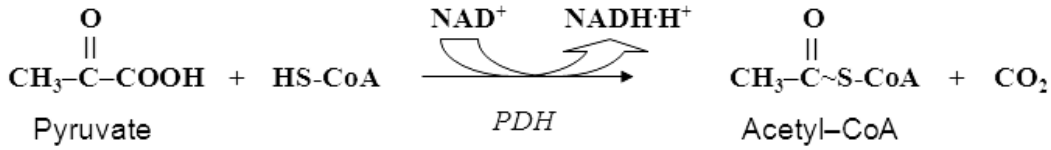



*Fig. 3.4.* The reactions catalyzed by pyruvate dehydrogenase complex

The following reaction summarizes the process:




Under physiological conditions the reaction is irreversible. The analogous multi-enzyme complex performs oxidative decarboxylation of α-ketoglutarate to produce succinyl-CoA in the citric acid cycle.

**Regulation of the process**

The rate-limiting step of the process is the reaction catalyzed by ***pyruvate decarboxylase***. Two mechanisms are participating in regulation: allosteric and covalent modification (phosphorylation and dephosporylation). The complex is **active when dephosphorylated** (in response to **insulin**), and **inactive when phosphorylated** (in response to **glucagon**). Increased levels of **acetyl-CoA and $NADH+H^+$** cause inactivation of the complex (feedback inhibition); **pyruvate, free CoA-SH, $NAD^+$** and **$Ca^{2+}$** (in muscles) cause its activation.

## TRICARBOXYLIC ACID CYCLE

Acetyl-CoA, produced from pyruvate or other metabolic sources, is finally oxidized to $CO_2$ in the **tricarboxylic acid (TCA) cycle**, also known as **citric acid cycle**, or the **Krebs cycle** (in honor of Hans Krebs, a Nobel Prize-winner, who postulated the essential features of this cyclic pathway). The enzymes of TCA cycle are located in mitochondria.

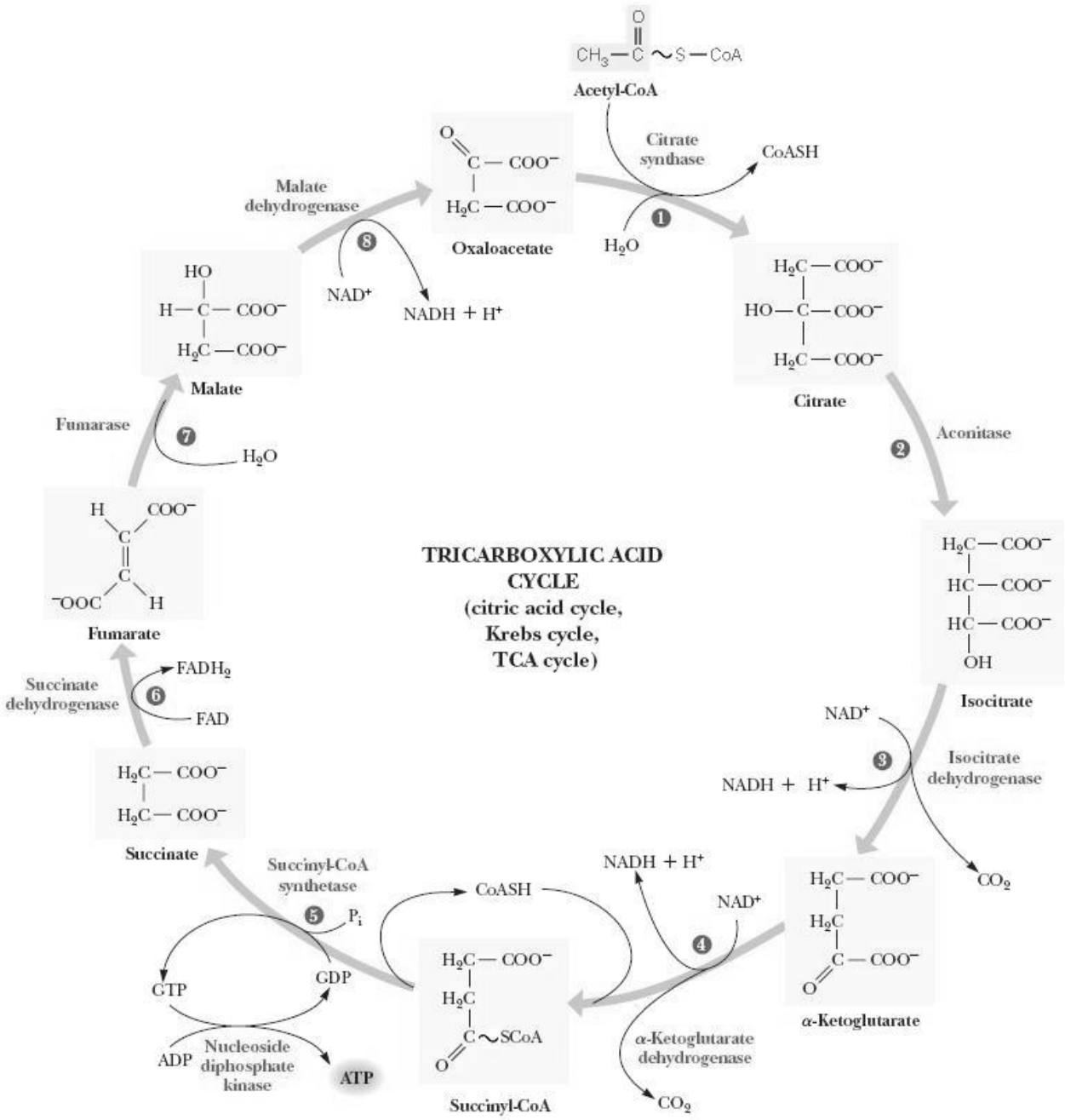



*Fig. 3.5.* The tricarboxylic acid cycle

The individual reactions of the TCA cycle are presented in figure 3.5.

1. The TCA cycle is generally considered to "begin" with addition of acetyl-CoA to oxaloacetate to form citrate. This initial step is catalyzed by *citrate synthase*.

2. Citrate is converted to isocitrate in a reversible reaction catalyzed by *aconitase*. Conversion of citrate to isocitrate occurs in two steps through the formation of intermediate product *cis*-aconitate, which determines the enzyme name.

3. *Isocitrate dehydrogenase* carries out the first oxidative decarboxylation in the cycle. Isocitrate is converted to α-ketoglutarate with concomitant release of $CO_2$ and reduction of $NAD^+$ to $NADH+H^+$.

4. Conversion of α-ketoglutarate to succinyl-CoA represents the second oxidative decarboxylation in the cycle. The reaction is catalyzed by *α-ketoglutarate dehydrogenase complex*, the multi-enzyme system analogous to *pyruvate dehydrogenase* and requiring the same vitamins as cofactors. In this reaction the second molecule of $CO_2$ and the second $NADH+H^+$ are produced.

5. The high-energy thiol ester linkage of succinyl-CoA is broken down, CoA-SH is liberated, and succinate is formed. The energy released is used for phosphorylation of GDP to GTP (substrate-level phosphorylation). *Succinyl-CoA synthetase* catalyzes this easily reversible reaction.

6. Succinate is oxidized to fumarate by *succinate dehydrogenase*, FAD is reduced to $FADH_2$.

7. Water is added across the double bond by *fumarase* (or *fumarate hydratase*), and fumarate is converted to malate.

8. The final reaction of the cycle is catalyzed by *malate dehydrogenase* and results in regeneration of oxaloacetate and reduction of $NAD^+$ to $NADH+H^+$.

Thus, two carbons from acetyl-CoA enter the citric acid cycle in each turn, and two carbons are lost as $CO_2$; 3 $NADH{\cdot}H^+$, $FADH_2$, and GTP are also produced.

**Functions of the TCA cycle**

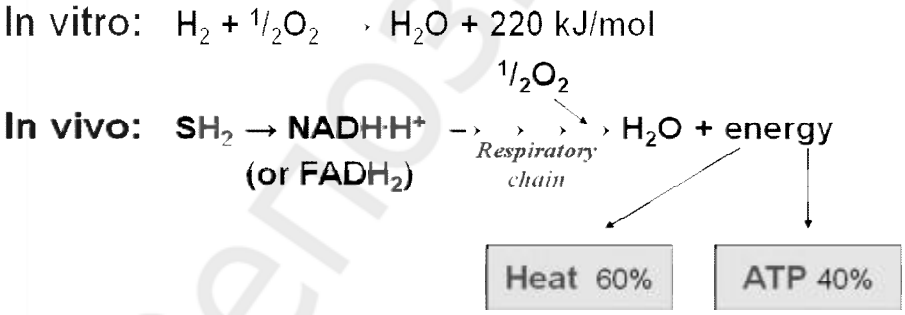
– Integration of metabolism
– Catabolic function (final degradation of fuel molecules to $CO_2$)
– Anabolic function (TCA cycle intermediates serve as precursors for biosynthetic processes)
– Energy production (synthesis of GTP by substrate-level phosphorylation)
– Hydrogen-donor (3 $NADH{\cdot}H^+$ and 1 $FADH_2$ produced in the reactions of TCA cycle donate hydrogens to the respiratory chain).

**Regulation of the TCA cycle**

The TCA cycle is regulated by the cell's need for energy shown by the **[ATP]/[ADP]** and **[NADH]/[$NAD^+$]** ratios. The most likely sites for regulation are irreversible reactions catalyzed by ***citrate synthase***, ***isocitrate dehydrogenase***, and ***α-ketoglutarate dehydrogenase***. In addition, citrate synthase is allosterically inhibited by fatty acids.

## TISSUE RESPIRATION AND OXIDATIVE PHOSPHORYLATION

**The energy obtained from degradation of food materials** (carbohydrates, lipids, and proteins) is partly lost as **heat**; a small part of this energy is stored in **ATP as a result of substrate-level phosphorylation**; and mainly it is funneled into formation of **reduced coenzymes** ($NADH+H^+$ and $FADH_2$). Reoxidation of these coenzymes by means of respiratory chain provides the energy for ATP synthesis through oxidative phosphorylation. The process involves the removal of both protons and electrons from the coenzymes, and their step-by-step transfer to molecular oxygen, $O_2$, which is the terminal acceptor of electrons in the chain. A series of redox reactions performed by the electron-transport chain is accompanied by the liberation of free energy, since the overall chemical reaction between hydrogen and oxygen is highly exergonic:




Thus, ATP synthesis in mitochondria is coupled with the reaction of water formation. Organic substrates ($SH_2$) serve as initial hydrogen donors (e. g., TCA cycle intermediates: malate, isocitrate, alpha-ketoglutarate, succinate).

The main definitions:

• **Tissue respiration** — electron transport from substrates to oxygen via respiratory chain resulting in (A) water formation; (B) generation of proton gradient across the inner mitochondrial membrane (IMM) (figure 3.6).

• **Respiratory chain** (**electron-transport chain**, ETC) — a sequence of electron carriers located in the IMM that perform transfer of $e^-$ to molecular $O_2$.

• **Oxidative phosphorylation** — ATP synthesis due to the energy released on substrate oxidation and subsequent electron transport via respiratory chain or ATP synthesis coupled to the $e^-$ transfer through the respiratory chain.

So, the processes of tissue respiration and oxidative phosphorylation are **membrane associated**.

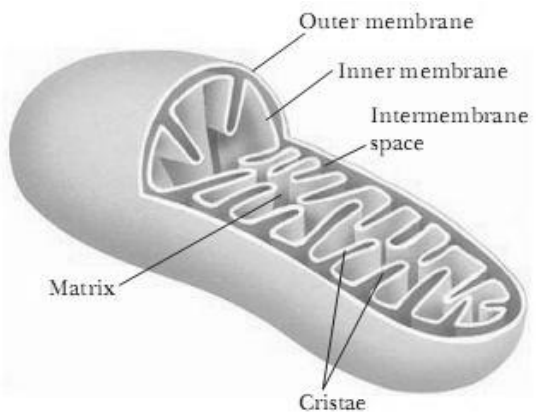



*Fig. 3.6.* The structure of the mitochondrion

**How is the respiratory chain organized?**

Electrons are passed through a highly organized chain of proteins and coenzymes. Each component of the chain can exist in (at least) two oxidation states, and each component is successively reduced and reoxidized as electrons move through the chain from NADH (or $FADH_2$) to $O_2$. The constituents of ETC involve:

– Flavoproteins — contain FMN or FAD as prosthetic groups, transfer $e^-$ and $H^+$.

– Iron-sulfur proteins (non-heme iron proteins, FeS) — transfer $e^-$ only.

– Cytochromes *b, $c_1$, c, a, $a_3$* (hemoproteins) — contain either iron or both iron and copper atoms, transfer $e^-$ only:

$$Fe^{3+} + \bar{e} \leftrightarrow Fe^{2+}$$
$$Cu^{2+} + \bar{e} \leftrightarrow Cu^{+}$$

– Ubiquinone (Coenzyme Q) — lipophilic molecule, freely diffuses within the membrane, transfers $e^-$ and $H^+$.

The position of the carrier in the ETC depends on its reduction (or redox) potential. Reduction potential charachterizes the ability of molecules to donate (and accept) $e^-$. The substances with negative redox potentials are reducers; those with positive reduction potential — oxidizers. Molecules along the electron-transport chain have reduction potentials between the values for the $NAD^+$/NADH couple (-0,32 V) and the oxygen/$H_2O$ couple (+0,81 V), so electrons move down the energy scale toward progressively more positive reduction potentials (figure 3.7).

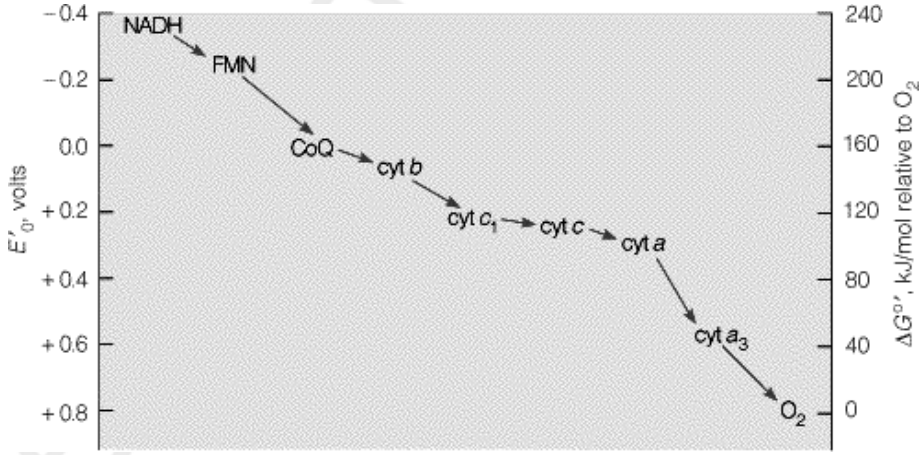



*Fig. 3.7.* Standard reduction potentials of the major electron carriers

In other words, *the every following carrier in the chain is the more potent oxidizer than the preceding one.* This is the factor that determines the direction of electron transfer along the chain.

Two components of ETC (CoQ and cytochrome *c*) are mobile. The others are grouped into 4 lipid-protein complexes which are embedded in the inner mitochondrial membrane (figure 3.8):

I. **NADH-ubiquinone oxidoreductase.** Protons and electrons, taken by this complex from $NADH+H^+$, are passed to CoQ. FMN and FeS are used as intermediate carriers of electrons within complex.

II. **Succinate-ubiquinone oxidoreductase**. This complex takes protons and electrons from substrate (succinate) and transfers them to CoQ. Analogous complexes, containing FAD and FeS as prosthetic groups, can be found in the IMM for some other substrates (acyl-CoA, glycerol 3-phosphate).

III. **Ubiquinol-cytochrome *c* oxidoreductase**. This complex transfers electrons from CoQ through FeS, cytochromes *b* and $c_1$ to cytochrom *c* in intermembrane space. Protons are released into the intermembrane space.

IV. **Cytochrome *c* oxidase**. Electrons are passed through this complex to the terminal acceptor — molecular oxygen. $O_2$ undergoes full reduction (accepts 4 e-, accumulating by the complex, and 4 $H^+$ from mitochondrial matrix), thus endogenous water is produced.

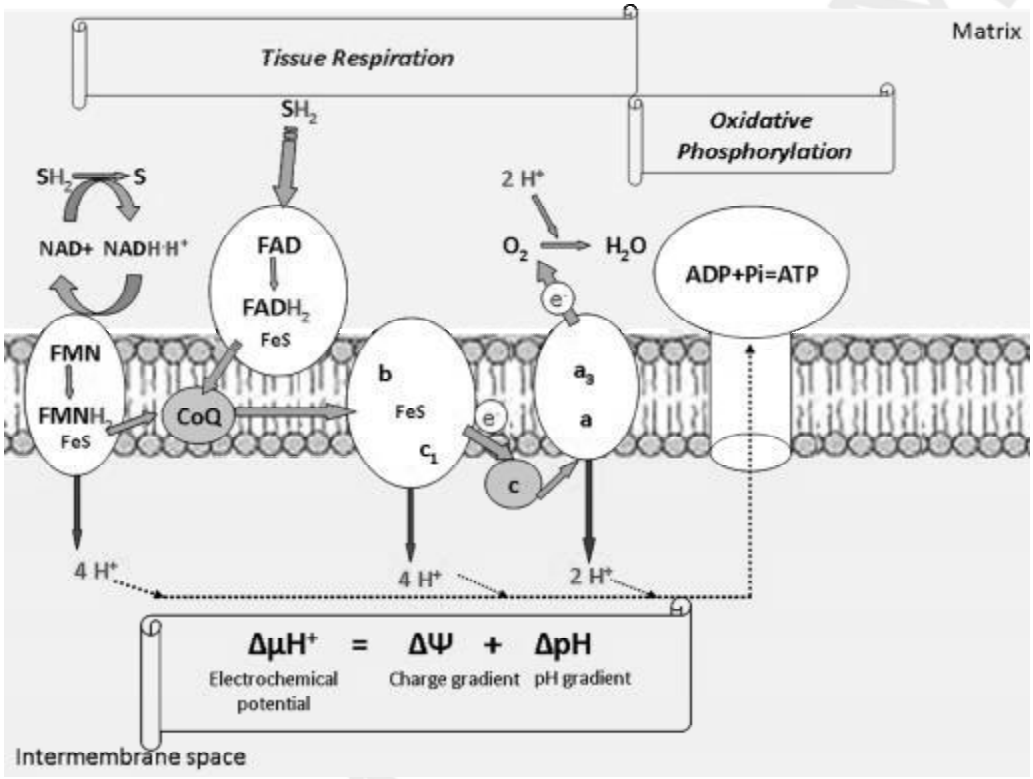



*Fig. 3.8.* The electron transport chain and oxidative phosphorylation

Electron transport is followed by the release of free energy. If the difference in redox potentials is less than 0,2 V, the energy is dissipated as heat. There are three sites in the chain (I, III, IV complexes), where the drop in redox potentials is more than 0,2 V. This energy is used for active transporting of protons from the matrix across the IMM to the intermembrane space. As a result, electrochemical potential (**$\Delta\mu H^+$**) is generated on the membrane. The presence of such potential on the IMM is an essential requirement for ATP production.

A large multisubunit transmembrane complex, which is responsible for ATP synthesis, is called **$H^+$-ATP synthase** (complex V of the IMM, figure 3.9). It consists of two parts — **$F_0$ and $F_1$**. **$F_0$** (a "base" or a "stalk") spans the membrane and forms a **proton channel**; **$F_1$** (a "knob") protrudes into the matrix and contains the active sites for ATP synthesis. The flow of protons through $F_0$ causes it to rotate, driving the conformational changes in $F_1$ and promoting formation of ATP from ADP and $P_i$ (**oxidative phosphorylation**).

The mechanism of coupling between tissue respiration and oxidative phosphorylation was predicted by Peter Mitchell (a Nobel Prize in 1978). The major postulates of Mitchells' **chemiosmotic theory**:

– the inner mitochondrial membrane is impermeable to ions, including $H^+$;

– I, III, IV complexes pump protons out of the matrix into the intermembrane space due to the energy obtained from $e^-$ transport;

– resulting electrochemical potential serves as the energy storage form;

– back-translocation of protons through the proton channel of complex V is a driving force for ATP synthesis.

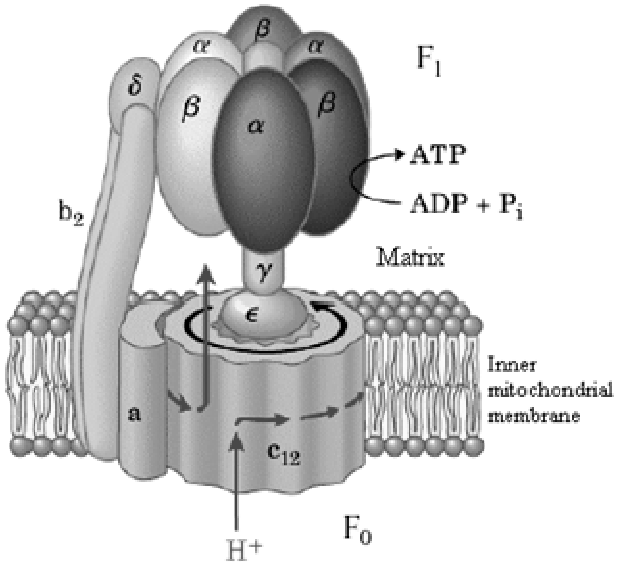



*Fig. 3.9.* The structure of ATP-synthase

So, **complexes I, III, IV** can be referred to as **"coupling sites"**, since they contribute to the creation of electrochemical potential, or "proton motive force". Estimates suggest that for each $NADHH^+$ oxidized, complexes I and III translocate four protons each, and complex IV translocates two.

ATP produced within mitochondria is transferred to the cytosol in exchange for ADP by a specific protein in the IMM — **ATP/ADP translocase (antiport)**. It brings ATP to the place of its main consumption and provides ADP in matrix for further phosphorylation. Phosphate is delivered to mitochondria through another transport system — **$P_i/H^+$ translocase (symport).**

**Phosphorylation Ratio (P/O)** — the number of ATP molecules synthesized per 1 atom of oxygen (½ $O_2$) consumed.

Synthesis of 1 ATP requires 4 $H^+$:

- 3 $H^+$ should pass through the proton channel of ATP synthase;
- 1 $H^+$ is used to transfer $P_i$ into mitochondrial matrix ($P_i/H^+$ translocase).

**Oxidation of $NADH{\cdot}H^+$** results in accumulation of 10 $H^+$ in the intermembrane space, enough for 2,5 ATP synthesis (10/4) **à P/O = 2,5.**

**Oxidation of $FADH_2$** results in accumulation of 6 $H^+$ in the intermembrane space, enough for 1,5 ATP synthesis (6/4) **à P/O = 1,5.**

Respiration is controlled by the availability of ADP ([ATP]/[ADP]+[Pi] ratio). $O_2$ consumption increases after addition of ADP to mitochondria. Such regulatory mechanism is known as **respiratory control.**

**Inhibitors of electron transport and oxidative phosphorylation**

The protein complexes of the IMM may be blocked by specific inhibitors. Many of such inhibitors are poisons.

- *Barbiturates, rotenone* block Complex I
- *Malonate* blocks Complex II
- *Antimycin A* blocks Complex III
- *Cyanide, azide, CO, $H_2S$* block Complex IV
- *Oligomycin* blocks $H^+$-channel of Complex V
- *Atractyloside* blocks ATP/ADP translocase

**Uncoupling agents**

The tight coupling between tissue respiration and oxidative phosphorylation occurs due to the formation of electrochemical potential on the IMM. Uncouplers decrease the proton gradient: being lipophilic "ionophores" they allow protons from the intermembrane space to reenter matrix without going through the proton channel of ATP synthase. Uncouplers cause the rate of $O_2$ consumption and the rate of the electron transport to increase, but ATP production does not occur, energy is dissipated as heat.

Natural uncouplers: *lipid peroxidation products, thermogenin* (protein found in brown adipose tissue).

Artificial uncouplers: *dinitrophenol, dicumarol, ether and some other anaesthetics.*

## PATHWAYS OF $O_2$ UTILIZATION BY CELLS

**1. Oxidase pathway**

– Cytochrome *c* oxidase utilizes > 80 % of cell oxygen for tissue respiration and coupled ATP production in mitochondria;

– Other oxidases (e. g., xanthine oxidase, amino acid oxidases).

**2. Oxygenase pathway** or **microsomal oxidation**

Oxygenases incorporate oxygen atoms from $O_2$ into a substrate (without ATP production). The microsomal oxygenase system represents an electron transport chain, which consists of flavoprotein and cytochrom p450. NADPHH+ serves as the initial donor of electrons. As a result of microsomal oxidation **hydroxyl group appears in a substrate** (figure 3.10).

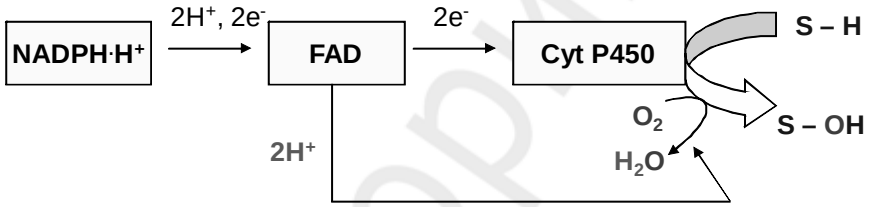



*Fig. 3.10.* The microsomal oxidation (scheme)

Biological role of microsomal oxidation:

- Detoxification of xenobiotics (drugs, dyes, toxic chemicals) — being hydroxylated a toxin becomes more hydrophilic and excretable with urine.
- α- and ω-oxidation of fatty acids.
- Synthesis of unsaturated fatty acids and steroids (including vitamin D).
- Collagen synthesis (hydroxylation of Pro, Lys).

## CLINICAL ASPECTS

**Impaired pyruvate oxidation**

Impaired pyruvate oxidation may occur due to **thiamine deficienc**y, **inherited pyruvate dehydrogenase deficiency**, **arsenic and mercury poisoning** (arsenite and mercuric ions react with –SH groups of lipoic acid and inhibit pyruvate dehydrogenase). Pyruvate will be converted mostly to lactate, and **lactic acidosis** develops. Urine contains increased amounts of pyruvate and lactate.

**Mitochondrial poisons**

Many drugs (barbiturates) and poisons (cyanide, azide) inhibit oxidative phosphorylation with fatal consequences and are commonly used for suicide. Effect of barbiturates depends on dosage, since they block electron passage through Complex I, but oxidation of FAD-dependent substrates is not affected. Cyanide and azide bind tightly to $Fe^{3+}$ of cytochrome $a_3$. Oxygen cannot receive electrons, respiration is totally arrested, energy production becomes impossible, and death occurs rapidly.

**Malignant hyperthermia**

The inhalation anesthetics (halothane, ether, methoxyflurane) may cause uncoupling of oxidative phosphorylation from electron transport in susceptible persons. Heat is generated instead of ATP, and temperature rises markedly. Stimulation of TCA cycle leads to excessive $CO_2$ production and acidosis.

**Cytochrome p450**

Cytochrome p450 is a name for a family of heme-containing proteins that perform hydroxylation reactions, as well as epoxidation, desulfuration, dealkylation, deamination, and dehalogenation reactions. Cytochrome p450 participate in metabolism of different compounds, including drugs, steroids, environmental carcinogens, alcohol. The synthesis of proteins of cytochrome p450 system may be induced by many of these agents. Such induction or competition between substrates for cytochrom p450 may change blood concentration of drugs and individual sensitivity to treatment.

# CHAPTER 4
# CARBOHYDRATE METABOLISM

## STRUCTURE AND FUNCTIONS OF MAJOR CARBOHYDRATES

**Carbohydrates** are aldehyde or ketone derivatives of polyhydroxy alcohols.

*Classification of carbohydrates*

**Monosaccharides** — are the simplest sugars, monomers, containing 3 and more carbons, one of which represents a keto-group (in ketoses) or aldehyde group (in aldoses), and others have attached OH-groups (figure 4.1).

According to the number of carbons they are termed trioses, tetroses, pentoses, hexoses, etc. The major representatives are given in the table 4.1.

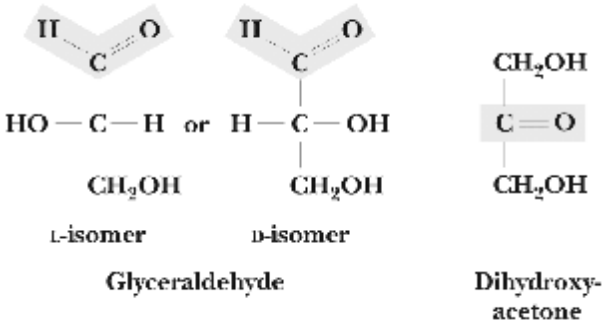



*Fig. 4.1.* Trioses

*Table 4.1*

**Major monosaccharides**

| Carbons | Category Name | Relevant Examples |
|---|---|---|
| 3 | Triose | Glyceraldehyde, Dihydroxyacetone |
| 4 | Tetrose | Erythrose |
| 5 | Pentose | Ribose, Deoxyribose, Ribulose, Xylulose |
| 6 | Hexose | Glucose, Fructose, Galactose |

The aldehyde and ketone moieties of the carbohydrates with five and six carbons spontaneously react with alcohol groups present in the same molecule and form five- or six-membered rings (**furanoses** or **pyranoses**, respectively). The rings can open and re-close.

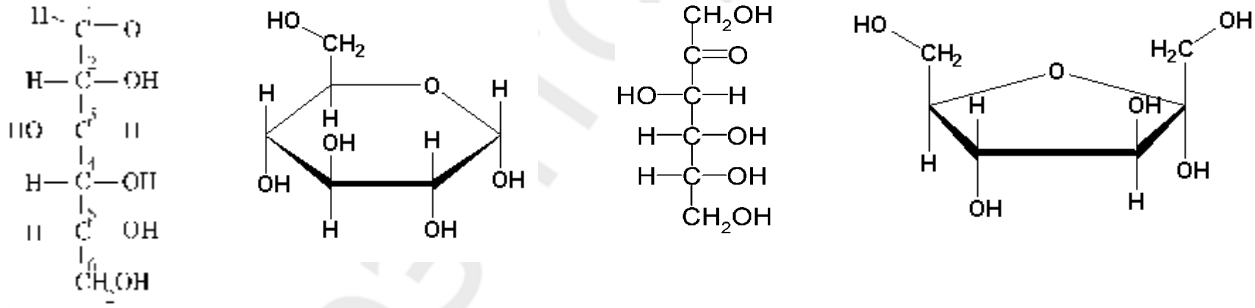



*Fig.4.2.* Open (Fisher) and closed (Haworth) representations of glucose and fructose

Sugars may form covalent **glycosidic bonds** between hydroxyls in neighboring molecules. Thus, oligo- and polysaccharides are produced.

**Oligosaccharides** — short chains of monosaccharides (contain 2–10 monomers). Several physiologically important disaccharides are sucrose, lactose and maltose (figure 4.3).

**Sucrose** is composed of **glucose** linked to **fructose** through α(1 **à** 2) glycosidic bond.

**Maltose** is composed of **two glucose** units joined α(1 **à** 4).

**Lactose** is composed of **glucose** and **galactose** linked through β(1 **à** 4) bond.

**Polysaccharides** — high molecular weight carbohydrate polymers. The monomeric building blocks used to generate polysaccharides can be varied; in all cases, however, the predominant monosaccharide found in polysaccharides is D-glucose. When polysaccharides are composed of a single monosaccharide building block, they are termed **homopolysaccharides**. Polysaccharides composed of more than one type of monosaccharide are termed **heteropolysaccharides.**

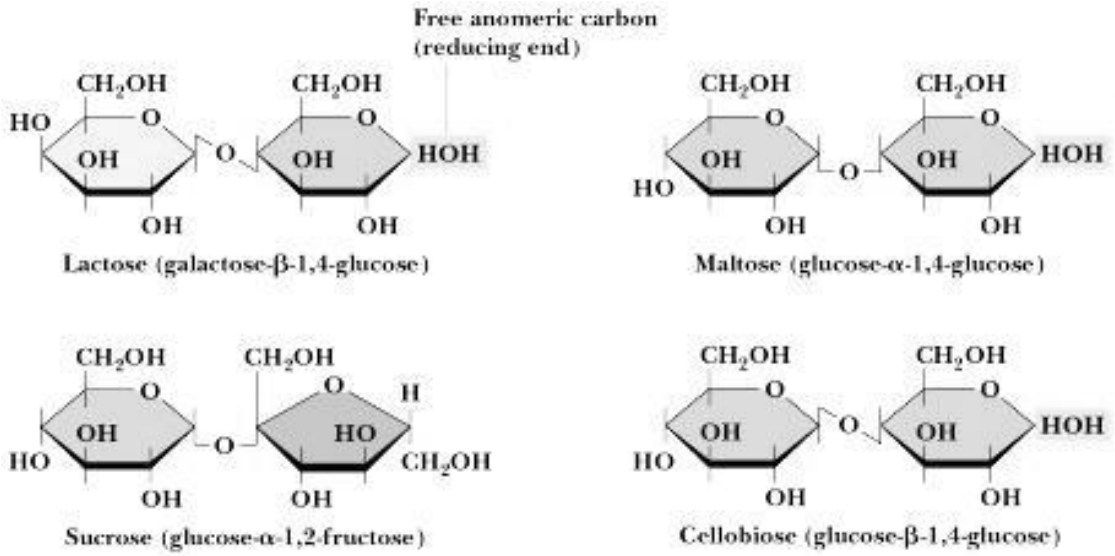



*Fig. 4.3.* Disaccharides

**Glycogen** is the major form of stored carbohydrate in animals. It is a homopolymer of glucose in α(1 à 4) linkage; it is also highly branched, with α(1 à 6) branch linkages occurring every 8–10 residues.

**Starch** is the major form of stored carbohydrate in plant cells. Its structure is identical to glycogen, except for a much lower degree of branching (about every 20–30 residues). Unbranched starch is called **amylose**; branched starch is called **amylopectin**. Long polymers like starch and glycogen have no influence on the osmotic pressure (in contrast to glucose) and may be present in large quantities in cells.

**Cellulose** is the major structural polysaccharide in plants and is the most abundant polymer in the biosphere. It is also a polymer of D-glucose, but the sugar residues are connected by β(1 à 4) linkages.

Another group of polysaccharides of importance is the **glycosaminoglycans**. These are heteropolysaccharides containing uronic acids and either N-acetylgalactosamine or N-acetylglucosamine as their monomeric units. Examples include chondroitin sulfates and keratan sulfates of connective tissue, dermatan sulfates of skin, heparin and hyaluronic acid.

**Biological roles of carbohydrates:**

– energy;
– detoxification;
– storage;
– structure;
– signals and their recognition.

## DIGESTION AND ABSORPTION OF CARBOHYDRATES

Carbohydrates constitute about 55 % of the daily calories. The major dietary carbohydrates are starch (> 60 %), sucrose and lactose. Small amounts of free glucose and fructose are also present in the diet.

Digestion of dietary carbohydrates begins in the mouth. *Salivary α-amylase* cleaves starch by breaking α-1,4 linkages between glucose residues within the chains. Dextrins (linear and branched oligosaccharides) are the major products, small amounts of maltose also are produced. The action of salivary amylase is limited to the area of the mouth and the esophagus; it is virtually inactivated by the strong acid in the stomach. In the stomach no enzymes for carbohydrate digestion are produced. The main carbohydrate digesting enzyme of the small intestine is *pancreatic α-amylase*. This enzyme is secreted by the pancreas and has the same activity as salivary amylase, producing disaccharides and trisaccharides. The latter are converted to monosaccharides by intestinal disaccharidases, located on the brush border of enterocytes. *Disaccharidases* include *maltase*,

*sucrase/isomaltase*, *lactase*, and *trehalase*. The net result is the almost complete conversion of digestible carbohydrate to its constituent monosaccharides. The resultant glucose and other simple carbohydrates are transported across the intestinal wall to the hepatic portal vein and then to liver parenchymal cells and other tissues.

**Indigestible polysaccharides (or fibers)** include cellulose, hemicelluloses, pectin, lignin. Unlike microorganisms, humans have no enzymes capable to break down linkages between monomers in these natural polymers. Nevertheless, indigestible fibers are essential components in human diet, since they favour the growth of bacterial flora in the colon; induce bile excretion; slow the movement of food through the system and ensure prolonged feeling full; control the release of glucose into the bloodstream; attract water, which causes it to "bulk up" within the large intestine and makes defecation easier; fibers have a cleansing effect on the large intestine and promote intestinal motility; bind with toxins, cholesterol, heavy metals and remove them from the body. Excellent sources of dietary fiber include beans, nuts, whole grains such as wheat, fibrous vegetables such as broccoli and dense fruits such as apples.

**Absorption of carbohydrates**

Monosaccharides produced by carbohydrate digestion are mostly absorbed in the upper part of small intestine. Efficiency of monosaccharide absorption decreases in the row "galactose > glucose > fructose".

Carbohydrate absorption requires participation of specific **transport proteins** (GLUT and SGLT1) since sugars can't freely pass through the membranes.

**GLUT** (**GLU**cose **T**ransporter) carries monosaccharides down a concentration gradient (facilitated diffusion).

**SGLT1** carries monosaccharide together with sodium ions. *$Na^+$-$K^+$-ATPase* then pumps $Na^+$ out of the intestinal cell, thus $Na^+$-gradient is maintained and glucose absorption through SGLT1 is continued (secondary active transport).

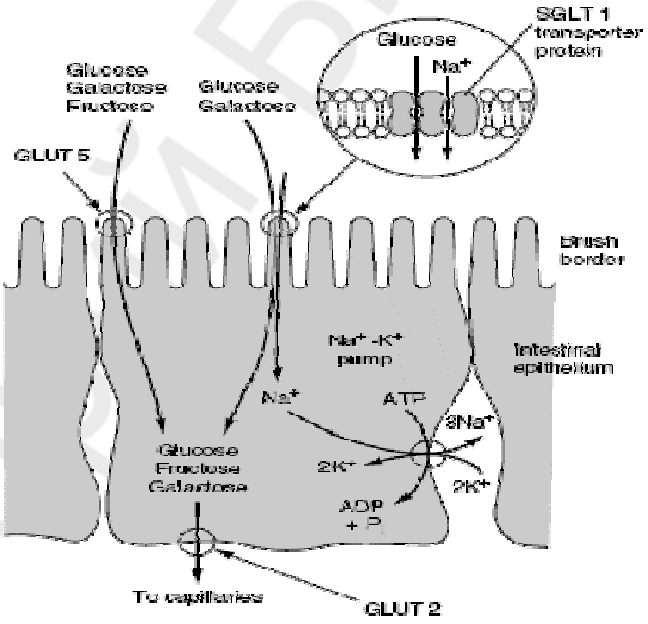



*Fig. 4.4.* Absorption of carbohydrates in intestines

Glucose uptake from the bloodstream by tissue cells is performed by GLUT transporters. The cells of some tissues are freely permeable to glucose (via the certain types of GLUT, shown in the table 4.2), whereas cells of adipose tissue, as well as skeletal and heart muscle, are relatively impermeable since their transporter (GLUT 4) depends on insulin.

*Table 4.2*

**Tissue distribution and functions of different types of GLUT**

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

## METABOLISM OF GLYCOGEN

**Glycogen biosynthesis (glycogenesis)**

Animals synthesize and store glycogen when the blood glucose levels are high. Glycogen synthesis occurs mainly in liver and muscle.

The pathway requires activation of glucose units before their transfer to preexisting glycogen chains (figure 4.5). At first glucose is phosphorylated to **glucose-6-phosphate** by *hexokinase* (in muscle) or *glucokinase* (in liver) using ATP as a source of phosphate. This reaction ensures solution of two tasks — activation of glucose and its retention inside of cell since only free glucose can pass through GLUTs.

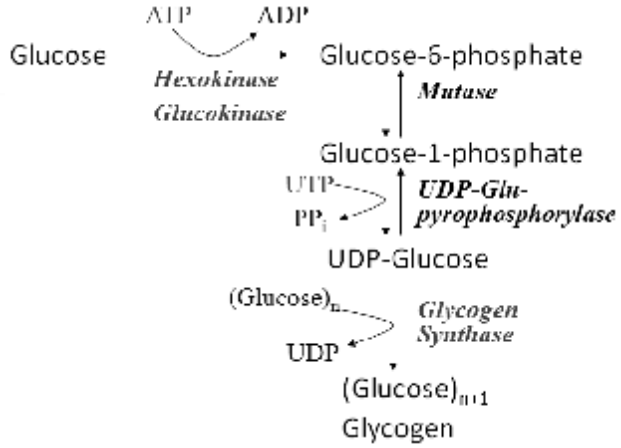



*Fig. 4.5.* Glycogen biosynthesis

Glucose-6-phosphate is isomerized to **glucose-1-phosphate** by *phosphoglucomutase*.

Next, glucose-1-phosphate reacts with uridine triphosphate (UTP). The active nucleotide form of glucose is generated (**UDP-glucose**), and pyrophosphate (PPi) is released. The reaction is catalyzed by *UDP-glucose pyrophosphorylase.*

Glucose residues are transferred from UDP-glucose to the end of a **glycogen primer** by the enzyme *glycogen synthase*. UDP is released and may be reconverted to UTP in the reaction with ATP.

*Glycogen synthase* catalyzes the formation of an α(1 à 4) glycosidic bond in a growing glycogen chain. The branches are formed by the transfer of the chain terminal fragment (6–8 glucose residues) to the 6-position of a glucose residue in the interior of the polymer. These two steps (removal and reattachment of the fragment via α(1 à 6) linkage) are catalyzed by *amylo-(1,4->1,6)-transglycosylase (branching enzyme).*

**Regulation of glycogen synthesis**

***Glycogen synthase*** is the major point of regulation of glycogen biosynthesis. Its' activity depends on covalent modification: phosphorylated form is less active, but can be allosterically stimulated by glucose-6-phosphate; dephosphorylated form is active and doesn't depend on glucose-6-phosphate.

**Glucagon and epinephrine** stimulate phosphorylation of glycogen synthase via the mechanism that involves cAMP (figure 4.6), and **inhibit the pathway** of glycogenesis.

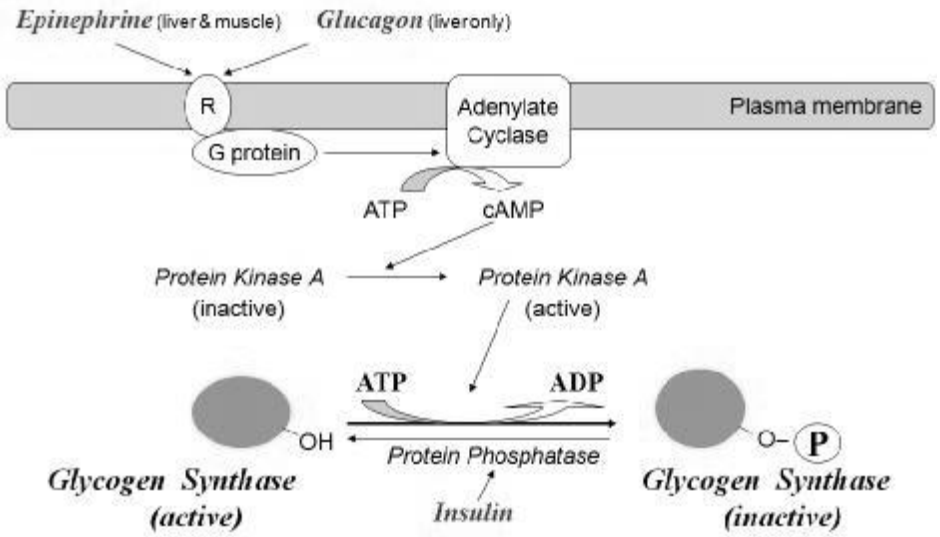



*Fig. 4.6.* Regulation of glycogen synthase activity (adenylate cyclase mechanism)

**Insulin** stimulates dephosphorylation of the enzyme, and **activates glycogen synthesis**.

**Glycogen breakdown (glycogenolysis)**

The glycogenolysis requires two principal enzymes: *glycogen phosphorylase* (figure 4.7) and "*debranching enzyme*" (figure 4.8). Glycogen phosphorylase catalyzes the phosphorolytic cleavage of α(1->4) bonds, generating glucose-1-phosphate in the process.

Glucose-1-phosphate can be converted to glucose-6-phosphate by *phosphoglucomutase*.

**In muscles** *glucose-6-phosphatase*, which removes phosphate and generates free glucose, is lacking. Thus, glycogen degradation ends with glucose-6-phosphate formation. Its further oxidation in muscle cells provides energy for contraction.

**In liver** *glucose-6-phosphatase* is present and produces free glucose which enters the bloodstream. So, liver glycogen is used to maintain blood glucose level between meals and in fasting (not more than 12–18 hours).

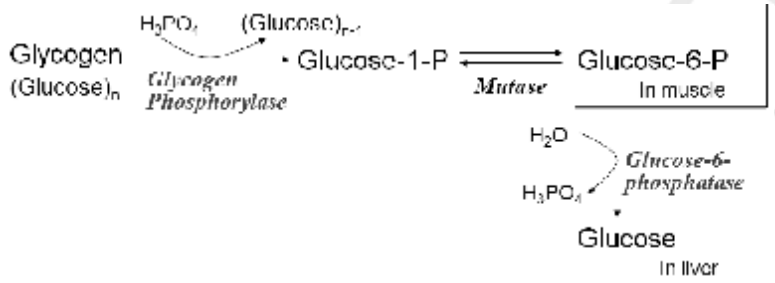



*Fig. 4.7.* Glycogen breakdown

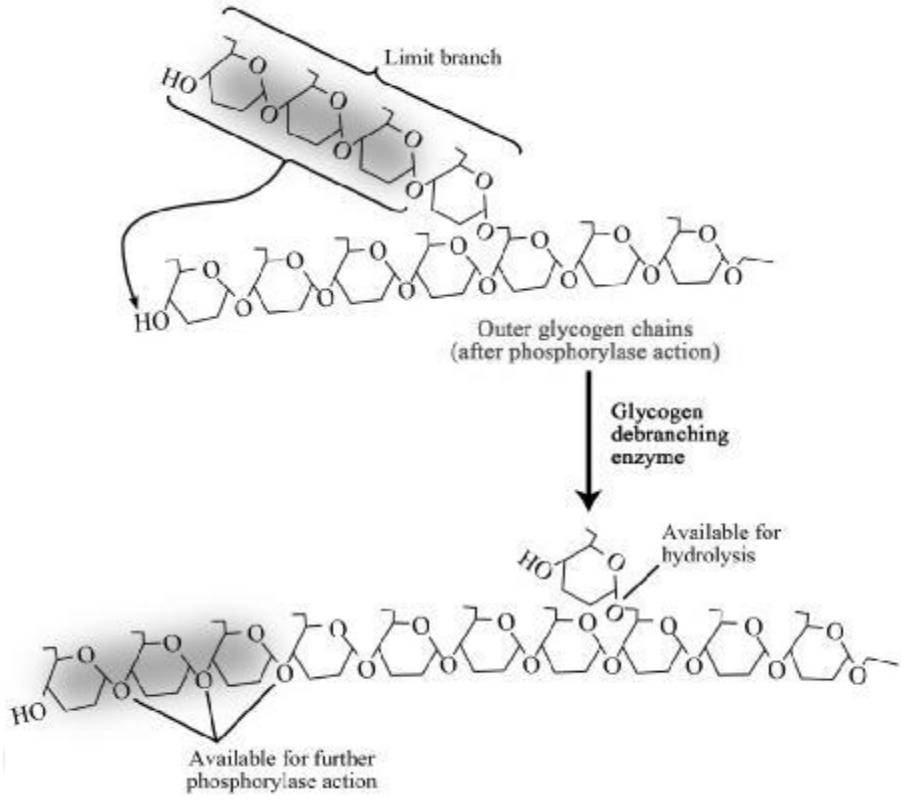



*Fig. 4.8.* The scheme of "debranching" enzyme action

**Regulation of glycogen breakdown**

The key enzyme of the pathway (***glycogen phosphorylase***) **is activated by phosphorylation** (stimulated by glucagon, epinephrine (see above)); and **inhibited by dephosphorylation** (caused by insulin action).

In muscles glycogen phosphorylase also may be **allosterically activated by $Ca^{2+}$ and AMP**, and **inhibited by ATP and glucose-6-phosphate**.

**Lysosomal degradation of glycogen**

Glycogen may be degraded by an *acid glucosidase* (or *γ-amylase*) located in lysosomes. In such case hydrolytic removal of free glucose from glycogen branches takes place.

## GLYCOLYSIS

**Glycolysis** is the pathway of glucose oxidation to pyruvate (under aerobic conditions) or lactate (under anaerobic conditions). It occurs in the cytosol of all cells in the body.

Glycolysis includes two phases:

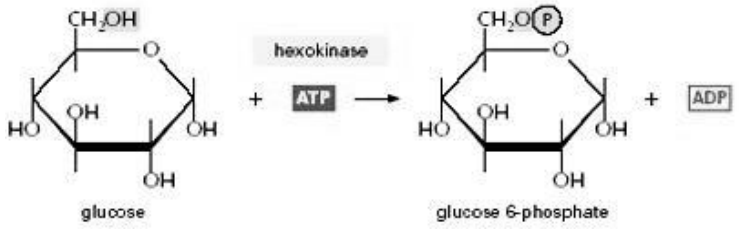
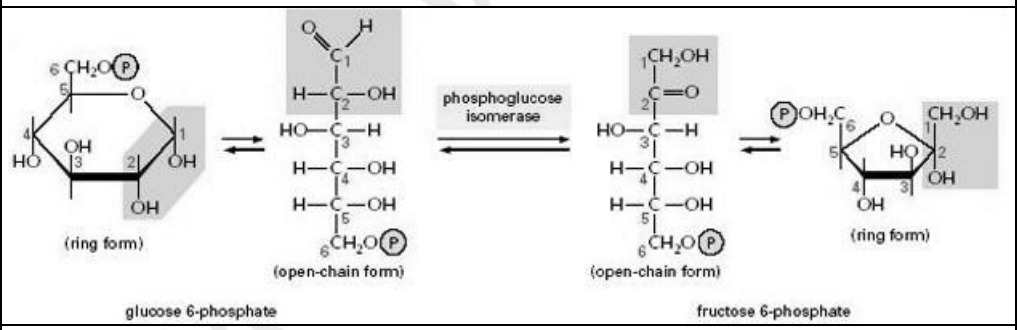
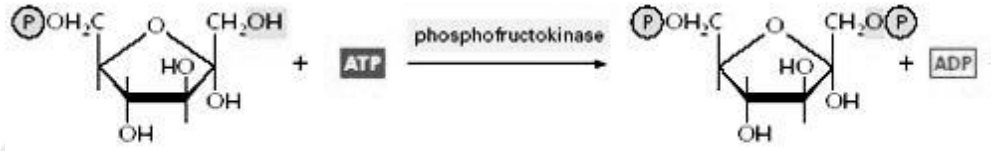
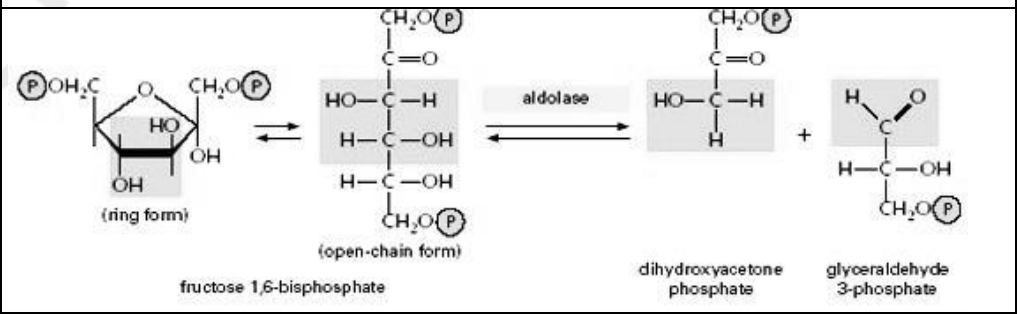
1) **Preparatory phase**, in which hexose is twice phosphorylated by ATP and then cleaved to yield two triose phosphates.

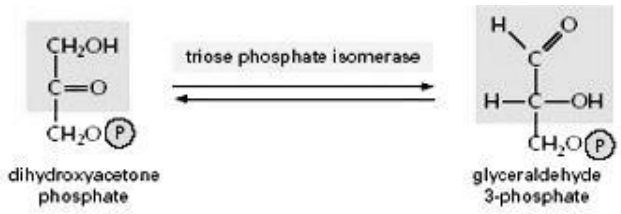
2) **Oxidative phase,** in which triose phosphates are oxidized to pyruvate with ATP production.

The first five steps of glycolysis comprising the **preparatory phase** are given in the table 4.3.

*Table 4.3*

**Preparatory phase of glycolysis**

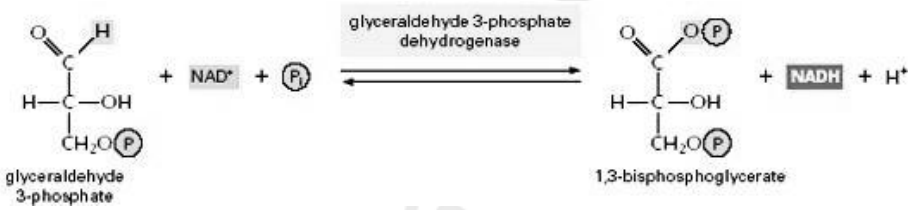
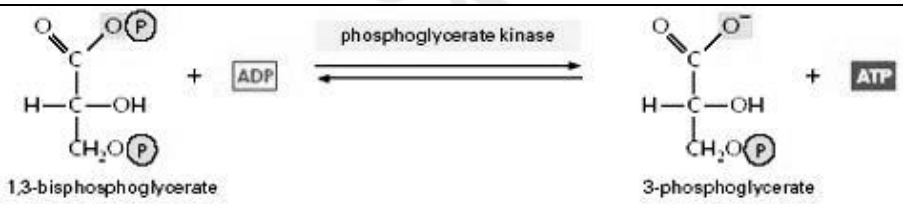
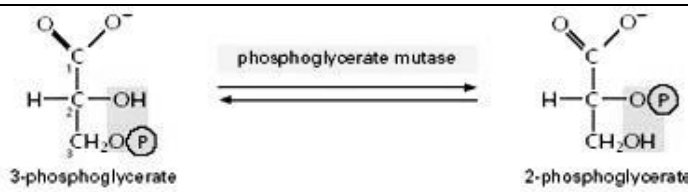
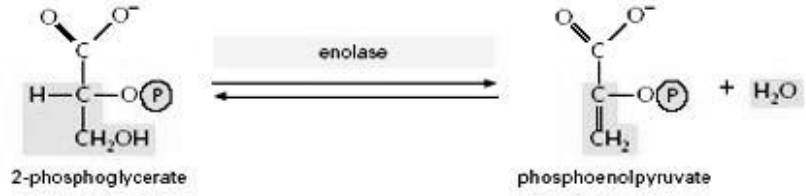
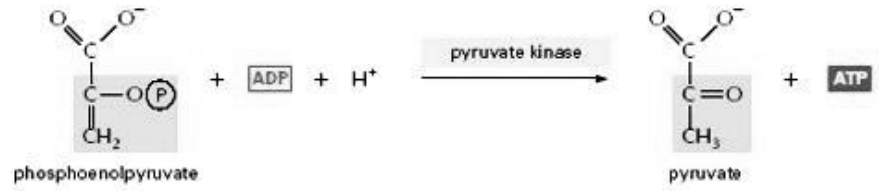
| | |
|---|---|
| Step 1<br>Glucose is phosphorylated by hexokinase (in all tissues) or by glucokinase (in liver) using ATP to form glucose-6-phosphate |  |
| Step 2<br>A readily reversible rearrangement of the sugar chemical structure (isomerization) that leads to conversion of aldose (glucose) to ketose (fructose) |  |
| Step 3<br>The new hydroxyl group on carbon 1 is phosphorylated by phosphofructokinase-1 using ATP, in preparation for the formation of two three-carbon sugar phosphates |  |
| Step 4<br>The six carbon sugar is cleaved by aldolase to produce two three carbon molecules. Only glyceraldehyde 3-phosphate can further proceed through glycolysis |  |

| | |
|---|---|
| Step 5<br>The other product of step 4, dihydroxyacetone phosphate, is isomerized to form glyceraldehyde 3-phosphate |  |

It can be concluded that the first phase results in formation of two molecules of glyceraldehyde-3-phosphate, both may undergo oxidation in the next steps. **Oxidative phase** (table 4.4).

*Table 4.4*

**Oxidative phase of glycolysis**

| | |
|---|---|
| Step 6<br>The glyceraldehyde 3-phosphate is oxidized by dehydrogenase and reacts with inorganic phosphate. NADH+H$^+$ and a new high-energy linkage to phosphate at carbon 1 are formed |  |
| Step 7<br>The transfer of high-energy phosphate group that was generated in step 6 to ADP forms ATP (substrate-level phosphorylation) |  |
| Step 8<br>The remaining phosphate group is moved from carbon 3 to carbon 2 to form 2-phosphoglycerate |  |
| Step 9<br>The removal of water from 2-phosphoglycerate by enolase creates a high-energy compound — phosphoenolpyruvate |  |
| Step 10<br>The transfer of high-energy phosphate group that was generated in step 9 to ADP forms ATP (substrate-level phosphorylation) |  |
| **Net products of 10 steps of glycolysis — 2 molecules of pyruvate, 2 molecules of ATP and 2 molecules of NADH+H$^+$. Under aerobic conditions NADH+H$^+$ can be reoxidized in the respiratory chain** | |
| Step 11 (under anaerobic conditions)<br>The transfer of hydrogen atoms from NADH+H$^+$ to pyruvate forms lactate and regenerates NAD$^+$ for step 6 |  |

**Entry of fructose and galactose to glycolysis**

Although glucose is the most abundant monosaccharide derived from the diet, fructose and galactose are also obtained, mainly from sucrose and lactose. These hexoses get involved in metabolism through the conversion to glucose or its metabolites (figure 4.9).

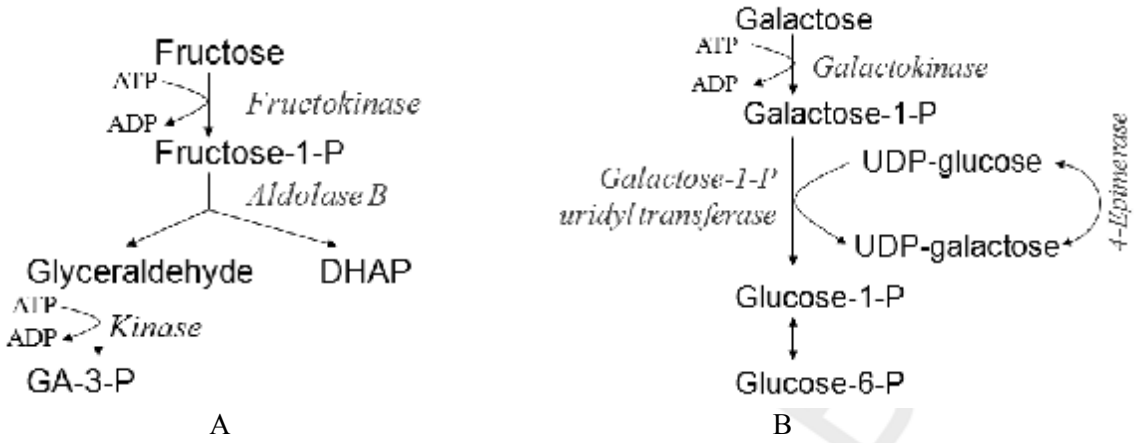



*Fig. 4.9.* Metabolism of fructose (A) and galactose (B) in the liver

In the liver specific enzyme *fructokinase* is present, generating fructose-1-phosphate. Fructose-1-phosphate is cleaved to glyceraldehyde and dihydroxyacetone phosphate by *aldolase B*. Glyceraldehyde undergoes phosphorylation to form glyceraldehyde 3-phosphate. The two triose phosphates, dihydroxyacetone phosphate and glyceraldehyde 3-phosphate, enter glycolysis, or may be substrates for gluconeogenesis. In extrahepatic tissues, some fructose can be metabolized by *hexokinase*, generating fructose 6-phosphate, which enters glycolysis.

Galactose is also readily metabolized in the liver. *Galactokinase* catalyses phosphorylation of carbon 1 in galactose. Galactose 1-phosphate reacts with uridine diphosphate glucose (UDP-glucose) to form UDP-galactose and glucose 1-phosphate in a reaction catalyzed by *galactose 1-phosphate uridyl transferase*. The enzyme *4-epimerase* catalyses conversion of UDP-galactose to UDP-glucose. The net result is that galactose is converted to the glucose moieties, UDP-glucose and glucose 1-phosphate, intermediates in pathways of glucose metabolism.

**Regulation of glycolysis**

There are 3 regulatory key enzymes in glycolysis: hexokinase (or glucokinase), phosphofructokinase-1 (PFK-1), and pyruvate kinase.

***Hexokinase*** is inhibited by its product, glucose-6-phosphate.

***Glucokinase*** is induced by insulin.

***PFK-1*** is stimulated by AMP and fructose-2,6-bisphosphate; inhibited by ATP and citrate.

***Pyruvate kinase*** is stimulated by fructose-1,6-bisphosphate; inhibited by ATP. Insulin causes *pyruvate kinase* to be activated via dephosphorylation, glucagon causes it to be inhibited via phosphorylation.

**Biological role of glycolysis**

During glycolysis, some of the potential energy stored in hexose structure is released and used for ATP synthesis. Glycolysis is a single energy producing pathway that can proceed under anaerobic conditions. In humans this is especially important for red blood cells and exercising muscle.

Glycolysis provides metabolic intermediates for other pathways, e. g. dihydroxyacetone phosphate for lipid biosynthesis.

Glycolysis supplies erythrocytes with 2,3-bisphosphoglycerate. This side product is formed from 1,3-bisphosphoglycerate by *bisphosphoglycerate mutase*. The special role of

2,3-bisphosphoglycerate is to lower the affinity of hemoglobin to oxygen. Later 2,3-bisphosphoglycerate is hydrolysed to form 3-phosphoglycerate which reenters glycolytic pathway.

Glycolysis is a universal pathway used in all living cells, not only in anaerobes. In aerobic cells, glycolysis is the initial phase of an overall degradation process that completely oxidizes carbohydrates to $CO_2$ and $H_2O$.

## GLUCOSE OXIDATION UNDER AEROBIC CONDITIONS

Complete carbohydrate oxidation to $CO_2$ and $H_2O$ occurs under aerobic conditions and includes next stages (figure 4.10):

- **Glycolysis** — glucose is converted to two molecules of pyruvate (cytosolic step).
- **Oxidative decarboxylation of pyruvate** — pyruvate is converted to acetyl-CoA (mitochondrial step).
- **Citric acid cycle** — acetyl moiety of acetyl-CoA is finally oxidized to form $CO_2$ (mitochondrial step).
- **Tissue respiration and oxidative phosphorylation** — reducing power generated in stages I–III in the form of $NADH+H^+$ and $FADH_2$ is used to produce ATP (mitochondrial step).

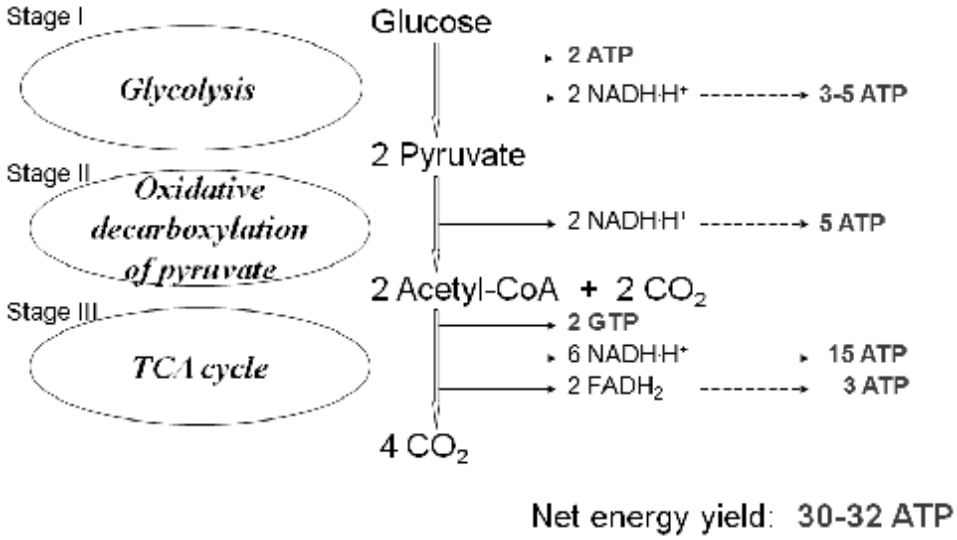


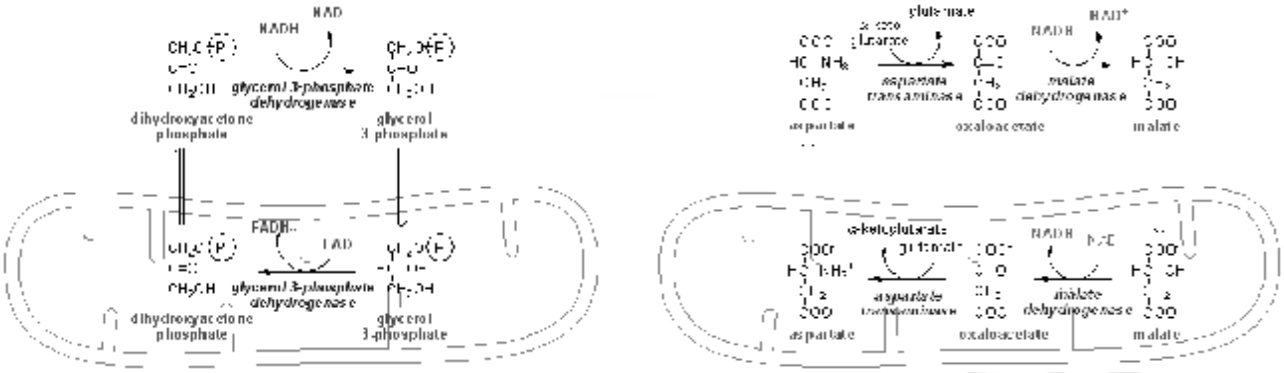

*Fig. 4.10.* Three stages of glucose oxidation under aerobic conditions

### Transfer of the cytosolic $NADH+H^+$ to the mitochondrion

$NADH+H^+$ produced in glycolysis cannot directly cross the mitochondrial membrane. Two shuttle mechanisms exist by which electrons can be passed to the respiratory chain (figure 4.11).

**Glycerol phosphate shuttle**. Cytosolic DHAP serves as acceptor of hydrogens from $NADH+H^+$ and glycerol 3-phosphate is created. Glycerol 3-phosphate will be oxidized by FAD-linked dehydrogenase in the inner mitochondrial membrane. DHAP is regenerated and carried back to the cytosol. Each mole of $FADH_2$ that is produced generates 1,5 moles of ATP via oxidative phosphorylation.

**Malate aspartate shuttle**. Cytosolic oxaloacetate serves as acceptor of hydrogens from $NADH+H^+$ and malate is created. Malate enters the mitochondrion and is oxidized by the mitochondrial malate dehydrogenase. $NADH+H^+$ and oxaloacetate are generated in matrix. Each mole of $NADH+H^+$ that is produced generates 2,5 moles of ATP via oxidative phosphorylation. Oxaloacetate cannot cross the mitochondrial membrane. It is carried back to the cytosol after transamination and conversion to aspartate.




A B

*Fig. 4.11.* Transfer of the cytosolic NADH+H+ to the mitochondrion:
A — glycerol phosphate shuttle; B — malate aspartate shuttle

## GLUCONEOGENESIS

**Gluconeogenesis** is the synthesis of glucose from compounds that are not carbohydrates. This pathway occurs mainly in the liver. The major precursors for gluconeogenesis are lactate, amino acids and glycerol.

Gluconeogenesis is almost reversal of glycolysis, but involves several enzymatic steps by which key reactions of glycolysis are bypassed. With the exception of the reactions shown below, the enzymes and reactions of gluconeogenesis and glycolysis are the same (figure 4.12).

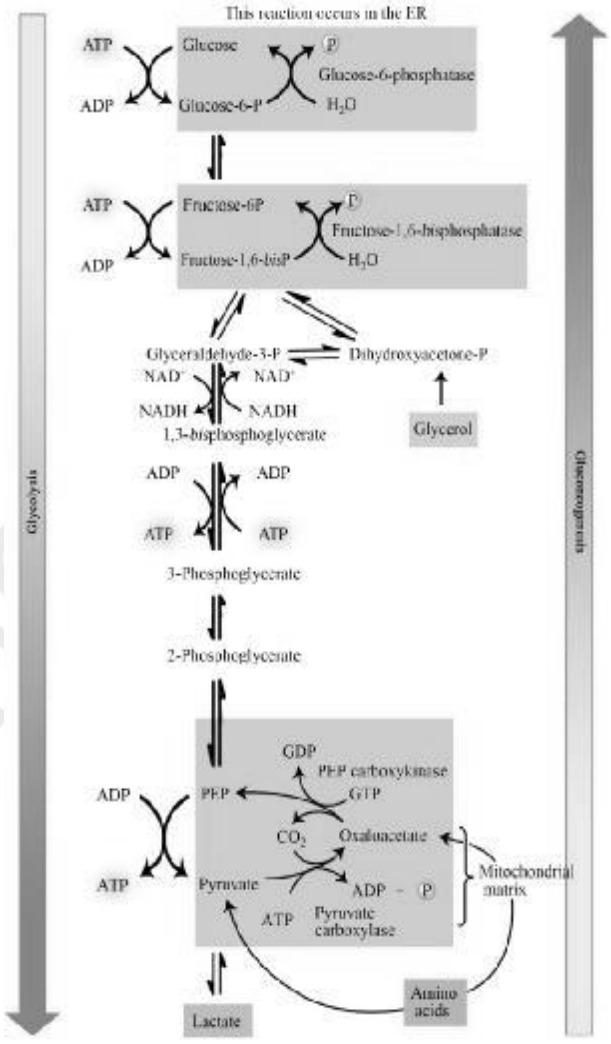
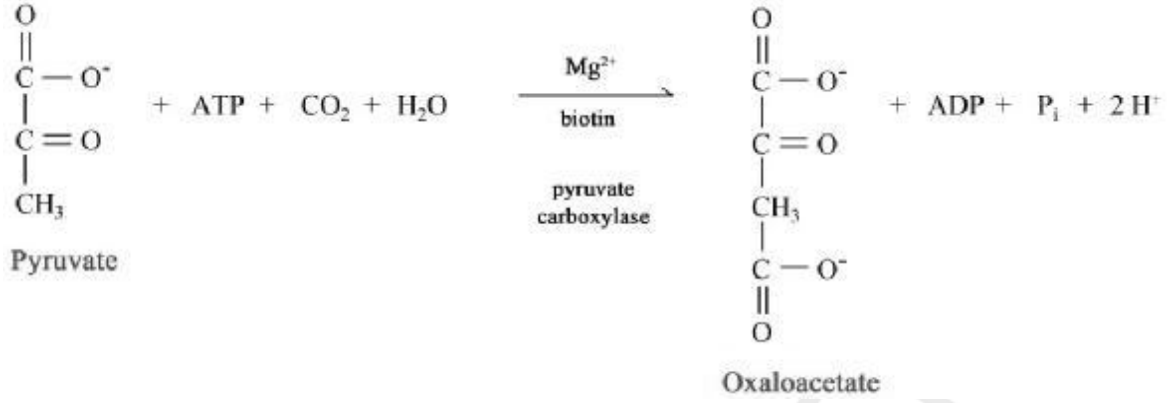



*Fig. 4.12.* Reactions of glycolysis and gluconeogenesis

Key reactions of gluconeogenesis:

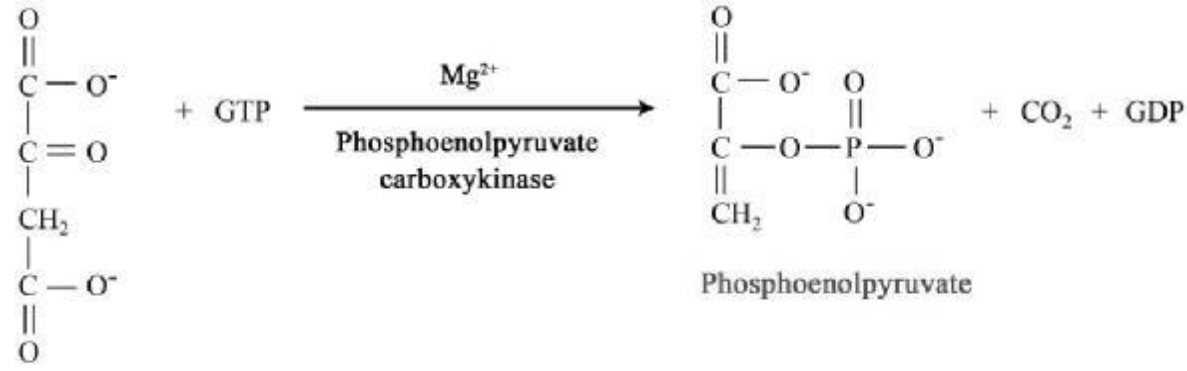
1. Conversion of **pyruvate** to **oxaloacetate** by *pyruvate carboxylase*, a mitochondrial enzyme that requires biotin and ATP.




Pyruvate

Oxaloacetate

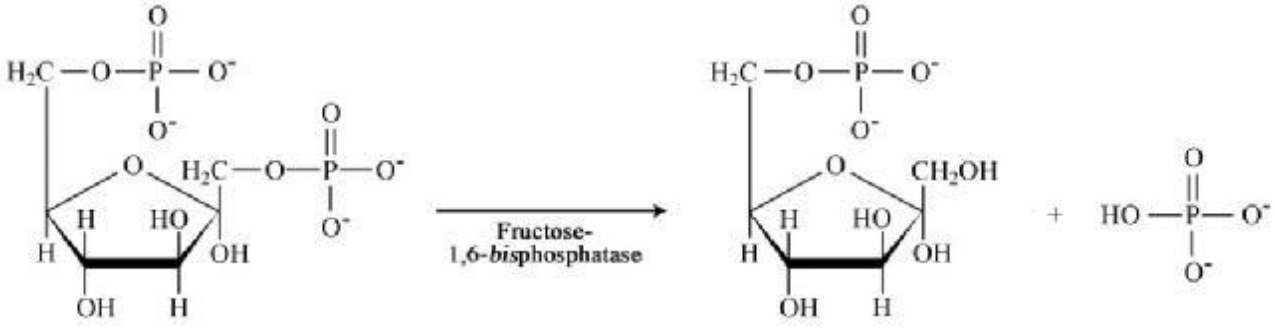
2. Conversion of **oxaloacetate** to **phosphoenolpyruvate** by *phosphoenolpyruvate carboxykinase*. Oxaloacetate is decarboxylated and phosphorylated, GTP is required.




Oxaloacetate

Phosphoenolpyruvate

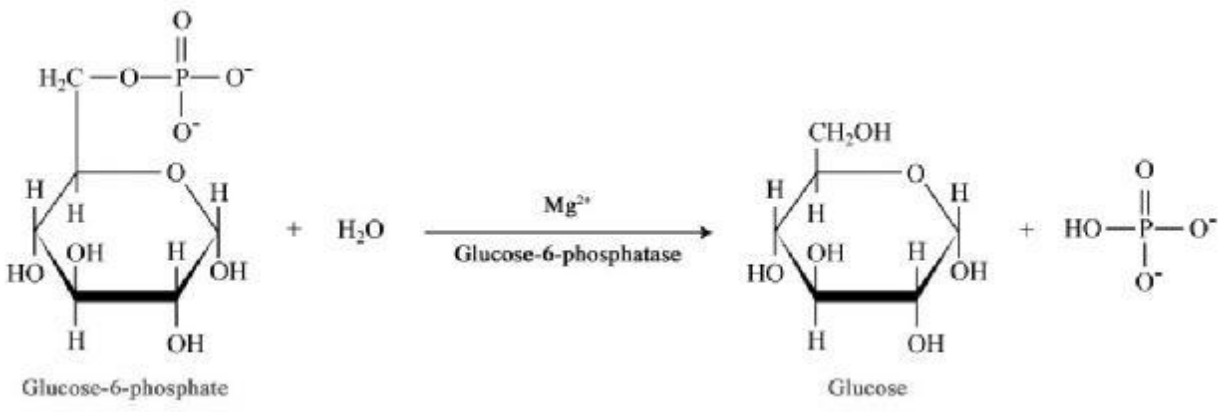
3. Conversion of **fructose 1,6-bisphosphate** to **fructose 6-phosphate** by *fructose 1,6-bisphosphatase*. Inorganic phosphate is released.




Fructose-1,6-*bis*phosphate

Fructose-6-phosphate

4. Conversion of **glucose 6-phosphate** to **glucose** by *glucose 6-phosphatase*. This enzyme is involved in both gluconeogenesis and glycogenolysis. Inorganic phosphate is cleaved from glucose 6-phosphate, and free glucose is released into the blood.




**Energy requirements for gluconeogenesis**

4 ATP and 2 GTP are required to form one glucose from lactate or pyruvate.

**Regulation of gluconeogenesis**

The opposing pathways, glycolysis and gluconeogenesis, are regulated in the reciprocal fashion. That is, the intracellular conditions that activate one pathway simultaneously inhibit the other.

Allosteric regulation of gluconeogenesis: ***pyruvate carboxylase*** is activated by acetyl-CoA; ***fructose 1,6-bisphosphatase*** is inhibited by AMP and fructose 2,6-bisphosphate; ***glucose 6-phosphatase*** is activated by glucose 6-phosphate (the substrate-level control).

Besides, hormonal regulation is used to regulate activity and synthesis of the key enzymes. Glucagon, epinephrine and glucocorticoids stimulate gluconeogenesis; insulin inhibits.

**Biological role of gluconeogenesis**

The pathway is important mechanism for maintaining the blood glucose level. Gluconeogenesis provides glucose when sufficient carbohydrate is not available from the diet or glycogen reserves. In addition, gluconeogenesis removes lactate from the blood.

## PENTOSE PHOSPHATE PATHWAY

The pentose phosphate pathway is a remarkable, multipurpose pathway that operates to varying extents in different cells and tissues (erythrocytes, phagocytes, liver, adipose tissue, gonads, adrenal glands, mammary glands, rapidly proliferating cells, others).

It is convenient to think of this pathway as operating in two phases — oxidative and nonoxidative. Both occur in the cytosol and do not require oxygen.

**Oxidative phase** (figure 4.13).

The oxidative phase of pentose phosphate pathway has two primary functions: 1) to provide reduced $NADP^+$ ($NADPH+H^+$) and 2) to provide ribose-5-phosphate.

The biological role of **$NADPH+H^+$** is to serve as electron source for the reductive reactions: biosynthesis of fatty acids, cholesterol, nonessential amino acids; reduction of ribonucleotides to deoxyribonucleotides; reduction of glutathione. In phagocytes NADPH is used by NADPH-oxidase for generation of reactive oxygen species ($O_2^-$) and phagocytosis. Microsomal oxidation for detoxification of xenobiotics in the liver also requires NADPH.

**Ribose-5-phosphate** is used for nucleotide and nucleic acid biosynthesis.

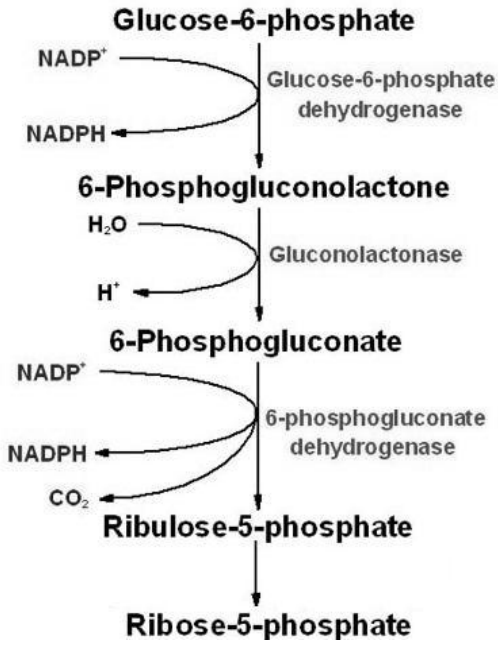



*Fig. 4.13.* The pentose phosphate pathway: reactions of the oxidative phase

**Nonoxidative phase**

In the nonoxidative phase some of pentoses produced in the oxidative phase may be converted to other sugars, mainly hexoses and trioses (intermediates of glycolysis). Reversible interconversions of sugars occur due to group-transfer reactions catalyzed by transketolase and transaldolase as shown in the table 4.5. *Transkletolase* transfers two-carbon unit, *transaldolase* transfers three-carbon unit from one sugar to another.

Nonoxidative phase is used to regulate the level of pentoses in the cell and to metabolize dietary pentose sugars, derived primarily from the digestion of nucleic acids.

*Table 4.5*

**Group-transfer reactions in the pentose phosphate pathway**

| | **Reactants** | **Enzyme** | **Products** |
|---|---|---|---|
| Two-carbon shift | $C_5$ + $C_5$ | **ß à** *Transketolase* | $C_7$ + $C_3$ |
| Three carbon shift | $C_7$ + $C_3$ | **ß à** *Transaldolase* | $C_6$ + $C_4$ |
| Two-carbon shift | $C_5$ + $C_4$ | **ß à** *Transketolase* | $C_6$ + $C_3$ |
| Net process | $3C_5$ | **ß à** | $2C_6$ + $C_3$ |

**Regulation of pentose phosphate pathway**

Oxidative phase is inhibited by the increased ratio **[NADPH+H$^+$]/[NADP$^+$]**. Synthesis of *glucose 6-phosphate dehydrogenase* and *6-phosphogluconate dehydrogenase* is induced by **insulin**.

Reactions of nonoxidative phase are regulated by substrate concentrations.

**URONIC ACID PATHWAY**

Uronic acid pathway is a secondary pathway of glucose utilization which occurs mainly in the liver and connective tissue. The sequence or reactions (figure 4.14) include UDP-glucose production and its oxidation to form **UDP-glucuronic acid** that is one of the most important metabolites in the pathway.

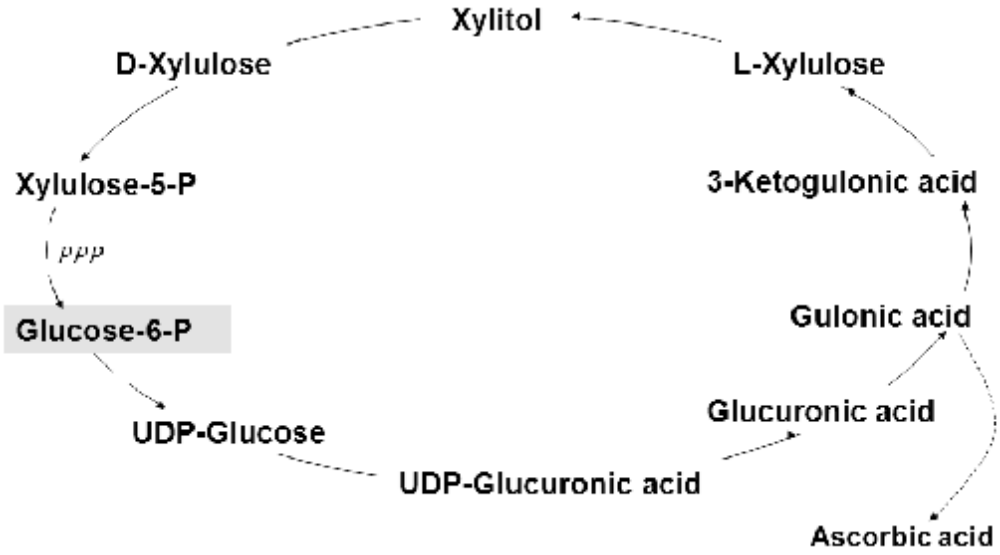



*Fig. 4.14.* The uronic acid pathway (schematic view)

*In the liver* this "activated" glucuronic acid participates in detoxification of nonpolar endogenous or exogenous substances by formation of glucuronide conjugates. Conjugation with glucuronic acid produces a strongly acidic compound that is more water-soluble and therefore can be easily excreted. For example, such mechanism is used for detoxification of bilirubin, steroids, a number of drugs. *In fibroblasts* UDP-glucuronic acid is used for biosynthesis of heteropolysaccharides, components of extracellular matrix.

Glucuronic acid may be reduced by NADPH to L-gulonic acid, which is then converted through L-gulonolactone to **L-ascorbic acid (vitamin C)** in plants and most higher animals (except humans, other primates and guinea pigs). Gulonic acid can also be oxidized to 3-ketogulonic acid and decarboxylated to form **pentoses** (L-xylulose, D-xylulose, xylulose 5-phosphate). **Xylitol** (sweetener) obtained from the diet may enter metabolism through this pathway, being oxidized to D-xylulose and phosphorylated to xylulose 5-phosphate. The later can enter the pentose phosphate pathway as described previously.

### ETHANOL METABOLISM

Ethanol metabolism occurs mainly in the liver. Two oxidations take place — first to form acetic aldehyde, and second to form acetic acid (figure 4.15). Acetic acid reacts with coenzyme A, thus acetyl-CoA is a product. High NADH levels from alcohol metabolism may inhibit the TCA cycle and cause unfavourable metabolic consequences: lactic acidosis, hypoglycemia, fatty liver.

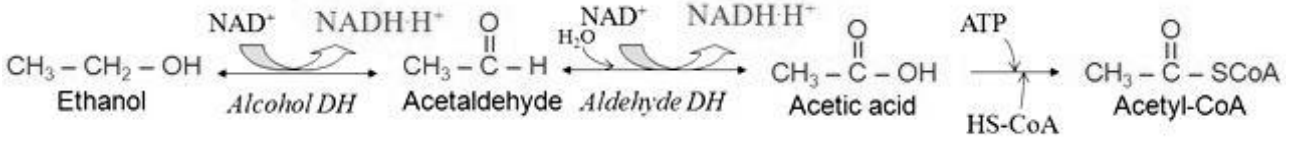



*Fig. 4.15.* The main pathway of ethanol metabolism

Commonly *alcohol dehydrogenase* performs 80 % of ethanol oxidation. In addition, *microsomal ethanol oxidizing system (MEOS)* exists, which oxidizes alcohol using cytochrom p450. Synthesis of the main components of this system is induced by alcohol intake. Minor alcohol metabolism pathway is its oxidation by *catalase* in liver peroxisomes.

### GLUCOSE REDUCTION PATHWAY (SORBITOL PATHWAY)

Glucose can be reduced to sorbitol by *aldose reductase*, which reduces the aldehyde group at carbon 1 to an alcohol (figure 4.16). Sorbitol is then reoxidized at carbon 2 by *sorbitol dehydrogenase* to form fructose.

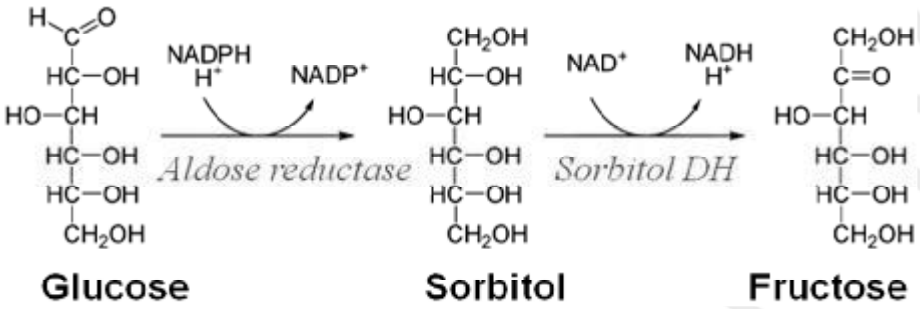



*Fig. 4.16.* The "sorbitol" pathway of glucose metabolism

Conversion of glucose to fructose, which serves as the major energy source for sperm cells, is particularly important in seminal vesicles.

If glucose levels are high the pathway becomes activated in insulin-independent cells. Sorbitol accumulates in the lens, peripheral nerves, and renal glomeruli, causing their osmotic damage.

### REGULATION OF BLOOD GLUCOSE LEVEL

Blood glucose levels are maintained within a rather narrow range 3,3–5,5 mmol/l (3,9–6,1 mmol/l in plasma).

After a meal, blood glucose is supplied by **dietary carbohydrate**. Excess of carbohydrate is stored as glycogen and may be converted to lipids. During fasting, blood glucose is maintained by the liver by the processes of **glycogenolysis** and **gluconeogenesis**. The key enzymes of these pathways are submitted to hormonal regulation. Hormones influence on their activity via phosphorylation and dephosphorylation, and their synthesis at a genetic level.

The major hormones that regulate blood glucose are insulin, glucagon, epinephrine and glucocorticoids (cortisol). **Insulin** causes blood glucose to be decreased by stimulation of glucose uptake in tissues through GLUT 4 and activation of glycogenesis and pathways of glucose utilization. **Glucagon, epinephrine, cortisol** cause elevation of blood glucose, since promote glycogenolysis and gluconeogenesis.

### CLINICAL ASPECTS

#### Lactase deficiency

In intestinal lactase deficiency lactose cannot be digested and is consumed by bacteria in the gut, producing gas, bloating, intestinal cramps, and watery diarrhea.

#### Glycogen storage diseases (glycogenoses)

Glycogen accumulates primarily in the liver or muscle, or both due to the deficiency of enzymes involved in glycogen breakdown. There are several types of such enzymopathies producing conditions ranging from mild disorders to severe liver or cardiorespiratory failure. Type I (Von Gierke's disease) is caused by deficiency of *glucose 6-phosphatase*. Liver and kidneys suffer being overloaded with glycogen. Hypoglycemia, lactic acidosis, ketosis develop. Type II (Pompe's disease) is caused by the absence of *lysosomal γ-amylase*. Generalized affection of internal organs occurs.

#### Hypoglycemia

Low blood sugar is caused by the inability of the liver to maintain blood glucose levels. It can result from high insulin (e. g., insulin-secreting tumors, inappropriate doses or usage of insulin), liver disease, impairment of glycogenolysis or gluconeogenesis. Excessive alcohol ingestion also

can cause hypoglycemia. Ethanol metabolism increases levels of NADH in the liver, which decreases gluconeogenesis because of pyruvate and oxaloacetate deficiency.

**Lactic acidosis**

Increase of lactate levels in the blood cause acidosis. This condition may develop as a result of hypoxia (stimulation of anaerobic glycolysis), alcohol ingestion (high NADH levels from alcohol metabolism cause pyruvate to be converted to lactate), thiamine deficiency (impaired oxidative decarboxylation of pyruvate, dominating pathway of pyruvate metabolism).

**Glucose 6-phosphate dehydrogenase deficiency**

In *glucose 6-phosphate dehydrogenase* deficiency NADPH is lacking. As a result, glutathione is not adequately reduced and, in turn, is not available to protect red blood cells from damage by reactive oxygen species, especially under certain conditions (e. g., when drugs or foods possessing oxidative properties are ingested). Hemolytic anemia develops.

**Pyruvate kinase deficiency**

Since only glycolysis provides energy for red blood cells, *pyruvate kinase* deficiency causes insufficient production of ATP for the membrane pumps, and hemolytic anemia results.

**Diabetes mellitus**

Blood glucose levels rise because of either insulin deficiency (insulin-dependent diabetes mellitus, IDDM), or inability of tissues to metabolize glucose in the presence of normal amounts of insulin (noninsulin-dependent diabetes mellitus, NIDDM, or insulin resistance). The body responds to insulin deficiency as if it is starving: lipid stores are degraded and ketoacidosis may occur. Many metabolic pathways are affected. High glucose is toxic. Two pathological processes seem to follow hyperglycemia: 1) stimulation of sorbitol pathway resulting in osmotic damage of cells and membranes, notably in the lens, microvasculature, and peripheral nerves; 2) spontaneous non-enzymatic glycosylation of proteins that leads to the conformational change and alteration of protein function. Glycosylated hemoglobin is known as $HbA_{1c}$. Normal values for $HbA_{1c}$ are about 5 % of total hemoglobin.

**Essential pentosuria**

*L-xylulose reductase* that converts L-xylulose to xylitol is deficient. Xylulose (a pentose) appears in the urine and gives a positive reducing-sugar test. The condition is benign.

**Essential fructosuria**

*Fructokinase* is deficient. Fructose cannot be metabolized, blood fructose levels rise, and fructose may appear in the urine. The condition is benign.

**Fructose intolerance**

*Aldolase B* is deficient. Fructose 1-phosphate accumulates and inhibits glucose production (glycogenolysis and gluconeogenesis). Fructose ingestion is followed by severe hypoglycemia.

**Galactosemia**

Galactosemia may be caused by inherited defects of *galactokinase*, *uridyl transferase* (most common) or *4-epimerase.* High concentrations of galactose are found in the blood after lactose ingestion. Excess galactose may be reduced to galactitol, causing cataract. Ultimately, liver failure and mental deterioration develop.

**Sugars and dental caries**

Mineral component of the teeth (apatite) is built on the base of calcium phosphate, which is easily dissolved in acidic medium. Caries develops as a result of action of acid-producing bacteria

(especially Streptococci mutans and Lactobacilli) on the tooth enamel. Bacteria synthesize extracellular polysaccharides (dextran, levan) which are used for adhesion to the enamel surface, and get energy (ATP) from easily fermentable diet carbohydrates such as glucose, fructose, and sucrose. Other carbohydrates are considered to be less cariogenic. Acid products of bacterial sugar metabolism (lactate, acetate, formate) decrease the pH of the plaque, and eventully cause demineralisation of the tooth enamel and the underlying dentine.

# CHAPTER 5
# LIPID METABOLISM

The organic compounds related to **lipids are**: 1) poorly soluble in water, and soluble in nonpolar solvents (acetone, chloroform); 2) natural or potential esters of fatty acids; 3) present in living organisms and utilized by them.

Lipids represent a diverse group of compounds (figure 5.1).

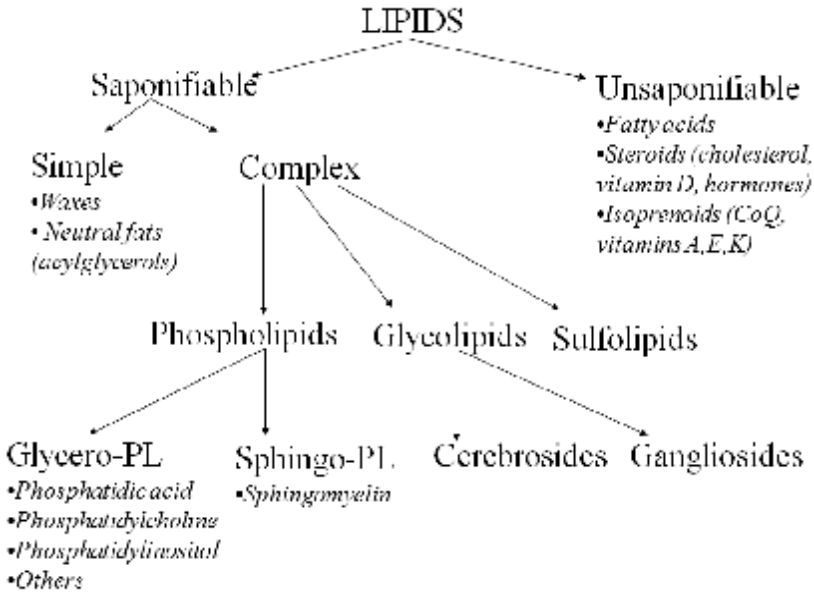



*Fig. 5.1.* Classification of lipids

### MOLECULAR STRUCTURE AND PROPERTIES OF LIPIDS

**Fatty acids** are the long chain carboxylic acids (figure 5.2). They are the simplest lipids, which rarely exist free, but mainly are the components of other more complex lipids (being esterified to alcohols). The residue of fatty acid is termed "acyl".

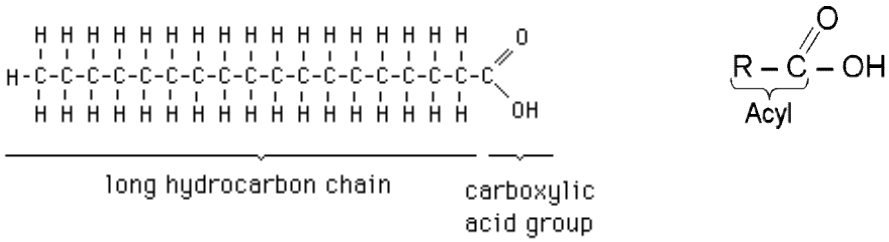



*Fig. 5.2.* The general view of fatty acids

The charge on the carboxyl group makes it extremely hydrophilic, whereas the long hydrocarbon tail is very hydrophobic. As a result, fatty acids are typical **amphipathic** substances.

In humans, fatty acids usually have an even number of carbon atoms, are 16–20 carbon atoms in length, and may be saturated or unsaturated (contain one or more double bonds). Examples are shown in the table 5.1.

*Table 5.1*

**The common fatty acids**

| Saturated Fatty Acids | Unsaturated Fatty Acids |
|---|---|
| Palmitic acid (C16)<br>Stearic acid (C18) | Oleic acid (18 : 1 ω9)<br>Linoleic acid (18 : 2 ω6)<br>Linolenic acid (18 : 3 ω3)<br>Arachidonic acid (20 : 4 ω6) |

Physical and physiologic properties of fatty acids reflect chain length and degree of unsaturation. The lipids containing saturated fatty acids are more solid, whereas those containing unsaturated fatty acids are liquid. Therefore membrane lipids, which must be fluid at all environmental temperatures, are more unsaturated than storage lipids.

Arachidonic acid is an important precursor for lipid hormones — eicosanoids (prostaglandins, thromboxanes, leukotrienes).

**Waxes** are esters of fatty acids with long chain monohydric alcohols. The waxes are completely water-insoluble, and usually serve as water repellents in nature, as in bird feathers or leaves of some plants.

**Neutral fats** are esters of fatty acids with glycerol. Mono-, di-, and triacylglycerols exist.

Triacylglycerols are highly **hydrophobic** (figure 5.3). In cells they serve as a lipid storage form and represent a highly concentrated reserve of metabolic energy and endogenous water. Besides, fats protect the internal organs, contribute to thermoregulation (both heat production and heat insulation), serve as a source of arachidonic acid, dissolve fat-soluble vitamins. Some animal fats show bactericidal effect.

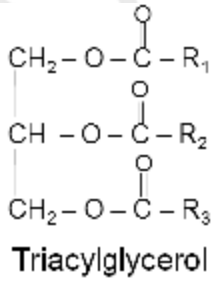
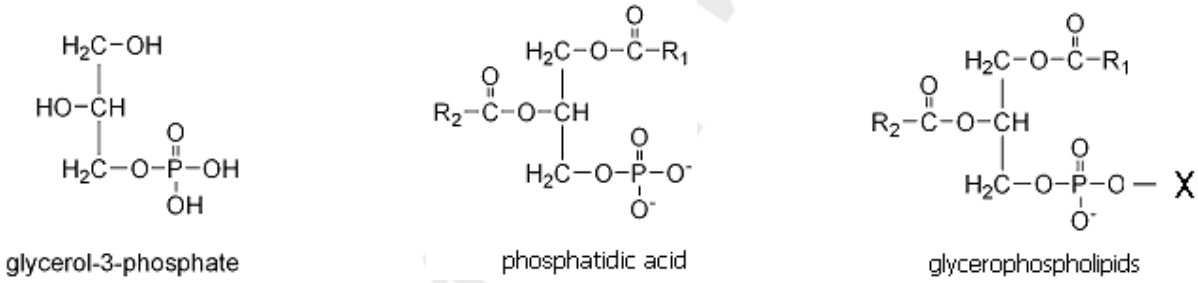



*Fig. 5.3.* General structure of neutral fat

**Phospholipids** are divided into glycerophospholipids and sphingophospholipids.

*Glycerophospholipids* can be considered to be derivatives of glycerol 3-phosphate (figure 5.4). The simplest member of the group is phosphatidic acid. Other glycerophospholipids are formed by the attachment of additional hydrophilic group (choline, serine, inositol, ethanolamine) to phosphate residue in the phosphatidic acid. Phosphatidylcholine, or lecithin, is the most abundant phospholipid in membranes.



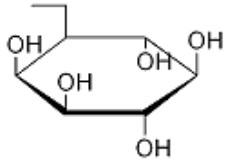

| name of X-OH | formula of X | name of phospholipid |
|---|---|---|
| ethanolamine | $-CH_2CH_2NH_3^+$ | **phosphatidylethanolamine** |
| choline | $-CH_2CH_2N(CH_3)_3^+$ | **phosphatidylcholine** |
| inositol |  | **phosphatidylinositol** |
| **serine** | $-CH_2CH(NH_3^+)COO^-$ | **phosphatidylserine** |

*Fig. 5.4.* Phospholipids — derivatives of glycerol 3-phosphate

*Sphingophospholipids* are built on the base of long-chain amino alcohol sphingosine linked to a fatty acid. (The combination of sphingosine plus fatty acid is known as ceramide, a structure also found in glycosphingolipids.) Sphingomyelin (its block structure is shown in figure 5.5) is an especially important example of sphingophospholipids.

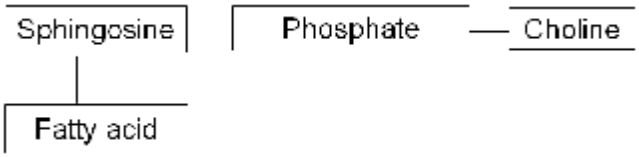


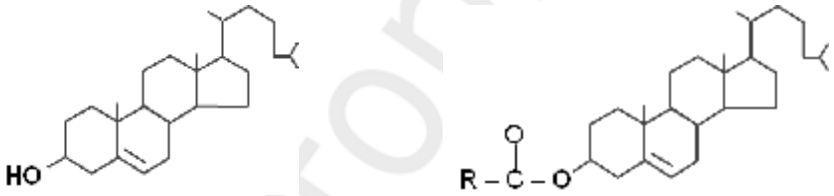

*Fig. 5.5.* Structure of sphingomyelin

All phospholipids are **amphipathic**. They are of great biological importance as the constituents of cell membranes, lung surfactant and blood lipid transport forms; activators of enzymes; precursors of second messengers; sources of arachidonic acid; detergents in intestines. These functions of phospholipids prevail over fuel.

**Glycolipids** in animal tissues are mainly sphingosine derivatives. They contain ceramide and one or more sugar residues. Glycolipids (**cerebrosides** and **gangliosides**) are membrane lipids present in every tissue of the body, particularly in nervous tissue. They participate in cell interconnections, recognition, adhesion, reception.

**Cholesterol** is the major steroid in animals. It is an important component of cell membranes, where it participates in regulation of membrane fluidity. Besides, cholesterol serves as precursor to all of steroid hormones, to vitamin D, and to the bile acids.

Cholesterol has a tetracyclic nucleus, aliphatic side chain and an OH-group (alcohol). Free cholesterol is a weakly amphipathic substance, which may be converted to virtually hydrophobic (**cholesterol ester**) by esterification with a fatty acid (figure 5.6).




A B

*Fig. 5.6.* Cholesterol (A) and cholesterol ester (B)

## DIGESTION OF LIPIDS

The major dietary lipid is triacylglycerol (90 %); phospholipids, cholesterol, cholesterol esters usually are present in the diet too. The poor water solubility of lipids presents problems for digestion because substrates are not easily accessible to the water-soluble digestive enzymes in the aqueous phase. Therefore, lipid digestion occurs in three phases:

1. Emulsification;
2. Enzymatic hydrolysis;
3. Formation of micelles and lipid absorption.

**1. Emulsification**, or dispersion of the lipid phase into smaller droplets, provides more sites for adsorption of enzyme molecules. Without such dispersion, the rate of enzymatic hydrolysis of lipids is extremely slow, and undigested lipids are excreted in the feces. Emulsification is aided by **peristaltic movements of intestines**, **$CO_2$ bubbles** and biological detergents synthesized by liver — **bile acids**. As derivatives of cholesterol, bile acids have both hydrophilic and hydrophobic groups (i. e. they are amphipathic) (figure 5.7). On exposure to a large aggregate of triacylglycerol, the hydrophobic portions of bile acids intercalate into the lipid, with the hydrophilic, negatively charged groups remaining at the surface. Such coating with bile acids contributes to breakdown of large lipid aggregates into smaller ones and causes stabilization of emulsion. The amphipathic initial lipid digestive products (free fatty acids, monoacylglycerols) also promote emulsification.

*Primary bile acids* (cholic acid, chenodeoxycholic acid) are synthesized in the liver from cholesterol. *Conjugated bile acids* are formed by conjugation of any bile acid with glycine or taurine (glycocholic acid, taurocholic acid, etc.). In the bile, the conjugated bile acids exist as $Na^+$ or $K^+$ salts known as bile salts. *Secondary bile acids* (deoxycholic acid, lithocholic acid) are formed through deconjugation and dehydroxylation in the intestines under the action of bacterial enzymes.

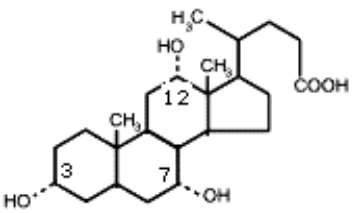
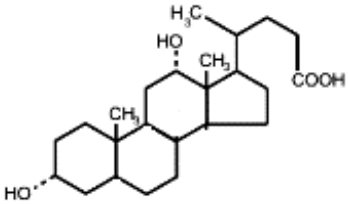
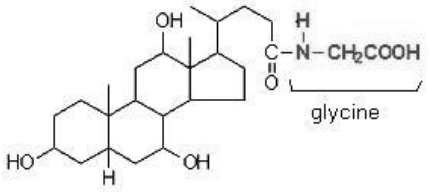
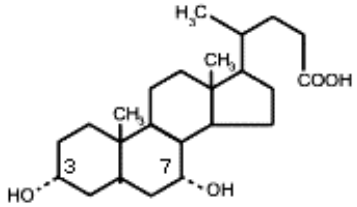
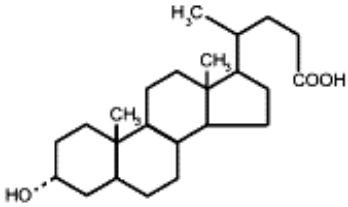
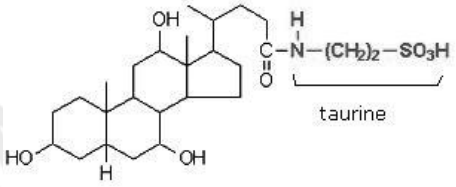
| **Primary Bile Acids** | **Secondary Bile Acids** | **Conjugated Bile Acids** |
|---|---|---|
| Cholic acid  | Deoxycholic acid  | **Primary or secondary bile acid + glycine or taurine, e. g.** Glycocholic acid  |
| Chenodeoxycholic acid  | Lithocholic acid  | Taurocholic acid  |

*Fig. 5.7.* Bile acids

The whole body pool of bile acids is about 3–5 g. In intestines most of secreted bile acids undergo reabsorption. Through the portal vein system they are returned to the liver and may be resecreted several times per day. Such secretion and reuptake is referred to as **enterohepatic circulation** of bile acids. Reabsorption of bile acids is quite efficient since only about 0,5 g of bile acids each day is lost with the feces.

**2.** The major enzyme for triacylglycerol hydrolysis is ***pancreatic lipase***. (Lingual and gastric lipases also exist. They play essential role in digestion of milk lipids in infants, but not in adults.) Pancreatic lipase becomes active at the water-lipid interface of emulsion droplets after formation of complex with colipase (a small protein secreted by the pancreas as procolipase and activated by trypsin).

Lipase preferentially hydrolyzes ester bonds formed by the long-chain fatty acids (> 10 carbons) in the positions 1 and 3 of glycerol. The products are free fatty acids and 2-monoacylglycerols. Free glycerol also may be produced through the further breakdown of monoacylglycerols.

Phospholipids are hydrolyzed by specific ***phospholipases.*** Pancreatic juice is especially rich in phospholipase $A_2$. The sites of action of different phospholipases on the phospholipid structure are shown in the figure 5.8.

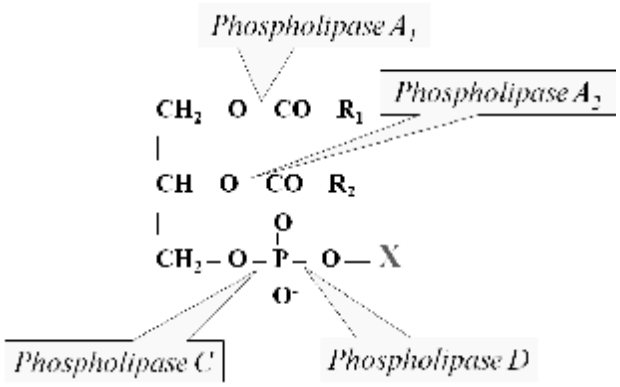
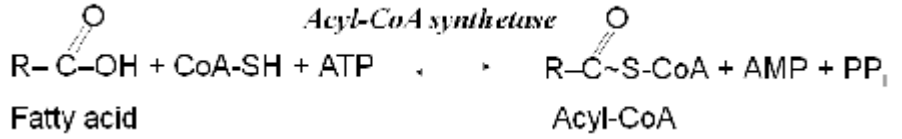



*Fig. 5.8.* Phospholipases and sites of their action

Cholesterol esters are hydrolyzed by ***cholesterol esterase***.

**3.** Mixed micelles are formed from the products of lipid digestion and bile acids. Bile acid micelles are the major vehicle for transferring lipids, including cholesterol and fat-soluble vitamins, from the intestinal lumen to the mucosal surface where absorption occurs.

Inside the enterocytes lipids undergo re-synthesis to form again triacylglycerols, phospholipids and cholesterol esters (figure 5.9). Fatty acids are used for lipid re-synthesis after activation by *fatty acyl-CoA synthetase*:




The re-synthesized lipids are packaged into chylomicrons, which are released to the lymph and eventually enter the blood (see "Lipid transport in the blood").

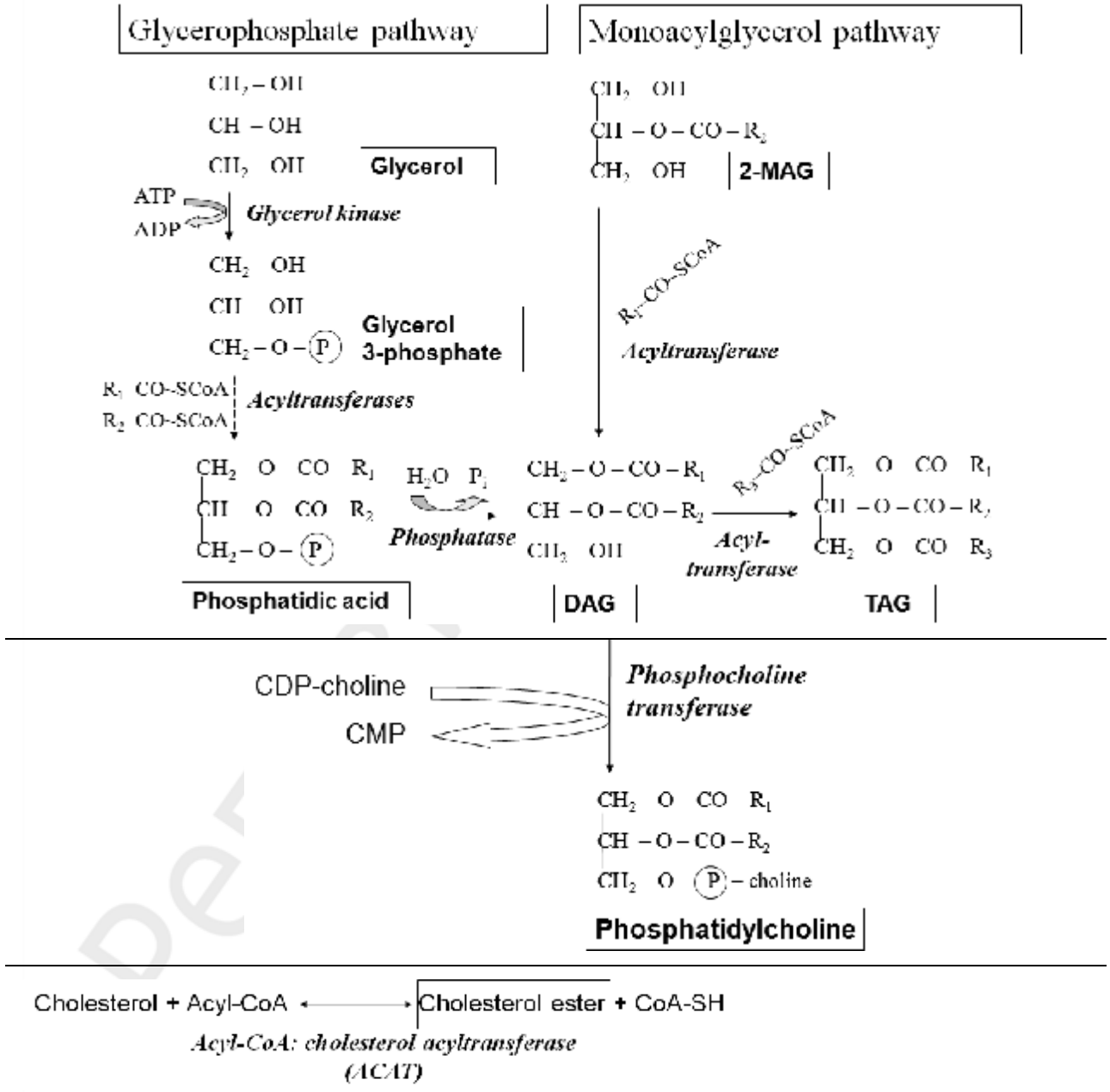



*Fig. 5.9*. Re-synthesis of triacylglycerols, phospholipids and cholesterol esters in enterocytes

## LIPID TRANSPORT IN THE BLOOD

To transport through the blood water-insoluble lipids from one tissue to another special transport forms exist — **lipoproteins**. There are 5 classes of blood lipoproteins:

1. Chylomicrons;
2. VLDL (Very Low Density Lipoproteins);
3. IDL (Intermediate Density Lipoproteins);
4. LDL (Low Density Lipoproteins);
5. HDL (High Density Lipoproteins).

Despite their differences in lipid and protein composition (table 5.2), all lipoproteins share common structural features, notably they have a spherical shape, and consist of a core of **triacylglycerols** or/and **cholesterol esters** surrounded by a single layer of **phospholipids**, into which a mixture of **cholesterol** and proteins (**apoproteins**) is inserted (figure 5.10).

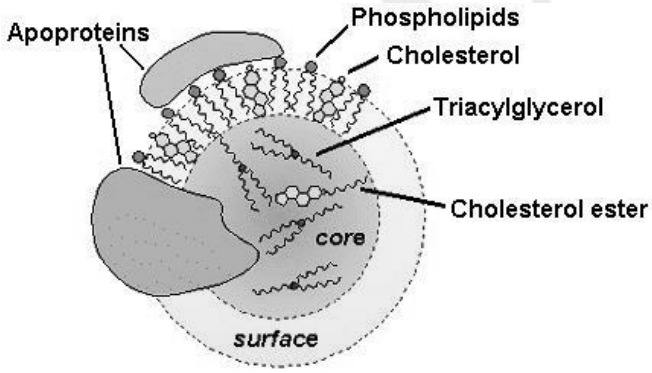



*Fig. 5.10.* The general structure of lipoprotein

Note that the phospholipids and cholesterol are oriented with their polar head groups facing outward to interact with solvent water and thus shield the hydrophobic lipids inside from the water outside. The proteins also contribute to the formation of lipoprotein polar surface, but additionally act as cofactors for enzymes, which take part in lipoprotein metabolism, and function as recognition sites for the various lipoprotein receptors throughout the body.

*Table 5.2*

**Approximate composition of blood lipoproteins**

| Component | Chylomicrons | VLDL | IDL | LDL | HDL |
|---|---|---|---|---|---|
| Triacylglycerol | 87 % | 55 % | 30 % | 6 % | 4 % |
| Cholesterol | 2 % | 7 % | 8 % | 9 % | 4 % |
| Cholesterol ester | 2 % | 12 % | 25 % | 43 % | 17 % |
| Phospholipid | 7 % | 18 % | 22 % | 22 % | 30 % |
| Protein | 2 % | 8 % | 15 % | 20 % | 45 % |
| Type | B-48, A, C, E | B-100, C, E | B-100, E | B-100 | A, C, E, D |

**Chylomicrons** serve to transport triacylglycerols and cholesterol esters from the intestines to other tissues and eventually to the liver. Chylomicrons are synthesized in intestinal epithelial cells. They are the largest and least dense of the blood lipoproteins because they have the most triacylglycerol (about 90 %) and rather low content of protein. Their triacylglycerols and cholesterol are derived from the dietary lipids, and their major protein is apo B-48.

Chylomicrons enter the lymphatic system and travel through the lymph into the blood. Additional apoproteins (apo CII and apo E) are transferred to nascent chylomicrons from HDL, and mature chylomictons are formed. In capillaries of peripheral tissues, particularly adipose and muscle, chylomicrons become the target for *lipoprotein lipase*. Lipoprotein lipase is activated by apo CII and hydrolyzes triacylglycerols to fatty acids and glycerol. Fatty acids are taken up and used by peripheral cells. As chylomicrons are degraded, chylomicron remnants are formed. Remnants interact with apo E receptors of hepatocytes, undergo endocytosis and lysosomal degradation.

**VLDL**, **IDL** and **LDL** form a group of related particles that deliver endogenous triacylglycerols and cholesterol from the liver to the peripheral tissues. **VLDL** is synthesized in the liver. Its' core is formed from triacylglycerols (about 55 %) and cholesterol esters. The phospholipids, cholesterol and apoprotein apo B-100 form the coat. VLDL particles are released into the blood and get additional apoproteins (apo CII and apo E) from HDL. In peripheral tissues, VLDL triacylglycerols are

hydrolyzed by *lipoprotein lipase*, and VLDL is converted to **IDL**. Most of IDL return to the liver, bind to apo E receptors, undergo endocytosis and lysosomal degradation. About 25 % of IDL in liver sinusoids are acted on by *hepatic lipase*. Further hydrolysis of triacylglycerols and removal of additional apoproteins results in **LDL** formation. LDLs react with apo B-100 receptors (LDL-receptors) on the cells of various tissues, are taken up by endocytosis and decomposed in lysosomes.

LDL is regarded as the vehicle delivering cholesterol to the peripheral tissues. Three regulatory mechanisms are used by cells to prevent excessive accumulation of cholesterol:

1. Cholesterol inhibits *HMG-CoA reductase*, the key enzyme of *de novo* cholesterol biosynthesis.

2. Cholesterol activates *acyl-CoA:cholesterol acyltransferase* (*ACAT*), which converts free cholesterol to cholesterol ester (cholesterol storage form).

3. Cholesterol inhibits synthesis of LDL-receptors thus reduces the amount of cholesterol taken up by the cell from the blood.

**The removal of modified LDLs** from the bloodstream occurs due to macrophages **through** their **scavenger receptors**. Scavenger receptors are not down-regulated by cholesterol, and unlimited uptake of LDL particles transforms macrophages to the **"foam cells"**. The accumulation and death of such foam cells in the intima of arteries cause **atherosclerotic plaque** formation, with cholesterol being its chief chemical constituent. Therefore, LDLs are referred to as "bad cholesterol", and prolonged elevation of LDL levels followed by their oxidative and other modifications lead to atherosclerosis.

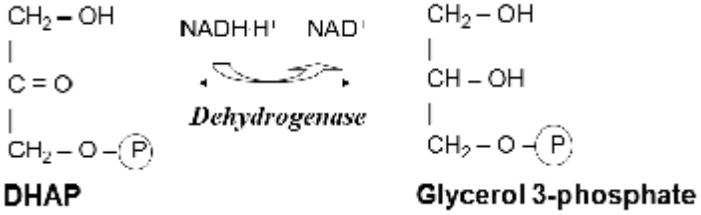
**HDL** is synthesized by the liver and released into the blood as disc-shaped particles. The major constituents of nascent HDL are phospholipids and proteins (apo A, C, D, E). HDLs participate in metabolism of chylomicrons and VLDLs providing apoproteins required for their metabolism. Besides, HDL takes cholesterol from the peripheral tissues and transports it back to the liver. Excessive cholesterol from the cells may be passed to HDL, converted to cholesterol ester by *lecithin:cholesterol acyltransferase* (*LCAT*) and shifted to the interior of the particle. LCAT is activated by apo A1. As cholesterol esters accumulate in the core of the lipoprotein, the particle becomes spherical (mature HDL). With the aid of apo D cholesterol esters partly may be transferred to the chylomicron remnants, IDL or other lipoproteins. Finally HDLs get unloaded of cholesterol in the liver, where it may be converted to the bile acids and secreted into the bile. Therefore, HDLs are often referred to as "good cholesterol" because they function to deliver cholesterol from peripheral tissues to the liver and help to lower total serum cholesterol.

## FATTY ACID METABOLISM

In the cells fatty acids mostly are used for 1) synthesis of structural lipids (phospholipids, glycolipids); 2) energy production; 3) storage as triacylglycerols.

## LIPID STORAGE AND MOBILIZATION IN THE ADIPOSE TISSUE

Lipids are stored in the adipose tissue as **triacylglycerols**. Synthesis of triacylglycerols in adipocytes occurs by the **glycerophosphate pathway**, which requires activated fatty acids (fatty acyl-CoA) and glycerol 3-phosphate. Since adipose tissue lacks glycerol kinase, dihydroxyacetone phosphate (DHAP, intermediate of glycolysis) serves as a source of glycerol moiety:




**Lipogenesis** in the adipose tissue **is stimulated in the well-fed state**, and is strongly favoured by high-carbohydrate diet. Insulin causes activation of the key regulatory enzymes of the pathways, involved in lipogenesis (glycolysis, oxidative decarboxylation of pyruvate, pentose phosphate

pathway, biosynthesis of fatty acids), as well as stimulates glucose and fatty acid delivery from the blood to adipocytes (figure 5.11).

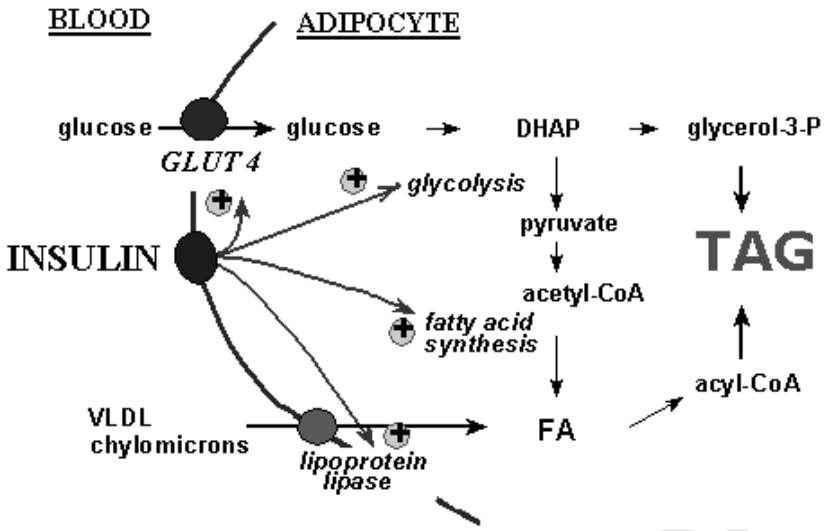



*Fig. 5.11.* Effects of insulin on lipogenesis

In the fasting state, **lipolysis** occurs in the adipose tissue. ***Hormone-sensitive lipase*** hydrolyzes triacylglycerols, free fatty acids and glycerol are released. Fatty acids are carried on albumins through the blood to tissues such as muscle, liver or kidney, where they are oxidized for energy. Glycerol can be used by the liver as a source of carbon for gluconeogenesis.

*Hormone-sensitive lipase* becomes active being **phosphorylated** by the mechanism that involves adenilate cyclase, cAMP and protein kinase A. Glucagon, epinephrine, and other hormones cause activation of adenilate cyclase, and thus promote lipolysis under different conditions.

### β-OXIDATION OF FATTY ACIDS

The major pathway of fatty acid catabolism in humans is β-oxidation in mitochondria. Prior to oxidation, fatty acids are activated, forming fatty acyl-CoA. Long-chain acyl-CoA, formed in the cytosol, cannot pass through the inner mitochondrial membrane. Fatty acyl-CoA is transported to mitochondria via a special transfer system — ***carnitine-acyltransferase*** (***CAT***) (figure 5.12). In the outer mitochondrial membrane, acyl-CoA reacts with carnitine, forming acyl-carnitine, which passes through the translocase, located in the inner mitochondrial membrane, and re-forms acyl-CoA on the matrix side. Thus acyl-CoA gains access to the mitochondrial system of β-oxidation enzymes.

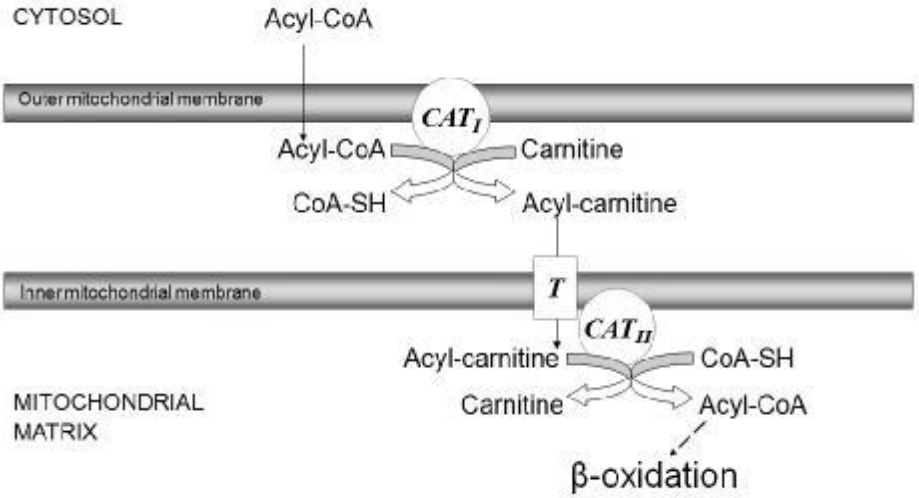



*Fig. 5.12.* Transport of fatty acids to mitochondria through carnithine acyltransferase (CAT)

The outer membrane contains $CAT_I$, the inner membrane contains translocase (T) and $CAT_{II}$

Oxidation represents a spiral metabolic pathway, each turn of the spiral consists of four sequential steps (figure 5.13):

1. Acyl-CoA is oxidized by ***acyl-CoA dehydrogenase***, **FAD** accepts hydrogens. $FADH_2$ interacts with the respiratory chain, generation of 1,5 ATP follows. A double bond is formed between the α- and β-carbons, and enoyl-CoA is produced.

2. $H_2O$ is added across the double bond by ***enoyl-CoA hydratase***, and β-hydroxyacyl-CoA is formed.

3. β-Hydroxyacyl-CoA is oxidized to form β-ketoacyl-CoA by ***β-hydroxyacyl-CoA dehydrogenase***, **$NAD^+$** serves as acceptor of hydrogens. $NADH+H^+$ interacts with the complex I of the respiratory chain, resulting in generation of 2,5 ATP.

4. The bond between α- and β-carbons in ketoacyl-CoA is cleaved by ***thiolase***, coenzyme A is added to the site of cleavage. Two carbons are removed as acetyl-CoA, therefore the remaining acyl-CoA is two-carbons shorter than the original one.

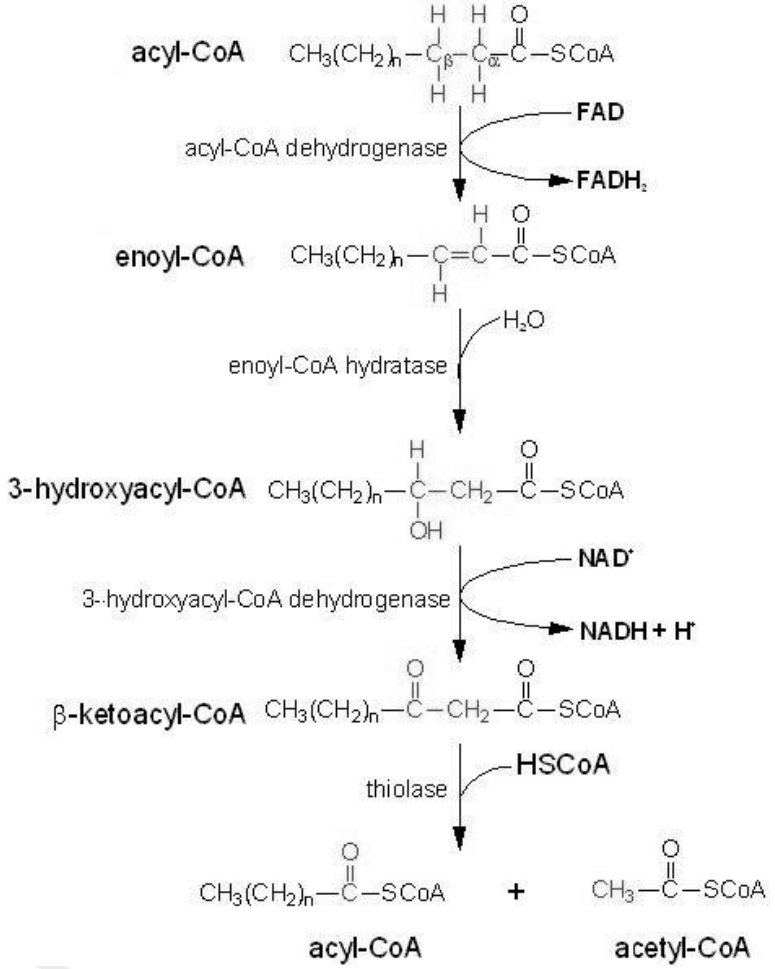



*Fig. 5.13* Reactions of β-oxidation

The shortened acyl-CoA repeats these four steps. In this way, a long-chain fatty acid may be degraded completely to acetyl-CoA (two-carbon units). Further oxidation of acetyl-moieties to $CO_2$ occurs in the TCA cycle.

**The pathway produces a large amount of ATP**

For example, the complete oxidation of palmitic acid (C16) yields 106 ATP:

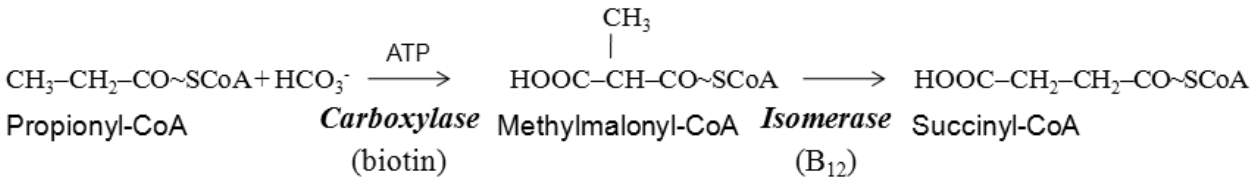
80 ATP are produced as a result of oxidation of 8 acetyl-CoA in the TCA cycle (each acetyl-CoA gives rise to 10 ATP);

28 ATP are produced as a result of electron transport from $FADH_2$ and $NADH+H^+$ via the respiratory chain (reduced coenzymes are generated in each turn of β-oxidation, producing 4 ATP; palmitate will be broken down during 7 turns);

2 ATP must be subtracted from the total amount of 108 ATP — that is the cost of the initial activation of the fatty acid.

**β-Oxidation of the odd-chain fatty acids**

The last cleavage of the odd-chain fatty acids results in the formation of a three carbon residue — propionyl-CoA. This compound is converted to succinyl-CoA, an intermediate of the TCA cycle:




**β-Oxidation of the unsaturated fatty acids**

Oxidation of unsaturated fatty acids requires two additional enzymes, *reductase* and *isomerase*. Following several turns of usual β-oxidation, a double bond in the natural unsaturated fatty acid appears near the carboxyl end of the acyl chain. The double bond will be found between carbons 3 and 4, or between 4 and 5, in *cis*-configuration. This position differs from that formed in enoyl-CoA by the enzymes of β-oxidation (between carbons 2 and 3, in *trans*-configuration). Reduction of the double bond by $NADPH+H^+$ to form saturated acyl-CoA, or isomerization with transfer of the double bond to the position accessible for enzymes of β-oxidation allows the pathway to proceed.

**Regulation of β-oxidation**

Fatty acid oxidation is regulated by the mechanisms that control oxidative phosphorylation — mostly by the demand for ATP. [Acetyl-CoA]/[Acyl-CoA], [NADH]/[$NAD^+$] and [$FADH_2$]/[FAD] ratios are of importance too. *Carnitine-acyltransferase I* located in the outer mitochondrial membrane is inhibited by **malonyl-CoA**, an intermediate in fatty acid synthesis. Therefore, when fatty acids are being synthesized in the cytosol, malonyl-CoA inhibits their transport into mitochondria, and, thus, prevents their immediate degradation.

## MINOR PATHWAYS OF FATTY ACID OXIDATION

**β-Oxidation in peroxisomes**

Very-long-chain, dicarboxylic and branched fatty acids are oxidized in peroxisomes in the pathway similar to mitochondrial. The process differs in that molecular oxygen is used as acceptor of hydrogens in the first step, and $H_2O_2$ is formed. The shorter-chain fatty acyls that are produced travel to mitochondria, where they undergo final oxidation.

**ω-Oxidation of fatty acids**

The ω (omega)-carbon (the methyl-carbon) of fatty acids may be oxidized to a carboxylic group in the endoplasmic reticulum. Dicarboxylic acids are produced.

**α-Oxidation of fatty acids**

Some branched fatty acids (e. g., phytanic acid, the product of chlorophyll metabolism) cannot be metabolized by β-oxidation because of the additional methyl-group present near the β-carbon. Such acids at first undergo α-oxidation in peroxisomes. ***α-Hydroxylase*** plays a central role in the pathway. Ultimately one carbon (as $CO_2$) is removed from the carboxyl end of the fatty acid. Such modification makes further β-oxidation possible.

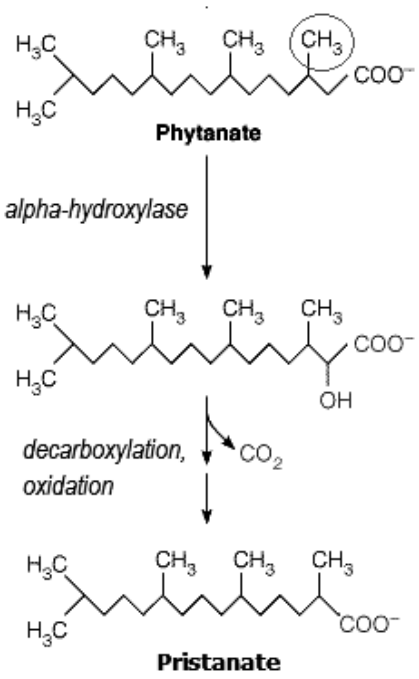



*Fig. 5.14.* α-Oxidation (scheme)

## FATTY ACID BIOSYNTHESIS

**Acetyl-CoA**, derived from glucose or other sources, serves as **precursor for fatty acid biosynthesis**. The pathway occurs in the **cytoplasm**, and acetyl-CoA is carried out of the mitochondrion by a **citrate shuttle** (figure 5.15): acetyl-CoA and oxaloacetate condense to form citrate, which can cross the mitochondrial membrane. In the cytoplasm, *citrate lyase* cleaves citrate to acetyl-CoA (used for biosynthesis of fatty acids) and oxaloacetate (returned to the mitochondria after conversion to malate or pyruvate). Pay attention to cytosolic *$NADP^+$-dependent malate dehydrogenase* (or ***malic enzyme***). Malic enzyme, together with oxidative reactions of pentose phosphate pathway of glucose metabolism, supplies the reactions of fatty acid biosynthesis with reduced equivalents ($NADPH+H^+$).

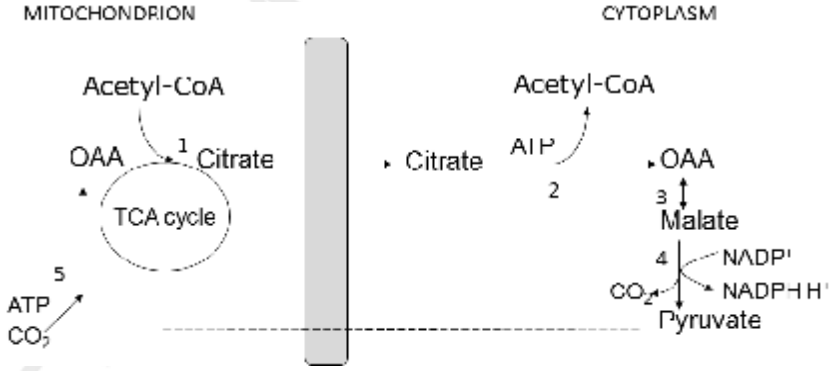
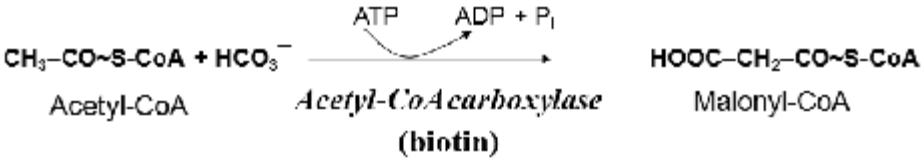



*Fig. 5.15.* Transfer of acetyl-CoA from the mitochondrion to the cytoplasm (citrate shuttle):
1 — citrate synthase; 2 — citrate lyase; 3 — malate dehydrogenase ($NAD^+$); 4 — malic enzyme; 5 — pyruvate carboxylase

Biosynthesis of fatty acids occurs on the **fatty acid synthase complex** — a multienzyme complex that incorporates an **acyl carrier protein (ACP)** and **seven catalytic domains**. An active enzyme is a dimer of two identical subunits. **ACP** contains a phosphopantetheine residue, derived from the vitamin pantothenic acid, as found in coenzyme A, thus, can form a thioester linkage with acyl groups. ACP operates as a "swinging arm" that carries the acyl through the multiple activities of the fatty acid synthase complex.

Initially, acetyl-CoA is converted to **malonyl-CoA** by ***acetyl-CoA carboxylase***. The reaction requires biotin as cofactor and ATP, and is a key reaction of the pathway:




The malonyl-CoA produced in this reaction, along with acetyl-CoA, provide substrates for the fatty acid synthase complex. First, to start reactions on the complex, acetyl and malonyl moieties are passed to ACP, with following transfer of the priming acetyl group to the enzyme cysteinyl residue. Like in fatty acid oxidation, one turn of fatty acid synthesis involves 4 enzymatic activities (figure 5.16):

1. The acetyl group condenses with malonyl-ACP under the action of *β-ketoacyl-ACP synthase*, the $CO_2$ is released, and a β-ketoacyl-ACP, containing four carbons, is produced.
2. The β-keto group is reduced to a hydroxy group by *β-keto-ACP reductase*, NADPH+$H^+$ is a source of hydrogen.
3. *3-hydroxyacyl-ACP dehydratase* cause dehydratation, and enoyl-ACP is produced.
4. Finally, the double bond is reduced by *enoyl-ACP reductase* and NADPH+$H^+$, and a four-carbon acyl group is generated.

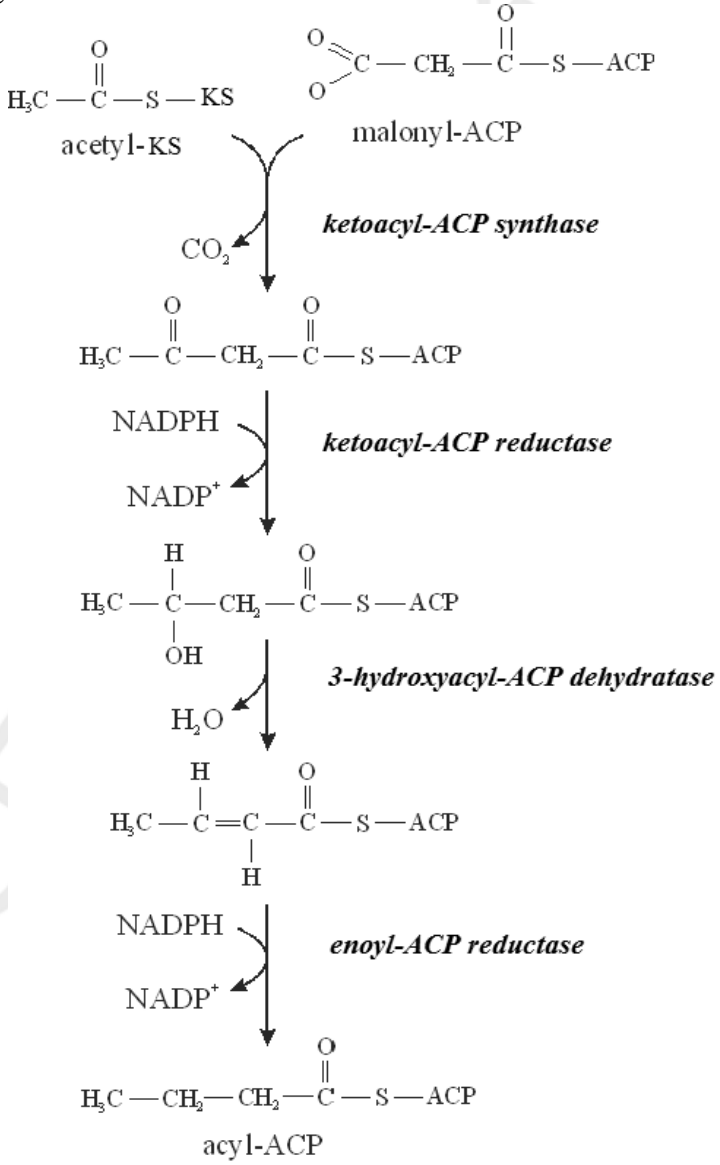



*Fig. 5.16.* Fatty acid biosynthesis. Reactions catalyzed by the fatty acid synthase complex

The two reduction reactions require $NADPH+H^+$. $NADPH+H^+$ is produced by the pentose phosphate pathway of glucose metabolism, and by malic enzyme.

Elongation of the growing fatty acid chain requires binding a new malonyl-CoA molecule to the ACP and repetition of the cycle. Each turn the chain becomes longer by two carbons.

The primary fatty acid synthesized by fatty acyl synthase is palmitate. Palmitate is then liberated from the enzyme, and can undergo separate elongation and/or desaturation to yield other fatty acid molecules.

– Elongation to produce long-chain fatty acids occurs in the endoplasmic reticulum. Malonyl-CoA provides the two-carbon units that are added to fatty acyl, the reactions are similar but not identical to those occurring on the fatty acid synthase complex.

– Desaturation of fatty acids is a complex process that requires O2, $NADPH+H^+$ and a microsomal enzyme system — *acyl-CoA desaturase*. In humans, desaturases may add double bonds at ω-9 position of a fatty acyl-CoA and between ω-9 carbon and the carboxyl group. Double bonds at the positions ω-6 and ω-3 can be produced in plants but not in animals. Therefore, certain unsaturated fatty acids (linoleic, linolenic, arachidonic) are essential components of the human diet. (Synthesis of arachidonic acid may occur from linoleic through the introduction of two additional double bonds and elongation of the chain.)

**Regulation of fatty acid biosynthesis**

The key regulatory enzyme of the pathway is ***acetyl-CoA carboxylase***. It is **activated by dephosphorylation (insulin)** and by **citrate**, **inhibited by phosphorylation (glucagon)** and by **acyl-CoA**. A number of enzymes involved in biosynthesis of fatty acids (citrate lyase, acetyl-CoA carboxylase, enzymes of fatty acid synthase complex) are **induced by insulin**.

## EICOSANOIDS

Physiologically and pharmacologically active compounds derived from C20 polyunsaturated fatty acids, mainly from arachidonic acid, are referred to as **eicosanoids.** Usually they exert paracrine and autocrine effects and act as local hormones. There are three groups of eicosanoids: **prostaglandins (PG), thromboxanes (TX), and leucotriens (LT)**.

Arachidonic acid can be either obtained from the diet, or synthesized in the body from linoleic acid. In the cells arachidonic acid usually is found esterified to position 2 of the glycerol moiety of membrane phospholipids, and can be easily cleaved by phospholipase $A_2$. Phospholipase C pathway also contributes to liberation of arachidonic acid due to subsequent cleavage of produced diacylglycerol (figure 5.17).

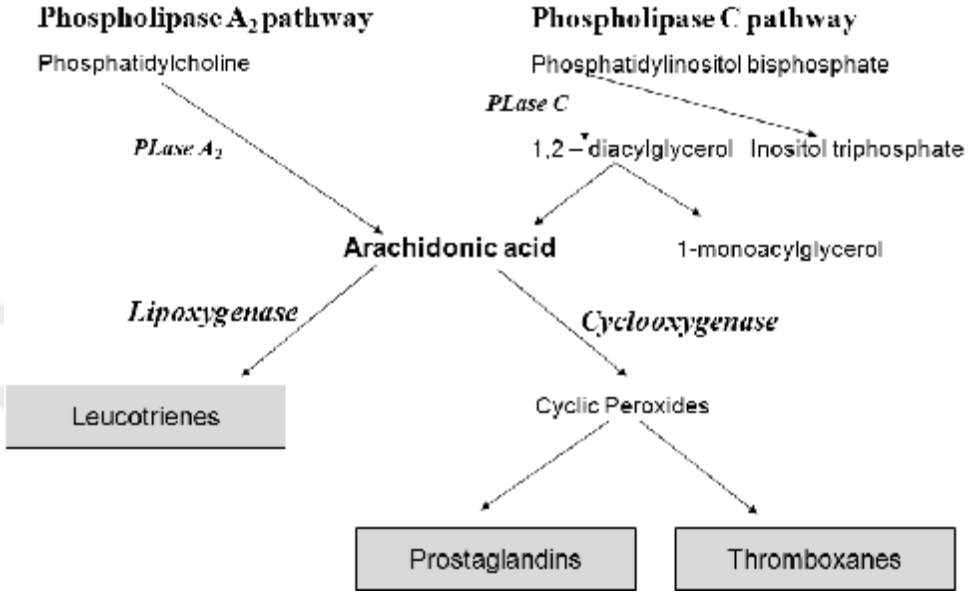



*Fig. 5.17.* Release of arachidonic acid from membrane phospholipids and biosynthesis of eicosanoids

Arachidonic acid is the substrate for the synthesis of prostanoids (prostaglandins and thromboxanes) by the ***cyclooxygenase*** pathway, or for the synthesis of leucotriens by ***lipoxygenase*** pathway. The two pathways compete for the substrate.

**PGs** have multitude of effects that differ from one tissue to another and include inflammation, pain, fever, lipolysis, alterations in vascular contractility and permeability. Certain prostaglandins exert specific effects: $PGI_2$, known as prostacyclin, is produced by vascular endothelial cells and inhibits platelet aggregation and relaxes arteries; $PGF_{2\alpha}$ causes uterine muscle contraction.

**TXs** promote platelet aggregation and vasoconstriction.

**LTs** participate in allergic reactions, inflammation, chemotaxis, cause contraction of smooth muscles in bronchi.

## CHOLESTEROL BIOSYNTHESIS

Cholesterol serves as a crucial component of cell membranes and as precursor to bile acids and steroid hormones. Cholesterol may be obtained from the diet and synthesized *de novo* in most tissues of the body from cytosolic acetyl-CoA.

The pathway can be divided into three principal stages (figure 5.18):

1. Condensation of three acetyl-CoA molecules to form 3-hydroxy3-methylglutaryl-CoA (HMG-CoA) and its reduction to mevalonic acid (C6).
2. Formation of isoprenoid units (C5) and their polymerization to squalene.
3. Cyclization of squalene, formation of lanosterol and its transformation to cholesterol.

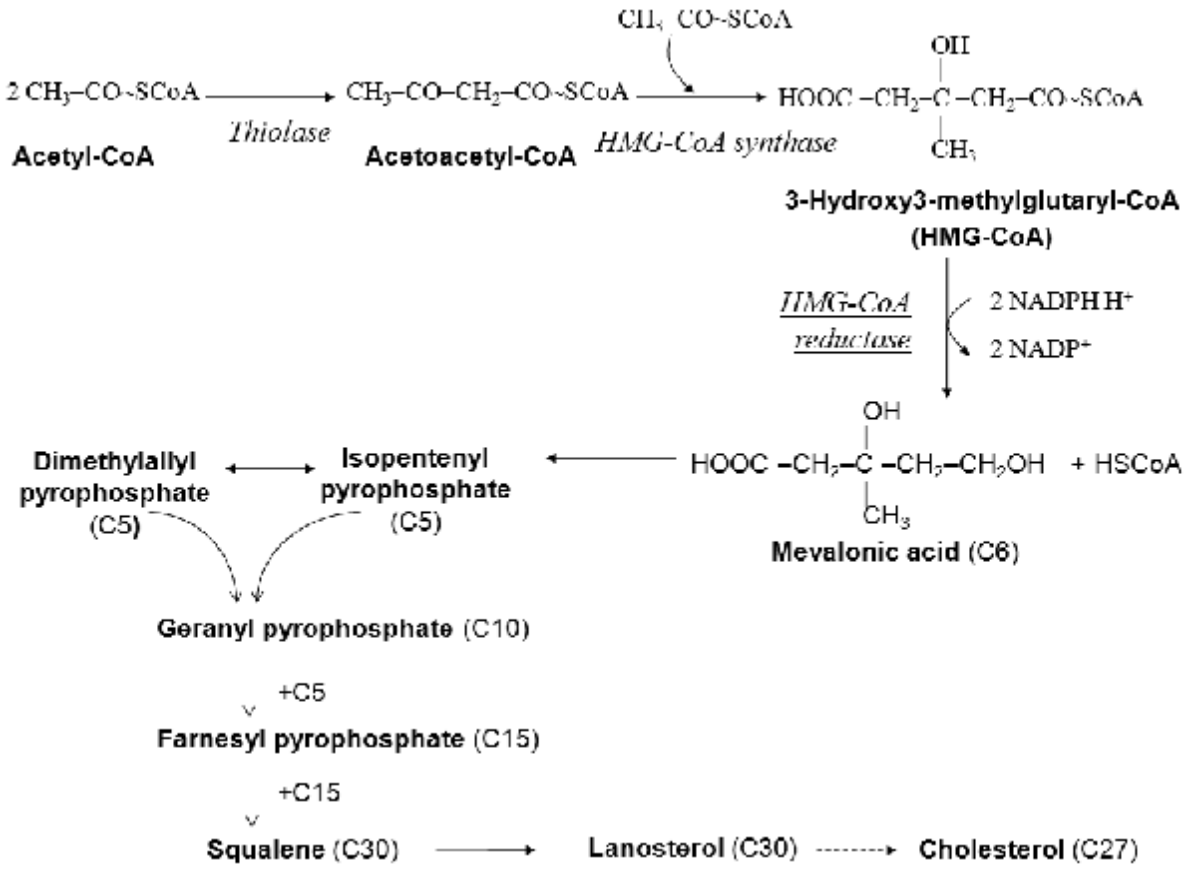



*Fig. 5.18.* Cholesterol biosynthesis

The pathway, as well as fatty acid biosynthesis, requires reductive equivalents in the form of $NADPH{\cdot}H^+$.

The ring structure of cholesterol cannot be degraded in the body. The bile salts excreted in the feces are the major form in which the steroid nucleus is excreted.

**Regulation of cholesterol biosynthesis**

The regulatory step of cholesterol biosynthesis is the reaction catalyzed by ***3-hydroxy3-methylglutaryl-CoA reductase***. *HMG-CoA reductase* is **inhibited by cholesterol** and bile salts in the liver; **induced by insulin**. The enzyme activity can be modulated more rapidly by **phosphorylation** (cause inhibition) and **dephosphorylation** (cause activation). This reaction is the site of action of cholesterol-lowering drugs — **statins**, which are competitive inhibitors of HMG-CoA reductase.

## KETONE BODIES.

Three substances, **acetoacetate**, **β-hydroxybutyrate**, and **acetone**, are collectively known as the **ketone bodies** (figure 5.19). Acetoacetate and 3-hydroxybutyrate are produced by the process of ketogenesis. Acetone is the product of spontaneous (nonenzymatic) decarboxylation of acetoacetate. The concentration of total ketone bodies in the blood does not normally exceed 0,2 mmol/l.

Acetoacetate $CH_3–C(=O)–CH_2–COOH$

β-Hydroxybutyrate $CH_3–CH(OH)–CH_2–COOH$

Acetone $CH_3–C(=O)–CH_3$

*Fig. 5.19.* Ketone bodies

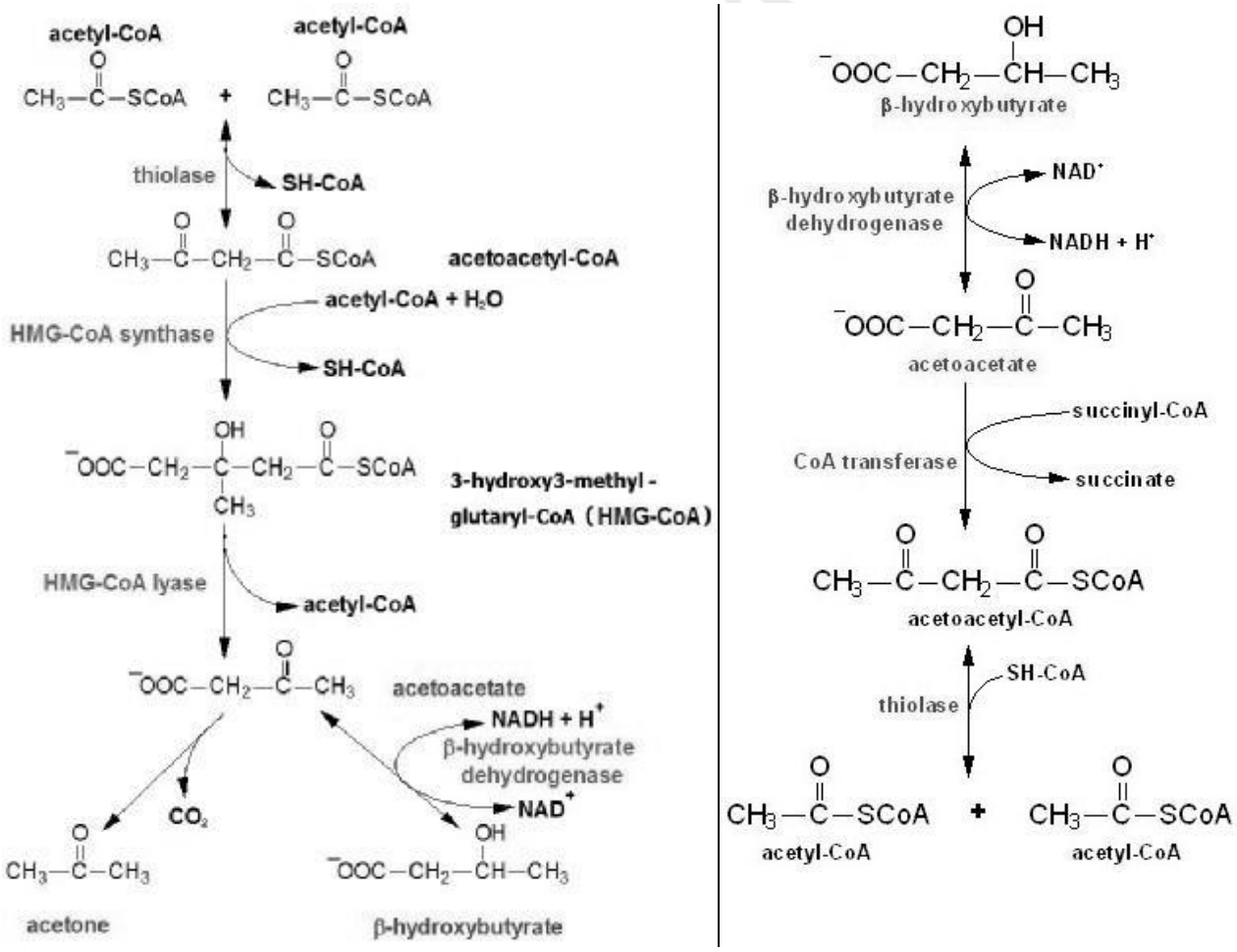



*Fig. 5.20.* Ketone bodies synthesis (A) and utilization (B)

Liver appears to be the single organ to produce ketone bodies and release them to the blood. The **ketogenesis occurs in liver mitochondria from acetyl-CoA** (figure 5.20):

1. Two molecules of acetyl-CoA condense to produce acetoacetyl-CoA. This reaction is catalyzed by *thiolase*.
2. Acetoacetyl-CoA and acetyl-CoA form HMG-CoA in a reaction catalyzed by *HMG-CoA synthase*.
3. HMG-CoA is cleaved by *HMG-CoA lyase* to form acetyl-CoA and acetoacetate.
4. Acetoacetate may be reduced by an $NAD^+$*-dependent dehydrogenase* to β-hydroxybutyrate. The reaction is reversible.
5. Acetoacetate may also undergo spontaneous decarboxylation, forming acetone.

When ketone bodies are released from the liver into the blood, they are taken up by peripheral tissues, such as **muscles** and **kidney**, where they are oxidized for energy. During starvation, the brain also oxidizes ketone bodies. Utilization of ketone bodies requires conversion of β-hydroxybutyrate to acetoacetate in the reversible dehydrogenase reaction, and activation of acetoacetate in the reaction with succinyl-CoA to form acetoacetyl-CoA and succinate (the enzyme is *succinyl-CoA:acetoacetate-CoA transferase*). Then acetoacetyl-CoA is cleaved by thiolase to form two acetyl-CoAs, which enter the TCA cycle and are oxidized to $CO_2$ and $H_2O$.

Ketogenesis increases under metabolic conditions associated with a high level of free fatty acids in the blood and increased rate of fatty acid oxidation, when acetyl-CoA accumulates beyond its capacity to be oxidized.

## CLINICAL ASPECTS

### Malabsorption of fats

Effective fat digestion and absorption requires sufficient secretion of many components present in the bile and pancreatic juice. Blockage of the bile duct by gallstones or duodenal or pancreatic tumors can lead to an inadequate concentration of bile salts in the intestine. Certain diseases affecting pancreas can lead to a decrease in bicarbonate and digestive enzymes in the intestinal lumen. In both types of disorders, digestion and absorption of dietary lipids is diminished, and steatorrhea may result. Malabsorption of fats leads to caloric deficiency, lack of fat-soluble vitamins and essential fatty acids.

### Fatty liver

Fatty liver is a result of fat accumulation in hepatocytes. It is considered to be a reversible condition, however, it can progress to cirrhosis and liver failure. Lipids — mainly triacylglycerols — accumulate in the liver if the production and secretion of VLDL particles do not meet the body requirements. This may occur either due to increased lipogenesis, or because of insufficient synthesis of phospholipids and/or protein for VLDL coating. Lipotropic factors (choline, methionine, inositol, essential fatty acids, vitamins $B_6$, $B_9$, $B_{12}$) enhance phospholipid biosynthesis, prevent triacylglycerol accumulation in the liver and aid in recovery.

### Cholelithiasis

Bile salts are responsible for maintaining the substances which are poorly soluble in water (cholesterol, bilirubin) in the solubilized state in the bile. As a result of bile acids deficiency, these substances may precipitate in the bile and form cholesterol (or bilirubin, or mixed) gallstones. This may occur due to the defective reabsorption of bile acids in the intestines, or impaired liver function. Decreased bile evacuation (cholestasis) also contributes to gallstones formation.

### Familial hypercholesterolemia

Familial hypercholesterolemia (FH) is a hereditary disease caused by mutations affecting LDL receptor. Cells from FH individuals have an impaired ability to take up cholesterol via receptor mediated endocytosis. The result of these mutations is a higher than normal level of serum

LDL and cholesterol. High LDL levels favor oxidation of their components and ultimately formation of atherosclerotic plaques. Individuals who are homozygous for the disease have very high levels of cholesterol in the blood and usually die of heart disease before age 20. People heterozygous for the disease have higher than normal cholesterol levels and are at high risk for heart attacks and cerebral infarcts.

**Atherosclerosis contributing factors and treatment strategies**

The incidence of atherosclerosis and coronary heart disease correlates with the serum total cholesterol and LDL cholesterol, and is in inverse relationship with HDL concentrations. The major factors affecting the blood lipoprotein levels are heredity, age, gender, nutritional and life style. Regular exercise and the diet rich in fibers, essential fatty acids and other lipotropic factors are effective in lowering plasma LDL and raising HDL. Widely used cholesterol-lowering drugs inhibit HMG-CoA reductase and decrease endogenous cholesterol biosynthesis (statins), or inhibit the absorption of cholesterol and bile acids in intestines (resins, ezetimibe). Other drugs used (fibrates) mainly decrease plasma triacylglycerols and VLDL.

**Impaired oxidation of fatty acids**

Impaired oxidation of fatty acids may occur owing to carnitine deficiency, carnitine-acyltransferase (CAT) deficiency, treatment with sulfonylurea drugs (inhibit $CAT_I$), inherited defects or inhibition of the β-oxidation enzymes by toxins (e. g., hypoglycin). The major sequences of impaired fatty acid oxidation are hypoglycemia (when severe, cause coma and death), lipid accumulation, and muscle weakness.

**Refsum's disease**

Refsum's disease is a rare inherited disease caused by deficiency of α-hydroxylase (or its peroxisomal receptor). Phytanic acid cannot undergo α-oxidation and accumulates. This leads to neurological manifestations and death in early adulthood.

**Anti-inflammatory drugs**

Many anti-inflammatory drugs interfere in the synthesis of eicosanoids. Aspirin, ibuprofen, indomethacin (non-steroidal anti-inflammatory drugs, NSAID) inhibit cyclooxygenase, aspirin by irreversible covalent acetylation, others noncovalently. These drugs reduce pain, fever, and inflammation associated with the action of prostaglandins; decreased production of thromboxane A2 reduces platelet aggregation and provides antithrombotic effect. Anti-inflammatory effect of corticosteroids is mediated by inhibition of phospholipase $A_2$.

**Respiratory distress syndrome in the newborn**

Lung surfactant is composed mainly of phospholipids and prevents alveoli from collapsing. Dipalmitoylphosphatidylcholine is the major component which decreases surface tension at the air-liquid interface. Since this phospholipid develops in the fetus after week 30 of gestation, premature infants do not have an adequate amount of it. As a result, acute respiratory distress syndrome develops, — a leading cause of morbidity and mortality in premature newborns. Administration of either natural or artificial surfactant is of therapeutic benefit.

**Ketosis**

Syndrome of ketosis includes **ketonemia, ketonuria,** and **the odor of acetone** in the breath. Ketosis is the common metabolic problem in **starvation** and uncontrolled **diabetes mellitus**. Low insulin and high glucagon levels cause adipose tissue to release increased amounts of fatty acids, which are converted to acetyl-CoA, and, subsequently, to ketone bodies by the liver. Prolonged excessive production of ketone bodies by the liver results in **acidosis** that, if not treated rapidly and effectively, may lead to coma and death. A high-fat and low-carbohydrate diet also can lead to increased ketone bodies production.

# CHAPTER 6
# PROTEIN AND AMINO ACID METABOLISM

**Nitrogen** is an essential component of many important biological molecules, and proteins (amino acids) are the main source of this element for humans. **Nitrogen balance** is a generalized characteristic of protein metabolism and can be calculated as the difference between nitrogen intake (with food proteins) and nitrogen excretion (mainly in urine). Healthy adults usually are in **nitrogen equilibrium**. **Positive nitrogen balance** is characterized by the retention of nitrogen in the body, e. g. in pregnancy, growth, recovery from metabolic stress (illness or starvation). **Negative nitrogen balance**, when more nitrogen is lost than is taken in, is observed in senility, certain disease states, starvation, poor or inadequate protein nutrition.

Individual **protein daily requirements** vary greatly depending on the age, gender, and activity level. On average, 0,8–1,0 g of protein per kg of ideal (!) body weight is enough to maintain nitrogen equilibrium. However, it should be kept in mind that under certain conditions (e. g., pregnancy, lactation, intense physical exercise) protein requirements increase. In addition, adequacy of protein nutrition strongly depends on the **amino acid composition** of the dietary proteins, particularly on the contents of **essential amino acids**, since they can't be synthesized in the human body in sufficient amounts. Eight of twenty proteinogenic amino acids are essential: **Leu, Ile, Val, Lys, Met, Phe, Trp, Thr** (+ **Arg, His** for children). Bioavailability and possibility of digestion also influence on the nutritive value of food proteins. Animal proteins (eggs, milk, meat) are the best source of amino acids for humans.

## DIGESTION OF PROTEINS

In the digestive tract dietary proteins are broken down to amino acids, which can be absorbed, by the enzymes called ***proteases*** or ***peptidases.*** *Endopeptidases* attack internal bonds in the proteins and produce smaller peptide fragments, while *exopeptidases* remove terminal amino acids from either carboxyl (*carboxypeptidases*) or amino terminus (*aminopeptidases*).

Peptidases possess **specificity** and hydrolyze peptide bonds formed by a certain type of amino acids.

Peptidases are synthesized and secreted as inactive **zymogens** (pepsinogen, trypsinogen, etc.). Activation of such proenzymes requires **selective proteolysis** of their polypeptide chains by other enzymes, or autocatalytically.

Protein digestion can be divided into a gastric, a pancreatic, and an intestinal phase, depending on the source of peptidases (table 6.1).

*Table 6.1*

**Gastric, pancreatic and intestinal peptidases**

| Source | (Pro)enzyme | Activator |
|---|---|---|
| Gastric juice | Pepsin(ogen) | HCl, pepsin |
| Pancreatic juice | Trypsin(ogen)<br>Chymotrypsin(ogen)<br>(Pro)elastase<br>(Pro)carboxypeptidases A and B | Enteropeptidase, trypsin<br>Trypsin<br>Trypsin<br>Trypsin |
| Intestinal cells | Aminopeptidases, dipeptidases | |

*Gastric phase*. Essential components of gastric juice involved in protein digestion are HCl and pepsinogen, precursor of pepsin.

**HCl** serves to kill off microorganisms, to denature proteins and make them more susceptible to proteases, to induce activation of pepsinogen and create strong acidic environment, which is optimal for pepsin.

Active **pepsin** is generated from pepsinogen at pH below 5 (spontaneously) and by the action of pepsin. Pepsin is the endopeptidase, and cleaves proteins mainly into large peptide fragments.

It has rather broad specificity with a preference for peptide bonds formed by the large hydrophobic (Leu), aromatic amino acids (Phe, Tyr, Trp), and, to less extent, Glu.

*Pancreatic phase*. Pancreatic juice is a source of endopeptidases (**trypsin, chymotrypsin, elastase**) and exopeptidases (**carboxypeptidases A and B**). All of these enzymes are released to duodenum in the inactive form. *Enteropeptidase*, a protease produced by duodenal epithelial cells, activates trypsinogen to trypsin. Trypsin activates more trypsinogen (autocatalysis) and also acts on the other proenzymes, producing active chymotrypsin, elastase, and carboxypeptidases.

– Trypsin hydrolyzes peptide bonds formed by carboxyls of basic amino acids (Lys, Arg).

– Chymotrypsin hydrolyzes peptide bonds formed by carboxyls of aromatic amino acids (Tyr, Trp, Phe).

– Elastase hydrolyzes peptide bonds formed by carboxyls of small aliphatic amino acids (Gly, Ala, Ser).

– Carboxypeptidase A cleaves C-terminal neutral amino acids.

– Carboxypeptidase B cleaves C-terminal basic amino acids.

The combined action of pancreatic enzymes in small intestines results in the formation of free amino acids and small peptides of 2–8 amino acids.

*Intestinal phase.* Final digestion of oligopeptides depends on enzymes of the small intestine (**aminopeptidases** and **dipeptidases**). Final break down occurs near the luminal surface of enterocytes (brush border). The enzymes produce free amino acids, which can be absorbed via specific transport systems (figure 6.1).

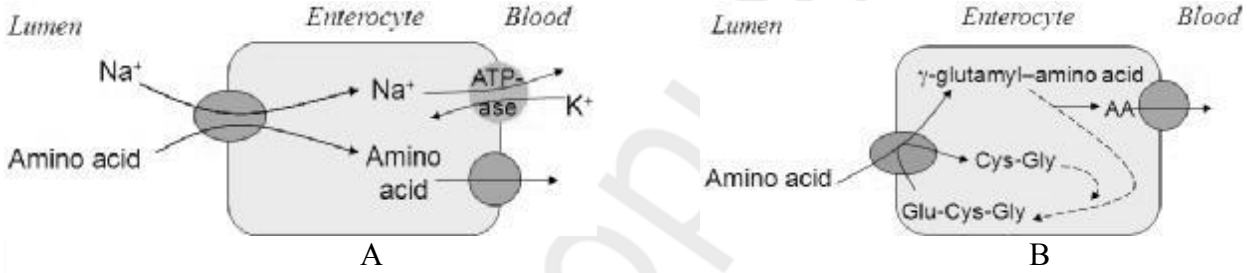



*Fig. 6.1.* Amino acid absorption via A) $Na^+$-dependent transport; B) glutathione-dependent transport (γ-glutamyl cycle). Glutathione = Glu-Cys-Gly

Evidently, total hydrolysis of dietary proteins requires a whole range of peptidases.

In many developed countries the daily intake of proteins exceeds real requirements. Excess amino acids cannot be effectively absorbed. Their decay caused by bacteria in the colon is known as "**protein putrefaction**" and results in generation of **toxic intermediates** (phenol, scatol, indole, putrescine, etc.). Such xenobiotics usually undergo detoxification in the liver by **conjugation** either **with UDP-glucuronic acid** (see "Uronic acid pathway", chapter 4) or **with phosphoadenosine-phosphosulfate** (active sulfate, PAPS). Non-toxic conjugates leave the body with urine.

## AMINO ACID POOL

Sources of amino acids and pathways of their utilization in cells are summarized in the figure 6.2.

Figure 6.3 shows the major nitrogen-free intermediates, which are produced from amino acids. According to the ways of carbon skeleton utilization, amino acids can be classified as follows:

**Glucogenic** amino acids give rise to the intermediates of carbohydrate metabolism, such as pyruvate, fumarate, oxaloacetate, α-ketoglutarate, succinyl-CoA. These products may be converted to glucose by gluconeogenesis. All non-essential amino acids are glucogenic in character.

**Ketogenic** amino acids give rise to the intermediates, which cannot be used for gluconeogenesis, such as acetyl-CoA and acetoacetate. These products are switched over fat metabolism and ketone bodies production. Lys and Leu are ketogenic.

Some amino acids (Ile, Phe, Tyr, Thr, Trp) are both glucogenic and ketogenic.

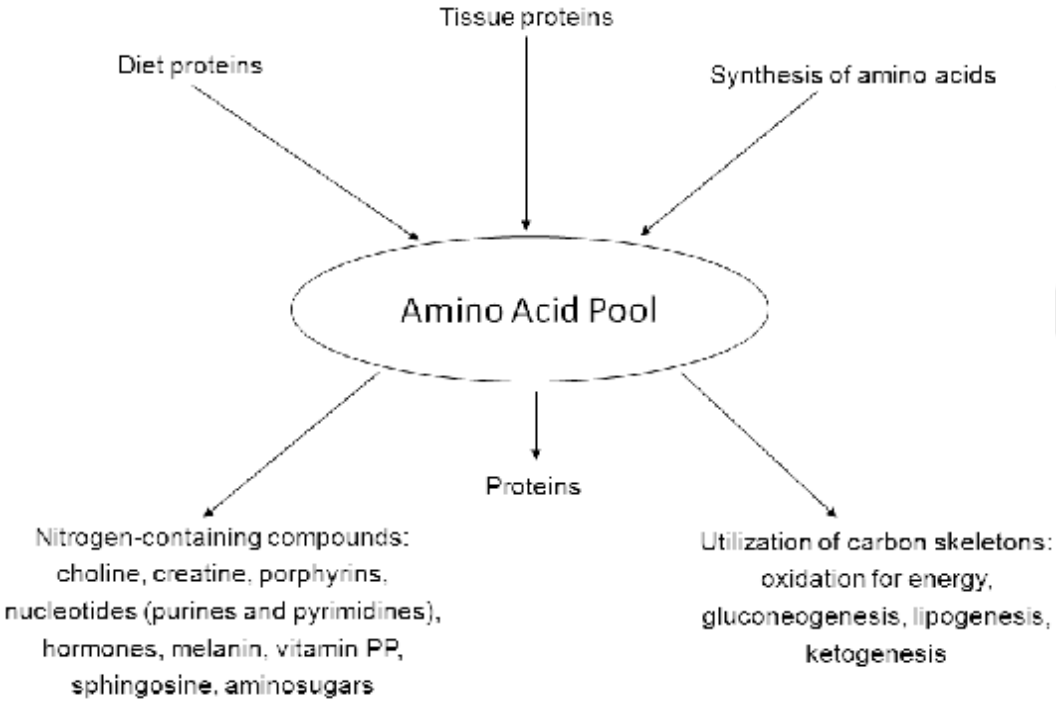



*Fig. 6.2.* Amino acid pool, sources of renewal and pathways of utilization

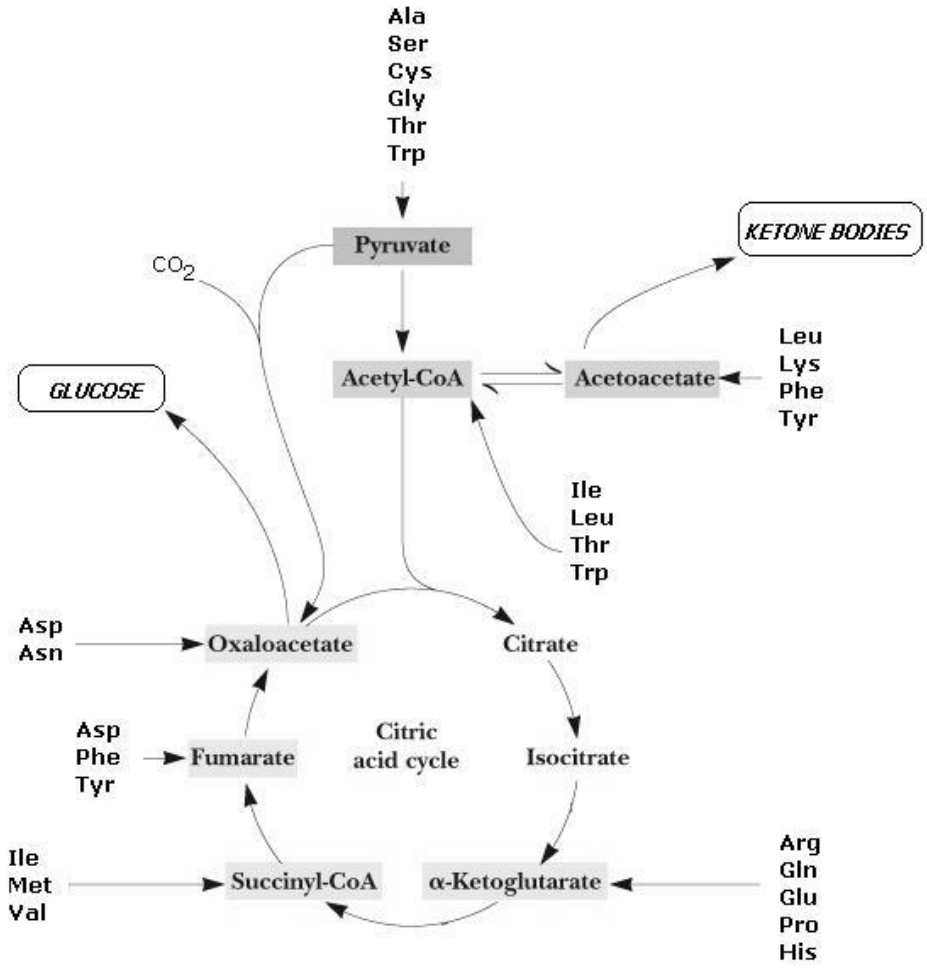



*Fig. 6.3.* Common products of amino acid degradation

## TRANSAMINATION

**Transamination** involves transfer of α-amino group from an amino acid to the α-keto position of an α-keto acid. In the process, the amino donor becomes an α-keto acid while the α-keto acceptor becomes an α-amino acid (figure 6.3). Transamination reactions are catalyzed by ***aminotransferases*** (the preferred name for enzymes formerly termed *transaminases*). A required coenzyme — **pyridoxal phosphate** — is derived from vitamin $B_6$.

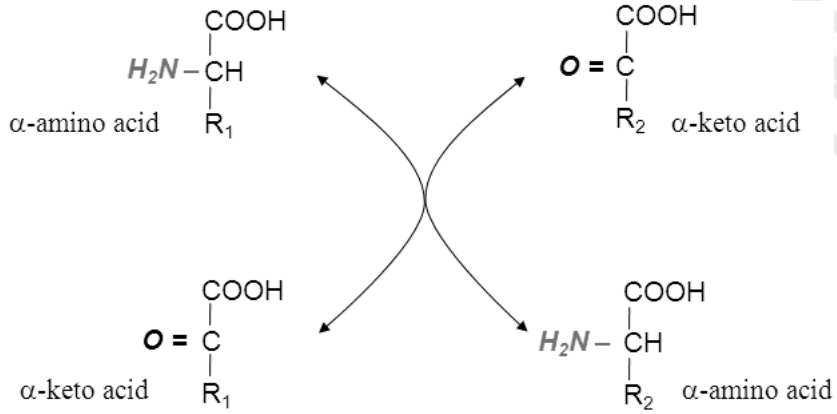



*Fig. 6.3*. Transamination

Aminotransferases are named according to their amino acid substrates and use α-ketoglutarate as the predominant acceptor of amino group. So, glutamate/α-ketoglutarate is one of the two amino/keto acid pairs involved in transamination (see examples in figure 6.4). The reactions are readily reversible, and the direction in which reaction proceeds in large part is a function of the intracellular concentrations of the reactants. This means that transamination can be used not only for **amino acid synthesis**, but also for **degradation of amino acids** that accumulate in excess of need. Transamination involving essential amino acids is normally unidirectional since the body cannot synthesize the equivalent α-keto acid.

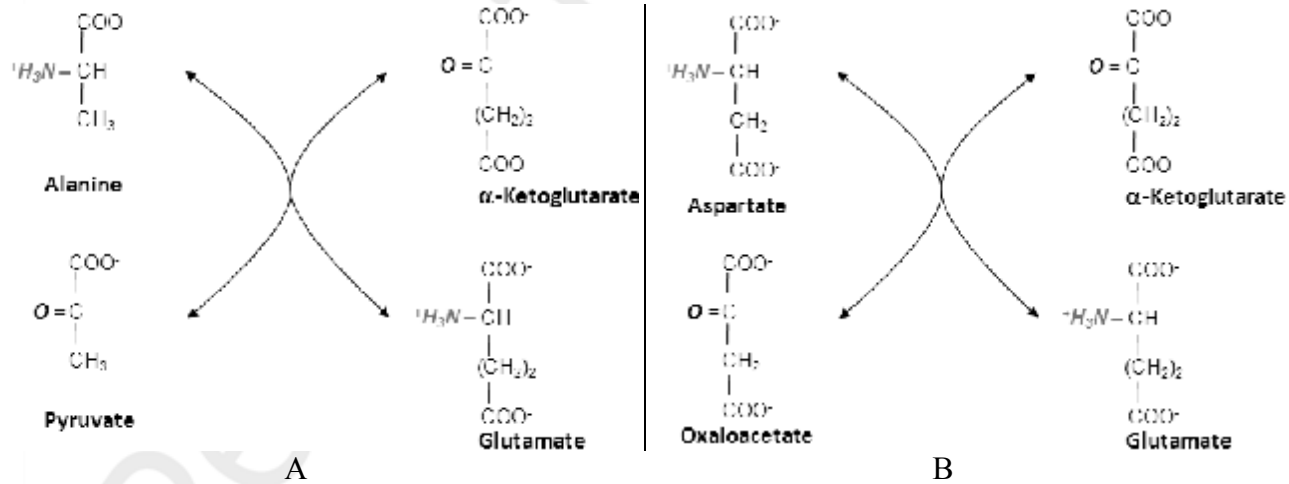



*Fig. 6.4.* Reactions catalyzed by *alanine aminotransferase* (A) and *aspartate aminotransferase* (B)

Usually transamination is the primary step of amino acid metabolism. The produced keto acids may be oxidized for energy, and provide an important link between the protein and carbohydrate or fat metabolism.

Tissue distribution of some of the aminotransferases is used diagnostically (see "Clinical Aspects").

**DEAMINATION**

**Deamination** results in the removal of the amino group as ammonia ($NH_3$). Figure 6.5 shows the existing principal types of amino acid deamination.

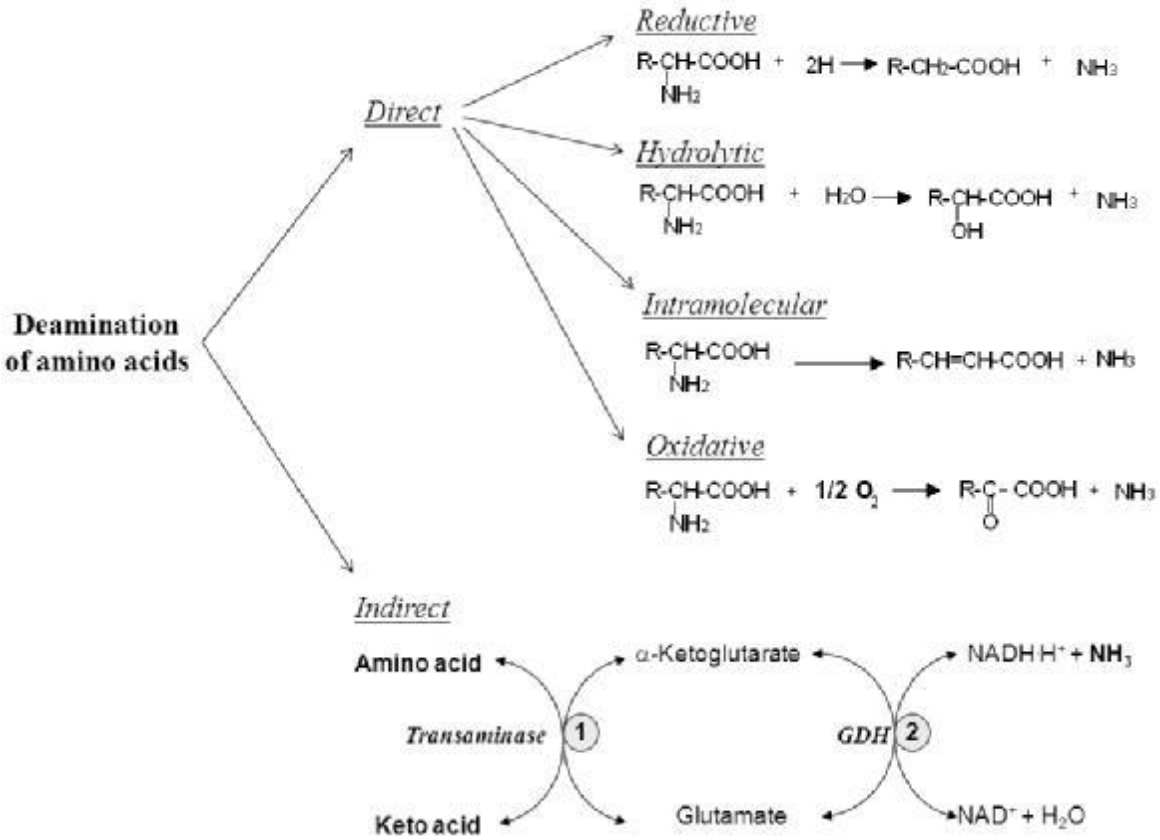
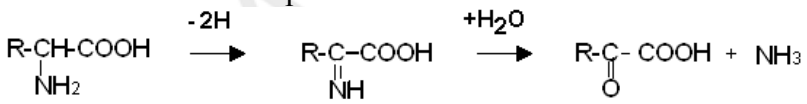



*Fig. 6.5.* Deamination of amino acids. GDH — glutamate dehydrogenase

In humans predominant types of amino acid deamination are oxidative (direct) and indirect.

In **oxidative deamination** amino acid is converted to the respective keto acid when ammonia is liberated. The process involves two steps:




The first step is an enzymatic one and leads to the formation of imino acid; the second step proceeds spontaneously, imino acid is cleaved in the presence of water into ammonia and α-keto acid.

Two types of enzymes may oxidize amino acids in the cells during the first step:

1. ***Oxidases***, which contain FMN or FAD as a prosthetic group. Oxidases are specific to L- and D-amino acids. The hydrogens, removed by amino acid oxidase, eventually are transferred to oxygen, and $H_2O_2$ is produced. The significance of amino acid oxidases in human metabolism seems to be small.

2. ***L-glutamate dehydrogenase***, which can use $NAD^+$ or $NADP^+$ as coenzymes. In the reaction, ammonia is liberated, glutamate is converted to α-ketoglutarate, and $NAD^+$ serves as the acceptor of hydrogens (figure 6.6). The reverse reaction (reductive amination) also may occur; in such synthetic process glutamate dehydrogenase utilizes $NADPH+H^+$.

Reaction catalyzed by glutamate dehydrogenase **occupies a central place in amino acid metabolism**:

– Glutamate collects nitrogen from other amino acids by means of transamination, and this nitrogen may be easily released as $NH_3$ by glutamate dehydrogenase. The process is often termed "**transdeamination**", or **indirect deamination** (figure 6.5), and most of amino acids are deaminated in such manner.

– The reaction connects the amino acid metabolism with TCA cycle (via α-ketoglutarate) and respiratory chain (via NADH+$H^+$).

The reverse reaction solves two problems: synthesis of non-essential amino acids and fixation of toxic ammonia in tissues.

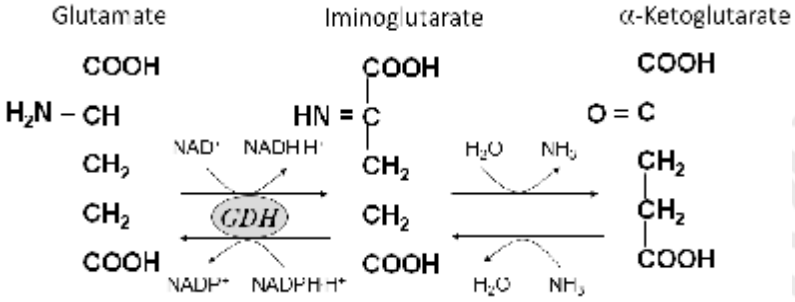



*Fig. 6.6*. Glutamate dehydrogenase reaction

**DECARBOXYLATION OF AMINO ACIDS**

Biologically active molecules termed **biogenic amines** are produced by decarboxylation of amino acids. *Amino acid decarboxylases* use **pyridoxal phosphate** as a coenzyme.

**γ-Aminobutyric acid (GABA),** an inhibitory neurotransmitter, is produced by α-decarboxylation of glutamate.

**Histamine** is produced by decarboxylation of histidine. Histamine causes vasodilatation and increased vessel permeability, bronchoconstriction, in the stomach stimulates the secretion of HCl.

**Serotonin** is produced by decarboxylation of 5-hydroxy-tryptophan. Serotonin plays multiple roles in the nervous system, including neurotransmission. It is a precursor to melatonin in the pineal gland. In the intestine, serotonin regulates intestinal peristalsis. It is also a potent vasoconstrictor and stimulator of platelet aggregation.

**Catecholamines (dopamine, norepinephrine, epinephrine**) are derived from tyrosine as shown in figure 6.7. Catecholamines participate in regulation of blood pressure, cause vasoconstriction, act as neurotransmitters, influence on the metabolic processes (e. g., lipolysis, glycogenolysis).

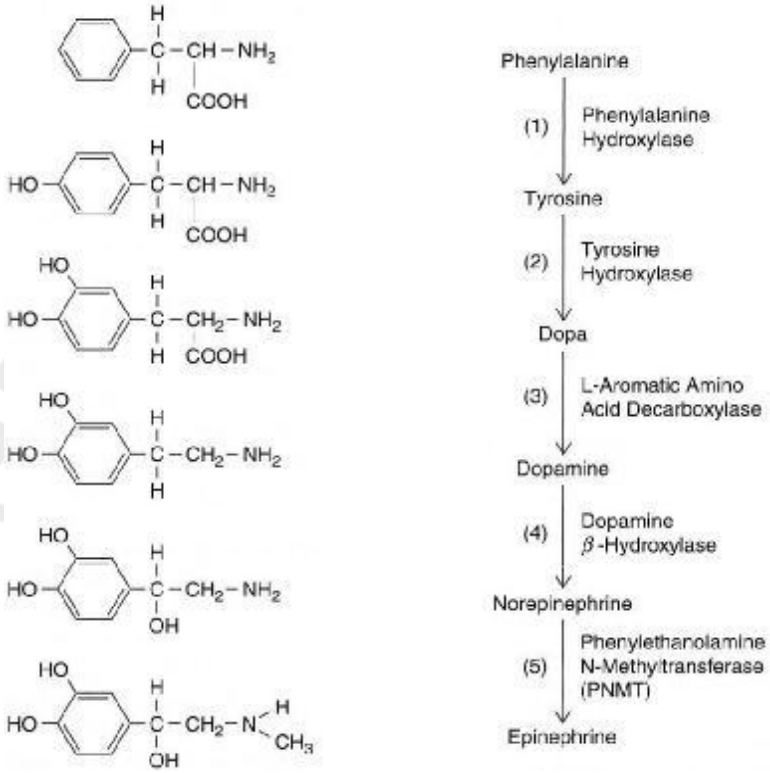



*Fig. 6.7.* Synthesis of catecholamines

Catecholamines are inactivated by *monoamine oxidase* (MAO) and *catecholamine O-methyltransferase* (COMT).

### AMMONIA DETOXIFICATION

Essential amounts of ammonia are produced from different nitrogen-containing compounds, mostly from amino acids, by intestinal bacteria and by tissues. Ammonia is very toxic, particularly affecting the central nervous system, therefore should be rapidly removed from circulation. Symptoms of ammonia intoxication include tremor, slurred speech, blurred vision, coma, and ultimately death. Normally only traces of ammonia are present in peripheral blood due to the existing efficient mechanisms of its detoxification:

– *in extrahepatic tissues*: synthesis of temporal nontoxic transport forms, which deliver ammonia to the liver (for final detoxification), or to kidneys (for excretion as $NH_4^+$);

– *in the liver*: synthesis of urea.

Two reactions may help **to fix ammonia in peripheral tissues**: 1) **reductive amination** of α-ketoglutarate catalyzed by *glutamate dehydrogenase*; 2) **synthesis of glutamine** from glutamate catalyzed by *glutamine synthetase* (figure 6.8). Formation of glutamine is coupled to hydrolysis of ATP to ADP and $P_i$.

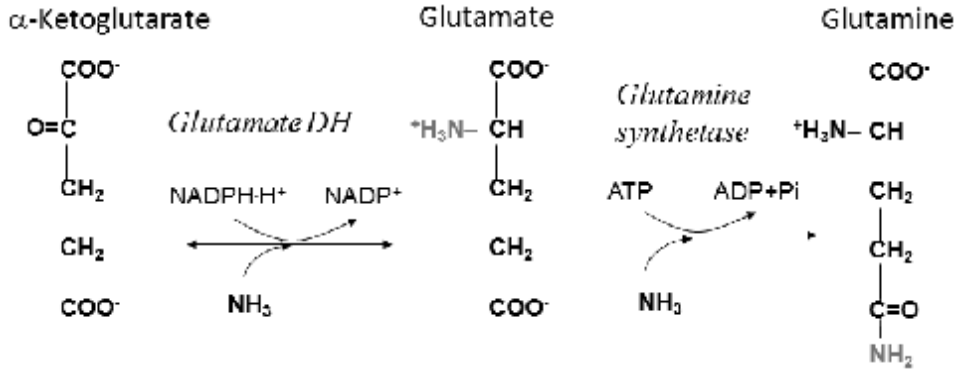



*Fig. 6.8.* Ammonia fixation in extrahepatic tissues

**Glutamine** is readily formed in peripheral tissues, and is regarded as a main transport form of ammonia. **Alanine** carries ammonia from muscles (figure 6.9).

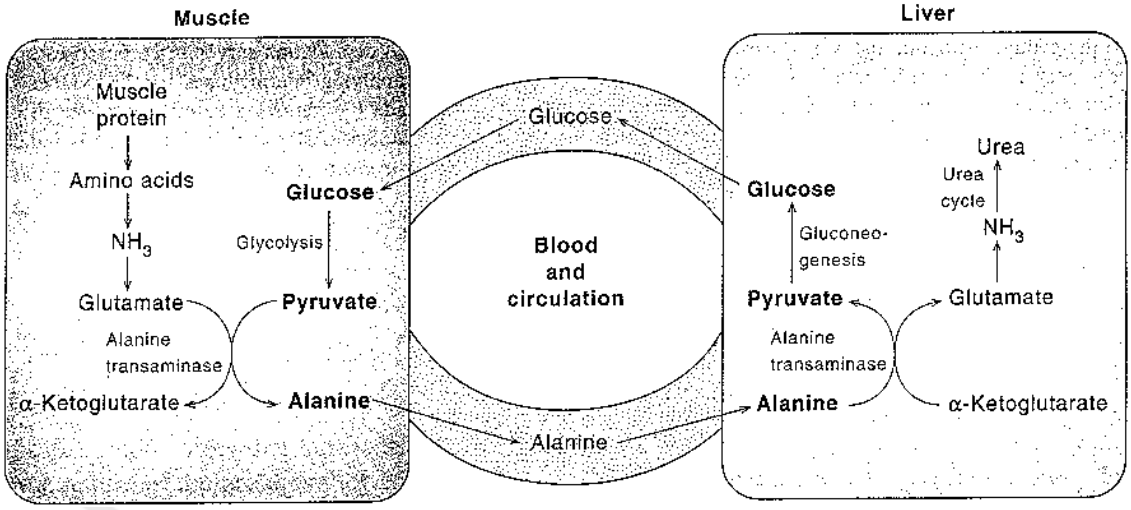



*Fig. 6.9.* Glucose-alanine cycle

**Urea synthesis** is the central pathway of ammonia detoxification. The whole set of enzymes for urea synthesis is found only in the liver. Ammonia and aspartate provide the nitrogen, and $CO_2$ provides the carbon to produce urea. The pathway is often termed "ornithine cycle", since ornithine serves as a carrier that is regenerated in the cycle (figure 6.10).

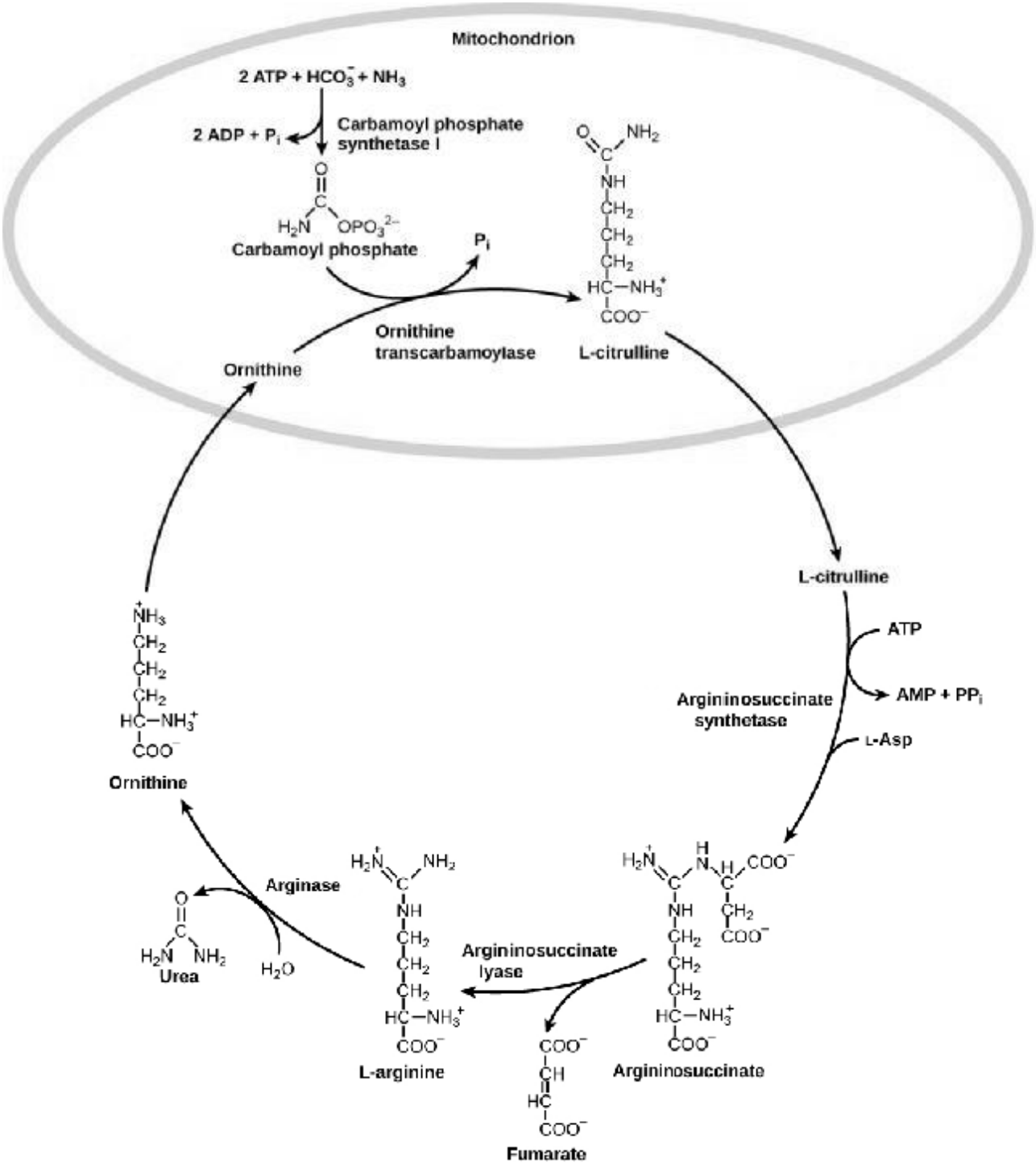



*Fig. 6.10.* The urea synthesis (ornithine cycle)

1. In the first reaction, **carbamoyl phosphate** is synthesized from $NH_3$ and $CO_2$ by *carbamoyl phosphate synthetase I*. Two ATP are utilized.

2. Carbamoyl moiety is transferred from carbamoyl phosphate to ornithine by *ornithine transcarbamoylase*, **citrulline** is formed, inorganic phosphate is released.

3. Aspartate is added to citrulline by *argininosuccinate synthetase*. The reaction is driven by hydrolysis of ATP to AMP and pyrophosphate ($PP_i$), **argininosuccinate** is formed.

4. Argininosuccinate is cleaved to form **arginine** and **fumarate** by *argininosuccinate lyase*. Fumarate may be used for regeneration of aspartate: reactions of TCA cycle (up to oxaloacetate) and transamination will be involved.

5. Arginine is hydrolyzed by *arginase* to form **urea** and regenerate **ornithine**.

Urea is extremely soluble, passes into the blood, and is readily excreted by kidneys. Urea accounts for about 90 % of the nitrogenous excretory products in human.

**Regulation of the urea cycle**

**N-Acetylglutamate** is an allosteric activator of ***carbamoyl phosphate synthetase I***. Synthesis of N-acetylglutamate from acetyl-CoA and glutamate is stimulated by arginine. The high-protein diet consumed for several days, causes induction of the urea cycle enzymes.

## CLINICAL ASPECTS

**Protease inhibitors in treatment of pancreatic diseases**

If inflammatory or other pancreas injuries occur, the proteases may become active within tissue, which results in pancreas autodigestion. Protease inhibitors (contrykal, gordox) are used for treatment.

**Serum aminotransferases**

Some aminotransferases are tissue specific. For instance, the highest activity of *alanine aminotransferase* (ALT) is found in the liver cells, whereas *aspartate aminotransferase* (AST) — in the heart tissue. Increased serum activity of the enzyme is a sign of cell injury: ALT is elevated in hepatitis, AST — in myocardial infarction.

**Inborn defects of amino acid metabolism**

In **phenylketonuria** (PKU), *phenylalanine hydroxylase*, which catalyzes conversion of phenylalanine to tyrosine, is defective. Phenylalanine accumulates and is converted to toxic intermediates such as phenylpyruvate. Mental retardation occurs. Restriction of phenylalanine in the diet is essential to prevent irreversible brain lesion.

In **albinism**, *tyrosinase* is defective, and tyrosine cannot be converted to melanin. Albinism is associated with a number of vision defects and high susceptibility to sunburn and skin cancers.

**Blood urea is a diagnostic marker**

Normal blood urea values are 2,5–8,3 mmol/l. Urea is removed from the body in the urine. In renal failure, urea is retained, and the level of blood urea will rise. In liver disease, urea production may decrease, and blood urea level will be low.

# CHAPTER 7
# HEMOSTASIS

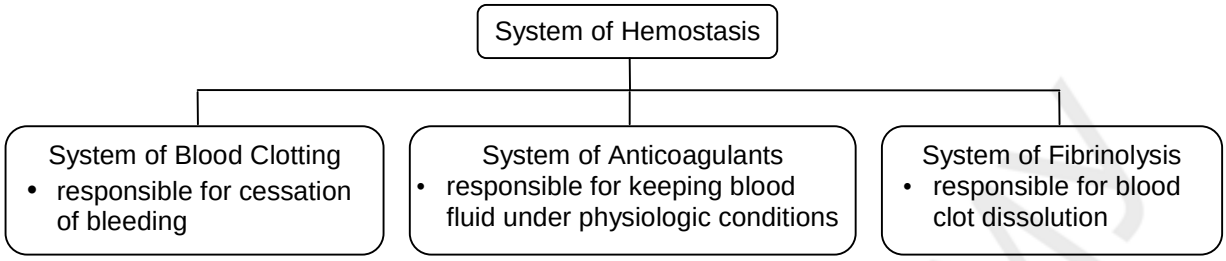



*Fig. 7.1*. System of hemostasis

**Participants of Hemostasis:**

1. Blood vessel wall;
2. Blood cells (mainly platelets);
3. Blood plasma (mainly proteins).

*Under physiologic conditions* the action of all participants provide the net *anti*thrombotic effect:

1. Endothelium produces inhibitors of platelet aggregation and coagulation: *NO, prostacyclin, ADP-ase, tissue plasminogen activator (t-PA), thrombomodulin, heparan sulfate.*
2. Platelets have discoid form and neutral charge.
3. Plasma participants are in the form of zymogens (inactive).

*An injury of blood vessels* causes the system of blood clotting to be activated.

## BLOOD CLOTTING

**2 types of hemostasis** can be defined:

– primary hemostasis (mediated mainly by blood vessel wall and platelets);
– secondary hemostasis *or* coagulation (mediated mainly by plasma factors and platelets).

### Primary Hemostasis

Phases:

1. Spasm of the vessel (at first reflectory, later supported by serotonin & epinephrin, released by activated platelets).
2. Platelet adhesion to subendothelial collagen.
3. Platelet activation and secretion.
4. Platelet aggregation and formation of the platelet plug.

Mechanism:

– von Willebrand factor (vWf) forms bridges between collagen and membrane receptor of platelets (GPIb/IX) **à** enables platelets to adhere to the injured vessel wall;

– receptor drives a signaling pathway that results in release of intracellular $Ca^{2+}$ (initial activation of platelets).

↑ Cytosolic $Ca^{2+}$ leads to the:

– change of cell shape and charge (becomes /-/ outside);
– release of a number of platelet stimulants (ADP, serotonin, epinephrin, thromboxane $A_2$);
– expression of membrane receptors GPIIb/IIIa promoting platelets to stick together via fibrinogen (aggregation) **à** platelet plug formation (unstable!).

### Secondary Hemostasis (Coagulation)

– involves a cascade of proteolytic reactions causing activation of specific proteases, and resulting in the conversion of soluble fibrinogen into insoluble fibrin.

*Major participants*:

1. Proteins:
– zymogens;
– cofactors (factors V, VIII, tissue factor III).
2. $Ca^{2+}$ (factor IV).
3. Phospholipids (PL) of activated platelet membrane.

Plasma factors are concentrated and activated on the surface of activated platelets! Being activated, a zymogen becomes an enzyme (protease) that performs proteolytic cleavage and activation of the next participant in the clotting cascade.

Phases of Coagulation:
1. Formation of ***prothrombinase complex*** (5–7 min).
2. Formation of ***thrombin*** (2–5 sec).
3. Formation of ***fibrin*** (2–5 sec).
4. Postcoagulation phase — clot retraction (> 1 hour).

Extrinsic and Intrinsic Pathways of Coagulation Differ in Triggers (figure 7.2).

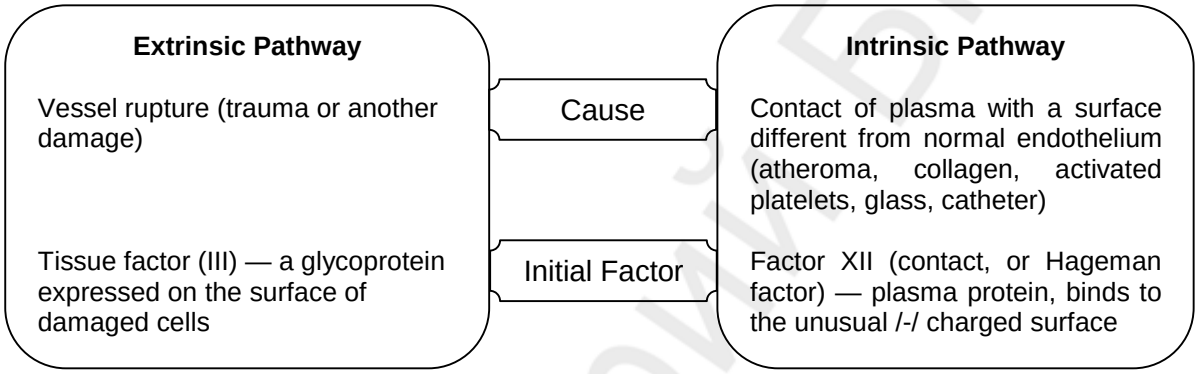



*Fig. 7.2.* Extrinsic and intrinsic pathways of coagulation: causes and prime factors

The sequence of factors and reactions involved in blood clotting cascade is shown in the figure 7.3.

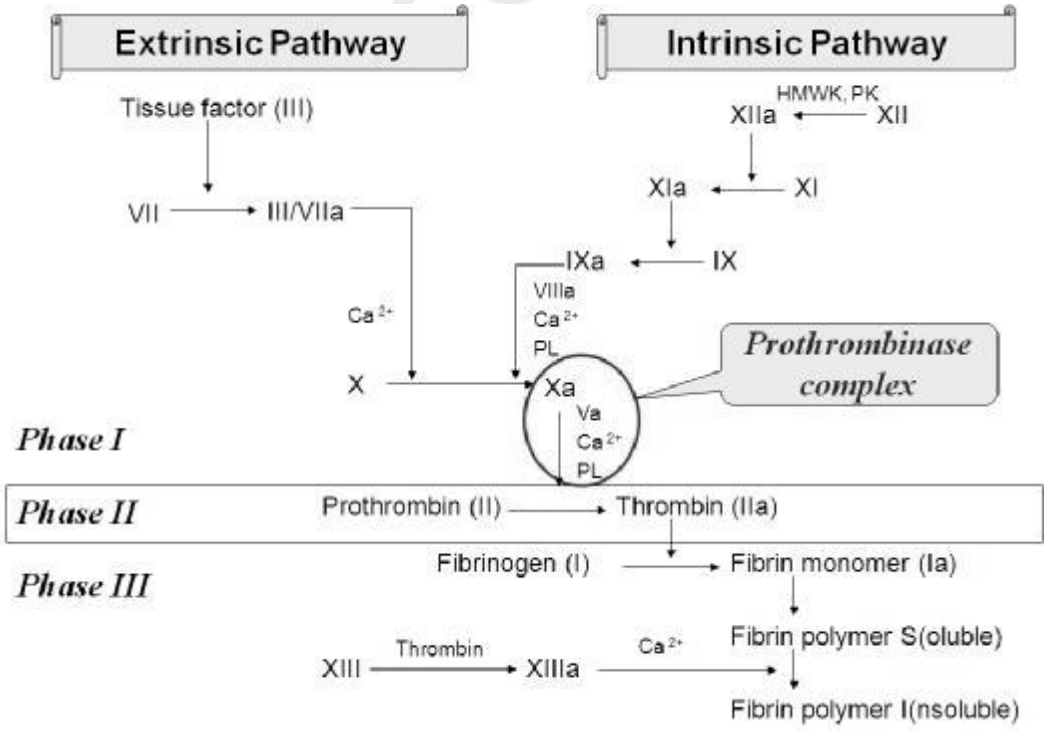



*Fig. 7.3.* Blood clotting cascade

**Fibrinogen (Factor I)**

– A soluble plasma glycoprotein produced in the liver.

– Consists of 6 chains (2αA, 2βB, 2γ).

– Additional fibrinopeptides A and B have excess /-/ charges **à** contribute to the solubility of fibrinogen in plasma and prevent aggregation.

***Thrombin*** removes FPA and FPB, thus *fibrin monomer* is produced.

Monomers readily stick together and form *fibrin polymer S* (still unstable).

Factor XIIIa (***transglutaminase***) covalently cross-links fibrin molecules and generates *fibrin polymer I* (stable).

**Role of $Ca^{2+}$ in Blood Clotting**

– Factor IV:
  Total plasma $Ca^{2+}$ 2,2–2,6 mmol/l;
  Free (ionized) $Ca^{2+}$ 1,1–1,3 mmol/l.

– Contributes to the platelet activation.

– Cofactor of the XIIIa.

– Binds factors II, VII, IX, X on the plasma membrane of the activated platelets.

**Role of Vitamin K in Blood Clotting**

– Essential for the carboxylation of factors II, VII, IX, X in the liver. Such modification allows the protein to bind calcium. Vitamin K serves as cofactor of γ-*glutamyl carboxylase* (figure 7.4).

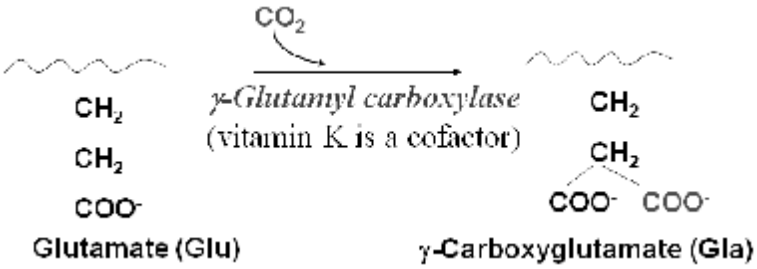



*Fig. 7.4.* Reaction catalyzed by γ-glutamyl carboxylase

**ANTICOAGULANT SYSTEM**

Anticoagulants are the inhibitors of coagulation. Classification of anticoagulants is presented in the figure 7.5.

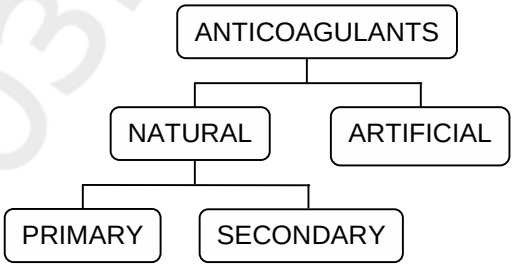



*Fig. 7.5.* Classification of anticoagulants

*Primary Anticoagulants* are constantly produced in the liver or endothelial cells and exist in normal plasma.

– Antithrombin III (ATIII) — a protein, the most potent inhibitor of thrombin and other serine proteases. 75 % of total anticoagulant activity.

– Heparin — GAG, causes 1000-fold activation of ATIII.

– α2-Macroglobulin — a large plasma glycoprotein, "panproteinase" inhibitor.

– α1-Antitrypsin.

– Protein C and S — cause degradation of factors Va and VIIIa.

Tissue factor pathway inhibitor (TFPI) — a protein associated with plasma lipoproteins, forms inactive complex TFPI+Xa+VIIa/Tf.

*Secondary Anticoagulants* are formed in coagulation and fibrinolysis.

– Fibrin — binds and inactivates thrombin.

– Fibrin degradation products.

*Artificial Anticoagulants* are used in the treatment and prophylaxis of thromboses.

– Heparin — prompt action ("at the point of the needle").

– Coumarin drugs (eg, warfarin) — vitamin K antagonists, require several days to reach full effect.

## FIBRYNOLYSIS

**Fibrinolysis** is a process of fibrin degradation.

***Plasmin*** is a serine protease responsible for degrading fibrin. It is present in plasma in the form of inactive zymogen — ***plasminogen***.

System of fibrinolysis (*plasmin system*) includes (figure 7.6):

1. Plasminogen;
2. Plasminogen activators;
3. Plasmin inhibitors;
4. Plasminogen activator inhibitors (PAI).

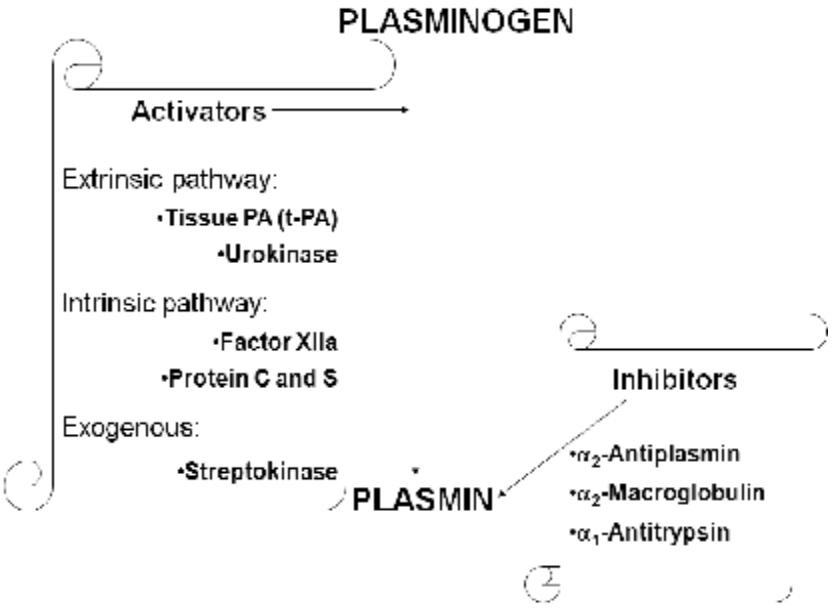


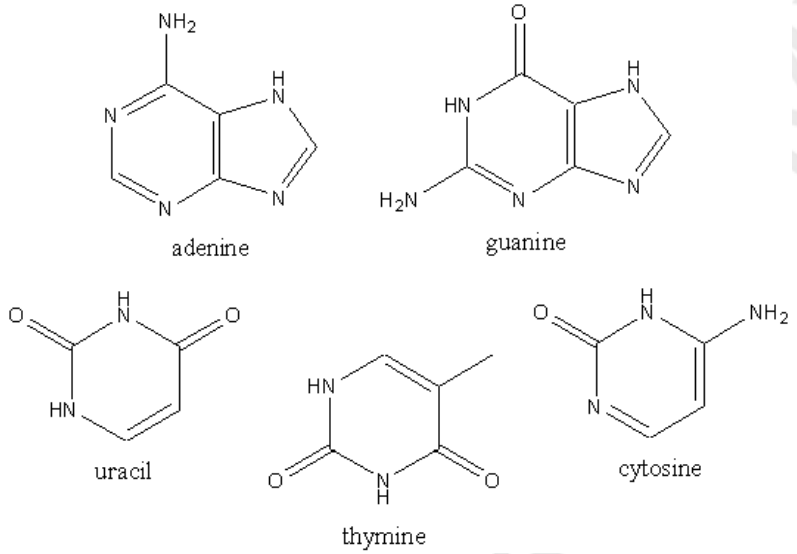

*Fig. 7.6.* Plasmin system

# CHAPTER 8
# CHEMISTRY OF NUCLEIC ACIDS

**Nucleic acids** (DNA and RNA) are **biopolymers** which **consist of nucleotides**.

A nucleotide consists of: 1) a nitrogenous base; 2) sugar (pentose); 3) phosphoric acid residues.

**Nitrogenous bases** are heterocyclic compounds which are divided into two groups: purines (adenine, guanine) and pyrimidines (uracil, thymine, cytosine).




The main properties of the nitrogenous bases: they are hydrophobic, planar, absorb UV at 260 nm.

**Nucleoside** = nitrogenous base + pentose (ribose or deoxyribose). Between the nitrogenous base and C1-atom of pentose **N-glycosidic bond** is formed. Note, that the atoms in the nitrogenous base are numbered 1, 2, 3, etc., whereas in the pentoses — 1', 2', 3', etc.

**Nucleotide** = nucleoside + 1–3 residues of phosphoric acid (fig. 8.1).

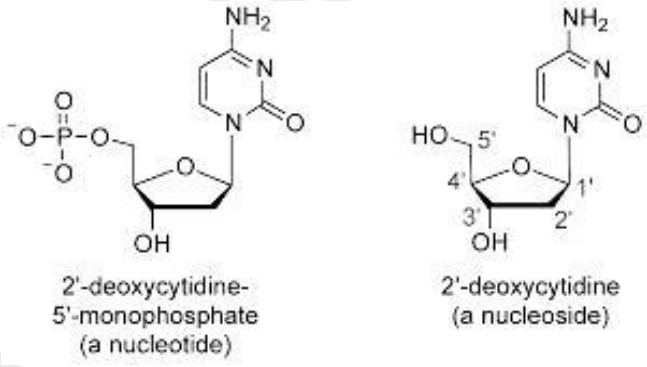



*Fig. 8.1.* Nucleoside and nucleotide structure

Properties of nucleotides: acidity, negative charge.

Nomenclature of nucleosides and nucleotides is presented in table 8.1.

The biological role of nucleotides:

1. Universal sources of energy in the cell (ATP, GTP).
2. Activators and carriers of monomers in the cell (eg, UDP-glucose, CDP-choline).
3. Allosteric regulators of enzyme activity.
4. Constituents of the coenzymes ($NAD^+$, $NADP^+$, FAD, CoA-SH).
5. Cyclic purine mononucleotides (cAMP, cGMP) serve as second messengers in signal transduction pathways.
6. Monomeric units of nucleic acids.

*Table 8.1*

**Nomenclature of nucleosides and nucleotides**

| The nitrogenous bases | The nucleosides (nitrogenous base + ribose) | The nucleotides (nucleoside + phosphate) |
|---|---|---|
| *Purines* | | |
| Adenine | Adenosine* | Adenosine monophosphate (AMP)* |
| Guanine | Guanosine | Guanosine monophosphate (GMP) |
| Hypoxanthine | Inosine | Inosine monophosphate (IMP) |
| *Pyrimidines* | | |
| Uracil | Uridine | Uridine monophosphate (UMP) |
| Cytosine | Cytidine | Cytidine monophosphate (CMP) |
| Thymine | Thymidine (thymine + deoxyribose) | Thymidine monophosphate (TMP) |

* If the sugar is deoxyribose — deoxyadenosine, dAMP, etc.

## DNA STRUCTURE

DNA contains 4 types of nitrogenous bases: A, T, G, C; sugar — deoxyribose. In a polymer structure the nucleotides are linked together through the 3'-OH-group of one nucleotide and 5'-phosphoric acid residue of the other (**3'-5'-phosphodiester bond**). As a result, the polynucleotide molecule has direction: it has 3'-end and 5'-end. **The primary structure** of DNA refers to as a sequence of nucleotides in a polynucleotide chain.

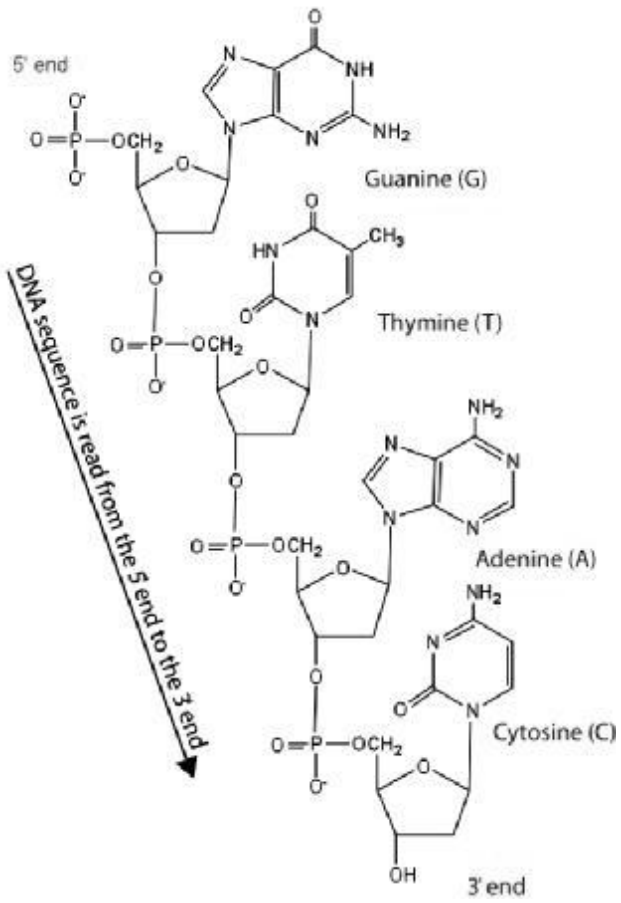



*Fig. 8.2.* The primary structure of DNA

**The secondary structure** of DNA (1953, J. Watson, F. Crick) is known as the **double helix**. Two strands of DNA are complementary (base pairing A – T, G – C) and antiparallel (3'-end of one chain corresponds to 5'-end the other). In the double helix hydrophobic nitrogenous bases are turned inward, to the axis of the helix, and polar pentoses and phosphates — out.

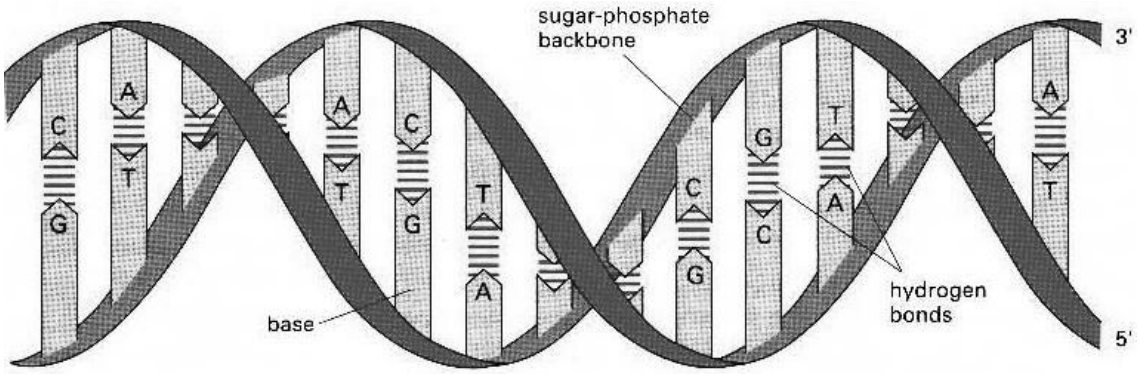



*Fig. 8.3.* The secondary structure of DNA

Double helix is stabilized by:

1. Hydrogen bonds between the nitrogenous bases (A = T, G ≡ C) ("horizontal" bonds);
2. Hydrophobic interactions between stacked nitrogenous bases ("vertical" bonds).

The surface of the double helix has two spiral grooves — major and minor. Nitrogenous bases protrude into a major groove, and pentose phosphates — into a minor one. Thus, the nitrogenous bases are available for a contact in the major groove without unwinding of the DNA strands. Through the major groove regulatory proteins can bind to specific sites in DNA.

The process of DNA chains separation and the formation of single-stranded molecules is called **DNA denaturation** (melting). In vitro this occurs by heating (70 °C). Gradual decrease of temperature may result in renaturation. In vivo local DNA denaturation takes place during replication and transcription.

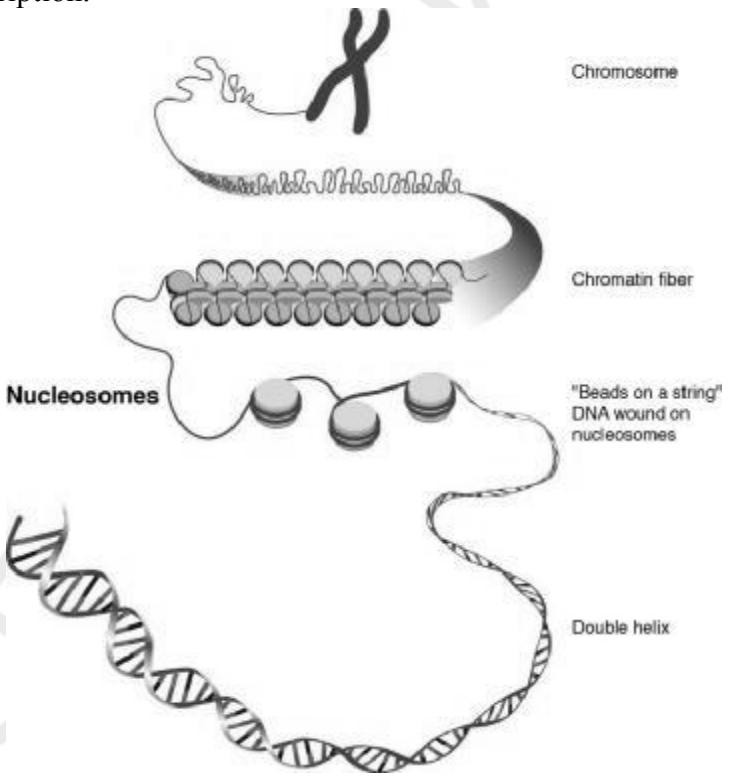



*Fig. 8.4.* Packaging of DNA

**The tertiary structure** of DNA is formed only in association with proteins and is used for efficient packaging of DNA in the nucleus. The proteins that make up the nucleoprotein:

1. *Histones*: basic proteins rich in arginine and lysine. Being "+" charged, histones easily bind to DNA by ionic bonds. Chemical modifications (phosphorylation, acetylation) lead to a reduction of the histones charge, resulting in their easier dissociation from DNA, thus, DNA

becomes available for replication and transcription enzymes. There are 5 classes of histones — H1, H2A, H2B, H3, H4. They participate in DNA packaging.

2. *Nonhistone proteins*: for DNA binding they have special domains (e. g., "zinc fingers") which "recognize" specific nucleotide sequences. These proteins regulate replication, DNA repair, transcription.

Levels of genetic material packaging:

1. *Nucleosome*. Nucleosome consists of a core — histone octamer (2 molecules of each histone class except H1), and 1,5–2 turns of DNA around this core;
2. *Solenoid.* Provided by histone H1;
3. *Loop.* Non-histone proteins are involved in the formation of loops;
4. The level of metaphase *chromosome* — the highest level of chromatin coiling.

DNA functions: storage, reproduction and transmission of heritable (genetic) information.

## RNA STRUCTURE

Differences from DNA:

1. Localization (cytoplasm).
2. Function (provides protein biosynthesis).
3. Size.
4. Structure. It contains U instead of T and ribose sugar. There are several types of RNA — messenger RNA (mRNA), transfer RNA (tRNA), ribosomal RNA (rRNA), and others.

In eukaryotes, RNA secondary structure always represents one strand which is spatially arranged due to hydrogen bonding between coplementary regions to form a special kind of structure, for example such as "cloverleaf" (for tRNA) (fig. 8.5).

The tertiary structure of tRNA is formed by itself, without protein assistance, and looks like a 3-D letter "L". Tertiary structures of rRNA and mRNA are formed in association with proteins (e. g., rRNA + protein = ribosome).

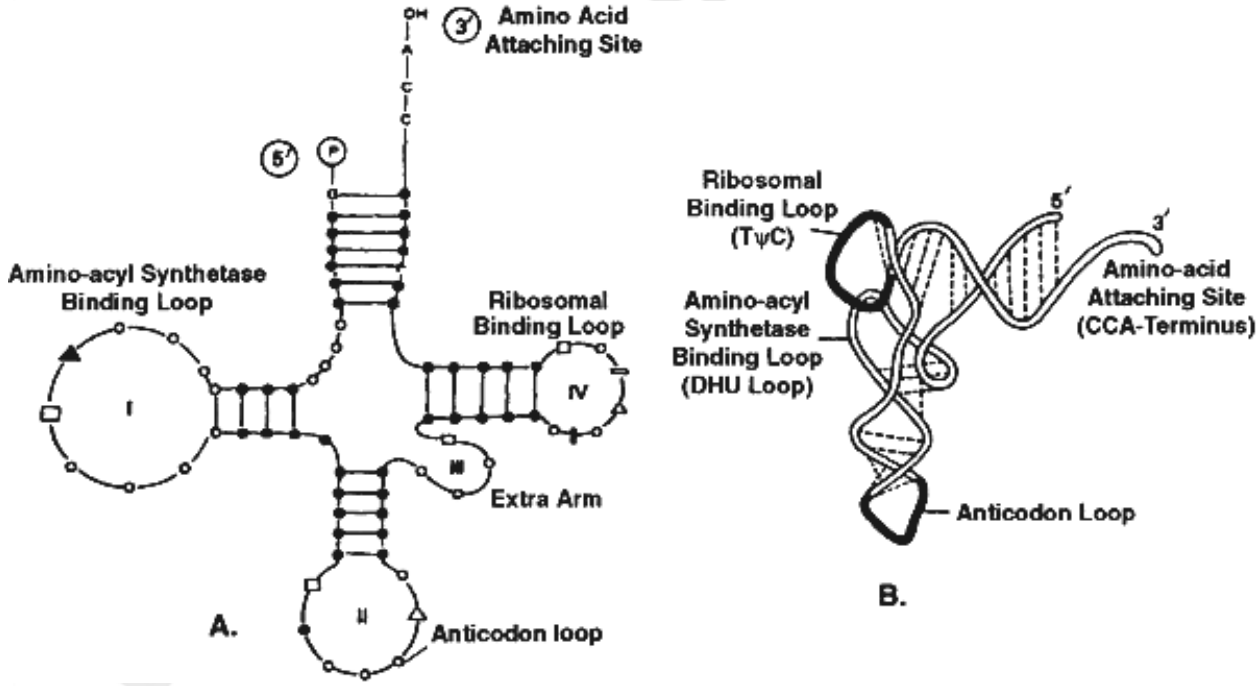



*Fig. 8.5.* Secondary structure and tertiary structure the tRNA.

## NUCLEOPROTEIN METABOLISM

The figure 8.6 shows a diagram of nucleoprotein digestion and absorption in the gastrointestinal tract.

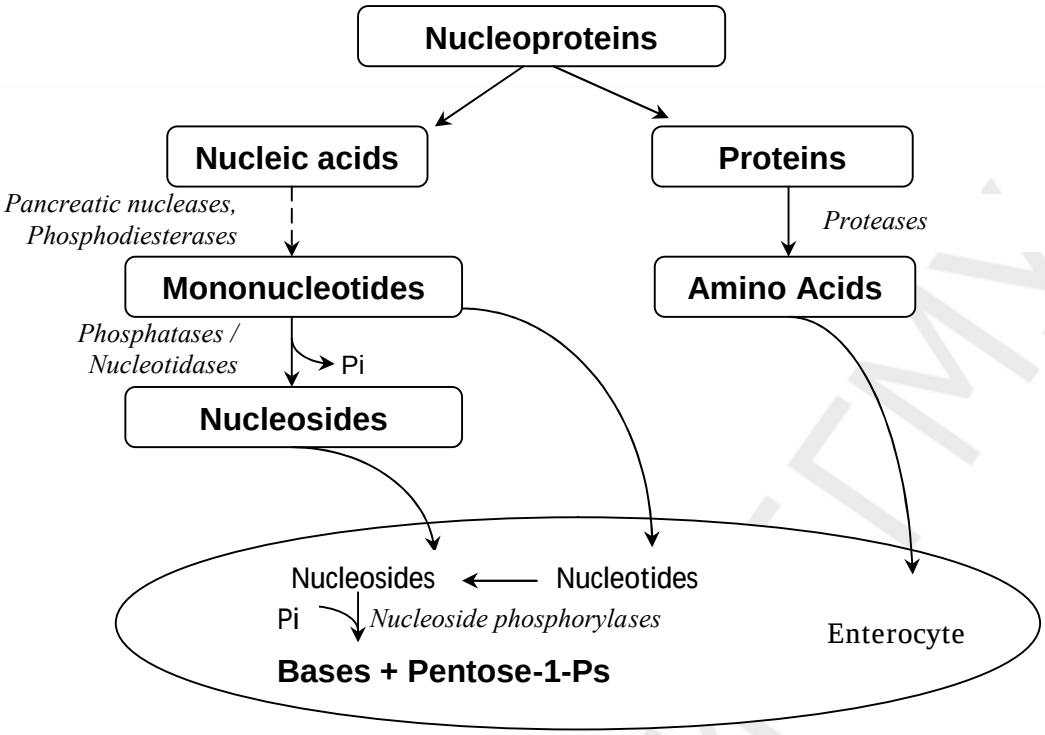



*Fig. 8.6*. Digestion and absorption of nucleoprotein in the gastrointestinal tract

Typically, exogenous nitrogenous bases, nucleosides and nucleotides are not used by the cells to synthesize their own nucleic acids. These compounds are destroyed to end products and excreted.

The end products of pyrimidine catabolism are β-alanine, β-amino isobutyric acid, $NH_3$, $CO_2$.

The final product of purine breakdown is uric acid (fig. 8.7).

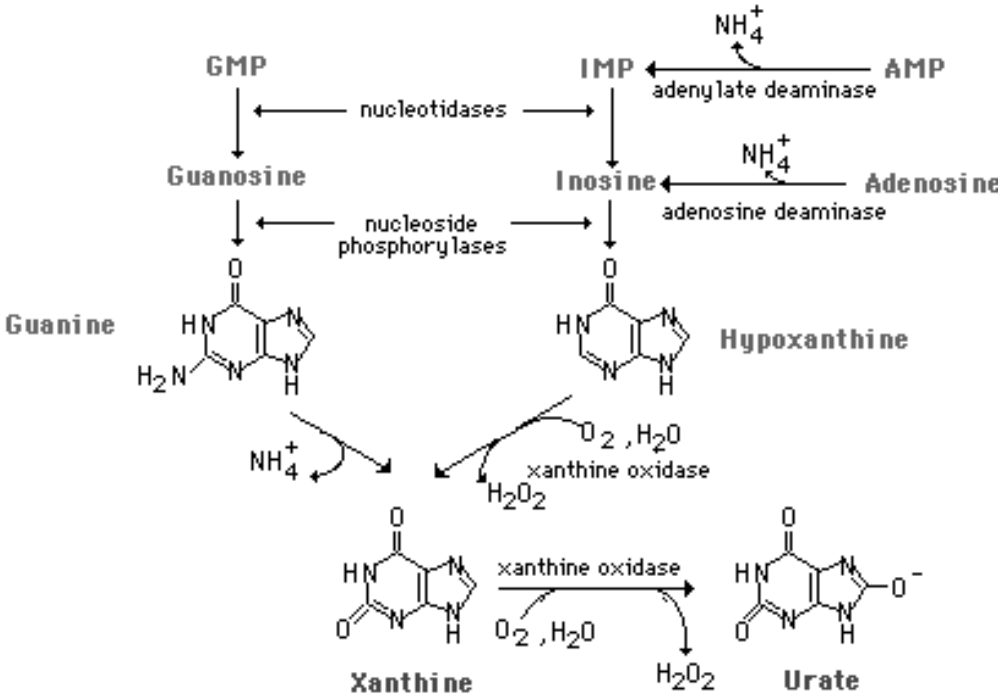



*Fig. 8.7.* Purine catabolism

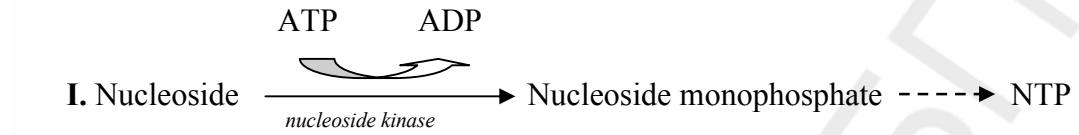
Uric acid contains unsplit purine ring, so is poorly soluble in water. In humans, uric acid is the end product of metabolism and is excreted in the urine.

## NUCLEOTIDES BIOSYNTHESIS

There are two ways for nucleotide biosynthesis in the cell:

1. *Salvage* pathways re-use nitrogen bases and nucleosides (not only exogenous but also those formed in the cell during DNA repair or breakdown of "used" RNA). These pathways are especially active in the rapidly proliferating cells (embryonic, regenerating, epithelial, germ, tumor).
2. Synthesis *de novo* from low-molecular-weight precursors.

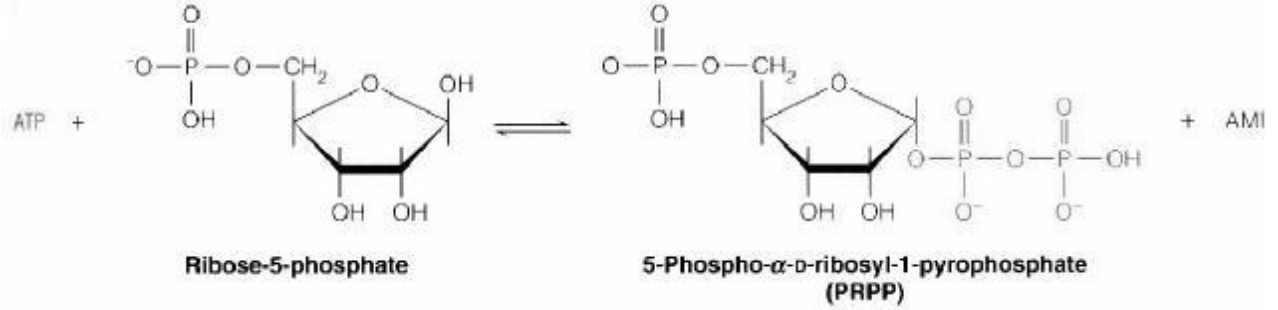
**Salvage pathways.** Chemotherapy of tumors and treatment of viral infections often are based on the existence of these pathways. They allow to produce nucleotides from administered to a patient analogs of nitrogenous bases and nucleosides (synthetic purines and pyrimidines, e. g., 5-fluorouracil, mercaptopurine, acyclovir). Incorporation of such preparations into the DNA molecule induces a cytotoxic effect.



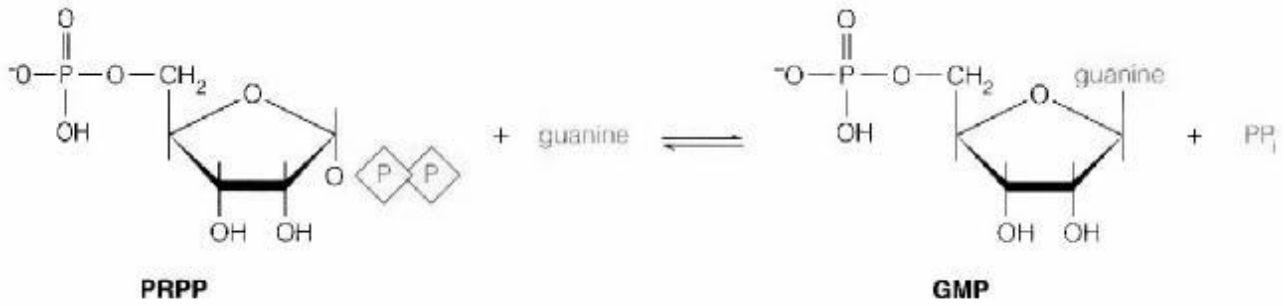

This path is more often used for re-utilization of pyrimidines (thymidine kinase, cytidine kinase).

**II.** The synthesis of nucleotides on the basis of nitrogenous bases is more typical for purines and occurs in two stages:

A) synthesis of the active form of ribose-5-phosphate, phosphoribosyl-pyrophophate (PRPP):




B) interaction of PRPP with the nitrogenous base:




Deficiency of the enzyme *hypoxanthine/guanine phosphoribosyl transferase* is a cause of the severe congenital disease — Lesch–Nyhan syndrome.

## DE NOVO SYNTHESIS OF PURINE NUCLEOTIDES

Low-molecular-weight precursors serve as the sources of atoms to purine ring. Such precursors are **glycine, glutamine, aspartate, $CO_2$, formyl-tetrahydrofolic acid (Formyl-THFA), methenyl-tetrahydrofolic acid (Methenyl-THFA**) (fig. 8.8). THFA corresponds to the active form of folic acid (vitamin $B_9$). The synthesis begins with ribose-5-phosphate, N-glycosidic bond is formed in the early stages of the synthesis, the reactions of purine ring formation occur afterwards. Inosine monophosphate (IMP) is a common intermediate in the synthesis of both adenine and guanine nucleotides (fig. 8.9).

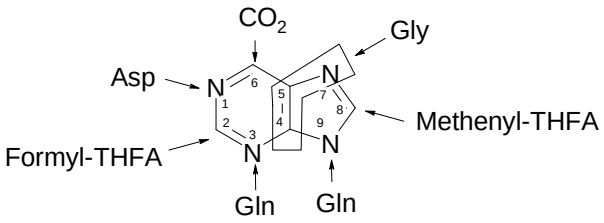



*Fig. 8.8* Precursors to the purine ring

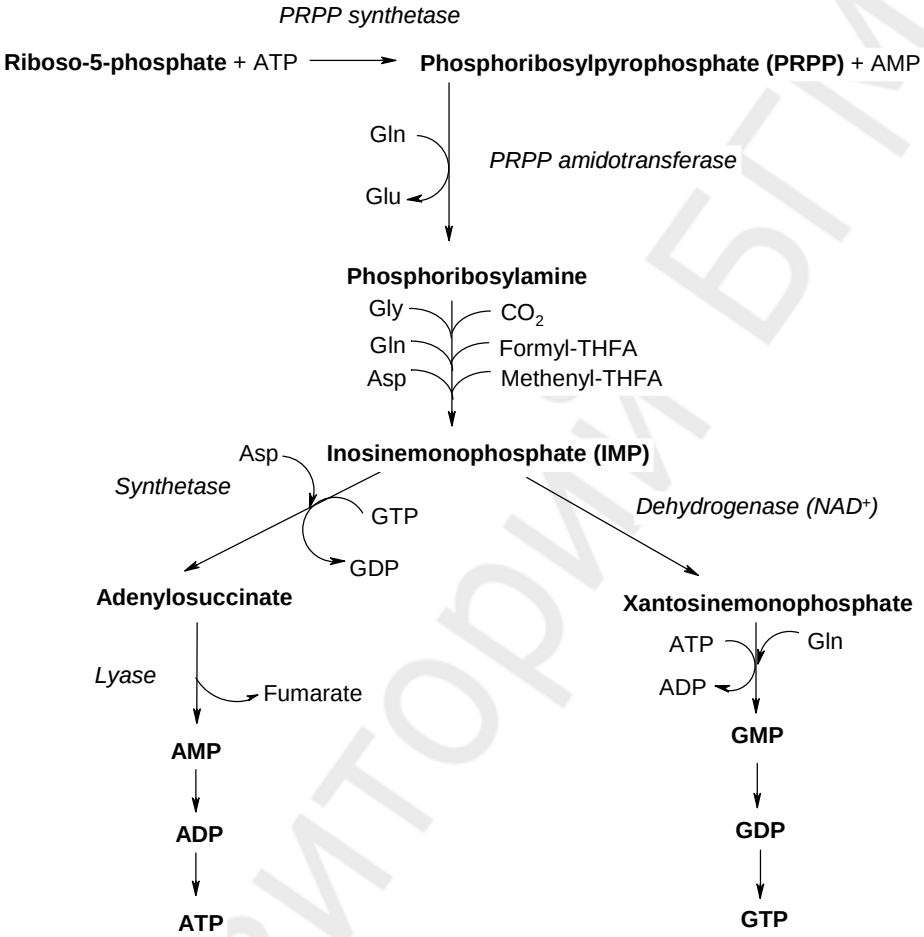



*Fig. 8.9.* De novo synthesis of purine nucleotides

A **key enzyme** in the synthesis of purines: *PRPP amidotransferase*.

**Regulation:**

1. Allosteric: excess of the end product (ATP, GTP) inhibits the key enzyme, and excess of PRPP activates it;

2. GMP inhibits formation of xanthosine monophosphate, AMP inhibits formation of adenylosuccinate;

3. Cross regulation: AMP synthesis requires GTP, and GMP synthesis requires ATP.

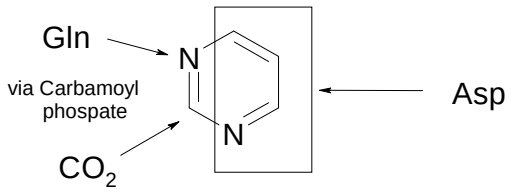
**Gout** is the most common pathology associated with altered purine metabolism. The main symptoms are the increased level of uric acid in the blood (hyperuricemia), and its deposition in the joints and kidneys. Reasons:

– excessive synthesis of purine nucleotides (insensitivity of enzymes to the regulators);
– the deficiency of purine salvage pathway enzymes;
– renal failure (insufficient elimination).

Contributing factor: excessive intake of purines with food. In the treatment of gout xanthine oxidase inhibitors are used (e. g., allopurinol is a structural analog of hypoxanthine).

## DE NOVO SYNTHESIS OF PYRIMIDINE NUCLEOTIDES

In the de novo biosynthesis of pyrimidines, unlike purines, initially the pyrimidine ring is formed, and afterwards ribose-5-phosphate is attached to it (fig. 8.10). **Glutamine, aspartate and $CO_2$** serve as the sources of atoms to the pyrimidine ring.




The synthesis begins with the formation of carbamoyl phosphate under the action of *carbamoyl phosphate synthetase-II* (*CPS-II*). Differences from *CFS I*:

| ***Carbamoyl phosphate synthetase-I*** | ***Carbamoyl phosphate synthetase-II*** |
|---|---|
| – *Participates in the synthesis of urea* | – *Participates in the synthesis of pyrimidines* |
| – *Localized in the mitochondria* | – *Localized in the cytoplasm* |
| – *Uses $NH_3$ as a nitrogen source* | – *Uses glutamine (Gln) as a nitrogen source* |

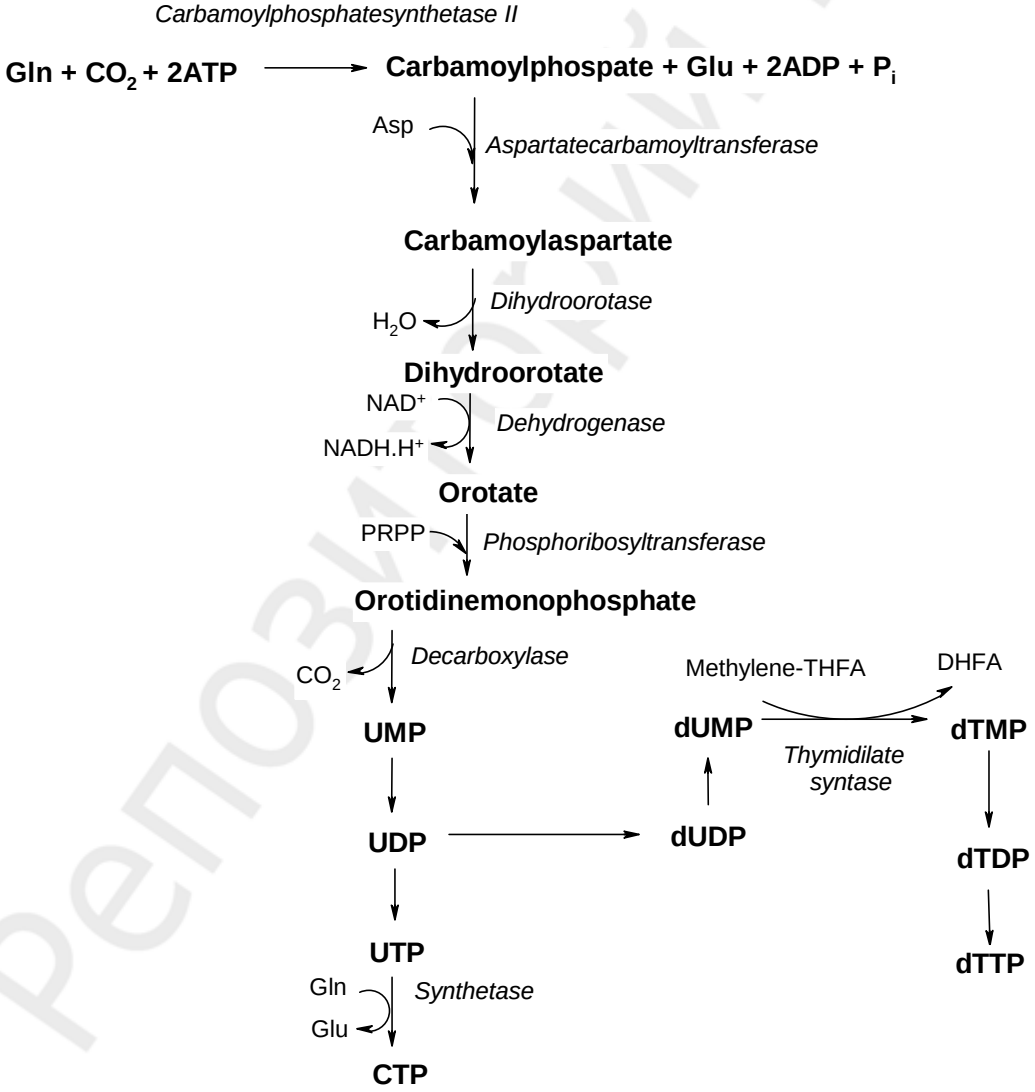



*Fig. 8.10.* De novo synthesis of pyrimidine nucleotides

**A key step** in the synthesis of pyrimidine nucleotides in mammals is considered to be the formation of carbamoyl phosphate.

**Regulation**: the process is inhibited by an excess of pyrimidine nucleotides, and is activated by excess of purines.

Orotic aciduria is a disease caused by defect in the enzymes that convert orotic acid to UMP. The disease is characterized by physical and mental retardation, megaloblastic anemia, excessive excretion of orotic acid in urine. Uridine is used for the treatment.

### FORMATION OF DEOXYRIBONUCLEOTIDES

Deoxyribonucleotides are necessary for DNA biosynthesis. They are formed from ribonucleotides at the **nucleoside diphosphate** level. Ribose residue in rNDP (e. g., ADP, GDP, CDP, UDP) is reduced under the action of a special enzyme (*rNDP reductase*) and protein thioredoxin (fig. 8.11). Thioredoxin contains free SH-groups, which donate hydrogen atoms for the reaction. Reduction of 2'-OH in rNDP results in dNDP formation, $H_2O$ release, and generation of a disulfide bridge in thioredoxin. Later dNDPs are phosphorylated to dNTP which are used for DNA synthesis.

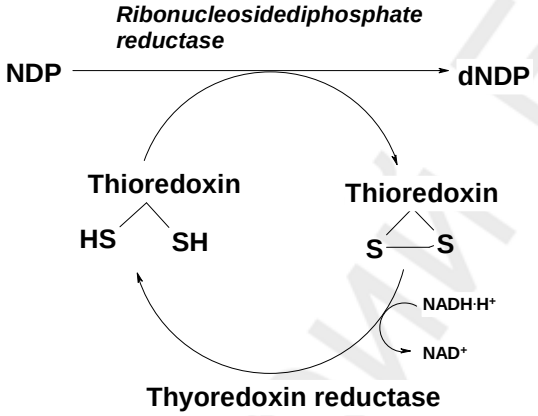
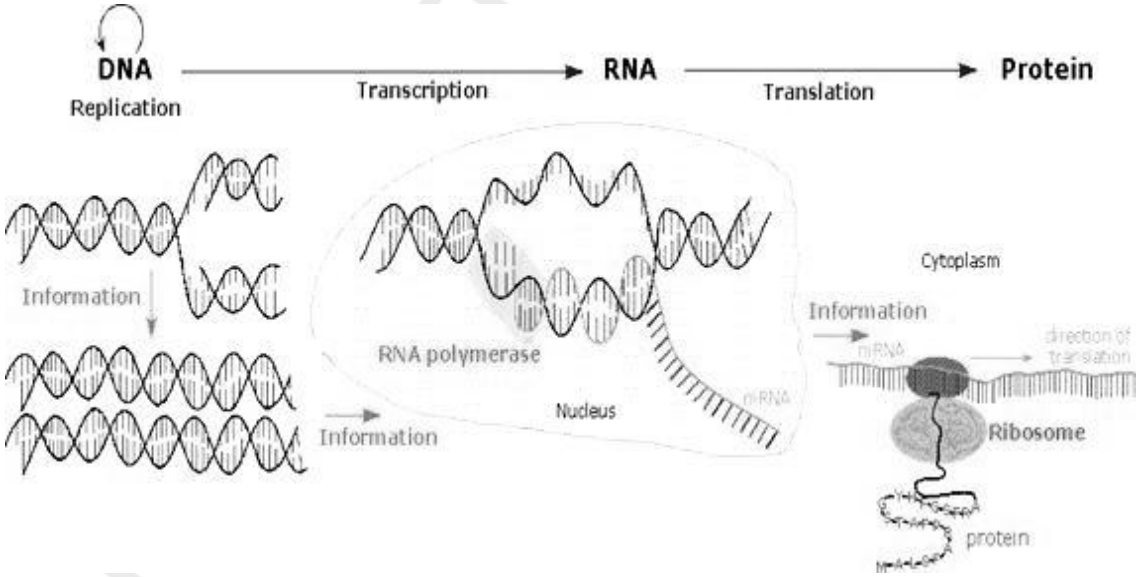



*Fig. 8.11.* Deoxyribonucleotide formation

### BIOSYNTHESIS OF DNA, RNA AND PROTEINS

The central dogma of molecular biology reflects the flow of information in the cell:




### BIOSYNTHESIS OF DNA

**Replication** — the process of DNA synthesis (duplication) (fig. 8.12).

DNA synthesis is semi-conservative, i. e. each daughter molecule of DNA contains one parental strand and one newly synthesized. Synthesis of each daughter DNA strand occurs complementary and antiparallel to the template strand and always in the 5' to 3'direction.

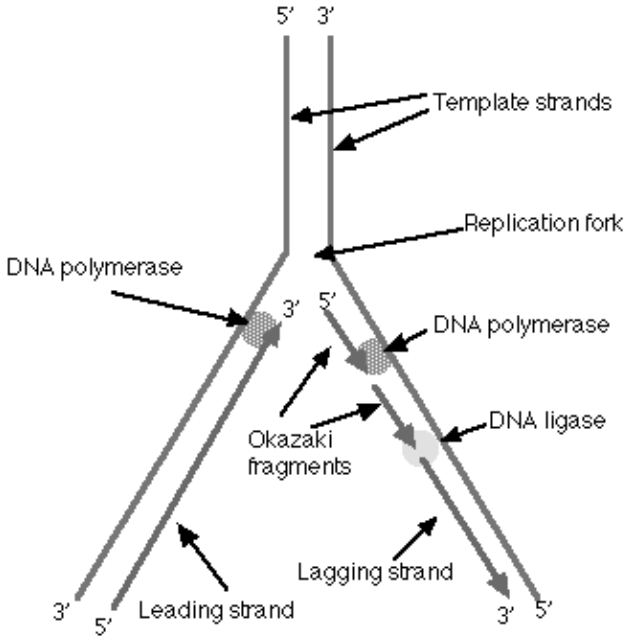



*Fig. 8.12.* Replication fork

Enzymes and proteins involved in replication are joined into a large complex — replisome. The major role belongs to the DNA polymerase. This enzyme adds nucleotides to the growing daughter chain, but cannot initiate synthesis. Therefore the short fragments of DNA (or RNA) are required as primers. Thus, initiation of the process includes separation of parental strands of DNA with formation of the replication forks and synthesis of primers.

*Helicase* unwinds the double helix of DNA at the replication fork.

*Topoisomerase* prevents the extreme supercoiling of the parental helix ahead of the replication fork.

*Primase* synthesizes the primers. Primase is the RNA polymerase, so the resulting primers are oligo*ribo*nucleotides.

***DNA polymerase*** is the main enzyme in the process. It needs the template, primer, substrates (activated nucleotides — dATP, dGTP, dCTP, dTTP), magnesium ions. DNA polymerase adds deoxyribonucleotide to the 3'-OH group in the existing chain (primer or the growing DNA strand) and catalyzes the formation of the phosphodiester bond. Removal of high-energy pyrophosphate from the substrate and its' subsequent cleavage provides energy that drives the reaction.

Most DNA polymerases have the ability to correct errors made during synthesis, by removing incorrectly attached nucleotide and replacing it with the correct one.

Since the strands of DNA are antiparallel, and the synthesis occurs only from 5'-end to 3'-end, one of the chains (leading chain) can be synthesized continuously toward the replication fork (fig. 8.12). The other strand will be synthesized discontinuously: when more of the helix is unwound, the synthesis of the lagging strand begins from another primer. The short fragments produced by this process are known as Okazaki fragments.

Subsequently RNA-primers are removed from the daughter chains, and the resulting gaps are filled with appropriate deoxyribonucleotides. Finally, fragments are connected together by *DNA ligase*.

After the end of replication, DNA undergoes methylation (protection from nucleases).

There are at least five DNA polymerases in eukaryotes — DNA polymerase α (responsible for the initiation of the process and synthesis of primers), β (reparative), γ (mitochondrial), δ (synthesizes the leading chain), ε (builds up the lagging strand). In eukaryotes, the synthesis of histones occurs along with replication.

The protection of the DNA structure from mutations is provided by:

1. High fidelity of replication.

2. The special mechanisms that repair the damages occurring in DNA. Direct repair (chemical reactions, restoring the original structure of the damaged nucleotides) and excision repair (excision of the damaged nucleotide or nitrogen base with subsequent filling in the gap) are available in the cell.

In nature, there is a way of DNA synthesis on a template of RNA with reverse transcriptase (or RNA-dependent DNA polymerase). Due to the presence of this enzyme, some RNA viruses have the ability to incorporate their genetic information into the DNA of the host cells.

## BIOSYNTHESIS OF RNA

**Transcription** is the biosynthesis of RNA on the DNA template. Unlike replication, the DNA molecule not wholly is subjected to transcription.

The main transcription enzyme is ***RNA polymerase***. It does not require a primer, synthesizes RNA in the direction from 5'-end to 3'-end, complementary and antiparallel to the template DNA strand. In the nucleus of eukaryotes, there are 3 types of RNA polymerases (I — synthesizes rRNA, II — for mRNA, III — for tRNA). Activated nucleotides (ATP, GTP, CTP, UTP) are used as the substrates for RNA synthesis.

*Initiation* of transcription: Prokaryotic RNA polymerase has a special subunit (σ-factor), which is responsible for the recognition of promoter. In eukaryotes, binding of RNA polymerase at the promoter site becomes possible in the presence of additional proteins — transcription factors. Being attached to the template, the enzyme causes the local melting of DNA at the promoter region and begins RNA synthesis (from the 5'-end). The primer for the initiation of synthesis is not required, strand selection and duplex unwinding and rewinding are carried out by the RNA polymerase itself.

*Elongation*: RNA polymerase moves along the template strand of DNA and catalyzes incorporation of ribonucleotides into the growing RNA chain with formation of phosphodiester bonds. The mechanism of the enzyme action is similar to DNA polymerase, however, the checking and correction of errors is not performed.

*Termination*: RNA polymerase detachment from the template occurs after copying the termination sequence of the gene. The exact mechanism of this process in eukaryotes is not established yet.

In prokaryotes, the resultant RNA molecule is immediately subjected to translation, it may contain information about several proteins. In eukaryotes, the resultant RNA molecule contains information about one protein; all kinds of RNA are synthesized as precursors and require the processing (maturation). Afterwards, RNA will be exported from the nucleus to the cytoplasm.

*Maturation of the mRNA*. Following the synthesis, pre-mRNA molecules undergo modifications — **capping** at the 5'-end and **polyadenylation** at the 3'-end. "Cap" stands for triphosphomethylguanosine added to the 5'-end in reverse orientation (5'-5'). Cap and poly(A) "tail" protect the mRNA from nucleases. **Splicing,** the excision of introns (non-informative inserts) and joining of informative exons, is the essential step in the mRNA maturation. In splicing, small nuclear RNAs participate, they contain sequences complementary to the intron-exon splice sites.

*Maturation* of tRNA. The precursor of tRNA is cleaved at the 3'-and 5'-ends to remove additional oligonucleotides, introns are cut out, acceptor site is built up, a loop of the anticodon is formed, the modification of nucleotides (formation of pseudouridine, dihydrouridine, etc.) occurs.

*Maturation* of rRNA. rRNA is synthesized as a large precursor. Then introns are removed, precursor is cut into fragments of different size, methylated, and combined with proteins to produce small and large ribosomal subunits.

## PROTEIN BIOSYNTHESIS

**Translation** is biosynthesis of protein on the mRNA template.

The sequence of nucleotides in the mRNA determines the sequence of amino acid incorporation into the protein. The sequence of three nucleotides (triplet or codon) corresponds to

one amino acid. There are $4^3$ = 64 codons. Three of them (UAA, UAG, and UGA) are nonsense codons and do not code for any amino acid, they serve as "stop" signals to end translation. The genetic code includes a whole set of codons.

Properties of the genetic code: it is triplet, specific (1 codon — 1 amino acid), degenerate (61 codon to 20 amino acids), unidirectional, non-overlapping, without punctuation, universal.

Role of tRNA in protein biosynthesis:

1. Transport of amino acid to the ribosome;
2. Adaptor function, i. e. tRNA serves as an intermediary between nucleic acids (the sequence of nucleotides) and proteins (amino acid sequence). An adaptor function is possible due to the presence in tRNA of both the acceptor site for amino acid and anticodon which matches the mRNA codon (genetic code).

*Recognition* is the process of specific coupling each amino acid to the appropriate tRNA. This coupling is catalyzed by enzymes called **aminoacyl tRNA synthetases**, which are specific to amino acids. The reaction occurs in two steps:

1. Amino acid + ATP → Aminoacyl-AMP + $PP_i$.
2. Aminoacyl-AMP + tRNA → **Aminoacyl-tRNA** + AMP.

Aminoacyl-tRNA is the active form of amino acid which is involved in protein biosynthesis.

Translation process can be divided into three stages: initiation, elongation and termination.

*Initiation*: 5'-end of the mRNA is delivered to the small ribosomal subunit; the first aminoacyl-tRNA (Met-tRNA) is attached to the initiation codon (AUG); the initiation complex assembling is completed after joining the large ribosomal subunit. Specific proteins — initiation factors (IF-1, 2, 3), and the energy of GTP are required.

*Elongation*: the next aminoacyl-tRNA comes to the aminoacyl site. The enzyme *peptidyl transferase* forms a peptide bond between the activated carboxyl group of the first amino acid and the amino group of the second amino acid, the resultant dipeptide temporarily is located at the aminoacyl (A) site (fig. 8.13). Then, the ribosome moves along mRNA by three nucleotides (*translocase* and GTP are required). Dipeptide occupies the peptidyl (P) site, aminoacyl site becomes free and readily binds the next incoming aminoacyl-tRNA. Elongation continues until stop codon enters the A site.

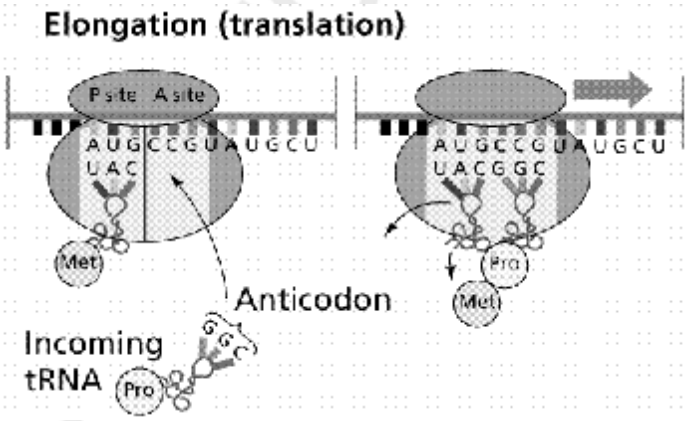



*Fig. 8.13*. Elongation process

*Termination* occurs when one of the three termination codons occupies the A site. These codons are recognized not by tRNAs, but by special proteins — release factors. Release factors bind at or near the A site and cause the release of synthesized peptide and dissociation of the ribosome subunits.

Many proteins are synthesized in the inactive form (as (pre)pro-proteins) and undergo postsynthetic modification. Types of protein modification are:

– partial proteolysis (removal of N-terminal Met and the signal peptide, the formation of active forms of hormones and enzymes);
– combining the protomers and the formation of the quaternary structure of proteins;
– the formation of intra- and inter-chain S-S bonds;

– covalent attachment of cofactors to the enzymes (pyridoxal phosphate, biotin);
– glycosylation (hormones, receptors, others);
– the modification of amino acid residues:
  *hydroxylation* of Pro and Lys (collagen);
  *iodination* (thyroid hormones);
  *carboxylation* (blood clotting factors);
– phosphorylation (milk casein, regulation of enzyme activity);
– acetylation (histones).

## REGULATION OF PROTEIN SYNTHESIS IN THE CELL

Protein synthesis in a cell can be regulated at the level of transcription, mRNA maturation and transport from the nucleus to the cytoplasm, by influence on the mRNA stability, translation, posttranslational protein modification. Regulation at the earliest possible stage (at the level of gene expression) is most widely used.

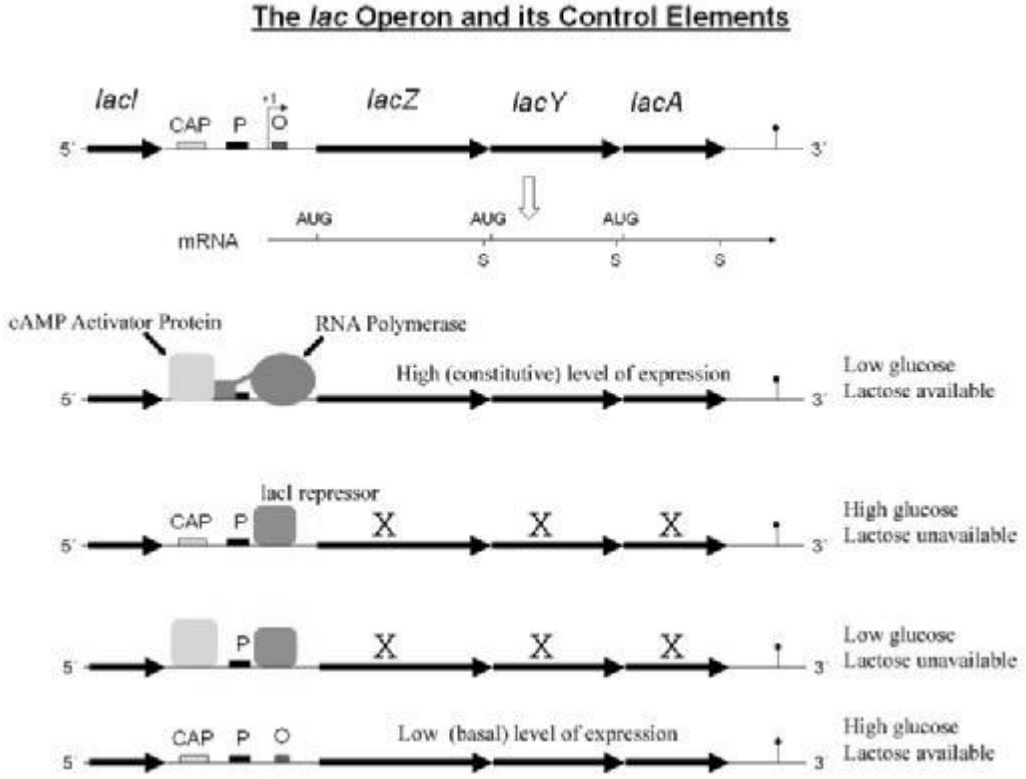



*Fig. 8.14. Lac-operon* in the presence and absence of lactose and glucose

An example of the regulation of gene expression is the work of lac-operon in *E. coli*. Lac-operon contains three structural genes of the enzymes involved in the metabolism of lactose. In the absence of lactose operon is blocked by binding of repressor protein to the operator (O) (fig. 8.14).

In the presence of inducer (lactose), the repressor changes its conformation, and dissociation of the inactive repressor-inducer complex from the operator permits transcription of the structural genes. However, if glucose can be found in the environment at the moment (glucose is an easily accessible source of energy), transcription does not occur. If the glucose level in the cell drops, cAMP level rises, triggering activation of a protein called catabolite activator protein (CAP). The cAMP-CAP complex binds to the site adjacent to the promoter (P) and allows the RNA polymerase to form a stable complex with the promoter and initiate transcription (fig. 8.14).

The regulation of eukaryotic genes is more complex. Different regulatory sites can be found upstream the gene: enhancers (elements that enhance transcription), silencers (attenuate transcription), hormone-response elements. The transcription factors can bind to any of these

elements, and thereby regulate the function of genes. Not only substrates can serve as inducers of protein biosynthesis at the genetic level (as lactose for lactase), but also the steroid hormones, vitamins D and A, thyroid hormones, metal ions. Activity of transcription factors may also be regulated by covalent modification (for example, by phosphorylation).

### INHIBITORS OF PROTEIN BIOSYNTHESIS

The mechanism of action of many toxins and antibiotics consists in the inhibition of the protein biosynthesis in the cells. Examples of such inhibitors are shown in table 8.2.

*Table 8.2*

**Inhibitors of protein biosynthesis**

| Inhibitor | Mechanism of action |
|---|---|
| **Antibiotics** | |
| Doxorubicin, Actinomycin D | Intercalate between the base pairs of DNA and disturb the unwinding of chains (replication and transcription) |
| Rifampicin | Inhibits RNA polymerase of prokaryotes |
| Streptomycin | Inhibits the initiation of translation (by binding to 30S-subunit) |
| Tetracyclines | Block entry of aa-tRNA to the A site |
| Chloramphenicol | Inhibits peptidyl transferase |
| Erythromycin | Inhibits translocation |
| **Toxins** | |
| α-Amanitin | Inhibits RNA polymerase II of eukaryotes |
| Ricin | Catalyzes the breakdown of the large subunit rRNA of eukaryotic ribosomes |
| Diphtheria toxin | Inhibits the translocation of ribosomes (catalyzes modification of translocase) |

# CHAPTER 9
# HORMONES

Hormones are the class of regulatory molecules that are synthesized by special cells.

Classification of hormones according to the chemical structure:

1. *Derivatives of amino acids:* tyrosine derivatives (thyroxine, triiodothyronine, dopamine, adrenaline, noradrenaline); tryptophan derivatives (melatonin, serotonin); derivatives of histidine (histamine).

2. *Protein and peptide hormones*: peptide hormones (glucagon, corticotropin, melanotropin, vasopressin, oxytocin, parathyroid hormone, calcitonin, hormones of stomach and intestines); simple proteins (insulin, growth hormone, prolactin); conjugated (glyco-) proteins (thyrotropin, follitropin, lutropin).

3. *Steroid hormones*: corticosteroids (aldosterone, cortisol, corticosterone), sex hormones (androgens, estrogens and progesterone), vitamin D.

4. *Fatty acid derivatives*: derivatives of arachidonic acid (prostaglandins, thromboxanes, leukotrienes).

Classification of hormones according to the mechanism of action:

1. Those interacting with receptors on the membrane surface (peptide hormones, adrenaline, eicosanoids);

2. Those interacting with intracellular receptors (steroid and thyroid hormones).

Major features of hormone action:

1. Low blood concentration ($10^{-6}$–$10^{-12}$ M).

2. Secretion of hormones is regulated: a) negative feedback mechanism; b) direct stimulation by other hormones or environmental changes.

3. Highly specific interaction with the receptor, which "turns *ON*" the cascade mechanism of signal amplification.

4. Hormones influence on the processes in target cells by altering the **amount** or **activity** of proteins (enzymes).

Hormonal signal can be "turned OFF" by several mechanisms: inactivation of the receptor by phosphorylation, removal of the receptor from the cell surface (endocytosis), relay proteins, breakdown of second messengers, opposite action on the target protein, etc.

Receptors are proteins by chemical nature, and are typically composed of multiple domains. Receptors can be divided into:

| MEMBRANE-BOUND | INTRACELLULAR |
|---|---|
| – 7-TMS;<br>– 1-TMS;<br>– Ion channels: ligand-, voltage-gated. | – Class I — nuclear and cytosolic receptors associated with heat shock proteins (hsp);<br>– Class II — nuclear receptors not associated with heat shock proteins. |

## MECHANISM OF SIGNAL TRANSDUCTION THROUGH THE 7-TMS RECEPTORS

7-TMS receptors represent the most common group of receptors (α-and β-adrenergic, histamine, serotonin, tropic hormones, glucagon, parathyroid hormone, calcitonin, hormones of hypothalamus). Mechanism of signal amplification implemented by this type of receptor involves synthesis of small molecules named second messenger (cAMP, $IP_3$, DAG, $Ca^{2+}$).

The receptor is composed of extracellular domain, seven transmembrane helical segments which are connected by hydrophilic extracellular and intracellular loops, and intracellular domain. The latter contains G-protein binding site, therefore this class of receptors is also known as G-protein coupled receptors (GPCR). In the inactive state, membrane-bound G-protein is a heterotrimer (αβγ), α-subunit of which is associated with GDP. There are about 20 known classes of G-proteins, but the most common are:

$G_s$ — activating adenylate cyclase;

$G_i$ — inhibiting adenylate cyclase;

$G_q$ — activating phospholipase C.

The binding of hormone to the receptor causes the conformational change of the latter, which induces the replacement of GDP in the α-subunit to GTP and subsequent dissociation of G-protein (α + βγ). Moving across the membrane α-subunit reacts with the effector protein — an enzyme (adenylyl cyclase, phospholipase C) or channel. The duration of effect depends on the GTPase activity of Gα-subunit.

*If $G_s$-protein is operating*:

– α-Subunit of $G_s$-protein contacts and activates the membrane enzyme — adenylate cyclase. Adenylate cyclase catalyzes formation of intracellular signal messenger, cAMP (fig. 9.1). The levels of cAMP in the cell may be quickly lowered by phosphodiesterases which breakdown cAMP to 5'-AMP.

– cAMP, in turn, activates the enzyme protein kinase A.

– Being activated catalytic subunits of protein kinase A phosphorylate target proteins in the cell. Phosphorylation inevitably alters protein conformation and activity:

• Following phosphorylation catalytic activity of enzymes changes, thus causing metabolic response;

• Phosphorylated transcription factor CREB interacts with cAMP-responsive element in DNA and enhances transcription of specific genes;

• Phosphorylation causes opening of cAMP-dependent ion channels.

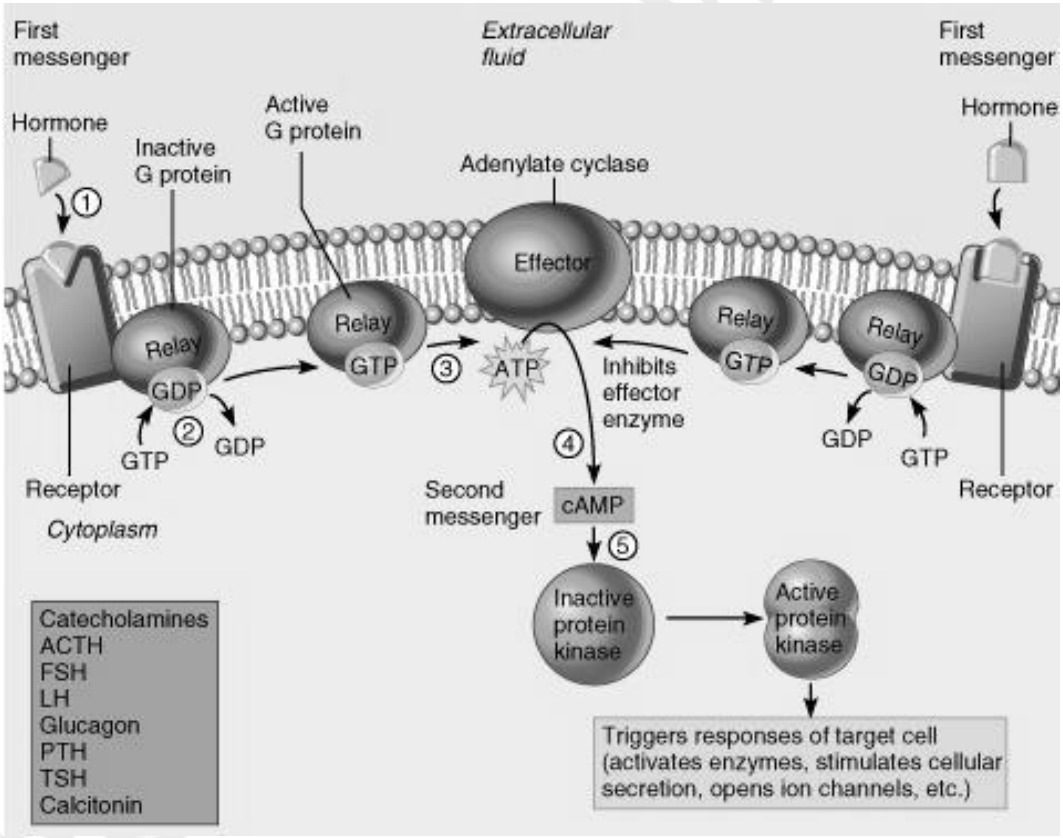



*Fig. 9.1.* Signal transduction through 7-TMS receptors and adenylate cyclase effector system

*If $G_q$-proteins is operating*:

– α-Subunit of Gq-protein contacts and activates the membrane enzyme — phospholipase C. Phospholipase C cleaves phosphatidyl inositolbisphosphate to inositol triphosphate ($IP_3$) and diacylglycerol (DAG).

– $IP_3$ binds to its receptor on calcisome (ligand-dependent calcium channel), resulting in the opening of the channel and $Ca^{2+}$ exit into the cytosol. These ions bind to special Ca-binding protein

— calmodulin. The resulting complex has an effect on a number of enzymes (adenylate cyclase, phospholipase $A_2$, many kinases, NO-synthase, etc.), and modifies the activity of $Ca^{2+}$-pumps.

– DAG remains in the membrane and either activates protein kinase C (which phosphorylates and thereby modulates the activity of various enzymes), or serve as a source of arachidonic acid, the substrate for prostaglandin synthesis.

Modification of G-proteins may be associated with pathological manifestations:

1. Cholera toxin inhibits GTPase activity of α-subunit of $G_s$-protein and leads to permanent activation of the adenylate cyclase.
2. Pertussis toxin causes modification of $G_i$-protein, preventing its interaction with receptors.

### MECHANISM OF SIGNAL TRANSDUCTION THROUGH THE 1-TMS RECEPTOR

1-TMS receptors are integral membrane proteins with a single transmembrane segment and the globular extra- and intracellular domains. These receptors can be subdivided into:

1) receptors possessing catalytic activity (guanylate cyclases, tyrosine kinases, protein phosphatases, serine/threonine kinases);

2) receptors without catalytic activity, but associated with the cytosolic tyrosine kinases.

**Tyrosine kinases**

The most common group of 1-TMS receptors are tyrosine kinases. Through them effects of insulin and various growth factors (insulin-like, epidermal, platelet-derived, etc.) are mediated. The special features of signal transduction by tyrosine kinases include:

1. Receptor dimerization (except insulin and insulin-like growth factors). After ligand binding, receptor dimerizes, and one receptor catalyzes the phosphorylation of Tyr residues in the other.
2. Direct contact between the participants, carried out by means of specific adapter proteins. They contain SH-domains and are able to interact with phosphorylated amino acids. The adapter protein Grb2 has two SH-domains: SH2-domain for interaction with the phosphorylated receptor and SH3-domain for interaction with the SOS-protein — guanine nucleotide exchange factor. SOS causes change in inactive Ras-GDP protein, thereby forming an active Ras-GTP. Ras-protein is a protein-relay: possessing GTPase activity it can "turn off" the signal transduction with the assistance of special protein that activates the GTPase and enhances the degree of GTP hydrolysis. From the active Ras-protein, signal is transmitted to Raf-protein (Ser/Tre-kinase), then to MEK (Tyr/Ser/Tre-kinase), and finally to MAP kinase (**m**itogen-**a**ctivated **p**rotein kinase). MAP kinase phosphorylates a variety of cytosolic and nuclear target proteins (ribosomal proteins, transcription factors), thus stimulating cell growth, differemtiation and proliferation.

Being a classical tyrosine kinase, insulin receptor has a number of features:

1. Even in the absence of insulin the receptor represents a dimer composed of α and β chains held together by disulfide bonds. β-Chain of the insulin receptor has the tyrosine kinase activity.
2. Upon binding the ligand and autophosphorylation, insulin receptor phosphorylates special proteins — insulin receptor substrates (IRS1 and IRS2). Activation of IRS2 triggers a cascade of reactions described above, while activated IRS1 associates with phosphatidyl inositol-3-kinase which evokes the potein kinase B dependent signaling pathway. Activation of protein kinase B promotes GLUT 4 translocation to the plasma membrane and thus accelerates the transmembrane glucose transport in muscle cells and adipose tissue; activates phosphodiesterase reducing the intracellular cAMP concentration, thus lipolysis in adipocytes is inhibited (fig. 9.2).

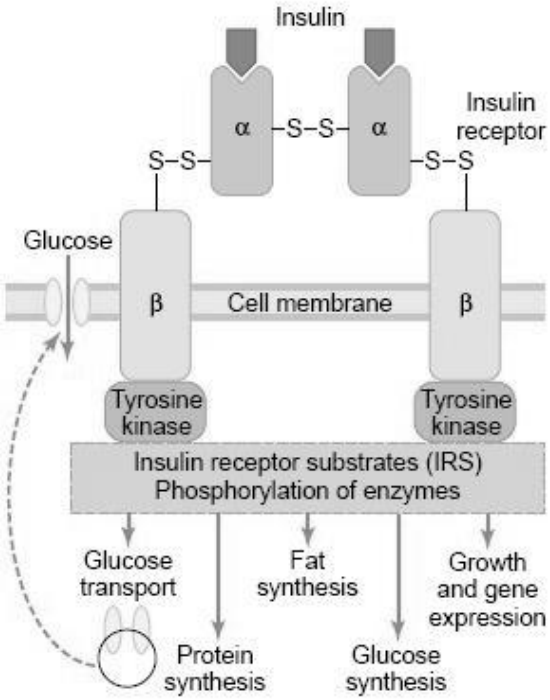



*Fig. 9.2*. Insulin effects

**Guanylate cyclase**

Another type of 1-TMS receptor is guanylate cyclase (GC). It is found in the heart, lungs, kidneys, adrenal glands, intestines, endothelium, retina and other tissues and organs. Guanylate cyclase receptor also is known as a "membrane-bound GC". In addition, soluble (cytosolic) form of the enzyme exists.

1. Cytosolic GC is activated by nitric oxide — NO, formed in the body from arginine by the enzyme NO-synthase. Other sources of NO are nitrate and nitroglycerine.

2. Membrane-bound GC is activated by atrial natriuretic peptide produced by cardiomyocytes in response to stretching of the atria and the stimulation of β-adrenergic receptors.

The function of guanylate cyclase is the formation of cyclic GMP from GTP. cGMP in turn:

1. Causes activation of protein kinase G and further phosphorylation of target proteins:

a) Phosphorylation of some smooth muscle proteins causes relaxation of blood vessels and reduction of blood pressure.

b) In the distal convoluted tubules of the nephron, the cGMP-dependent phosphorylation of the epithelial sodium channel inhibits the reabsorption of sodium ions and thus enhances diuresis.

2. Opens cGMP-dependent $Na^+/Ca^{2+}$ ion channels for light and odorants.

3. Increases the activity of cAMP-phosphodiesterase (i. e. cGMP is an antagonist of cAMP).

### 1-TMS RECEPTOR ASSOCIATED WITH CYTOSOLIC TYROSINE KINASES

Through this signaling pathway growth hormone, prolactin and cytokines exert their effects. After binding the ligand and dimerization of receptor, intracellular domains react with Janus kinases (JAK) having two domains with tyrosine kinase activity. The first JAKs' domain phosphorylates the receptor, whereas the other domain phosphorylates Tyr residues in STAT-proteins (**S**ignal **t**ransducer and **a**ctivator of **t**ranscription). Phosphorylated STATs dimerize and translocate to the nucleus where activate transcription of specific genes (fig. 9.3). In the liver, JAK-STAT signaling pathway induces the synthesis of insulin-like growth factor (IGF-1), the primary mediator of the growth hormone effects. The growth promoting action on almost all tissues involves activation of the MAP kinase pathway by IGF-1 via insulin-receptors.

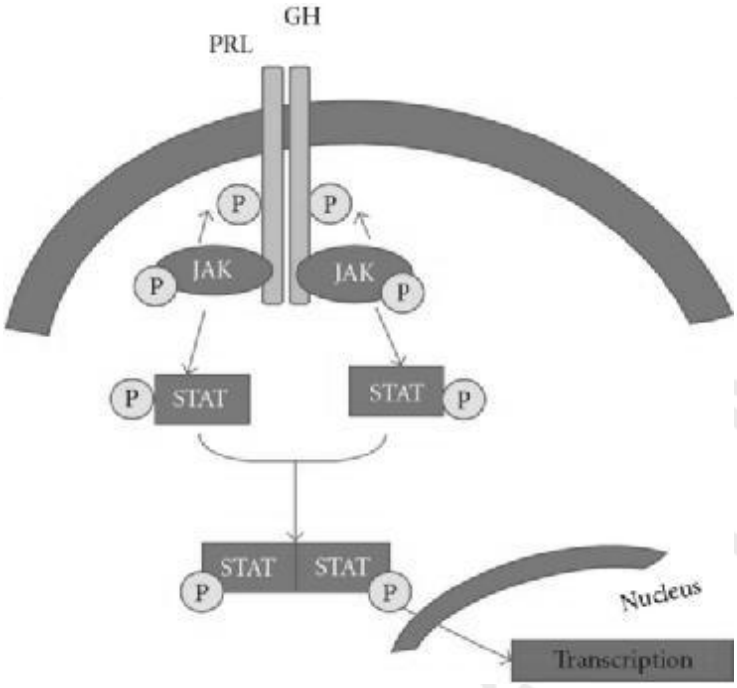



*Fig. 9.3*. A schematic representation of JAK-STAT signaling pathway mediating growth hormone and prolactin action

**MECHANISM OF SIGNAL TRANSDUCTION THROUGH THE INTRACELLULAR RECEPTORS**

*Class I*

This class includes nuclear and cytosolic receptors associated with **h**eat **s**hock **p**roteins (hsp 90). Corticosteroids, androgens and progesterone exert their action through the **cytosolic** class I receptors, and estrogens — through the **nuclear** class I receptors.

Above mentioned hormone receptors have similar structure and contain (fig. 9.4):

– domain A/B — regulatory domain that activates transcription factors;

– domain C — DNA binding domain — has two Zn-fingers involved in receptor dimerization, recognition and specific binding to a hormone response element on the DNA;

– domain E — ligand binding domain. It interacts with the hormone, binds heat shock proteins (chaperones), and along with the domain C contributes to the dimerization of the receptor.

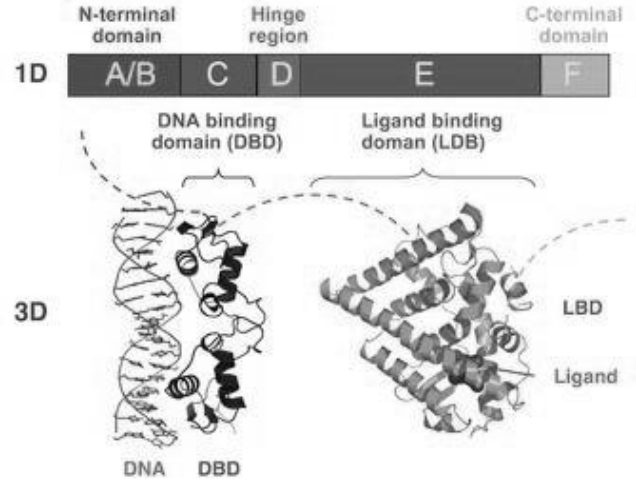



*Fig. 9.4.* Structure of intracellular receptors

In the "silent" (ligand-free) state, heat shock proteins cover Zn-fingers, and inactivate the receptor.

The interaction with the hormone binding site at the C-terminal part of the polypeptide chain of the receptor causes a conformational change in the receptor and the release of the chaperone. Two receptor molecules join to form a homodimer (fig. 9.5). Dimerized receptor translocates into the nucleus (in case of cytosolic receptors), and recognizes specific nucleotide sequence located near the promoter region of the gene. A/B domains of the receptor react with transcription factors and coactivators and stimulate RNA polymerase II, thus activating transcription.

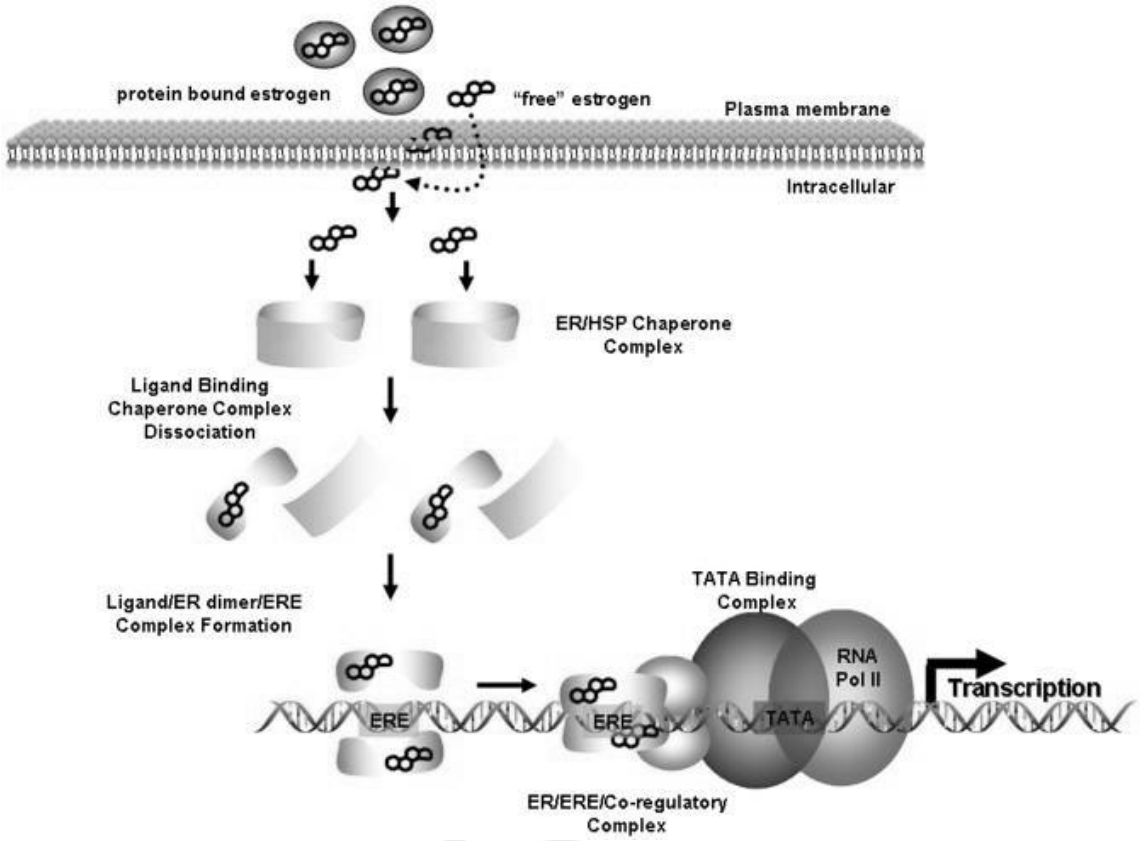



*Fig. 9.5.* The mechanism of estrogen signal transduction

*Class II*

Nuclear receptors not associated with heat shock proteins belong to this class. Thyroid hormones, retinoic acid, and vitamin D exert their action through class II receptors. Thyroid hormone receptors are always associated with DNA. In the absence of hormone, receptors inhibit the expression of the corresponding genes. Interaction with hormone is accompanied by formation of the heterodimer complex (hormone receptor + retinoic acid receptor), attachment of coactivator, and initiation of transcription.

## HYPOTHALAMIC HORMONES

All hormones of the hypothalamus are peptides by chemical structure. They can be divided into three subclasses:

– **releasing hormones** — stimulate the secretion of anterior pituitary hormones. This group includes thyrotropin-releasing hormone, prolactin-releasing hormone, corticotropin-releasing hormone, growth hormone-releasing hormone, gonadotropin-releasing hormone. Their effects are implemented through 7-TMS receptor associated with Gs-protein;

– **statins** inhibit the secretion of anterior pituitary hormones — somatostatin, prolactostatin and melanostatin. Effects are implemented through 7-TMS receptor associated with Gi-protein;

– **posterior pituitary hormones**:

• *vasopressin* (antidiuretic hormone) influences mainly on the cells of renal distal tubules and collecting ducts, and vascular smooth muscle cells. ADH receptors in the kidney are known as

the $V_2$-receptors coupled with Gαs-protein (for details, see "Water and mineral metabolism"). In blood vessels, vasopressin activates the $V_1$-receptors associated with Gq-protein which activates phospholipase C and causes an increase in intracellular $Ca^{2+}$ concentration resulting in vasoconstriction. Thus, vasopressin participates in homeostatic maintenance of blood pressure;

• *oxytocin* is acting through activation of 7-TMS receptors associated with Gq-protein. It causes an increase in intracellular $Ca^{2+}$ concentration and increase the contractile activity of smooth muscle of the pregnant uterus. In the lactating mammary gland oxytocin causes milk ejection due to contraction of the myoepithelial cells surrounding the alveoli and ducts of the breast. Receptors to oxytocin have been also found in the amygdala, the ventral part of the hypothalamus, the brain stem; they are responsible for the formation of sexual behavior, monogamy, the maternal instinct.

## HORMONES OF THE ADENOHYPOPHYSIS

This group includes next hormones (proteins and peptides):

1. Corticotropin, gonadotropins (follitropin and lutropin), thyrotropin — they implement their action via 7-TMS receptors.
2. Prolactin and growth hormone — they act through 1-TMS receptors associated with JAK.

*Thyroid-stimulating hormone* acts on the 7-TMS receptors of thyrocytes initiating the adenylate cyclase or phospholipase C mechanism. Activation of the receptor and the subsequent increase in cAMP levels increase the uptake of iodine by thyroid cells and increase the activity of thyroid peroxidase involved in the biosynthesis of $T_3$ and $T_4$. Activation of the phospholipase C is necessary to secrete the synthesized hormones into the bloodstream. TSH also enhances hexokinase-1 gene expression. A few hours after TSH administration the increased synthesis of DNA, mRNA, proteins and phospholipids is observed (necessary compounds for the growth, proliferation of thyrocytes and formation of follicles). In addition, thyrotropin acts on peripheral tissue receptors and causes activation of selen-dependent monodeiodinase and enhancement of thyroid hormone receptor sensitivity.

*ACTH* acts on the adrenocortical cells. ACTH receptor is 7 TMS-(R) associated with Gαs-protein, triggering the mechanism of cAMP synthesis. Processes controlled by this hormone, include: the delivery of LDL cholesterol into the cells, hydrolysis of cholesterol esters, cholesterol transport into mitochondria (synthesis of STAR-protein), synthesis of steroidogenic enzymes (desmolase).

*Gonadotropins* act on 7-TMS receptors associated with Gs-protein i. e. activate the adenylate cyclase system. In women, FSH stimulates the growth and maturation of ovarian follicles and the formation of LH receptors on the surface of the theca cells and granulosa cells, thus stimulating the estrogen synthesis. LH contributes to the synthesis of androgens in the theca cells, which are then converted to estrogens in granulosa cells under the action of FSH. In large doses, LH stimulates the rupture of dominant follicle, i. e. ovulation. After ovulation, LH promotes the development and maintenance of the function of the corpus luteum and stimulates the synthesis of progesterone. In men, FSH stimulates testicular tubule epithelium and actively influence on spermatogenesis. It acts on Sertoli cells and stimulates the production of proteins and nutrients that are required for the maturation of sperms. LH triggers the development and maturation of the interstitial cells (Leydig cells) and stimulates the biosynthesis of androgens by them.

*Growth hormone (GH)* — mediates its action through the 1-TMS receptors by the mechanism described above. Prompt effect of growth hormone is manifested in the increased lipogenesis and glycolysis (insulin-like action). Prolonged effect of growth hormone, however, consists in activation of lipolysis and gluconeogenesis, reduction of glucose uptake but enhancement of amino acid transport into the cells, increase of DNA polymerase activity, protein synthesis in bone, cartilage, internal organs. The effect of growth hormone hyperproduction depends on the age of the individual. In children (before the closure of growth plates in the skeleton), it causes gigantism. In adults, excess of growth hormone is called acromegaly. The condition is characterized by

enlarged hands, feet, nose, lips, ears, coarse facial features, hypertension, diabetes. Growth hormone deficiency in children results in growth retardation (dwarfism) and delayed puberty. Laron syndrome is caused by a defect in the growth hormone receptor, African Pygmies — alterations in postreceptor signal transduction.

*Lactotropin (prolactin)* — also interacts with the 1-TMS receptor, signal is transduced similar to GH. Prolactin is an anabolic hormone that enhances the synthesis of lactalbumin, casein, triacylglycerols, phospholipids, and reduces water excretion. Prolactin stimulates the immunoprotective response, extends luteal phase of the menstrual cycle, high levels inhibit ovulation and pregnancy. Through the receptors on the Leydig cells, prolactin regulates synthesis of testosterone.

## IODINE CONTAINING THYROID HORMONES

The basis structure of these hormones is iodinated thyronine nucleus consisting of two fused molecules of L-tyrosine. In the thyroid follicles, two main hormones are formed: $T_3$ (3,5,3'-triiodothyronine) and $T_4$ (3,5,3',5'-tetraiodothyronine, or thyroxine).

Entrance of $I^-$ into the thyroid follicle cell occurs through $Na^+/I^-$ channel. It is driven by the $Na^+$ gradient maintained by the $Na^+/K^+$-ATPase. Further thyroid peroxidase catalyzes the oxidation of iodide ions to iodine and iodination of tyrosine residues in thyroglobulin to form mono- and diiodotyrosine (MIT and DIT). MIT and DIT condensation results in the formation of $T_3$ and $T_4$, still bound to the thyroglobulin molecules. Thyroglobulin accumulates in the follicle lumen (colloid) and serves as the store of thyroid hormones. Hormone secretion occurs in response to TSH and involves endocytosis of colloid by follicle cells, its lysosomal hydrolysis and liberation of $T_3$ and $T_4$.

In peripheral tissues $T_4$ becomes more metabolically active $T_3$ under the action of selenium-containing 5'-deiodinase. In the placenta, under the action of 5-deiodinase the inactive $rT_3$ is formed, this mechanism protects the fetus from maternal thyroxine.

Thyroid hormones act on intracellular receptors class II according to the above mechanism. The result is the activation of the following genes: fatty acid synthase, malic enzyme, phosphoenolpyruvate carboxykinase, uncoupling proteins, growth hormone, and at the same time, the inhibition of prolactin, TSH, and TRH genes.

Biological response of the body to the action of thyroid hormone is the increase of basal metabolic rate (increased $O_2$ consumption by cells other than the brain, gonads), heat production in the cold, enhanced effect of catecholamines on the heart, increased gastrointestinal motility, cell growth and differentiation. Thyroid hormones are essential for normal development of the central nervous system of the fetus. The symptoms of hyperthyroidism may be nervousness, irritability, increased perspiration, tachycardia, hand tremors, increased body temperature and intolerance to heat, weight loss, exophthalmos. Autoimmune disorder — Grave's disease — is the most common cause of hyperthyroidism. Hypothyroidism in infancy causes cretinism, in adults — myxedema characterized by fatigue, feeling cold, slow puls rate, weight gain, swelling of the limbs.

## PANCREATIC HORMONES

*Insulin* is anabolic hormone that promotes the storage of glucose, fatty acids and amino acids, and *glucagon* — catabolic hormone, it causes mobilization of glucose, fatty acids and amino acids from tissue stores into the bloodstream.

Insulin consists of two polypeptide chains (A-chain comprises 21 amino acids, B-chain — 30) connected by two disulfide bridges.

Insulin receptors are 1-TMS-receptors with tyrosine kinase activity, the hormonal signal is transmitted as described above. Effects of insulin are tissue-specific, diverse, time-dependent:

1. Fast effects (seconds) — acceleration of glucose, amino acid and potassium transport into the insulin-dependent tissues.

2. Intermediate effects (minutes) — stimulation of protein synthesis and inhibition of protein degradation, activation of enzymes of glycolysis and glycogen synthesis, inhibition of enzymes of gluconeogenesis, glycogen breakdown, lipolysis.

3. Long-term effects (hours) — increase in the synthesis of messenger RNA (induction of enzymes involved in carbohydrate metabolism, lipogenesis, etc.).

The complex disorder caused by deficiency of insulin function is called diabetes mellitus. The two forms of diabetes:

1. Insulin-dependent diabetes mellitus (diabetes type 1), develops due to insulin deficiency caused by autoimmune destruction of the β-cells of the pancreas.

2. Non-insulin dependent diabetes mellitus (diabetes type 2) is characterized by reduced sensitivity to insulin. It occurs more frequently in elderly and typically is associated with obesity.

A characteristic feature of diabetes is hyperglycemia that occurs due to inability to transport glucose into the cells of insulin-dependent tissues. In diabetic, the concentration of blood glucose after glucose load is significantly higher and returns to its initial level slower than in healthy individual. Hyperglycemia > 10 mmol/L exceeds renal reabsorption possibility (renal threshold) and causes glucosuria. Excretion of osmotic active glucose molecules results in the loss of large amounts of water (osmotic diuresis). Dehydration leads to deficiency of cations like sodium, potassium, calcium and magnesium. The patient suffers from thirst, polyuria, weakness, fatigue, muscle spasms, cardiac arrhythmias, etc.

Because of insulin deficiency utilization of glucose in the cells is disturbed. Energy needs are met by increasing the rate of lipolysis and, as a result, the increased formation of ketone bodies in the liver, their accumulation in the blood, as the rate of production exceeds the capacity of cells to use them. This leads to the development of metabolic acidosis. Despite the hyperglycemia, gluconeogenesis is stimulated. Precursors for glucose synthesis are amino acids, supplied by the activation of protein catabolism. Intensive catabolic response results in the weight loss.

Long-term hyperglycemia also leads to sorbitol production and non-enzymatic glycosylation of proteins. As a result of osmotic tissue damage and impaired functioning of many vital proteins, numerous pathological changes develop in various organs, e. g., diabetic nephropathy, retinopathy, neuropathy. Determination of the glycosylated hemoglobin — HbA1c — is used for evaluating the effectiveness of insulin treatment.

*Glucagon* is a peptide (29 amino acids), synthesized by α-cells of the pancreas. Its effect is mediated mainly through specific 7-TMS receptors of the liver, initiating the adenylate cyclase mechanism and leading to the activation of glycogen phosphorylase and inhibition of glycogen synthase. Thus, glucagon in liver stimulates glycogen breakdown, helps to maintain blood glucose at a constant level. Glucagon also activates gluconeogenesis, lipolysis and ketogenesis in the liver.

## STEROID HORMONES

Steroid hormones are synthesized from cholesterol chiefly in the adrenal cortex, testis, ovary and placenta, and act on intracellular receptors (I class). The first two reactions in synthesis of all steroid hormones are common:

1. The conversion of cholesterol to pregnenolone by a 6-carbon cleavage of a side chain fragment (key enzyme — $C_{20-22}$ desmolase, activated by ACTH).

2. Oxidation and isomerization of pregnenolone to progesterone.

Progesterone is then subjected to hydroxylation at C-17 or C-21, resulting in formation of different functional classes of steroids.

*Glucocorticoids* (cortisol and hydrocortisone):

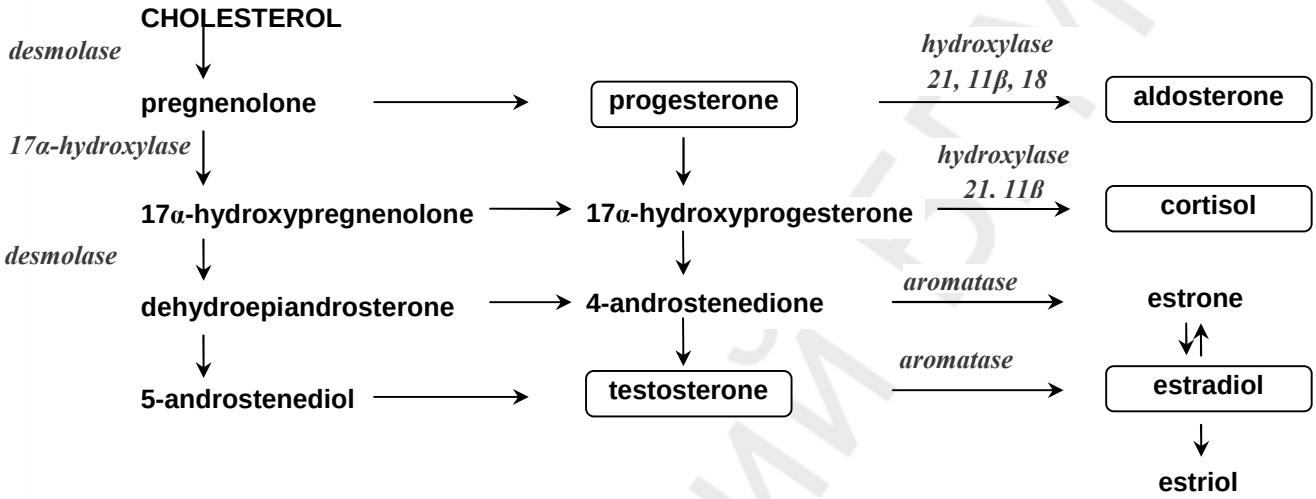
– stimulate glucose production in the liver, increasing gluconeogenesis, and simultaneously increasing the rate of release of amino acids (substrates for gluconeogenesis) from peripheral tissues;

– in hepatocytes stimulate the synthesis of proteins and nucleic acids, whereas in muscle, adipose and lymphoid tissue, skin and bones their synthesis, by contrast, is inhibited;

– excess cortisol stimulates lipolysis in the limbs and lipogenesis in other parts of the body (face and upperparts);

– high concentration of glucocorticoids suppress the inflammatory response, reducing the number of circulating leukocytes and inducing synthesis lipocortin which inhibits phospholipase $A_2$ and inhibit the synthesis of mediators of inflammation — prostaglandins and leukotrienes;

– high concentration of glucocorticoids also cause inhibition of growth and division of fibroblasts, collagen and fibronectin production, thus hypersecretion of glucocorticoids is typically accompanied by thinning of the skin, poor wound healing, muscle weakness and atrophy of muscles.




*Mineralocorticoids* (aldosterone) increase reabsorption of $Na^+$ and water, and cause the secretion of $K^+$, $H^+$ in kidneys. Water and sodium retention in the body leads to the increase of blood pressure (see section "Water and mineral metabolism").

*Male sex hormones* (testosterone, dihydrotestosterone) influence on the tissue differentiation, the development of secondary sexual characteristics, are involved in the process of spermatogenesis. They have a pronounced anabolic effect, enhancing the process of protein synthesis, resulting in the increase of muscle mass, bone growth. They cause retention of sodium, potassium, water, calcium, sulfate and phosphate, stimulate erythropoiesis.

*Female sex hormones — estrogens* (estradiol, estrone, estriol) and *progesterone*. Estrogen synthesis is carried out from androgens under the action of aromatase. These hormones are involved in the formation of women's appearance, improve trophic of the skin, determine the growth of breast, endometrial proliferation, increase the calcification of bones, induce the synthesis of clotting factors. *Progesterone* is the precursor of all steroids. In females, it is involved in menstrual cycle (luteal phase) and pregnancy (supports gestation).

# CHAPTER 10
# BIOCHEMISTRY OF THE LIVER

The exceptional importance of the liver in metabolism is primarily associated with its function of intermediary distributor of bloodstream between the portal and general circulations. Average liver mass in the body of an adult healthy human is about 1,5 kg; water accounts for over 70 % of its mass. Proteins account for a larger half of the dry liver weight, over 90 % of them being globulins. The liver is also rich in enzymes, lipids, and glycogen.

## BIOLOGICAL ROLE OF THE LIVER

The function of the liver is that of a primary regulator for maintaining the concentration of nutritive substances, supplied to the organism in the food, at an appropriate level. Despite the intermittent and loosely regulated regimen for the uptake of nutrients from the intestine into the blood, the variations in concentration of these components in the general circulation are insignificant. The liver has a unique ability to maintain not only its own homeostasis but also the homeostasis of the whole organism because of participating in:

***1. Protein metabolism.*** Most amino acids supplied to the liver via portal vein are utilized for:

– synthesis of plasma proteins (albumins, globulins, blood coagulation factors), "acute phase" proteins, antitrypsin, ceruloplasmin, α-phetoprotein;
– α-keto acids formation;
– gluconeogenesis;
– ketogenesis;
– energy production by conversions in Krebs cycle;
– urea synthesis;
– synthesis of creatine and choline.

***2. Lipid metabolism.*** The enzyme systems of the liver are capable of catalyzing the majority, or even all, lipid metabolism reactions:

– fatty acids synthesis and oxidation;
– TAG synthesis and degradation;
– VLDL and HDL synthesis;
– cholesterol synthesis;
– ketone bodies production.

***3. Carbohydrate metabolism.*** The major function of the liver in carbohydrate metabolism is to provide a constant level of glucose in the blood. This is achieved by:

– regulating the rate ratio for synthesis and breakdown of glycogen;
– regulation of gluconeogenesis;
– conversions of other hexoses (galactose and fructose) in glucose.

***4. Vitamin metabolism*:**

– vitamin A forms interconversions;
– vitamin D3 precursor — 25(OH)-cholecalciferol — synthesis;
– vitamin $B_{12}$ deposition.

***5. Excretory function.*** Different substances are excreted from the liver with the bile and then with feces or secreted in the blood and then, through the renal system, are excreted in the urine.

***6. Detoxification of different substances.*** Extraneous compounds (xenobiotics), potent physiological agents, normal metabolites and drugs are detoxyfied (metabolized) in the liver. The detoxification is performed by:

– methylation (vitamin PP);
– acetylation (sulphanilamide drugs);
– oxidation (alcohol, aromatic hydrocarbons, catecholamines, biogenic amines);
– reduction (nitrobenzene);
– urea synthesis (ammonia);

– conjugation with UDP-glucuronate and PAPS (phosphoadenosine phosphosulphate): steroid hormones, bilirubin, soporifices, products of proteins intestinal putrefaction;
– microsomal oxidation enzymes: drugs, aldehydes, ketones.
– peroxisomal oxidation enzymes: D-amino acid oxidase, catalase;
– cysteine-rich protein — metallothioneine. It binds heavy metals: Cd, Cu, Hg, Zn;
– hydrolysis — peptide and protein hormones, aspirin.

***7. Pigment metabolism.*** The lifetime of erythrocytes is 120 days; after this period of time the erythrocytes suffer degradation to release hemoglobin. The major organs responsible for the erythrocytolisis and hemoglobin breakdown are liver, spleen and marrow. Degradation of hemoglobin (fig. 10.1) and metabolism of bile pigments (fig. 10.2) may be represented by schemes below:

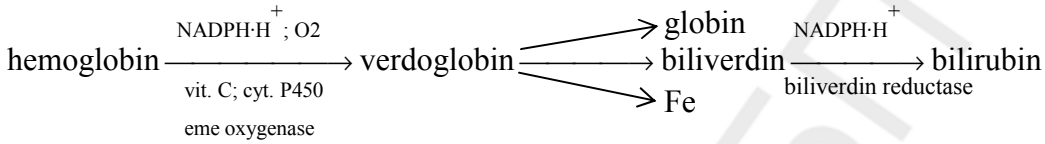



*Fig. 10.1.* Degradation of hemoglobin

Bilirubin thus formed is known as indirect (unconjugated) bilirubin. Bilirubin glucuronide is referred to as direct (conjugated) bilirubin.

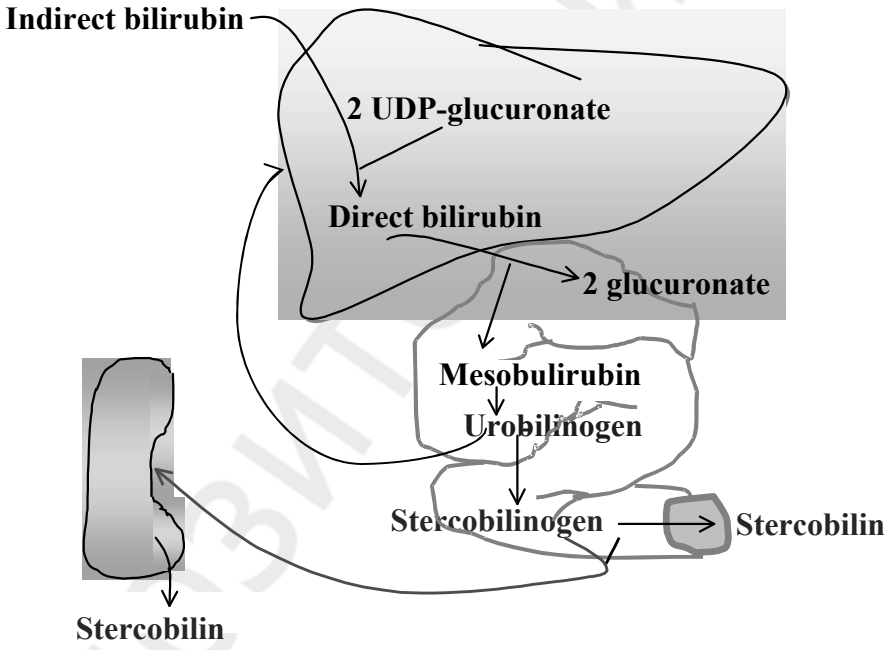



*Fig. 10.2.* Metabolism of bile pigments

The normal concentration of total bilirubin in blood plasma — 8,55–20,52 μmol/L; indirect bilirubin accounts for about 75–80 % of this concentration. An increase in the total bilirubin concentration in the blood to 25 μmol/L leads to the development of jaundice. Quantitative determination of both bilirurubins in the blood is essential for clinical differential diagnosis of various forms of jaundices (table 10.1).

***8. Hemoglobin biosynthesis.*** Hemoglobin is a conjugated protein. It is composed of a simple protein (globin) and a bound therewith non-protein component (heme). Globin is synthesized in much the same manner as all proteins. Heme synthesis takes place in all cells but occurs to the greatest extent in the liver and marrow (fig. 10.3).

*Table 10.1*

**Differential diagnosis for jaundice**

| Bile pigments / Jaundice, type of | Blood | | Urine | | | | Feces |
|---|---|---|---|---|---|---|---|
| | **Direct bilirubin** | **Indirect bilirubin** | **Direct bilirubin** | **Indirect bilirubin** | **Stercobilin** | **Urobilin** | **Stercobilin** |
| Norm | ± | + | – | – | + | – | + |
| Hemolytic | ± | ↑↑↑ | – | – | ↑↑ | – | ↑↑ |
| Hepatic | ↑↑↑ | ↑ | + | – | ± | ↑↑ | ± |
| Obstructive | ↑↑↑ | ↑ | + | – | – | – | – |

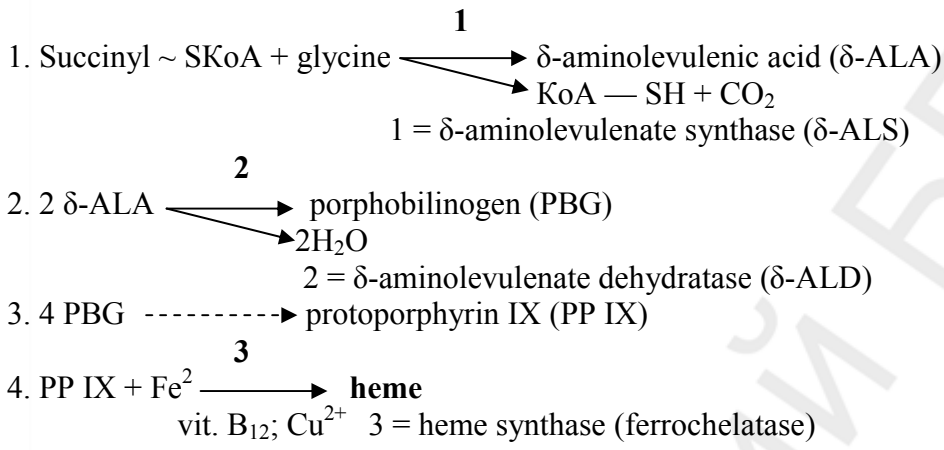



*Fig. 10.3.* Heme biosynthesis

**Heme synthesis regulation.** δ-ALS is inhibited via the feedback mechanism by the end synthetic product heme and is activated by steroid hormones. δ-ALD and ferrochelatase are also inhibited by heme. Furthermore they are extremely sensitive to inhibition by heavy metals.

### LIVER FUNCTION TESTS

The liver function tests (LFT) are the biochemical investigations to assess the capacity of the liver to carry out the functions it performs. LFT will help to detect the abnormalities and the extent of liver damage.

The major LFT may be grouped as follows:

**I. Tests based on synthetic functions** — determination of serum proteins. Albumins and globulins are synthesized in the liver, therefore their concentrations in plasma reflect the functional capacity of the organ. The normal concentration of albumins in the blood plasma is 40–50 g/L, of globulins — 20–30 g/L. Hence, normal albumin/globulin ratio is equal to 1,5–1,7. This ratio is always decreased in liver disease: in acute liver damage because of decreased albumin concentration and in chronic liver disease because of increased globulin concentration.

**II. Tests based on excretory functions** — measurement of bile pigments (see above), utilizing marker substances that are excreted or metabolized by the liver. We can measure either the rate of their removal from the blood (e. g., bromsulphthalein test) or the rate of formation of a metabolite.

**III. Tests based on detoxification** — determination of hippuric acid, indican and urea.

A. Hippuric acid is produced in the process of benzoic acid detoxification through its conjugation with glycine. Synthesis of hippuric acid in humans proceeds mainly in the liver, therefore in the clinical practice, in order to diagnose the antitoxic function of the liver, Quick's test is of common use. This test is based on the administration of sodium benzoate followed by a determination of hippuric acid in the urine. In the case of liver parenchyma damage the hippuric acid synthesis is reduced.

B. Determination of indican concentration in the urine allows estimating the rate of protein intestinal putrefaction as well as the functional state of the liver. In the latter case we can measure the indicant level in the urine after loading with diet protein. In the liver, indole (the putrefaction product) is initially oxidized to indoxyl which then undergoes conjugation with PAPS to form indican.

C. Urea synthesis occurs in the liver. Normally, the ratio *urea nitrogen/nonprotein blood nitrogen* is equal to 0,5 and decreases in liver damage.

**IV. Tests based on metabolic functions** — galactose tolerance test.

**V. Tests based on determination of serum enzymes derived from the liver.**

A. Liver cells contain several enzymes, which may be released into the circulation in the liver damage (**indicator enzymes**). In hepatitis, serum activity of the following liver enzymes significantly increases: *fructose-1-phosphate aldolase (aldolase B), ALT*, *AST*, *γ-glutamyl transpeptidase, $LDH_5$* (total LDH activity may be normal).

B. Normally, liver produces and releases some enzymes to the bloodstream (*choline esterase, blood clotting factors*). The serum level of such **secretory enzymes** may decrease in liver disease.

C. Some enzymes normally are excreted with the bile (*alkaline phosphatase*). The increased serum activity of **excretory enzymes** is a sign of obstructive disorders.

# CHAPTER 11
# INTEGRATION OF METABOLISM

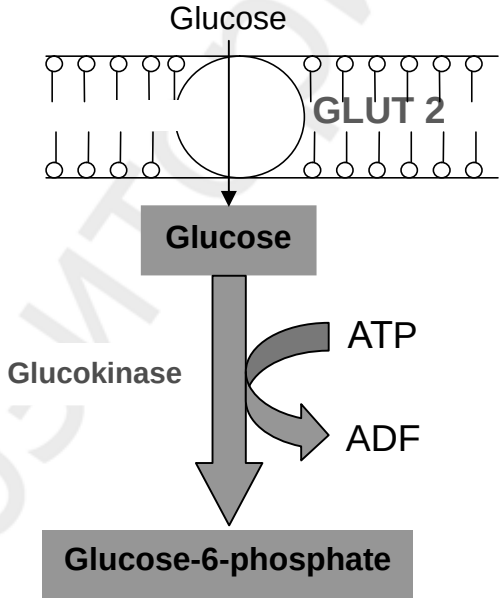
The metabolic processes in the human organism are not chaotic, they are highly integrative, coordinated and "finely tuned" to the major goal: preservation and continuation of the species. All transformations are united in an indivisible metabolic process subordinate to the laws of interdependence and interrelation. Cells are supplied with energy and substrates due to the integration of metabolic pathways, which is necessary for the maintenance of vital activity of organism. Liver plays the central role in maintaining the homeostasis of the body. Destination of metabolic pathways in the liver and other organs varies depending of their supplying with nutrients.

### PRINCIPAL COMPONENTS OF METABOLISM INTEGRATION

1. Presence of common intermediate products.
2. Use of common coenzymes.
3. Presence of a common catabolic way and unified system of releasing and using energy.
4. Presence of similar regulation mechanisms: substrate concentration, allosteric regulation, covalent modification of enzyme, enzyme concentration, compartmentation, i. e. differential localization of mutually exclusive metabolic processes.
5. Organ specialization.

### MAJOR PECULIARITIES OF METABOLISM IN THE LIVER IN THE WELL FED STATE

*Glucose concentration in the blood increases up to 6–8 mmol/L.* Insulin level in the blood increases. Glucose is transported to the liver and converted in glucose-6-phosphate by glucokinase; the latter activated by insulin in turn.




To reduce the blood glucose level the processes of its use in the liver are stimulated and glucose excess is converted to fatty acids:

1. Glycogen is synthesized as glycogen synthase activity increases under the action of high concentrations of insulin and glucose-6-P.

2. Glycolysis is stimulated since the action of insulin and allosteric regulators increases the activity of key enzymes (fig. 10.4):

– high concentration of fructose-2,6-PP activates allosterically phosphofructokinase-1;
– pyruvate kinase is activated by fructose-1,6-PP and insulin.

3. Intensive glycolysis and insulin stimulate pyruvate dehydrogenase complex and the formation of acetyl-CoA.

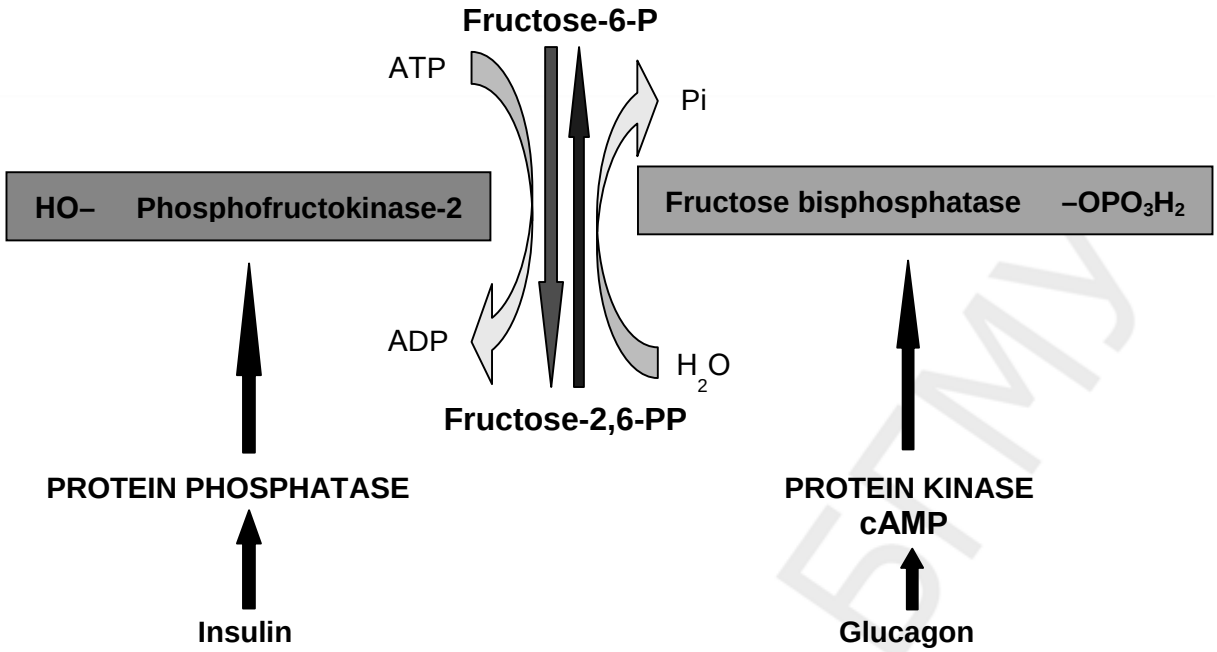



*Fig. 10.4.* Regulation of fructose-2,6-PP concentration by bifunctional enzyme

4. The pentose phosphate pathway is activated (insulin induces glucose-6-phosphate dehydrogenase).

5. Fatty acid synthesis is activated and β-oxidation is inhibited since:

– acetyl-CoA carboxylase is activated by insulin and high concentrations of citrate;

– insulin induces fatty acid synthase;

– high malonyl-CoA concentration inhibits acyl-carnitine transferase which transports fatty acids into the mitochondria for oxidation.

6. Fatty acids are used for synthesizing TAG, phospholipids secreted into the bloodstream as constituents of VLDL and HDL.

7. The supply of amino acids in the liver exceeds the consumption of them. Some of the amino acids are partially degraded, i. e. deaminated. The carbon skeletons derived from glucogenic amino acids are fed into intermediary metabolism, while the others are used for lipid synthesis. Protein synthesis is also increased to some extent.

### EXTRAHEPATIC TISSUE METABOLISM PECULIARITIES IN THE WELL FED STATE

**Muscle tissue:**

1) GLUT 4 insertion into the cell membrane is stimulated under the action of insulin and glucose uptake increases;

2) glycogen synthesis is activated;

3) amino acids are converted to α-keto acids via deamination; the latter are oxidized in Krebs cycle;

4) major fuels: fatty acids, ketone bodies.

**Adipose tissue:**

1) intensive TAG synthesis occurs;

2) the source of glycerol-3-phosphate is glucose which enters the adipose tissue involving GLUT 4;

3) lipolysis is inhibited as insulin dephosphorylates hormone-sensitive lipase.

**Nerve tissue.** Major sources of energy are glucose (120 g/day) and amino acids

**For kidney** basic energy sources are fatty acids (β-oxidation).

**Red blood cells:**

1) do not have mitochondria therefore aerobic metabolism is impossible;

2) glucose is an exclusive fuel; oxidized to lactate;

3) pentose phosphate pathway is intensified and provides reduced coenzymes for the reactions involved in maintaining the iron in hemoglobin in reduced state.

**Blood plasma.** Lipoprotein lipase is activated. TAG are hydrolyzed in chylomicrons and VLDL. Thus released fatty acids are taken up by adipose tissue and stored.

### ORGAN SPECIALIZATION OF METABOLISM IN FASTING

Glucose concentration decreases between meals to 4 mmol / l; it leads to:

– reduced secretion of insulin;
– decreased glucose utilization by all tissues except the nervous;
– increased secretion of glucagon.

Metabolic processes occurring at this time in the body are rearranged so as to maintain a normal blood glucose level.

**Liver:**

1. Liver prevents further decreasing of glucose concentration in the blood:
– glycogen degraded to glucose and the latter is released in the bloodstream;
– gluconeogenesis rate increases;
– glycolysis and glycogen synthesis are inhibited.
2. Major source of energy — fatty acids.
3. Acetyl-CoA is used for ketone bodies synthesis.
4. Intensive deamination of amino acids occurs.
5. Urea synthesis increases.
6. Oxydative decarboxylation of pyruvate is also inhibited.

**Skeletal muscles** (no glucagon receptors):

1) energy sources — fatty acids, ketone bodies.

2) storage of glycogen is mobilized under the influence of adrenalin; anaerobic glycolysis is activated.

**The heart muscle** (no glycogen present):

1) aerobic metabolism exclusively;
2) the main source of energy — fatty acids;
3) ketone bodies and lactate may be used.

**Adipose tissue:**

1) does not use glucose (Glut 4 doesn't function);
2) lipolysis occurs (glucagon activates hormone-sensitive lipase).

**Nerve tissue:**

1) major fuel is glucose (120 g/day);
2) glucose transporter — Glut 3. It provides permanent supply of glucose;
3) no glycogen present;
4) it's impossible to use FA since they do not penetrate the blood-brain barrier.

### INTERORGAN METABOLISM AFTER 12 HOUR FAST

**Blood plasma:**

– decreased insulin/glucagon ratio;
– glucose level decreases;
– fatty acids and ketone bodies levels increase 4-fold.

**Adipose tissue:**

– does not extract glucose from the bloodstream;
– increased activity of hormone-sensitive lipase due to glucagon. Releasing of fatty acids and glycerol.

**Muscle tissue:**

– protein degradation; release of high amounts of amino acids in the blood;

– decreased utilization of glucose (~ 40 % undergoes aerobic oxidation; ~ 40 % degraded to lactate; ~ 20 % leaves muscle as alanine);
– energy sources — fatty acids and ketone bodies.

**Liver:**
– gluconeogenesis is stimulated (amino acids, lactate and glycerol are used as substrates);
– β-oxidation of fatty acids is activated and an excess of acetyl-CoA used for the formation of ketone bodies.

**Kidney** continues to use fatty acids and ketone bodies as fuels.

**Nerve tissue** continues to use glucose as a source of energy.

### INTERORGAN METABOLISM AFTER A 3-DAY FAST

**Blood plasma:**
– decreased insulin/glucagon ratio (~ 10 % well fed state);
– increased cortisol level;
– increased fatty acids (8–10-fold);
– glucose decreased (~ 60 %);
– ketone bodies elevated (~ 20-fold).

**Adipose tissue.** Hormone-sensitive lipase activity increased by the action of glucagon and cortisol: fatty acids and glycerol released.

**Muscle tissue:**
– increased protein degradation to amino acids;
– no glucose used for fuel;
– fuels — fatty acids and ketone bodies.

**Liver:**
– no glycogen present;
– active gluconeogenesis occurs (amino acids, lactate and glycerol are used as substrates);
– ketone bodies synthesis;
– urea synthesis increases.

**Kidney:**
– fuels — fatty acids and ketone bodies;
– active gluconeogenesis (amino acids, mainly glutamine, are used as substrates).

**Nerve tissue:**
– continue to use glucose as major fuel;
– ~ 1/3 of energy derived from ketone bodies.

### INTERORGAN METABOLISM AFTER 3 WEEKS OF STARVATION

**Blood plasma:**
– decreased insulin/glucagon ratio (10-fold lower than in the well fed state);
– increased cortisol level;
– ketone bodies elevated ~ 100-fold; leads to metabolic acidosis;
– urea decreased.

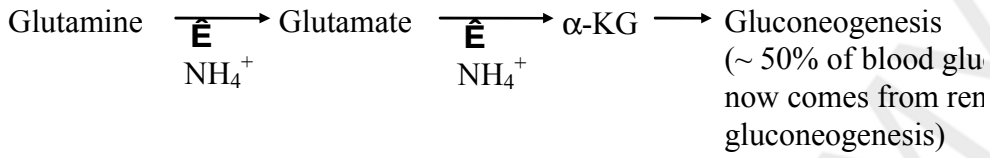
**Adipose tissue:** increased activity of hormone-sensitive lipase. Lipolysis intensified; TAG being degraded to fatty acids and glycerol.

**Muscle tissue:**
– protein degradation to amino acids, but decreasing;
– fuels — fatty acids and ketone bodies.

**Liver:**
– gluconeogenesis occurs (amino acids, lactate and glycerol are used as substrates);
– ketogenesis intensified.

**Kidney:**
– increased ketone bodies in the blood — ketonemia; it leads to metabolic acidosis;
– metabolic acidosis leads to cation depletion;

– concentration of ketone bodies in the blood exceeds renal reabsorption threshold; ketone bodies being lost in the urine — ketonuria;
– fuels — fatty acids and ketone bodies;
– increased utilization of glutamine for expendable cations ($NH_4^+$).




**Nerve tissue:** ~ 2/3 of energy derived from ketone bodies; ~ 1/3 from glucose.

## CHAPTER 12
## BIOCHEMISTRY OF NUTRITION. VITAMINS AND OTHER ESSENTIAL NUTRITIVE FACTORS. PROTEIN-ENERGY MALNUTRITION

The science of **nutrition** examines the qualitative and quantitative requirements of the diet necessary to maintain good health.

**Nutrient** is an integral part of food, which provides the body with structural-functional components or energy.

Main categories of nutrients:

– energy-yielding (macronutrients — proteins, carbohydrates, fats);
– micronutrients — vitamins, minerals;
– dietary fibers — indigestible fiber polysaccharides.

*Essential nutritive factor* is a substance provided to the body with food as the body itself cannot form it in the sufficient amount.

**Essential nutritive factors:**

– water;
– energy (calories) from carbohydrates, fats or proteins;
– 8–10 essential amino acids;
– essential fatty acids;
– 13 vitamins;
– 16–20 minerals.

Energy needs of the adults at rest — 1300–1800 kcal. They increase with burns, trauma, infectious disease, in the postoperative period. At fasting they decrease. The main sources of energy are carbohydrates — 42 %, fats — 40 % proteins — 15 % alcohol — 3 %. Preferably, 55 % of the ATP formed in the cleavage of carbohydrates, 30 % — lipids, 15 % — proteins.

**Protein** is a significant source of **nitrogen.** Animals cannot synthesize some amino acids — essential amino acids.

| Essential amino acids | Nonessential amino acids |
|---|---|
| Arginine[1] | Alanine |
| Histidine[1] | Aspartate |
| Leucine | Asparagine |
| Isoleucine | Glutamate |
| Valin | Glutamine |
| Methionine | Glycine |
| Phenylalanine | Serine |
| Threonine | Cysteine[2] |
| Tryptophan | Tyrosine[3] |
| Lysine | Proline |

1 — only during rapid growth
2 — formed from food methionine
3 — formed from food phenylalanine

Part of proteins consumed is the energy source to provide metabolism in the cells of organs and tissues:

– amino acids that form pyruvate or intermediates of the TCA cycle are **glucogenic** (or gluconeogenic) since they provide carbon for synthesis of glucose — Ala, Asp, Asn, Glu, Gln, Pro;
– amino acids that form acetyl-CoA or acetoacetate are **ketogenic** since they form primarily ketone bodies — Lys, Leu;
– some amino acids are both glucogenic and ketogenic — Ile, Trp, Phe, Tyr;

– in case when amount of carbohydrates and lipids is sufficient proteins are not necessary for producing energy, so they are converted into TAG and stored in fatty depots.

**Carbohydrates.** The most important function is **energy production:**

– monosaccharides undergo glycolytic oxidation in the presence and absence of oxygen producing energy;

– poly- and oligosaccharides (except dietary fibers) are split to monosaccharides which are oxidized by glycolytic pathway;

– intracellular metabolism of carbohydrates leads to releasing energy from various monosaccharides but the brain specifically uses **only glucose** as fuel;

– when the amount of carbohydrates exceeds the necessary requirement in energy they are converted into glycogen and TAG;

– but in case they are not enough ketone bodies are synthesized from acetyl-CoA acting as fuel (including for the brain).

**Fatty acids and lipids.** Fatty acids and TAG are used as a source of energy by the cells of the most organs and tissues. Phospholipids are constituents of cell membranes. Excess of the dietary fat is stored as TAG. It is necessary to avoid deficiency of dietary lipids supply since some fatty acids cannot be synthesized in the body therefore must be consumed in the diet. They are referred to as essential fatty acids (more frequently called polyunsaturated fatty acids — PUFA). PUFA — linoleic (ω6) and linolenic (ω3) — cannot be synthesized by humans. In a strict sense, only these two are essential fatty acids. Arachidonic acid (ω6) can be synthesized from linoleic acid but nutritionists recommend that it is better to include some amount of arachidonic acid also in the diet.

The energy released during the digestion of food in the body is stored in the form of ATP. At rest, 36 % of the formed ATP is consumed by the enzymatic reaction; 22 % — $Na^+$, $K^+$-ATPase; 21 % — biosynthesis of proteins, 11 % — muscle contraction and 10 % — transport of calcium ions through biological membranes.

## VITAMINS

Vitamins are referred to as organic substances necessary in small amounts for the body to function properly and for its metabolic integration; cannot be synthesized by the human body and must, therefore, be supplied by the diet. Deficiency of vitamins leads to characteristic symptoms and diseases; compensation of the deficit results in their correction. The currently accepted classification is based on the physico-chemical properties (in particular, solubility) and chemical nature of vitamins and makes use of letters of the alphabet. Each vitamin, alongside its letter notation, is supplemented with a succinct characteristic of its biological activity, occasionally with the prefix "anti", indicative of the capacity to prevent or check the course of the corresponding disease. In terms of solubility, ***water-soluble*** and ***fat-soluble*** vitamins are distinguished.

### Water-soluble vitamins

Distinctive feature of water-soluble vitamins and their derivatives is the involvement in the build up of **coenzymes.**

### Vitamin $B_1$ (thiamin, antineuritic vitamin)

Coenzyme (active) form — *thiamin pyrophosphate (TPP).*

**Biological role:**

– thiamin in the form of TPP is involved in oxidative decarboxylation of pyruvate and α-ketoglutarate;

– TPP also acts as a coenzyme of transketolase in the pentose phosphate pathway.

**Deficiency.** "Dry" beriberi. Symptoms include peripheral neuropathy, fatigue and impaired capacity to work. "Wet" beriberi develops with a severe deficiency. Edema is the important feature. In addition to neurologic manifestations, cardiovascular symptoms are more apparent, including heart enlargement and tachycardia. Wernicke–Korsakoff syndrome is seen primarily in alcoholics

because of their poor nutrition. Wernicke's encephalopathy consists of nystagmus, ataxia, ophthalmoplegia, impaired consciousness. Korsakoff's psychosis consists of mental confusion, listlessness, dysphonia, and confabulation with impaired memory of recent events.

Daily requirement — 1.1–1.5 mg. Sources — wheat coarse bread, cereal, grain husk, soybean, haricot bean, peanut. Of the products of animal origin — liver, lean meat, kidney, brain, egg yolk.

**Vitamin $B_2$ (riboflavin)**

Coenzyme forms — *FMN, FAD.*

**Biological role:**

– FMN and FAD are constituents of enzymes known as flavoproteins. They play role in the electron transport system in mitochondria (complexes I and II);

– FMN and FAD are coenzymes of L- and D-amino acid oxidases respectively;

– FAD is also a coenzyme of acyl-CoA dehydrogenase, which is involved in the oxidation of fatty acids.

**Deficiency.** Vascularization of cornea, keratitis, cataract, lesions of the lips, mouth skin and genitalia, especially angular stomatitis, cheilosis, glossitis (magenta tongue).

Daily requirement — 1–3 mg. Sources — liver, kidney, egg yolk, cottage cheese, sour milk, cereals.

**Pantothenic acid**

Coenzyme form — *4′-phosphopantetheine*.

**Biological role.** 4′-phosphopantetheine is a functional part of coenzyme A (CoA-SH) and acyl carrier protein (ACP-SH).SH-group acts as carrier of acyl radicals in both CoA-SH and ACP-SH. This occurs with CoA-SH in reactions of the citric acid cycle, fatty acid synthesis and oxidation, acetylation reactions (e. g. drugs) and cholesterol synthesis. ACP-SH participates in reactions concerned with fatty acid synthesis.

**Deficiency.** The vitamin is widely distributed in all foods, and deficiency has not been unequivocally reported. However, the burning foot syndrome has been ascribe to pantothenate deficiency in prisoners of war and in associated with reduced capacity for acetylation.

Daily requirement — 10–15 mg. Sources — royal jelly, yeast, liver, egg yolk, buckwheat, oats liver, kidney, egg yolk, cottage cheese, sour milk, cereals.

**Nicotinic acid (niacin, PP — "pellagra preventive")**

Coenzyme forms — *$NAD^+$ ($NADH{\cdot}H^+$), $NADP^+$ ($NADPH{\cdot}H^+$)*.

**Biological role.** $NAD^+$ and $NADP^+$ play a widespread role as coenzymes of many dehydrogenases occurring both in cytosol and within mitochondria. They are therefore key components of many metabolic pathways affecting carbohydrate, lipid and amino acid metabolism. Generally, NAD-linked dehydrogenases catalyze oxidoreduction reactions in oxidative pathways; NADP-linked dehydrogenases or reductases are often found in pathways concerned with reductive synthesis (fatty acids, cholesterol and steroid hormones syntheses). $NADPH.H^+$ is also involved in microsomal oxidation of xenobiotics.

**Deficiency** of niacin results in pellagra. Clinical manifestations include the "three Ds": diffuse, pigmented rash (dermatitis); gastroenteritis (diarrhea) and widespread neurologic deficits, including psychosis and cognitive decline (dementia).

Daily requirement — 15–25 mg. Sources — liver, meat, rice, bread, potatoes. The essential amino acid tryptophan present in the dietary proteins is converted into niacin in the body. Thus in order to produce niacin deficiency a diet must be poor in both available niacin and tryptophan.

**Vitamin $B_6$, (pyridoxine, pyridoxal, pyridoxamine, antidermatitic vitamin)**

Coenzyme forms — *pyridoxal phosphate*, *pyridoxamine phosphate.*

**Biological role:**

– transamination reactions;

– decarboxylation of amino acids;
– biogenic amines detoxification (oxidation);
– glycogen breakdown (pyridoxal phosphate acts as coenzyme for glycogen phosphorylase);
– heme synthesis;
– thyroid hormones synthesis and degradation in peripheral tissues;
– synthesis of niacin from tryptophan.

**Deficiency.** Skin lesions — dry seborrheic dermatitis. Hypochromic anemia (impaired heme synthesis). Convulsions due to depletion of γ-amino-butyric acid content. Neurological symptoms such as depression, irritability, nervousness. In tuberculous patients: in treating this disease the patients are prescribed isoniazid which has been found to be antagonistic to vitamin $B_6$.

Daily requirement — 2.0–2.2 mg. Sources — meat, fish, beans, nuts, whole grains, potatoes.

**Folic acid ($B_c$, $B_9$)**

Coenzyme form — *tetrahydrofolate.*

**Biological role.** THF acts as a carrier of one-carbon fragments: methyl (–$CH_3$), methylene (=$CH_2$), methenyl (≡CH), formyl (–CHO), formimino (–CH=NH).

One-carbon fragments are necessary for:
– nucleotide synthesis → DNA synthesis;
– conversion of glycine to serine;
– methylation of homocysteine to form methionine. Elevated blood homocysteine is an associated risk factor for atherosclerosis, thrombosis and hypertension.

**Deficiency.** Macrocytic megaloblastic anemia. Maternal deficiency increases the risk of fetal neural tube defects (spina bifida) and other brain defects (anencephalia).

Daily requirement — 400 μg. Sources — green leafy vegetables, particularly salad greens, yeast, liver, meat, tomatoes, egg yolk, strawberries. Folic acid is very important for all women who may become pregnant. Adequate folate intake during the periconceptual period, the time just before and just after a woman becomes pregnant, protects against neural tube defects.

**Vitamin $B_{12}$ (cobalamine, antianemic vitamin)**

Coenzyme forms — *5-deoxyadenosylcobalamine and methylcobalamine.*

**Biological role.** Vitamin $B_{12}$ is one of the most complex metabolites in nature. It contains a complicated ring system with cobalt as central atom. Vitamin $B_{12}$ can only be absorbed when the gastric mucosa is secreting the so-called intrinsic Castle's factor. This glycoprotein binds cobalamine and thus protects it from degradation. Substantial amounts of vitamin $B_{12}$ can be stored (!) in the liver. Active forms of cobalamine are involved in rearrangement reactions:
– 5-deoxyadenosylcobalamine functions as coenzyme of methylmalonyl-CoA mutase. It converts methylmalonyl-CoA in succinyl-CoA essential for heme synthesis;
– methylcobalamine acts as coenzyme of homocysteine methyltransferase, which catalyzes methylation of homocysteine to methionine.

Vitamin $B_{12}$ function is related to folic acid metabolism; lack of methylcobalamine leads to a deficiency in the folate coenzyme pool.

**Deficiency.** Pernicious macrocytic megaloblastic anemia (Addison — Biermer anemia). Damage to the white matter of the spinal cord and brain; demyelinating and axonal peripheral neuropathy. Mucosal atrophy and inflammation of tongue (glossitis).

Daily requirement — 3 μg. Sources — cobalamine is the only vitamin whose synthesis is carried out exceptionally by microorganisms; both animal and vegetable tissues lack this capacity. Major dietary sources — liver meat and meat products, seafood (crabs, salmon, sardines), milk, eggs.

**Biotin (vitamin H, antiseborrheic vitamin)**

Coenzyme form — *carboxybiotin.*

**Biological role.** Carboxybiotin acts as a coenzyme in carboxylation reactions:
– Pyruvate → Oxaloacetate (gluconeogenesis);

– Acetyl-CoA → Malonyl-CoA (fatty acid synthesis);
– Propionyl-CoA → Methylmalonyl-CoA (subsequently is converted to succinyl-CoA, which enters citric acid cycle or initiates heme synthesis).

**Deficiency.** Biotin deficiency due to inadequate dietary intake is very rare unless accompanied by other factors such as following. Antibiotics that inhibit the growth of intestinal bacteria eliminate this source of biotin. Ingestion of unusually large amounts of avidin, a protein present in raw egg whites, prevents biotin absorption because it has a very high affinity for biotin. Seborrheic dermatitis, hair loss (alopecia), erythematous rash, muscular pain, drowsiness, fatigue.

Daily requirement — 150–200 μg. Sources — intestinal bacteria are capable of synthesizing biotin in sufficient amounts. Legumes, cauliflower, mushrooms; of the products of animal origin — liver, kidneys, egg yolk, milk.

**Vitamin C (ascorbic acid, antiscorbutic vitamin)**

The most important property of *ascorbic acid* is its capacity to be oxidized reversibly to dehydroascorbic acid thereby it is involved in the oxidation-reduction reactions of the cell. *No coenzyme form.*

**Biological role:**
– hydroxylation of proline and lysine in collagen synthesis;
– hydroxylation of tryptophan to hydroxytryptophan in serotonin synthesis;
– hydroxylation reactions in the syntheses of epinephrine, norepinephrine (adrenal medulla hormones), corticosteroid hormones;
– participates in thyroxin synthesis;
– acts as prooxidant in microsomal oxygenase system;
– stabilizes vitamin E functioning as antioxidant;
– it is required for the absorption of iron and icorporation of plasma iron in ferritin; it is also essential for mobilization of iron from ferritin.

**Deficiency.** Scurvy: abnormal bone development in infants and children, easy bruising and bleeding due to fragile capillaries, loosening of teeth and swollen gums, easy fracturability of bones, poor wound healing, osteoporosis. Iron-deficiency anemia. Increasing of reactive oxygen species generation and reducing of the body immune protection.

Daily requirement — 100–200 mg (2.5 mg/kg body weight). Sources — ascorbic acid is an essential food factor for man, monkey and guinea pig. All the other animal species can synthesized it. Citrus fruits and their juices, strawberries, gooseberry, black currant guava, green leafy vegetables (lettuce, spinach), cabbage, green pepper, fresh potatoes, fresh raw fish.

**Fat-soluble vitamins**

*Differences from water-soluble vitamins:*
– act at genetic level as steroids: inducers of protein synthesis;
– structural components of cellular membranes; act as antioxidants (may act as prooxidants);
– are not coenzymes (except vitamin K).

**Vitamin A (retinol, antixerophtalmic, growth vitamin)**

Retinol is a cyclic unsaturated monobasic alcohol, containing a β-ionone ring and side polyisoprenoid chain. In the body there are 3 forms (vitamers) of vitamin A: retinol, retinal and retinoic acid. In vegetables and fruits vitamin A exists as provitamin in the form of β-carotene. Only retinol has full vitamin A functions, the others fulfilling some, but not all, vitamin A functions (fig. 12.1).

**Biological role of β-carotene:** antioxidant, stimulates immunogenic and antitumor activity.

Daily requirement — 2–5 mg. Sources — all pigmented vegetables and fruits: carrots, pumpkin, red pepper, tomatoes, papayas, apricots, peaches and leafy green vegetables.

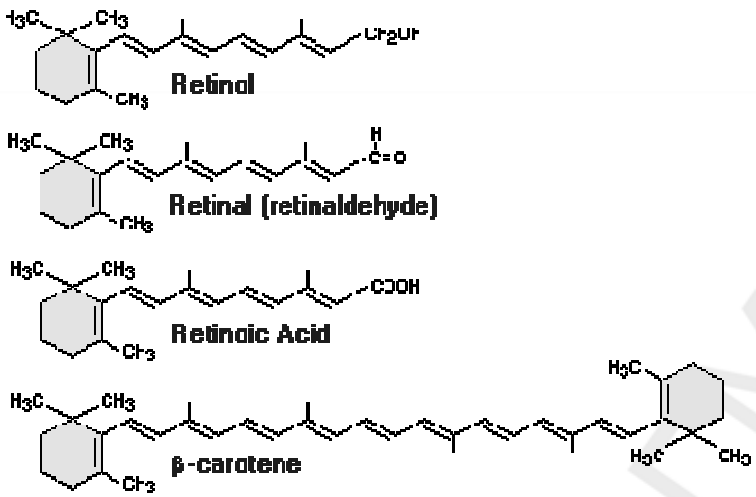



*Fig. 12.1.* β-Carotene and vitamin A vitamers

**Vitamin A metabolism.** Enters the liver with chylomicrons remnants and deposited as retinol esters. Esters are hydrolyzed for being transported to peripheric tissues and free retinol is bound with plasma retinol-binding protein. In cytoplasm of extrahepatic cells: retinol → cis-retinoic acid → binding with protein → nucleus → binding to the receptor connected with DNA → change in transcription (in complex with hormones).

**Biological role:**

– specific role in the physiologic mechanism of vision;

– as antioxidant: enhances vitamin E antioxidant activity; includes selenium into the active center of glutathione peroxidase together with tocoferol and ascorbic acid; prevents from oxidation SH-containing proteins of keratin, i. e. prevents keratinization;

– regulates cell growth and differentiation;

– participates in glycoprotein synthesis (glycosylation reactions) which are required for growth and mucus secretion;

– stimulates cellular immunity (increases T-killers activity).

**Deficiency:**

– impairment of dark adaptation progressing to night blindness (nyctalopia);

– keratinizing metaplasia of epithelium of nose, respiratory mucosa, esophagus and genitourinary tract;

– xerophthalmia — thickening and loss of transparency of the bulbar conjunctiva with yellowish pigmentation;

– follicular conjunctivitis;

– keratomalacia — softening of the cornea, in advanced cases, with ulceration and necrosis;

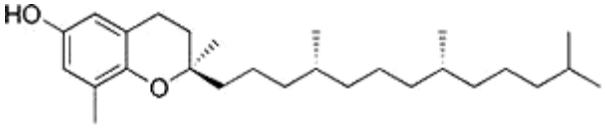
– follicular hyperkeratosis of the skin.

**Toxicity.** Vitamin A toxicity in children may result from taking large doses, usually accidentally. In adults, acute toxicity has occurred when arctic explorers have ingested polar bear or seal livers: inflammation of the cornea, hyperkeratosis; loss of appetite, nausea, vomiting, diarrhea; headache, pain in joints; hepatomegaly.

Daily requirement — 1.5–2.5 mg. Sources — fish (cod or shark) liver oil, bovine and pig liver, egg yolk, dairy products.

**Vitamin E (tocoferol, fertility vitamin)**

From the Greek «*tokos*», offspring + «*pherein*», to carry.



Contains a chromanol ring, with a *hydroxyl* group that can donate a *hydrogen* atom to *reduce free radicals* and a *hydrophobic side chain* which allows for penetration into *biological membranes*.

**Metabolism.** Enters the gastrointestinal tract as part of oil → enterocytes → chylomicrons → lipoprotein lipase → remnants → liver → binding with proteins → export into the blood as part of VLDL → supply to extrahepatic tissues as constituent of LDL.

**Biological role:**

1. The major antioxidant:
– membrane-stabilizing activity;
– prevention from oxidation of membrane proteins SH-groups;
– prevention from oxidation of double bonds in carotene molecules and in vitamin A;
– includes selenium into the active center of glutathione peroxidase together with ascorbic acid;
– inhibits vitamin C prooxidant activity;
– antihypoxic activity (leads to increasing of coupling between tissue respiration and oxidative phosphorylation).
2. Inhibits lysosomal phospholipase $A_2$ activity.
3. Controls nucleic acid synthesis.

**Deficiency:**

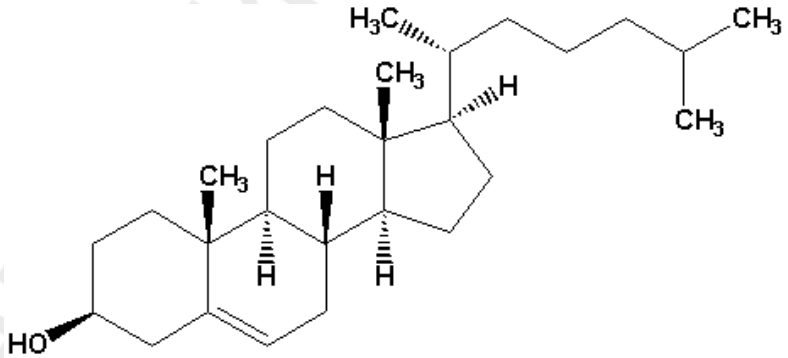
– increased membrane permeability;
– muscular dystrophy;
– infertility, abortion;
– liver necrosis and cerebrospinal degeneration (particularly of cerebellum).

**Toxicity.** Many adults take relatively large amounts of vitamin E for months to years without any apparent harm.

Daily requirement — 20–40 mg. Sources — fresh vegetable oils, nuts, buckwheat, wheat germs, lettuce, cabbage; of the products of animal origin — butter, lard, egg yolk, caviar.

**Vitamin D (calciferol, antirachitic vitamin)**

The vitamins D are a group of compounds. All are sterols occurring chiefly in animal organisms but also in plants and yeast. Vitamin D is a prohormone with several active metabolites that act as hormones. The most important D vitamins are $D_2$ (ergocalciferol, is formed in plants) and $D_3$ (cholecalciferol). $D_3$ is formed from 7-dehydrocholesterol in exposed skin.




**7-Dehydrocholesterol**

**Ultraviolet light**

**Vitamin $D_3$ (cholecalciferol)**

**Metabolism.** In the liver, dietary vitamin $D_3$ (or $D_2$) is taken up from the blood (bound to a specific plasma $\alpha_2$-globulin) after absorption from micelles in the intestine. It is hydroxylated to form 25-hydroxycholecalciferol. In the kidney the latter is further hydroxylated to 1,

25-dihydroxycholecalciferol (calcitriol; 1, 25$(OH)_2$-$D_3$), the most potent vitamin D metabolite (active form of vitamin $D_3$).

**Biological role:**

– the main function of vitamin D is to maintain plasma calcium and phosphate concentration. Calcitriol increases calcium and phosphate concentration in the blood (for more details see chapter 13 "Biochemistey of nutrition. Water and mineral metabolism");

– regulates marrow cells growth and differentiation;

– calcitriol is involved in insulin secretion, synthesis and secretion of parathyroid and thyroid hormones;

– acts as antioxidant and anticarcinogen.

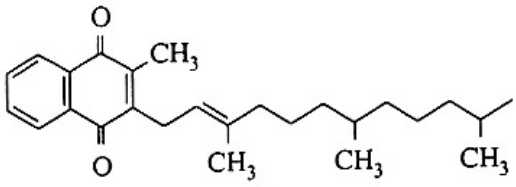
**Deficiency.** In children — rickets. In young infants rickets causes softening of the entire skull (craniotabes). When palpated, the occiput and posterior parietal bones feel like a ping pong ball. In older infants with rickets, sitting and crawling are delayed, as is fontanelle closure; there is bossing of the skull and costochondral thickening. Costochondral thickening can look like beadlike prominences along the lateral chest wall (rachitic rosary). In children 1 to 4 year epiphyseal cartilage at the lower ends of the radius, ulna, tibia, and fibula enlarge; kyphoscoliosis develops, and walking is delayed. In older children and adolescents walking is painful; in extreme cases, deformities such as bowlegs and knock-knees develop. In adults — osteomalacia. There is an increased radiolucency of bones and tendency for fractures to occur. In the elderly, hip fractures may result from only minimal trauma.

**Toxicity.** Usually, vitamin D toxicity results from taking excessive amounts. The main symptoms of vitamin D toxicity result from hypercalcemia. Anorexia, nausea, and vomiting can develop, often followed by polyuria, polydipsia, weakness, nervousness, pruritus, and eventually renal failure. Proteinuria, azotemia, and metastatic calcifications (calcium deposition in organs and tissues, particularly in the kidneys) can develop.

Daily requirement: infants and children — 10–25 μg, adults — 5 μg. Sources — liver and viscera of fish and liver of animals which feed on fish, cod liver oil, egg yolk, butter.

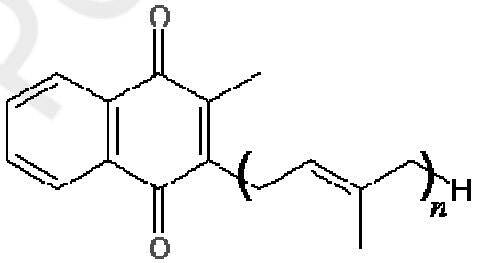
**Vitamin K (naphthoquinones, antihemorrhagic vitamin)**

Three compouds have the biologic activity of vitamin K: phylloquinone (vitamin $K_1$), the normal dietary source found in green vegetables; menaquinone (vitamin $K_2$), synthesized by intestinal bacteria; menadione (vitamin $K_3$), synthetic compound. All three vitamins are naphthoquinone. Isopprenoid side chain is present in vitamin $K_1$ and $K_2$. Vitamin $K_3$ is water insoluble and has been used for the synthesis of water-soluble derivative named vicasol.

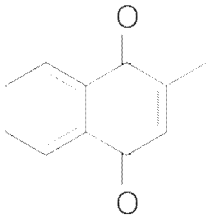



***Vitamin K1***

**Phylloquinone**




**Menaquinone (vitamin $K_2$)**




**Menadione (vitamin $K_3$)**

**Metabolism.** Vitamin K is absorbed only in presence of bile acids, like other lipids, and is distributed in the bloodstream via the lymphatics in chylomicrons. Vicasol, being water-soluble, is absorbed in the absence of bile acids. Vitamin K is stored mainly in the liver, to lesser extent in other tissues.

**Biological role:**

– participates in the synthesis of blood clotting factors II, VII, IX, X, all of which are synthesized in the liver initially as inactive precursor proteins. Vitamin K acts as a coenzyme (!) of γ-glutamate carboxylase. The latter converts glutamate to γ-carboxyglutamate. The γ-carboxyglutamatic acid residues are negatively charged ($COO^-$) and they combine with positevely charged calcium ions ($Ca^{2+}$) to form complex (fig. 12.2);

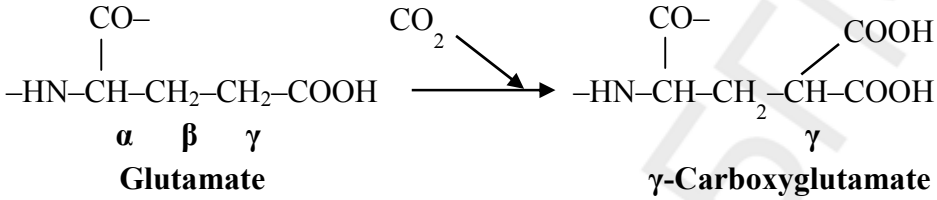



*Fig. 12.2.* γ-Carboxyglutamate synthesis

– vitamin K is structurally related to ubiquinone, the main mitochondrial antioxidant, therefore vitamin K is also considered to act as antioxidant;

– it is also involved in electron transport and photosynthetic phosphorylation.

**Deficiency.** Vitamin K deficiency is of rare occurrence in adults. This is due to two major reasons: firstly, the mixed diet is rich enough in vitamin K; secondly vitamin K is synthesized by the intestinal microflora. Vitamin K deficiency can be caused by fat malabsorption, which may be associated with pancreatic dysfunction, biliary disease, atrophy of the intestinal mucosa. In addition, sterilization of the colon by antibiotics can result in deficiency when dietary intake is limited. However newborn infants are vulnerable to the deficiency (hemorrhagic disease of newborn) because the placenta does not pass the vitamin to the fetus efficiently and the gut is sterile immediately after birth. Indirect artificial anticoagulants (dicumarin, synkumar, warfarin) are vitamin K antagonists.

Daily requirement: infants and children — 0.1 mg. Sources — green leafy vegetables, e. g. cabbage, spinach, green tomatoes, pumpkin, ashberry. Vitamin K practically never occurs in animal products, except pig liver.

## VITAMIN-LIKE SUBSTANCES

**Vitamin-like substances** — are not indispensable and are actually not vitamins. They are synthesized by the cells in humans (compared to vitamins) and are of utmost importance for normal metabolism of the body.

**Bioflavanoids (vitamin P)**

**Biological role:**

– stabilize the ground compound of connective tissue by inhibiting hyaluronidase activity;
– maintain normal capillary permeability;
– antioxidants.

**Choline**

**Biological role:**

– it is involved in membrane structure and lipid transport as a component of phospholipids (lecithins);

– prevents the accumulation of fats in the liver — lipotropic factor;

– precursor for the synthesis of acetylcholinestabilize the ground compound of connective tissue by inhibiting hyaluronidase activity.

**Inositol**

**Biological role:**

– inositol is required for the synthesis of phosphatidylinositol which is constituent of cell membrane;

– acts as lipotropic factor (along with choline) and prevents the accumulation of fats in the liver;

– acts as a second messenger ($IP_3$) for some hormones.

**Lipoic acid**

**Biological role.** Lipoic acid is involved in the decarboxylation reactions along with other vitamins (thiamin, niacin, riboflavin and pantothenic acid). The conversion of pyruvate to acetyl-CoA and α-ketoglutarate to succinyl-CoA require lipoic acid.

**Para-aminobenzoic acid (PABA)**

**Biological role:**

– PABA may be regarded as a vitamin in another vitamin (folic acid);

– it has been reported that PABA is essential for the normal process of pigmentation of hair;

– PABA is capable of producing a stimulating action on tyrosinase, the key enzyme in the biosynthesis of melanins, which impart normal color to the skin.

The sulfa drug sulfanilamide is a structural analogue of PABA. Sulfanilamide competes with PABA and acts as a bacteriostatic agent. Ingestion of large doses of PABA will compete with the action of drugs and therefore should be avoided during sulfanilamide therapy.

**Vitamin $B_{15}$ (pangamic acid)**

**Biological role:**

– it is involved in the biosynthesis of choline, methionine and creatine as a source of methyl groups;

– pangamic acid preparations are effective in the treatment of fatty liver and certain forms of oxygen deficiency.

**Vitamin U (methylmethionine, ulcer-preventive factor)**

**Biological role:**

– it is involved in the biosynthesis of choline, methionine and creatine as a source of methyl groups (along with pangamic acid);

– vitamin U is effective in the treatment of peptic ulcer (inhibits activity of histamine, which stimulates peptic juice secretion).

## PROTEIN-ENERGY MALNUTRITION

**Protein-energy malnutrition** is a pathological condition due to inadequacy of nutrition supply and expenditure, which causes body weight loss and changes in the body compounds (table 12.1).

**Causes:** social, economic, ecological, inappropriate food, production technologies, harmful habits, biological.

Biological causes — external (malnutrition, injuries, infections) and internal (impairment of digestion, malabsorption) factors. There are two common forms of *protein-energy malnutrition:* **kwashiorkor and marasmus (cachexia)**.

*Table 12.1*

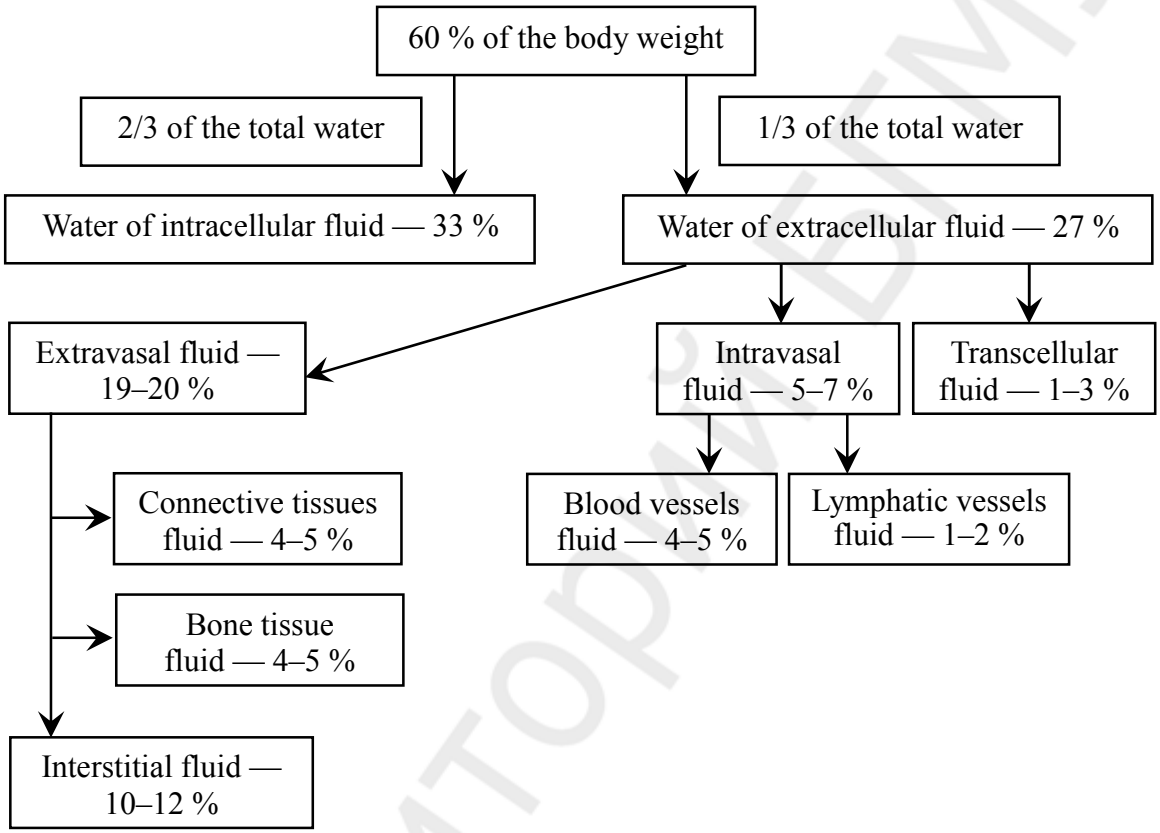
**Clinical forms of protein-energy malnutrition**

| **Clinical forms** | **KWASHIORKOR**<br>Early period after bad injuries burns, major surgical interventions | **MARASMUS**<br>Malignant tumors |
|---|---|---|
| **Primary cause** | Protein deficiency due to its lack in food, malabsorption | General deficiency of energy sources |
| **Edema** | *Occurs.* Develops as a result of the low oncotic pressure in the vessels *(hypoalbuminemia)* | Absent |
| **Hypo-albuminemia** | Albumin synthesis reduces in the liver (the compensatory reaction) | Absent |
| **Fatty liver** | *Occurs.* Proteins decrease in food, but carbohydrates consumption increases | Absent |
| **Level of insulin in the blood** | Normal | *Decreased.* Catabolic processes prevail, they being directed at getting energy from any depot |
| **Level of adrenalin in the blood** | Normal | *High* |
| **Loss of muscle weight** | No loss or slight loss | *Occurs.* May be marked as a result of protein catabolism |
| **Fat deposits** | Slight loss | Absent |
| **Temporary character of disease** | Develops in short time | *Gradual.* Unmarked catabolic response to starvation (may take a lot of time) |
| **Hypo-pigmentation** | Pallor | Absent |

# CHAPTER 13
# BIOCHEMISTRY OF NUTRITION. WATER AND MINERAL METABOLISM

## WATER

Water is an essential part of living organisms. In adults water content is about 60–65 % of the body weight. In the organs and tissues water content varies. Muscle tissue contains up to half of the total body water, in the skeleton — 1/8, in the skin — 1/16, in blood — 1/20. 2/3 of the body water is intracellular fluid water, 1/3 of the water located extracellularly:




**Biological role of water.** Water is universal solvent, contributes to dissociation of substances, participates in metabolism reactions (hydrolysis and hydratation) and transport of different substances, in biopolymer native structure formation and stabilization, fulfils structural (is part of supramolecular structures) and mechanical function (aids the cells and intercellular substance turgor), heat balance regulator.

Water requirement: adults — 30–40 g/kg/day; children — 3–4-fold more. Water intake: 2.5 L per day: 1.2 L with drinking water; 1.0 L — with food; 0.3 L — metabolic water. Water excretion: 1500 ml — with urine; 600 ml — by means of lungs; 300 ml — through the skin; 100 ml — through intestine with feces.

In healthy humans water balance is being maintained. Excessive consumption of water is accompanied by increased excretion from the body. Water retention in the body (*hyperhydration*) leads to high blood pressure and edema. *Dehydratation* (*hypohydration*) occurs when water excretion exceeds water consumption. 20 % loss of body water is incompatible with life. Vasopressin is the main regulator of water excretion from the body.

## VASOPRESSIN

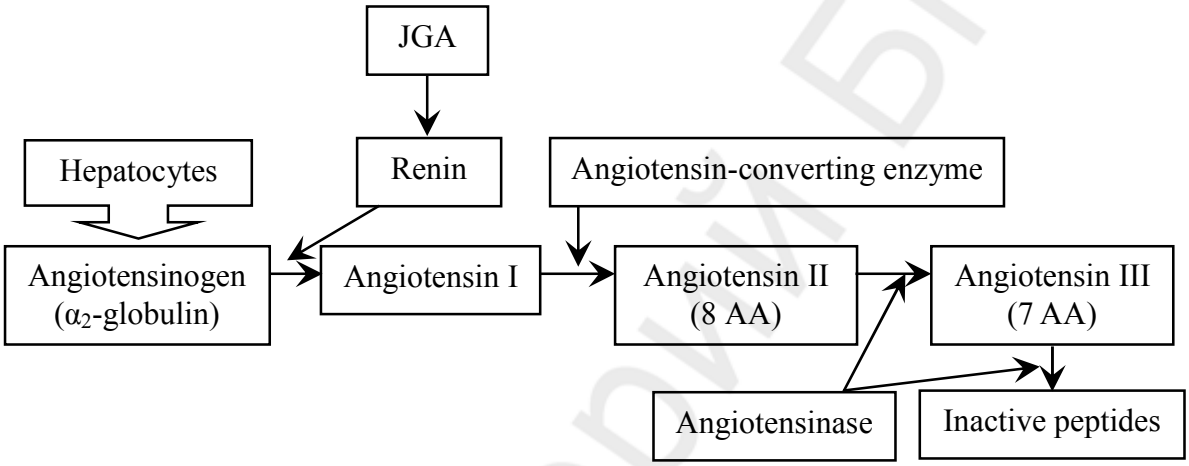
Vasopressin, or antidiuretic hormone (ADH) is nanopeptide (9 AA) produced by neurosecretory cells of the supraoptic (and paraventricular) nuclei of the hypothalamus.

Antidiuretic effect of vasopressin: it binds to its $V_2$ 7-TMS-receptor in distal collecting tubules of nephron → adenylate cyclase activation → cAMP → protein kinase A → stimulation of aquaporin-2 ($AQP_2$) translocation and its insertion in the luminal membrane of collecting tubules → water reabsorption from distal kidney tubules increases significantly.

Aquaporins are the cell membrane integral proteins forming channels. Phosphorylated aquaporins pass water and water diffuses into the extracellular space surrounding the collecting tubules.

The main stimulus for the secretion of vasopressin is increased osmolarity of blood plasma. Its secretion is also increased with a decrease in circulating blood volume. Decreased secretion of vasopressin, $V_2$-vasopressin receptor or renal aquaporins defects lead to the development of diabetes insipidus. Diabetes insipidus is characterized by a sharp increase in urine volume (10 l/day and more) and, accordingly, decrease urine osmolality and increased water consumption.

**RENIN-ANGIOTENSIN SYSTEM**




*Renin-angiotensin* system (RAS) or the renin-angiotensin-aldosterone system (RAAS) is a system of enzymes and hormones that regulate blood pressure and blood volume in the body. *Renin* is a proteolytic enzyme synthesized by juxtaglomerular apparatus of the kidney. It is often called the renin-hormone. In case of insufficient flow of blood in the glomerulus (due to the fall in blood pressure) juxtaglomerular apparatus cells begin to secrete renin. The release of renin is also controlled by such factors as changes in electrolyte balance, renal blood flow, the sympathetic activity, angiotensin II, prostaglandins and others. In the bloodstream renin is captured by *angiotensinogen*. Decapeptide cleaved is called "angiotensin I". Under the action of angiotensin-converting enzyme angiotensin I by cleavage of two amino acids converted to the octapeptide — angiotensin II, being the most active endogenous pressor. Angiotensin II decay is realized by angiotensinase synthesizing in many tissues.

A key role in this system belongs to angiotensin II. It binds to membrane receptors of kidneys, brain stem, pituitary gland, adrenal glands, the walls of the blood vessels and the heart (strong vasoconstrictive action causing a rapid increase in blood pressure). Under the influence of angiotensin II in the adrenal increases the synthesis and releasing of aldosterone and at high concentrations the releasing of vasopressin is stimulated in the pituitary gland. Angiotensin II also induces the feeling of thirst, stimulates appetite and sodium absorption in the intestine.

Since the main mechanism of angiotensin II action directly or indirectly adds up to elevated blood pressure and increased blood volume *angiotensin receptor blockers* are used in the treatment of hypertension. One of the angiotensin receptor blockers is a simple peptide **saralazin**. Its structure is similar to angiotensin II and it is a competitive antagonist. Nowadays a variety of synthetic inhibitors of *angiotensin-converting enzyme inhibitors* (**captopril, lisinopril, enalapril, ramipril, tsilazopril, pivalopril** and others) has been successfully synthesized.

## MINERALS

Minerals depending on their concentration are divided into macroelements and microelements (trace elements).

Macroelements are minerals, the content of which exceeds 50 mg/kg of the body weight — sodium, potassium, calcium, magnesium, phosphorus, sulfur and chlorine. Microelements is a group of chemical elements contained in very small amount in the body — less than 50 mg/kg of the body weight. Iron is one of them although its concentration exceeds the above-mentioned level.

### *Macroelements*

#### Sodium

Sodium is the major extracellular cation. The normal concentration of sodium in the blood plasma is 135–150 mmol/L. The body contains 80–100 g sodium: 50 % — in extracellular fluid, 40 % — in bones and cartilages, 10 % — in the cells.

**Biological role:** regulates the volume of extracellular fluid and osmotic pressure and the body's acid-base balance, participates in formation of electric membranous potential. It is involved in the intestinal absorption of glucose, galactose and amino acids, more often inhibits enzymes (but activates digestive enzymes).

**Deficiency (hyponatremia).** Causes: use of diuretics, fasting, renal diseases with reabsorption disturbance, Addison's disease. Symptoms: loss of appetite, hypotonia, nausea, tachycardia, muscle weakness, mental impairment.

**Excess (hypernatremia).** Causes: profuse perspiration, insufficient water intake in case of salty food excess, diabetes insipidus, Conn's disease (hyperaldosteronism), Cushing's syndrome. Symptoms: hypertonia, edema, depression, anxiety and fear.

Daily requirement — not more than 5–8 g NaCl.

#### Potassium

Potassium is the principal intracellular cation. The normal concentration of potassium is 3,6–5,4 mmol/L. The body contains 150–170 g potassium; 98 % of them is intracellularly and 2 % extracellularly.

**Biological role:** formation and maintenance of osmotic pressure in cells, maintains acid-base balance (is part of intracellular buffer systems — Hb, HbO, compare to sodium, which is part of the blood buffer systems), participates in formation of electric membranous potential, regulator of neuromuscular conductivity (contractility).

**Deficiency (hypokalemia).** Causes: vomiting, diarrhea, use of diuretics, Conn's disease, Cushing's syndrome, acute renal failure. Symptoms: weakness, apathy, drowsiness, reduced reflexes, muscle hypotonia, intestine atonia, arrhythmia, decreased arterial pressure (AP), diastolic cardiac arrest — «still death».

**Excess (hyperkalemia).** Causes: muscle traumas, muscle cells necrosis, malignant tumours, Addison's disease, acute and chronic renal failure. Symptoms: paresthesia, ascending paralysis of the limbs, arrhythmia, transverse blockade, fibrillation of ventricles, cardiac arrest.

Daily requirement — 2.5–3.5 g.

#### Chlorine

Chlorine (chloride ion) is the major extracellular anion. Chlorine body content is about 30 mmol/kg. 88 % of the total chlorine are inside the cells and 12 % outside the cells. The normal concentration of chlorine in the blood plasma is 96–105 mmol/L.

**Biological role:** participates in the regulation of osmotic pressure and water balance, gastric juice HCl formation, as a component of NaCl is essential for acid-base balance regulation, amylase activator.

**Deficiency:** vomiting, diarrhea, profuse perspiration.

**Excess:** practically the same causes which leads to Na excess.

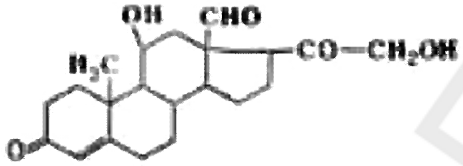
Daily requirement — not more than 5–8 g NaCl.

The difference between $Na^+$ and $K^+$ cations concentration on cell membranes maintains the plasma membrane **enzyme $Na^+/K^+$-ATPase**. It pumps three $Na^+$ ions out of the cell and carries two $K^+$ ions into the cell by the energy of ATP molecules hydrolysis.

*Cardiac glycosides* (**digitalis, strophanthin G (ouabain**) inhibits $Na^+/K^+$-ATPase. They possess cardiotonic effect: inhibition of cardiac myocytes Na/K-pump leads to increased levels of intracellular $Na^+$. The latter activates Na/Ca-exchanger, which in turn increases the amount of $Ca^{2+}$ in the cell stimulating heart contractions.

The main regulating factors of sodium and potassium excretion by the kidneys are aldosterone and natriuretic peptides.

**Aldosterone**




Aldosterone is a steroid hormone synthesized from cholesterol in the adrenal cortex. It is the most potent mineralocorticoid and the most important regulator of $K^+$-$Na^+$ balance.

Aldosterone secretion is predominantly adjusting by angiotensin II, but also depends on the content of adrenoglomerulotropin, ACTH, atrial natriuretic peptide, potassium, magnesium and sodium in the blood.

Aldosterone increases sodium reabsorption in the distal nephron and excretion of potassium and hydrogen ions. The mechanism of aldosterone action is the direct impact on the genetic apparatus of the cell nucleus that leads to the stimulation of the corresponding RNA synthesis and activation of cation transporting proteins. Aldosterone is a component of the renin-angiotensin-aldosterone system. The result of the mineralocorticoid action is an increase in blood volume and blood systemic pressure. In pathological cases of *hyperaldosteronism* (adrenal tumors, certain diseases of the heart, kidneys) hypernatremia, hypokalemia, increased blood volume, arterial hypertension, edema occur. *Lack of aldosterone* (removing of the adrenal gland, Addison's disease), which leads to a dramatic loss of sodium, threatens body‘s doom.

**Spironolactones** (aldosterone synthetic structural analogues) are antagonists of aldosterone and used as a *potassium-sparing diuretic.*

**Natriuretic peptides**

Natriuretic peptides (NUP) — is a group of peptides (21–35 AA) that stimulate the sodium and water excretion. Synthesized in the heart (the atria, the ventricles) (28 AC), brain (22 AA), the endothelium of blood vessels. Atrial natriuretic peptide (ANP) is synthesized mainly in the atria and secreted into the blood when the volume of blood and atrial stretch increase. Upon binding to its receptor (1-TMS-(R), membrane-bound guanilate cyclase) ANP stimulates natriuresis and diuresis: increases glomerular filtration, decreases the reabsorption of sodium in the renal tubules, causes peripheral vasodilation and diminishes renin, aldosterone and vasopressin secretion.

**Calcium**

Calcium body content is 1.0–1.5 kg. 99 % — in bones and cartilages, 1 % — in cells and in the blood plasma (2.2–2.7 mmol/L).

**Biological role:** structural component of bones and teeth, participates in neural conductivity and muscle contraction, blood clotting factor IV, second messenger for some hormones, strontium antagonist.

**Metabolism.** Calcium is absorbed into enterocytes by calcium binding protein — calbindine (facilitated diffusion) and $Ca^{2+}$-dependent ATPase (active transport). 1, 25-dihydroxycholecalciferol

(calcitriol; 1, 25$(OH)_2$-$D_3$)), stimulates their synthesis. Calcium supply into the cell is provided from extracellular space or from intracellular depots. *Extracellular* $Ca^{2+}$ is supplied into the cell via calcium ligand-gated channel. Ligang-gated channel is an integrative protein consisting of five subunits. It opens (phosphorylation) and closes (dephosphorylation) due to hormones. In the cell $Ca^{2+}$ is stored mainly in calcisomes, the minor part in mitochondria. $Ca^{2+}$ release from *intracellular* depots occurs due to hormones activating phospholypase C (ITP and DAG are second messengers). ITP binds to its calcisome receptor and $Ca^{2+}$ released from calcisome. Then $Ca^{2+}$ binds to calmodulin and this complex activates *protein kinase C*. DAG is also activates protein kinase C. The latter in its turn phosphorylates different proteins (enzymes) and alters their activity. Calcium release from the cell is provided by $Ca^{2+}$-ATPase and и $Na^{+}$-$Ca^{2+}$-antiport ($Na^{+}$ gets in the cell, and $Ca^{2+}$ out of the cell).

Three hormones play the major role in regulation of calcium level in the blood: parathyroid hormone (PTH), polypeptide, secreted by parathyroid glands; 1, 25$(OH)_2$-$D_3$, acting in a similar way as steroid hormones; calcitonin (CT), polypeptide, secreted by parafollicular cells of thyroid gland. The first two increase calcium concentration in the blood and CT decreases it. Target organs for these hormones are also three: intestine, bone and kidney.

PTH binds to 7-TMS-receptor → activation of adenylate cyclase and phospholypase C (cAMP, ITP, DAG are second messengers*). Intestine*: increases calcium and phosphate absorption by promoting the synthesis of 1, 25$(OH)_2$-$D_3$. *Bone*: increases bone resorption. Demineralization leads to an increase in blood levels of $Ca^{2+}$ and phosphate. *Kidney*: increases reabsorption of $Ca^{2+}$ but decreases reabsorption of phosphate.

In its actions calcitriol behaves like a steroid hormone, binding to a nuclear receptor. *Intestine*: stimulates $Ca^{2+}$-binding protein (calbindine) and $Ca^{2+}$-ATPase synthesis hence absorption of calcium and phosphate also increases. *Bone*: mobilizes bone mineral. *Kidney*: stimulates $Ca^{2+}$-ATPase synthesis in the membrane of renal tubules therefore increases reabsorption of calcium and phosphate.

CT binds to 7-TMS-receptor → activation of adenylate cyclase and phospholypase C (cAMP, ITP, DAG are second messengers). The action of CT on calcium metabolism is antagonistic to that of PTH and 1, 25$(OH)_2$-$D_3$. Has no influence on *intestine.* Bone: inhibits activity of osteoclasts and stimulates $Ca^{2+}$ and phosphate supply in bone tissue. *Kidney*: stimulates excretion of $Ca^{2+}$ and phosphate into urine

**Deficiency:** caries; muscle pain and cramps; skeleton decalcification → osteoporosis, deformations of vertebrae, easy fracturability of bones; decreased blood clotting, bleeding.

**Excess:** decreased muscle tone and blood clotting, calcinosis ($Ca^{2+}$ deposition in organs and tissues).

Daily requirement — 800–1000 mg. Women during pregnancy, lactation and post-menopause — 1.5 g.

**Phosphorus**

The body contains 500–900 g phosphorus.

**Biological role:** 85 % — extracellularly (bones, cartilages and phosphate buffer system), 15 % — intracellularly (inorganic compounds (phosphate-ion) and organic compounds). The normal of inorganic phosphorus in the blood plasma is 0.8–1.4 mmol/L.

Metabolism of phosphorus is closely interconnected with that of calcium (Ca × P = const (!) in blood plasma). Regulation of phosphorus level in the blood — see “Calcium”).

**Deficiency:** muscle weakness, increased fatigue, reduction of attention, reduction of resistance to infections, dystrophic changes in myocardium, hematomas on the skin and mucous membranes, osteoporosis.

**Excess:** decalcination of bone tissue, deposition of poorly soluble phosphates in organs and tissues, urolithiasis.

Daily requirement — 1200–1500 mg.

**Magnesium**

Magnesium cation takes second place in the quantity after Na cation. Mg body content is 20–30 g and it is mainly found in bones and muscles.

**Biological role:** skeleton and tooth formation, kinase and ATPase cofactor, activator of DNA polymerases, ribosome composition activator, membrane stabilizer, regulator of neuromuscular excitability, essential for parathyroid hormone secretion.

**Deficiency:** clinical manifestations are similar to those of calcium deficit: cramps, CNS hyperexcitability, irritability, anxiety and fear. Besides myocardium, contractibility and conductivity are impaired and chronic fatigue syndrome develops.

**Excess:** high doses of magnesium sulfate as laxative, analgesic, anticonvulsant leads to diarrhea.

Daily requirement — 0.3–0.4 g.

**Sulfur**

The body contains sulfur as anions (sulphate and sulphite) and organic compounds: sulfur-containing amino acids, proteins (enzymes, hormones: SH-groups of the active center, S-S bonds), sulphated geteropolysaccharides, sulfolipids, biologically active substances (CoA-SH, glutathione, vitamins $B_1$ and H, vitamin-like substances — lipoic acid, vitamin U, taurocholic acid, some antibiotics); PAPS and metallothioneine.

**Deficiency:** occurs mainly due to the insufficient protein consumption. Symptoms: various and multiple manifestations of deficit and metabolism disturbances of biologically active sulfur-containing substances.

**Excess:** skin irritation, rash, lacrimation, cough attacks resulting in bronchitis and in the onset of bronchial asthma.

Daily requirement — 0.5–1.0 g.

***Microelements***

**Iron**

Iron content in the body is 3–5 g. The highest level falls onto hemoglobin (2/3), 4.5 % — onto myoglobin, 2 % as enzymes (cytocromes, mono- and dioxygenase, NO-synthase, peroxidase, catalase), 20 % — as ferritin and 1 % as transferrin. Heme is the most predominant iron-containing substance. The nonheme iron is completely protein-bound which exists in the form of storage and transport. It is also utilized in the structure of aconitase and in the iron-sulfur proteins of the respiratory chain.

**Biological role:** binding, transport and storage of $O_2$ (hemoglobin and myoglobin), $CO_2$ transport, electron transport via respiratory chain (cytochromes). Participates in oxidation-reduction reactions (integral part of some oxidoreductases), collagen synthesis when proline and lysine hydroxylizing and in other hydrpoxylation reactions (cytochrome $P_{450}$), in hydrogen peroxide detoxification (catalase and peroxidase).

**Metabolism.** Most of the iron in the food occurs in the $Fe^{3+}$-state, but only $Fe^{2+}$ is soluble and readily absorbed. Under normal conditions only 10 % of dietary Fe is usually absorbed. Conversion of $Fe^{3+}$ into $Fe^{2+}$ occurs in the stomach under the action of HCl and vitamin C, or under the action of the duodenal membrane-bound cytochrome b (Fe-reductase). $Fe^{2+}$ is transfered from the intestinal lumen into the enterocyte through the *DMT 1* (divalent metal transporter 1) located at the brush border. *Ferroportin* serves as the "exporter" of $Fe^{2+}$ from enterocytes to the blood. The special protein *mobilferrin* is thought to be a transporter of iron inside of cells. In case of iron excess in plasma, Fe ions do not leave enterocytes, they are transferred to the iron-binding protein *apoferritin*. A molecule of apoferritin can accumulate 4,500 atoms of iron. Apoferritin bound with iron is called *ferritin*, and serves as the main storage form of iron in the tissues.

When needed iron is mobilized from the store and passed into the blood. $Fe^{2+}$ stimulates lipoperoxidation, therefore copper-containing plasma protein *ceruloplasmin* (*ferroxidase I*)

tranforms $Fe^{2+}$ into $Fe^{3+}$ (the same function belongs to *hephaestin*, the protein located on the basal membrane of enterocytes). In plasma, $Fe^{3+}$ is bound with another iron-binding protein *apotransferrin*, forming *transferrin*. It transfers $Fe^{3+}$ through the blood to the organs and tissues but mostly to red bone marrow, liver, kidney. Then iron either is utilized by the cells or is deposited in the form of ferritin. Another $Fe^{3+}$-storage protein is *hemosiderin*. It accumulates in the body when the supply of iron is in excess of body demands.

**Deficiency:** *iron deficiency anemia*. Several factors may contribute to its development. These include inadequate intake or defective absorption of iron, chronic blood loss, repeated pregnancies, and hookworm infections. Iron deficiency anemia mostly occurs in growing children, adolescent girls, pregnant and lactating women. It is characterized by microcytic hypochromic anemia, apathy (dull and inactive), sluggish metabolic activity, retarded growth and loss of appetite.

**Excess:** *hemosiderosis*. When excessive amounts of iron are released from red blood cells or introduced into the body beyond the capacity for its utilization, the excess is deposited in the various tissues, mainly in the liver. This may occur due to repeated blood transfusion, excessive breakdown of erythrocytes in hemolytic types of anemia and inadequate synthesis of hemoglobin as in pernicious anemia.

Daily requirement — for male — 10 mg, for female — 20 mg, in pregnancy — 40 mg (as 10 % of the ingested iron is only absorbed).

**Copper**

Contained in all tissues of the body. Total Cu content in the body — 40–80 mg.

**Biological role.** Copper is found in many enzymes: copper-containing oxidases: cytochrome oxidase, ferroxidase I, ascorbate oxidase, lysyl oxidase (collagen synthesis), tyrosinase (melanin synthesis), copper-zink SOD (cytoplasmic superoxide dismutase), monoamine oxidase, dopamine hydroxylase, catalase, heme synthase. Contributes to erythropoiesis participates in myelinization.

**Metabolism.** When absorbed from the mucous membrane in the portal blood copper is bound with albumins and *transcuprein* (special copper-binding protein) and enters the liver. There copper either becomes part of copper-containing enzymes or gets stored and only a small amount gets into the blood plasma via copper (ATP-dependent) pump. In blood plasma copper is tightly bound to blue in color *ceruloplasmin* ($\alpha_2$-globulin).

**Deficiency:** *iron deficiency anemia* since copper directly participates in iron metabolism.

**Excess:** *Wilson's disease (hepatolenticular degeneration).* The absorption of copper from the intestine is very high whereas ceruloplasmin formation is very less. Hence, a greater part of serum copper remains loosely bound to serum protein — notably albumin and therefore copper can be transferred to the tissues such as liver, lenticular nucleus of brain or to the urine. This may lead to hepatic cirrhosis and brain necrosis.

Daily requirement — 2.5 mg.

**Zinc**

Zinc content in the body — 2–3 g and 99 % intracellularly: β-cells of the pancreas, prostate, testes, sperm, iris and retina.

**Biological role:** receptor and other proteins bondage to DNA (zinc «fingers»), coenzyme of more than 300 enzymes (glutamate dehydrogenase, alcohol dehydrogenase, carbanhydrase, DNA- and RNA-polymerases, copper-zinc SOD, alkaline phosphatase). Participates in hemopoiesis and spermatogenesis, charge of the activity of taste and olfactory receptors, cadmium antagonist.

**Deficiency:** dermatitis, rash, growth and maturation delay, night blindness (nyctalopia), hair loss, tactile and sexual sensitivity disturbances, infertility, impotence.

**Excess:** vomiting, diarrhea, microcytosis, neutropenia, neurologic disturbances.

Daily requirement — 10–15 mg.

**Selenium**

The population of Belarus is characterized by selenium deficiency (50 %). It is provided into the body mainly as selenium-cysteine and selenium-methionine (90 % with food, 10 % — with water).

**Biological role:** the major antioxidant element, coenzyme of thioredoxin reductase, participates in synthesis of $T_3$ from $T_4$ (deiodinase), essential for testosterone synthesis, potent nonspecific immunomodulator (anticarcinogen) and is part of transducin (retinal protein).

**Deficiency:** growth and development retardment, infertility, premature aging, myopathy, risk of cancer development.

**Excess:** dermatitis, hair loss, brittle nails, peripheral neuropathy, decrease in calcium amount in tooth enamel, anemia.

Daily requirement — 50–200 μg.

**Iodine**

**Biological role.** Iodine content in the body — 20–30 mg and mainly as part of $T_3$ and $T_4$ as well as thyreoglobulin. More than 30 % of the Earth population undergets sufficient amount of iodine. The population of the Republic of Belarus gets 2–3 times less iodine than needed, besides, because of the Chernobyl catastrophe, there is excess of radioactive $^{131}I$ alongside with natural deficiency. Its accumulation in the thyroid results in cell destruction, hypothyreosis, hyperplasia. It is the main cause of the thyroid cancer. Iodine-prophylaxis: systemic — iodination of salt, drinking water, bread, dairy products and eggs; individual — antistrumin (KI).

**Deficiency:** iodine deficiency in the body leads to compensatory proliferation of the thyroid gland (*endemic goiter*) and *hypothyroidism*.

**The excessive** functional activity of the thyroid gland is conductive to hyperthyreosis (Basedow's disease). The triad of symptoms: tachycardia, exophthalmos and goiter is acknowledged as the most characteristic symptoms of this disease.

Daily requirement — 150–200 μg.

**Manganese**

**Biological role:** duplicates magnesium namely in DNA-polymerases (very active, increases DNA synthesis but with multiple mistakes), activator of hydrolases, transferases and isocitrate dehydrogenase, cofactor of manganese SOD (mitochondrial), arginase, pyruvate carboxylase, phosphoenolpyruvate carboxykinase, proteoglycans synthesis activator in bone and cartilage tissues.

**Deficiency:** anemia, calcification retardment and skeleton deformation, ataxia.

**Excess:** «metallic» taste, neurologic disturbances similar to Parkinson's disease, mental impairment.

Daily requirement — 8–10 mg.

**Cobalt**

Organism contains 5–8 mg Co and 40 % of them is in the liver.

**Biological role:** cobalt is part of $B_{12}$, constituent of enzymes, accelerates iron absorption, stimulates blood formation, contributes to muscle protein synthesis and participates in myelin membrane building.

**Deficiency:** anemia, degeneration of the spinal cord with involvement of posterior and lateral funiculi.

**Excess:** toxic in even low concentration. Symptoms: allergic dermatitis, polycythemia (increase in erythrocytes amount), thyroid hyperplasia.

Daily requirement — 0.15 μg.

# CHAPTER 14
# BIOCHEMISTRY OF MUSCLLE TISSUES

Muscle content in adult is about 40 % of body weight.

The muscle function is contraction and shortening with the following relaxation.

The role of muscles is of provision movement and resistance to mechanical action and static load.

The mechanism of muscle action is conversion of chemical energy into mechanical.

There are three types of muscle tissue in the body:

– skeletal muscle;
– cardiac muscle;
– smooth muscle.

Skeletal and cardiac muscle are cross-striated muscles. Muscular cells consist of *myofibrils* in which *sarcomere* is a functional unit (fig. 14.1).

When the myofibril is examined by electron microscopy, alternating dark and light bands (anisotropic bands meaning birefringent in polarizade light; and isotropic bands, meaning not altered by polarizade light) can be observed. These bands are thus referred to as A and I bands, respectively. The central region of the A band (the H band, or zone) appears less dense than the rest of the band. The I band is bisected by a very dense and narrow Z line. The sarcomere is defined as the region between two Z lines.

Proteins comprise 25 % of muscle weight. Muscle proteins are divided into 3 groups:

– myofibrillar (contractile) proteins;
– sarcoplasmic proteins;
– stromatic proteins.

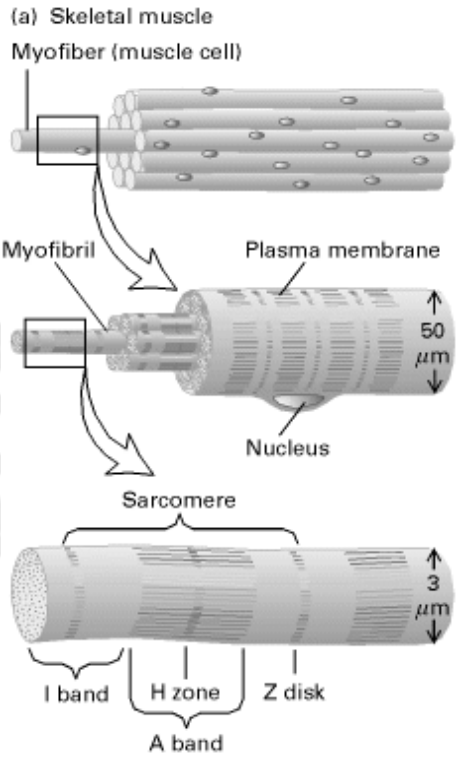



*Fig. 14.1*. Structure of the fibre of skeletal muscle

## MYOFIBRILLAR (CONTRACTILE) PROTEINS

1. *Myosin* is the basis of the thick filaments. Molecular weight ≈ 500,000 Da. Composed of six highly conserved polypeptide chains: two identical heavy chains and two each of two kinds of light chains. The heavy chains have long a-helical tails and globular head domains. The α-helical tails are interwound into a two-strand coiled coil and the light chains are bound to the globular head domains (fig. 14.2).

The myosin molecule can be cleaved by proteases. The tail domain is cleaved at a specific point by trypsin to yield fragments called *light meromyosin* and *heavy meromyosin*.

Myosin functions:

– structural — about 400 myosin molecules are connected to each other “tail” to “tail” and form a thick filament;
– catalytic — myosin head is able to cleave ATP;
– contact — connects to actin by their heads, which in this case are called “cross-bridges”.

2. *Actin* is a protein of the thin filament. It is long, helical polymer (fibrous actin, or F-actin) of a globular protein monomer (G-actin). The structure of the G-actin monomer is a two-domain molecule with a mass of 42,000 Da. The binding of ATP by a G-actin monomer leads to polymerization (i. e., the formation of F-actin). The ATP is hydrolyzed, but the ADP is retained in

the actin. Within F-actin filaments, the G-actin monomers are arranged in a two-strand helix. Actin filaments carry sites on each subunit that can bind to myosin (fig. 14.3).

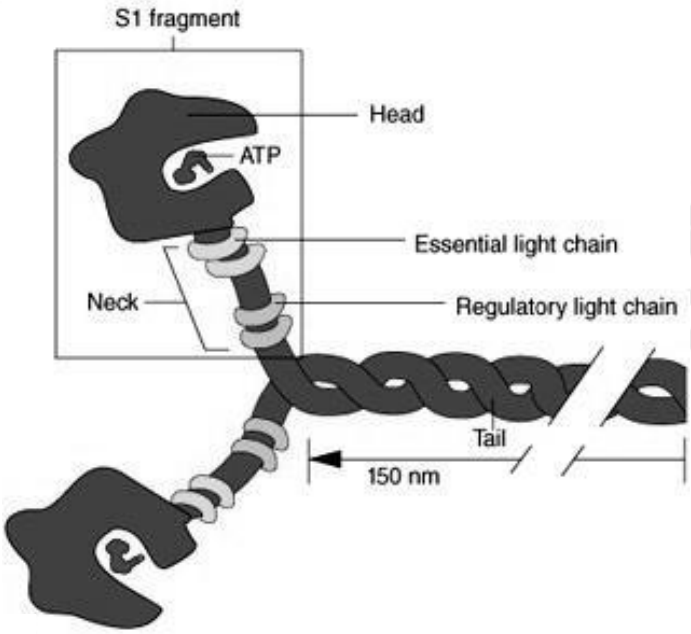



*Fig. 14.2*. Structure of the thick myosin filament

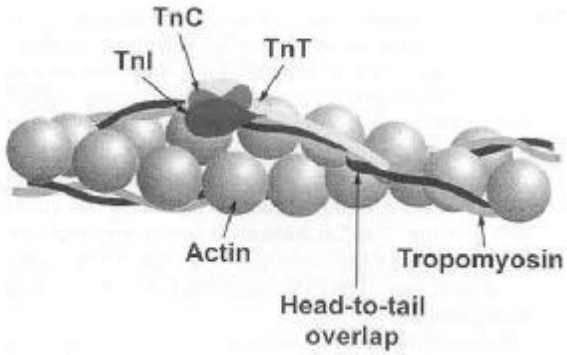



*Fig. 14.3.* Structure of the thin filament

3. *Tropomyosin* is also protein of the thin filament. It is a two chain fibrous protein that attach to F-actin in the groove between its filaments. Molecular weight — 62,000 Da.

4. *Troponin* is another protein of the thin filament. Molecular weight — 80,000 Da. Consists of three protein components: troponin C, a calcium binding protein like calmodulin; troponin I, which blocks the myosin binding site on F-actin; troponin T, which binds to tropomyosin and the other troponins

5. α-*Actinin*. Anchors actin to Z lines, stabilizes actin filaments.

6. β-*Actinin* acts as an actin-capping protein, specifically binding to the end of an actin filament.

7. *M-protein*. Anchors myosin to M lines.

8. *C-protein* inhibits myosin ATPase activity at low ionic strength but activates it at physiological ionic strength.

9. *Desmin* lies alongside actin filaments, attaches to plasmalemma. Provides coincidence boundaries of all sarcomeres.

10. *Nebulin* is associated with a thin filament from its (+) end at the Z disk to its (–) end and may be involved in controlling thin filament length.

11. *Titin* is the largest protein in the body. Molecular weight — 2, 8000,000 Da. Its filaments remain connected to thick and thin filaments during muscle contraction and generate a passive tension when muscle is stretched.

**Sarcoplasmic proteins.** These include myoglobin, glycolysis enzymes, tissue respiration enzymes, calmodulin and calsequestrin, which possesses ability to reversibly bind to $Ca^{2+}$.

**Stromatic proteins** are presented by collagen and elastin.

**Peculiarities of the smooth muscles:**

– contractile apparatus does not contain troponin system, and contains a special caldesmon protein that acts as troponin;
– myosin ATPase activity is 10 times lower;
– myosin can be connected with actin only if its light chain phosphorylated;
– its stroma rich in protein but poor in phospholipids and macroergs.

Smooth muscle are slow, but they can maintain long-term tension.

**ENERGY SOURCES AND ENERGY PROVISION MECHANISMS OF MUSCULAR CONTRACTION**

| | |
|---|---|
| At rest | Fatty acids (FA) and ketone bodies (KB) |
| In moderate exercise | FA + KB + blood glucose |
| In maximal exercise | FA + KB + blood glucose + muscle glycogen |

1. The major regulator of muscle cell energy metabolism is ATP/ADP. At rest ATP concentration is high which brings about the activity inhibition of glycolysis and Krebs cycle key enzymes and also the activity of respiratory chain enzymes. As the muscles start its work the ATP concentration decreases and ADP concentration increases that leads to above-mentioned processes activation and ATP formation to make muscles work.

2. During exertion the rate of glycolysis in muscle exceeds that of the citric acid cycle: lactate accumulates and is released. Another metabolic product is alanine (transamination from pyruvate in the glucose-alanine cycle). Both lactate and alanine are transported through the bloodstream to the liver, where they are reconverted (in gluconeogenesis) to glucose — the Cori cycle.

3. Adenylate kinase (myokinase) reaction:

$$2\ ADP \overset{\textbf{АК}}{\leftrightarrow} ATP + AMP$$

**АК** = adenylate kinase (myokinase)

ATP is used for muscle activity whereas AMP stimulates glycolysis and gluconeogenesis.

4. Creatine kinase reaction — Lohman reaction:

$$Creatine + ATP \overset{\textbf{CK}}{\leftrightarrow} Creatine\ phospate\ (CP) + ADP$$

**CK** = creatine kinase

Resting muscles contain 5–10-fold as much CP as ATP but CP in contrast to ATP cannot be used for muscle contraction. The role of CP is not only to be a reserve and transport energy form in the muscle but also to give up its energy-rich bond to ADP to form ATP, which is spent for muscle contraction. It is emergency reaction, which is triggered first in ATP deficiency. CP supply is sufficient for 10 seconds of muscle activity but the $1^{st}$–$3^{rd}$ mechanisms are switched on during this interval. This system is of particular importance to the myocardium.

# CHAPTER 15
# BIOCHEMISTRY OF CONNECTIVE TISSUES

Connective tissues consist of extracellular matrix (ECM) and different types of cells (fibroblasts, fibroclasts, melanocytes, macrophages, endotheliocytes, mast cells, mesenchymal cells).

Connective tissue **specifics:**

– most common;
– prevalence of ECM over cellular elements;
– well-organized system of substances synthesis — rich in endoplasmic reticulum and Golgi apparatus;
– rich in mitochondria;
– numerous regulators controlling different processes.

**Biomedical value** of ECM:

– provides cellular transfer in the process of embryogenesis;
– is connected with the onset of acute and chronic inflammation;
– is connected with tumor cells metastasis;
– is connected with ageing;
– ECM cells can be influenced which enables us to use this property in cosmetology;
– a great number collagen diseases (collagenoses) are caused by genetic disturbances in ECM cells metabolism;
– genetically predetermined defects of lysosomal hydrolases which catalyze ECM cells destruction lead to severe disorders (mucopolysaccharidoses).

The major components of ECM are **proteoglycans**, **structural proteins** (***collagen, elastin***), **adhesive proteins** (***fibronectin and laminin families***).

## PROTEIN-CARBOHYDRATE COMPLEXES

Connective tissue proteins contain carbohydrates in its structure so they are called protein-carbohydrate complexes (PCC) and classified according to chemical content:

– proteoglycans (95 % carbohydrates and more);
– mucoproteins (10–50 % carbohydrates);
– glycoproteins (less than 10 % carbohydrates).

### Proteoglycans

**Proteoglycans** are proteins that contain covalently linked glycosaminoglycans (GAG). They vary in tissue distribution, nature of core protein, attached GAG, and function. GAG are constructed of disaccharide units (uronic acid — hexoseamin) which are blocked monomers. The proteins bound covalently to GAG are called "core proteins". *Aggrecan*, the major type found in cartilage, with its overall structure resembling that of a bottlebrush. It contains a long strand of hyaluronic acid (one type of GAG) to which link proteins are attached noncovalently. In turn, these latter interact noncovalently with core protein molecules from which chains of other GAGs (keratin sulfate and chondroitin sulfate in this case) project (fig. 15.1).

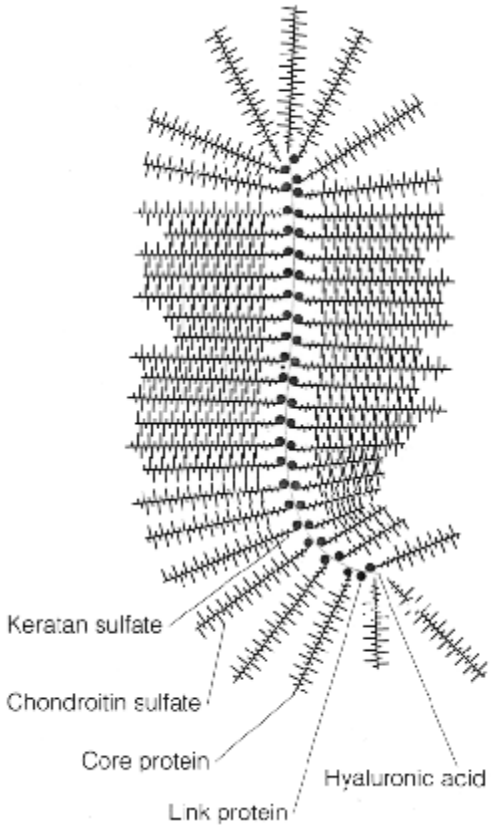



*Fig. 15.1*. Proteoglycan (aggrecan) structure

**Function of proteoglycans.** The biological functions of proteoglycans are the direct consequence of their chemical and physical properties. In the ECM they can act as:

– lubricants (hyaluronat is an excellent lubricant because it carries such a large shell of water);

– shock absorbers (the large aggrecan complex of cartilage is an excellent shock absorber. Remember that when you jog);

– molecular sieves;

– heparan sulfate binds growth factors; it can serve as a reservoir of these factors as well as a co-receptor or activator;

– heparin is the only proteoglycan to be stored intracellularly, in the granules of mast cells. When released into the circulation, it has anticoagulant activity because of binding and activation of antithrombin;

– keratan sulfate and dermatan sulfate ensure transparency of the cornea;

– ion exchangers.

**Mucoproteins**

Carbohydrate component is oligosaccharide, which monomers are minor monosaccharides: mannose, galactose, hexosamines, arabinose, xylose. At the end of the oligosaccharide is one more monosaccharides derivative located — sialic acid (acyl derivative of neuraminic acid). Increased concentration of sialic acid in the blood indicates disintegration of ECM that occurs during inflammation.

**Function of mucoproteins:**

– being components of mucous membrane secrets mucoproteins possess defense properties (saliva) and reduce friction of contacting surfaces (in the joints);

– provide group, specific and tissue characteristics;

– possess enzyme activity.

**Glycoproteins**

**Glycoproteins** are similar to mucoproteins by chemical structure but contain a less amount of carbohydrates.

**Function of glycoproteins:**

– structural components of cellular membranes, collagenous and fibrin fibers, bone matrix;

– transport molecules for vitamins, lipids, microelements;

– provide immunity;

– possess hormonal and enzyme activity (TSH, FSH, LH; blood clotting factors).

## METABOLISM OF PCC

**Synthesis of PCC**

There are two types of PPC depending on the kind of linking between the protein component and sugar.

**1.** ***PCC with O-glycoside bond.***

The protein portion is synthesized on ribosomes (matrix) synthesis.

Carbohydrate portion is synthesized by successive addition of monosaccharides to the OH-group of serine, threonine or hydroxylysine.

Carbohydrate portion is synthesized by sequential addition of monosaccharides to the OH-group of serine, threonine or hydroxylysine.

Enzymes involved in the synthesis — glycosyltransferases and substrates used — activated monosaccharides (UDP-glucose or UDP-galactose).

**2.** ***PCC with O-glycoside bond.***

The protein portion is synthesized on ribosomes (matrix synthesis).

Carbohydrate is synthesized on a special carrier — dolichol (a compound of polyisoprene), and then attached to the nitrogen atom of the amide group of asparagine by oligosaccharidtransferase.

**Decay of PCC**

Lysosomal enzymes destruct PPC. The protein part is cleaved by proteinases, and carbohydrate chain — by glucosidases and sulfatases.

Congenital defects in the structure and function of these enzymes leads to ***mucopolysaccharidoses*** (PPC storage diseases, or lysosomal diseases). Inherited with the X-chromosome and autosomal. The frequency of occurrence — 1 : 100,000 newborns. In lysosomes of these patients products of incomplete splitting of PPC accumulated. Accumulated GAG seen in the cells of the cartilage, fascia, tendons, periost, skin, meninges, blood vessels, spleen, liver, and excreted in the urine.

*Diagnosis:* GAG excretion in the urine; enzyme defects; accumulation of GAGs in culture of fibroblasts; genetic counseling; prenatal diagnosis — amniocentesis.

## COLLAGENS

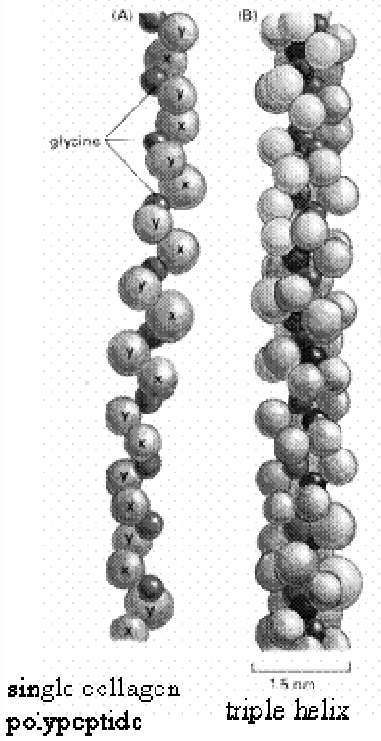
Collagens — are the major glycoproteins of connective tissues (25 % of all proteins of the human body) and provide tensile strength in contrast to PG, which counteract the contraction.

In the human genome there are 30 genes encoding collagen α-chain. More than 20 types of collagen molecules (isocollagens) have been detected.

Types I–III isocollagens were called fibrilforming and isocollagens IX and XII — fibrillassociating as they are usually linked with collagen fibers, which formed by fibrilforming collagens. Fibrillassociating collagens provide connections with other molecules of the matrix. Type IV and VII collagens called netforming. They form a reticular structure and are most commonly found in basal membranes, providing linkage between the cell layers of the epithelium and the underlying connective tissue. This is of particularly importance for the skin.

**Structural organization of collagen**

***Amino acid and carbohydrate composition***




- GLY (1/3).
- PRO and OH-PRO — 1/5.
- OH-LYS.
- No sulfur and aromatic amino acids.
- Carbohydrate — a disaccharide (glucose + galactose).

**Primary structure**

Left-twisted polypeptide chain consisting of a triad of amino acids. In the triads, the third amino acid glycine always, the second — proline or lysine, the first — any other amino acid, except the three listed. Because of the large number of imino acids, it is not possible to form hydrogen bonds in the polypeptide chain.

**Secondary structure**

Three polypeptide chains fold into the collagen triple, linear, right-twisted spiral. Such a molecule is called tropocollagen. Stabilized by hydrogen bonds between peptide groups and disulfide bonds between cysteine residues at the N-and C-termini of the molecule. Tropocollagen molecule is soluble in water and capable of forming fibrils.

**Tertiary and quaternary structures of collagen** formed extracellularly. After removal of the rich in cysteine additional peptides triple helix collagen becomes insoluble and capable of aggregation. It promotes the formation of fibrous structures, which are formed by numerous triple helix collagen. Cross-links are formed between the collagen molecules, whereby the fibers become transverse striation.

**Processing of preprocollagen**

Polypeptide chains are synthesized on polysomes as preprocollagen ("pre" indicates a signal, leading peptide; "pro" — the presence of additional peptides on the N-and C-termini, which are necessary for proper formation of the spatial structures). Then starts preprocollagen processing, which consists of two phases — intra- and extracellular.

**Intracellularly:**

– removal of signal peptide upon entry into lumen of endoplasmic reticulum, giving "procollagen";

– hydroxylation of PRO and LYS residues (prolyl hydroxylase, lysyl hydroxylase, cofactors — $O_2$, $Fe^{2+}$, α-ketoglutarate, ascorbic acid);

– glycosylation from UDP-galactose and UDP-glucose to several OH-LYS residues;

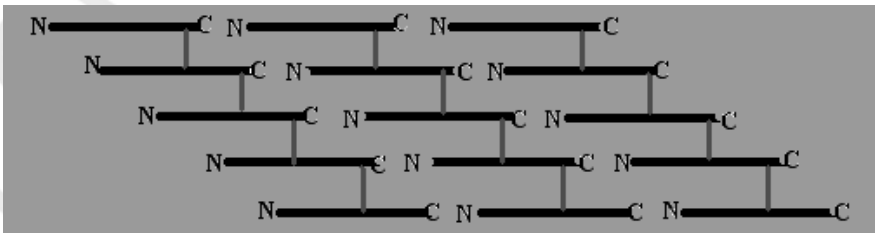
– formation of S-S bonds at N- and C-termini, both intra-chain and interchain;

– formation of triple helix. Triple helix formation stops glycosylation. The polypeptide is now ready for secretion.

**Extracellularly:**

– once the collagen is outside the cell, the terminal propeptides are cleaved by specific amino- and carboxypeptidases;

– the resulting "tropocollagen" spontaneously assembles in parallel fashion and in a staggered array. Oxidase (they require $Cu^{2+}$ and vitamin $B_6$) catalyze the oxidative deamination of ε-amino groups of lysine and hydroxylysine in collagen molecules with the formation of aldehyde groups. The condensation of aldehyde groups is accompanied by the formation of cross-linking in collagen fibers. The tropocollagen chains are 3000 nm long and in lining up they leave "holes" of 400 nm. These holes are potential nucleation sites for hydroxyapatite.



**ELASTIN**

Elastin — is the most durable of proteins known in the human body. *Unlike collagen elastin has one genetic type, small amount of OH-PRO, no OH-LYS, additional peptides, carbohydrates, does not form a triple helix.*

Elastin molecule consists of two types of fragments, alternating along the chain: hydrophobic (fibrillar) segments, which are responsible for the elastic properties of the molecule and α-helix globular segments, rich in ALA and LYS, and participating in the formation of cross-links between elastin molecules. Elastin is synthesized as a monomer, and extracellulalyr fibrillogenesis occurs with the formation of transverse links. Four residues of LYS take part in the formation of cross-links. Then oxidative deamination of LYS with the participation of oxidases and subsequent intermolecular condensation of 4-aldehyde groups take place. As a result, formed a special type of intermolecular cross-links — desmosine that is unique to elastin (fig. 15.2).

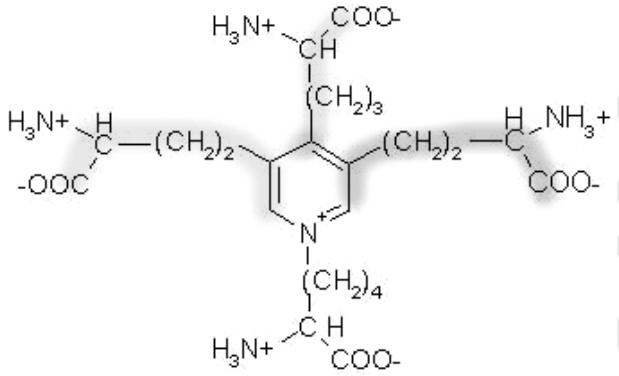


*Fig. 15.2.* Desmosine

### ADHESIVE PROTEINS

The extracellular matrix contains a large number of non-collagenous adhesive proteins. Their structural feature is the presence of domains that specifically bind to other molecules and receptors on the cell surface. Indispensable component of domains that enable interaction with cells is the sequence of amino acids — ARG-GLY-ASP.

**Fibronectin** is a high-molecular glycoprotein. There are multiple forms of fibronectin. One of them — plasmatic fibronectin and other — tissue fibronectin. Plasmatic fibronectin participates in the mechanisms of blood coagulation and wound healing. Tissue fibronectin located on the cell surface accelerates cell migration by providing cell-matrix interactions.

**Fibrillin** is a structural component of microfibrils, ensuring the formation of elastin fibers. Found in the lens, periost and the aorta. Mutation of the gene coding synthesis of fibrillin, develops *Marfan syndrome*: lens ectopia, arachnodactyly ("spidery" fingers), joint disease, cardiovascular problems.

**Laminin and entactin** are of basal glycoproteins lamina. They bind not only among themselves but also with isocollagen IV, heparan sulfate, the surface of epithelial cells. For binding to different substances, they have their own domains.

*Each type of connective tissue has its own specific set of molecules:* in addition to the relevant isocollagens, there are specific **non-collagenous proteins.**

***In cartilaginous tissue:*** *major proteoglycan and major proteoglycans (fibromodulin* is a regulator of fibrillogenesis; *biglican* — its role is not identified yet; *decorin* is able to bind to isocollagen II and inhibits fibrinolysis; *proteins with different molecular mass* and not well-identified functions; well-knows function is binding to chondrocytes, hydroxyapatite crystals, isocollagen II for its fixation to chondrocytes.

***In bone tissue:*** osteogenesis inductors and inhibitors, mineralization initiators — *osteocalcin, osteonectin, osteopontin, bone acid glycoprotein, bone sialoprotein, thrombospondin.*

# CHAPTER 16
# BIOCHEMISTRY OF TEETH AND ORAL FLUID

## TEETH

Tooth — is a complex organ, derivative of epithelial and connective tissues. Consist of three types of dental hard tissues: enamel, dentin, cement and the sort of loose connective tissue, part of the tooth pulp. The main mineral (inorganic) component of the teeth hard tissue are *apatite crystals*. At their base — calcium phosphate. Any mineral component is formed as follows:

$$\underset{\text{brushite}}{Ca(HPO_4)_2 \times 2H_2O} \longrightarrow \underset{\text{octacalcium phosphate}}{Ca_8(PO4)_6 \times 5H_2O} \rightarrow \underset{\text{hydroxyapatite (HA)}}{\mathbf{Ca_{10}(PO_4)_6(OH)_2}}$$

General formula of apatite — $A_{10}(BO_4)_6X_2$, where A — Ca, Ba, Sr, Cr, Pb, Cd; B — P, As, Si; X — F, Cl, $OH^-$, $CO_3^{2-}$. HA is the most common in the mineralized tissues, the most resistant to acids — fluorapatite $Ca_{10}(PO_4)_6(F)_2$. When the concentration of F in oral care aids up to 500 mg/l *hydroxyfluorapatite* produced — $Ca_{10}(PO_4)_6(OH)F$; 500–2000 mg/l — *fluorapatite*, more than 2000 mg/l — $CaF_2$, but it is not apatite crystals.

In an "ideal" apatite Ca/P ratio = 1.67. The decrease of this ratio leads to adverse consequences, in particular to reducing of enamel resistance. With the substitution of Ca in the HA on Sr, especially on $Sr^{90}$, which is a β-emitter, *strontium rickets* developed, which is characterized by fragility and brittleness of bones and teeth, fractures, skeletal deformities.

Exchange of ions of apatite crystals on the ions in the solution called isomorphic substitution. The predominant factor in the possibility of change is the similarity of the size of the atom, and the similarity of the charge is of secondary importance. In addition to an isomorphic substitution, the composition of the apatite crystal can be changed by other ions filling of vacant seats in the apatite crystal lattice.

Steps of various elements penetration in HA crystals:

- penetration of elements in the water hydration shell of the crystal (lasts several minutes);
- exchange of ions between the hydration shell and the surface of the crystal (lasts a few hours);
- penetration of ions in the crystal (lasts for months or years).

**Mineralization of dental hard tissues.**

The basis of this process is the formation of apatite crystals with calcium phosphate participation. In the body extracellular fluid is oversaturated with calcium phosphate, and it begins to precipitate. There are two stages of calcium phosphate precipitation:

- nucleation — the formation of dense deposit (nucleus);
- crystal growth from the nucleus — epitaxis.

*Nucleation* can be *homogeneous* (crystals are formed without the involvement of the other phase) and *heterogeneous* (another phase initiates crystal formation and plays the role of matrix). The matrix can guide the growth of crystals. Proteoglycans, glycosaminoglycans, Ca-binding proteins: phosphoproteins and proteins, containing γ-carboxyglutamic acid (γ-CGA), which is needed vitamin K for its synthesis, perform the role of the matrix.

Theories of dental hard tissues mineralization:

- physio-chemical, which is based on the above-mentioned two stages;
- enzymatic: alkaline phosphatase hydrolyzes bone phosphoorganic esters, resulting in phosphate ion releasing that in the presence of calcium and the matrix causes growth of HA crystals;
- mixed: first synthesized extracellular matrix, and then starts the stage of mineralization due to a oversaturated status of calcium phosphate solution and the presence of the matrix.

**Teeth tissues**

*Table 16.1*

**Chemical composition of dental tissues (% by weight)**

| Tissues | Mineral (inorganic) substances | Organic substances | Water |
|---|---|---|---|
| Enamel | 95 | 1–1,5 | 4 |
| Dentin | 70 | 20 | 10 |
| Cement | 60 | 25 | 15 |
| Pulp | 5 | 40 | 55 |

**Enamel.** Water is here in two forms: free and bound (hydration shell of apatite crystals).

Mineral foundation — apatite crystals: HA — 75 %, the rest — fluorapatite, carbonate apatite, chlorapatite. The outer layer contain Ca, P, and F 10-fold more than in the underlying layers, so it is more resistant to acids. Besides F, Zn, Pb, Sb and Fe presented. In deep layer there are many Na, Mg and carbonate ion. Sr, Cu, Al, K are evenly distributed throughout the entire thickness of enamel. Chemical composition of enamel mineral component is shown in table 16.2.

*Table 16.2*

**Chemical composition of enamel mineral component**

| Component | Concentration, % by weight | Component | Concentration, parts per million |
|---|---|---|---|
| Ca | 33,6–39,4 | Fluorine | 50 and > 5000 — on the surface |
| P | 16,1–18 | Iron | 8–218 |
| CO2 | 1,95–3,66 | Strontium | 50–400 |
| Sodium | 0,25–0,90 | Copper | 10–100 |
| Magnesium | 0,25–0,56 | Manganese | 0–18 |
| Chlorine | 0,19–0,30 | Silver | 0–100 |
| Potassium | 0,05–0,30 | Ca/P (mole) | 1,5–1,67 |

The organic component — non-collagenous proteins, peptides, lipids, monosaccharides.

Non-collagenous proteins — amelogenins, enamelins, enamel Ca-binding protein. In the process of the enamel maturation amelogenin concentration decreases and that of enamelin — increases. Enamelins firmly attached to the crystals of apatite.

Ca-binding protein plays a major role in the formation of the protein matrix — the basis of the enamel. The three-dimensional enamel network is formed by combining in the space molecules of Ca-binding protein with $Ca^{2+}$. This network (matrix) is the zone of nucleation for the growth of HA crystals. It is fixed to the fibers of amelogenin.

**Dentin.** *Primary* dentin formed during the eruption and the formation of the teeth and makes up the bulk of dentin; *secondary* (*physiological secondary*) formed in the tooth after the eruption and is a continuation of the primary; *tertiary* (*reparative secondary*) formed in response to irritating factors across from enamel lesion. Processes of odontoblasts pass through the enamel and dentin to form channels for the trophic of the tooth. They are filled with dentinal fluid that carries out mineralizing and sensory function.

*The mineral component* — HA, but the ratio of Ca/P is not 1.67 and 1,5–1,67. F concentration is 2-fold that of in enamel and Mg concentration is 3-fold higher than in the bones.

*The organic component* — isocollagen type I and non-collagenous proteins (proteoglycans and phosphoproteins). They are able to bind calcium and connect with isocollagen I.

In the dentin amorphous (non-crystalline) phase presented, in which there are phosphate and calcium carbonate.

**Cement.** Similar to the bone but unlike it has no blood vessels and is not subject to constant adjustment.

*The mineral component* — mainly HA.

*The organic component* — isocollagen type I, proteoglycans and lipids.

**Pulp.** Contains blood vessels and nerves, and performs trophic, protective, reparative functions.

## ORAL FLUID

In the oral cavity there is oral fluid, or mixed saliva (unlike pure saliva, which is produced by salivary glands).

Oral fluid mixed, or saliva — is the total secret of salivary glands, the detritus of oral cavity, gingival fluid, dental fluid, microflora and the products of its life, white blood cells and their degradation products, food residue, toothpaste, rinse fluids, bronchial and nasal secretions. In the Russian language the term "saliva" is used, in the medical and scientific literature — "oral fluid" (table 16.3).

*Table 16.3*

**Chemical composition of oral fluid**

| | | | |
|---|---|---|---|
| Water, % | 98 | Mucin, g/l | 3 |
| Dense matter, % | 1,4–1,5 | Glucose, mg/l | 10–100 |
| Organic substances, % | 1 | Amylase, mg/l | 380 |
| Density, kg/m$^3$ | 1002–1017 | Immunoglobulin A, mg/l | 190 |
| pH | 6,4–7,3 | Immunoglobulin G, mg/l | 14 |
| Volume, l/day | 0,7–1,5 | Immunoglobulin M, mg/l | 2 |
| Chlorides, g/l | 2,5–3, | Urea, mg/l | 200 |
| Calcium ions, mg/l | 40–50 | Cholesterol, mg/l | 80 |
| Phosphates, mg/l | 190–200 | Residual nitrogen, mg/l | 100–200 |
| Fluorine, mg/l | 0,6–1,8 | Pyruvate, mg/l | 9 |
| Protein, g/l | 2–3 | Lactate, mg/l | 33 |
| Protein fractions (electrophoresis), %: | | Glycoprotein carbohydrates, mg/l: | |
| albumins | 7–8 | hexoseamines | 100 |
| α-globulins | 11–12 | | 90 |
| β-globulins | 45 | | 12 |
| γ-globulins | 18 | | 195 |
| lysozyme | 18–20 | | |

*Unstimulated saliva* — this is the secret of the salivary glands in the absence of external stimulation, *stimulated* — due to external stimulants.

### Functions of oral fluid

1. Protective.
2. Mineralizing.
3. Purifying.
4. Digestive.
5. Regulatory.
6. Excretory.

### Proteins of oral fluid and their functions

*Mucins* are protective proteins. Protect the tooth surface from bacterial contamination and calcium phosphate dissolution, add to viscosity saliva, binding a lot of water.

*Cystatins*: inhibit bacterial proteases and proteases of periodontal tissues.

*Histatins*: rich in GIS and are potent inhibitors of Candida albicans and Streptococcus mutans growth.

*Protein rich in PRO*: they contain a lot of $H_3PO_4$ and because of the "–" charge inhibit the growth of crystals in the saliva, binding $Ca^{2+}$.

*Lactoferrin*: able to bind iron ions, depriving bacteria of this important element and limiting their growth, although some bacteria are able to digest iron associated with lactoferrin.

### Enzymes of oral fluid and their functions

Enzymes of oral fluid are divided by origin into: 1) glandular; 2) leukocytic; 3) microbial.

*Lysozyme*: bactericide action is based on the fact that it hydrolyses the glycosidic linkage of microbial shell heteropolysaccharides and causes the aggregation of bacteria decreasing their adhesion to the tooth surface.

*Peroxidase*: an indispensable condition of its action is the presence of $H_2O_2$ and anions $CNS^-$ and $Cl^-$, which formed $OCNS^-$ and $NOCl^-$, acting on microorganisms amino acids. These amino acids are transformed into toxic aldehydes and have a damaging effect on microbes.

*Nucleases* — acidic and alkaline *DNase* and *RNase*. Inhibit the growth and reproduction of many microorganisms in the oral cavity.

*Gingival fluid* is the contents of the gingival groove. It is physiological environment of complex composition containing white blood cells, epithelium, microorganisms, electrolytes, proteins, enzymes. 0.5–2.5 ml of gingival fluid pass in the oral cavity per day. In a healthy periodont gingival fluid is transudate of blood serum, in the affected periodont — fluid, which is formed due to the increase vascular permeability and contain products of bacteria and plaque metabolism.

*Dental liquor* is a fluid that fills the voids of dental tissues. Includes dentin and enamel (enamel free water) fluid. It is through dental liquor do all the necessary substances pass for the trophic of dental tissues. The protein composition similar to blood plasma proteins. Dental liquor comprises and other organic and inorganic molecules. Dentine and enamel fluids are closely linked: various substances filtered from dentin fluid to enamel fluid.

## ENAMEL SURFACE FORMATIONS

*Cuticle* is lost after the eruption of the teeth.

Pellicle — a thin layer of organic material containing a small amount of bacteria. Plays a protective function: repeatedly reduces the solubility of enamel and protects enamel against the damaging action of the organic acids. Completely restored in 20 minutes after brushing, does not disappear during chewing.

Plaque. Covered with mucoid film so resistant to washing with saliva and to oral cavity insing. Easily removed by toothbrush, does not erase when chewing food (exception — hard food). Begins to accumulate within 2 hours after brushing. It is a layer of organic matrix and bacterial cells on the surface of the pellicle. Plaque contains 80 % of water, 20 % — dry residue, from which 40 % — mineral substances and 60 % — organic. The mineral substances — hydroxy- and fluorapatite, $CaF_2$. Of organic matter — polysaccharides: glucans, levans and heteropolysaccharides. Plaque is a prerequisite for caries. Rapid plaque formation contributes to the presence of sucrose in the diet. Saliva stimulation by chewing cheese, nuts, sugar-free chewing gum and urea can compensate plaque pH decreasing after ingestion. They increase the strength of saliva neutralizing effect by hydrocarbonate buffer and nitrogen compounds (urea): plaque is converted to alkaline products.

*Dental tartar* — is a mineralized plaque. The mineralization process lasts ≈ 12 days, but the first signs of mineralization appear after 1–3 days. Dental tartar formed because of the saturation of plaque by calcium phosphate crystals. Amino acids, monosaccharides, phospholipids, trace elements, enzymes, food ingredients, decomposition products of leukocytes and epithelial cells found in the tartar. A local increase in the saliva pH is required for the formation of tartar. The tartars are a common cause of periodontal disease.

*Caries* is a pathological process, which manifests itself after the eruption of the teeth and is characterized by demineralization of dental hard tissues and the formation of a defect in the form of a cavity under the action of microorganisms (streptococcus).

There are several theories of caries initiation. Currently the dominant theory is *acidogenic*, or *chemical-parasitic* theory: the mechanism of tooth decay is the production of organic acids that dissolve the mineral component of teeth. There are *common* (defective and incorrect nutrition, diseases of organs and tissues, ionizing radiation, stress) and *local* (the presence of plaque, microorganisms, carbohydrate residues of food, decreased saliva pH and the speed of salivation) cariogenic factors. An undeniable connection between the development of dental caries and the role

of carbohydrates in foods and oral microflora is proved. Refined carbohydrates — sugar, glucose, fructose, maltose, lactose — are cariogenic and natural food — polysaccharides — practically not dangerous for the teeth because of slow hydrolyze. The rate of organic acids formation is minor and they are neutralized by saliva.

*Cariogenic bacteria (Str. mutans, salivarius, sanguis, mitis and lactobacilli)* are characterized by the following features:

– able to metabolize carbohydrates to organic acids that reduces the pH of oral fluid to 4–5 and enamel demineralization occurs;

– ability to synthesize intracellular carbohydrate stores and use them in the absence of carbohydrates in the diet for their livelihoods;

– ability to synthesize extracellular carbohydrates — glucans, levans, heteropolysaccharides — for permanent connection to the surface of the tooth.

## FLUORINE (F) AND ITS BIOLOGICAL ROLE

1. 99 % as F-apatite is part of bones and teeth, makes them hard and acid-resistant.
2. Stimulates bone and teeth remineralization (supplying with calcium and phosphorus).
3. Stimulates bone tissue synthesis, immunity (including oral cavity immunity), hemopoiesis.
4. Changes the electrical potential of the of the enamel surface and prevents the adhesion of bacteria to the enamel.
5. Inhibits bacterium glycolytic enzyme enolase (lactate synthesis decreases significantly) and synthesis of extracellular microbial polysaccharides.

F concentration expressed in mg/l and ppm (parts per million): 1 mg/l = 1 ppm; 1 % = 10,000 ppm.

World health organization (WHO) recommendations:

– optimal F requirement is about 4 mg/day;

– 1.2 mg F — with water (30 %); 2.0 mg — with food (50 %); 0.8 mg — from air (20 %);

– in hot countries F amount in water should be 0.5–0.8 mg/L; in countries with moderate climate — 0.8–1.0 mg/L; in northern countries — 1.0–1.2 mg/L.

Fluorine is mostly found in seafood, green and black tea, red wine.

In Belarus fluorine content in water is 0,2 ppm (mg/l), in consumed foods — 0,6 ppm, in the air — 0.5 mg. On average, we get (0.6 + 0.8 + 0.5) mg = 1.9 mg fluorine.

F-prophylaxis: systemic — fluorination of salt, drinking water, milk, tablets “Vitaftor”; individual — F-containing toothpastes, solutions, gels, varnishes, fissure sealants.

Recommended F-content in toothpaste for adults is 1,500 ppm, for children — 500 ppm.

F-deficiency leads to *caries*. F-excess causes fluorosis. The earliest signs are chalky-white, irregularly distributed patches on the surface of the enamel; these patches become stained yellow or brown, producing a characteristic mottled appearance. Severe toxicity weakens the enamel, pitting its surface. Other clinical manifestations are cartilage and ligament mineralization, skeleton osteosclerosis, premature aging, decreases in immunity and hormonal activity.

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