Yu. Gubsky

BIOLOGICAL CHEMISTRY

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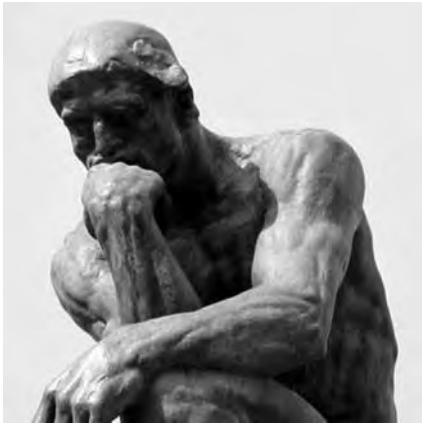


Auguste Rodin “Thinker”

What is Biological Chemistry?

I’l do my best to give you the answers and explanations you need!

Yu. Gubsky

CONTENTS

PREFACE

"This is a wonderful time in biochemistry".
Lubert Stryer, 1994

Medicine is a rapidly developing branch of human knowledge which amazing achievements of the last decades are based predominantly on the fundamental discoveries in biochemistry and molecular biology, especially the recombinant DNA technologies and protein chemistry. The disentangling of intricate interplay and transformations of thousands of diverse biomolecules, including nucleotides, peptides, amino acids, lipids, sugars etc., has laid the foundation for the deep insight into molecular mechanisms of the most grave human diseases origin and the development of high-performance medicines. The new, more sophisticated understanding of spatio-temporal organization and functioning of principal cellular biomolecules resulted in formulating of such up-to-day, generalized notions as Proteomics, Genomics and Metabolomics.

Today, it is impossible to conceive the process of principal human diseases diagnostics and treating without modern achievements of clinical biochemistry based on clear understanding of molecular and cellular mechanisms of general pathology and intricate disorders of cellular metabolism. Namely the developments in constructing recombinant DNA molecules resulted in constructing of principally new and highly effective medicines of protein nature. From the other viewpoint, biochemistry became the essential basis of such fundamental biological sciences and academic disciplines as cellular biology, molecular physiology, microbiology, genetics.

This is the academic course of Biological Chemistry for medical students worked out on the basis of abundant educational experience of the author and the scholastic staff in the whole of the Bioorganic, Biological and Pharmaceutical Chemistry Department of the National O. O. Bogomolets Medical University, the principal approaches and features of which were presented in the manual "Біологічна хімія" (Yu. I. Gubsky "Biologichna Chimia", Kyiv,

2011). The academic schedule of lectures, seminars and laboratory assignments in bioorganic and biological chemistry, advanced by Prof. Yu. Gubsky, was reviewed during the joint meetings of the Scientific Commission in Biological and Medical Chemistry of the Ministry of Public Health of Ukraine, which were held in the last years and were discussed and approved in the meetings of XI Ukrainian Biochemical Congress (Kyiv, 2014).

The presented study course of Biological Chemistry for the 2-nd year medical students consists of 30 chapters made up of text material and plentiful illustrations, including chemical formulas, reaction equations, drawings, depicting complicated intracellular transformations of biomolecules, and metabolic "charts". The study guide of the lecture course in bioorganic chemistry, intended for the 1-st year medical students, was published in 2009 (Yu. Gubskyi "Bioorganic Chemistry". – "Nova Knyha").

The author is profoundly indebted to his colleagues, especially Prof. I. Nyzhenkovskaya (O. Bogomolets National Medical University), Prof. H. Erstenyuk (Ivano-Frankivsk National Medical University), Prof M. Korda (I. Horbachevsky Ternopil State Medical University), M. Velikiy (Palladin Institute of Biochemistry, NAS of Ukraine), for their valuable comments and benevolent criticisms during the preparation of the course. No doubt, the author would also welcome any further suggestions from students and biochemistry teachers.

Yu. Gubsky
Kyiv, 2016

Das Sein ist ewig; denn Gesetze
Bewahren die Lebendgen Schatze,
Aus welchen sich das All geschmuckt.
Johann Wolfgang Goethe

Буття є вічним, тому що існують
Закони, що оберігають скарби Життя,
якими прикрашає себе Всесвіт
Йоган Вольфганг Гете

Part 1

BIOMOLECULES. CELLS. METABOLISM

Chapter 1.

BIOCHEMISTRY: BIOMOLECULES, CELLS METABOLISM

1.1. Biochemistry as fundamental biomedical science

Biological Chemistry (Biochemistry) is the modern biomedical science. The essence of Biochemistry, or Biological Chemistry, is the study of the molecular basis of life.

The fundamental scientific foundations of Biochemistry are Bioorganic Chemistry and Physiology ("Physiologic Chemistry", which means the study of chemical bases of physiogical phenomenae).

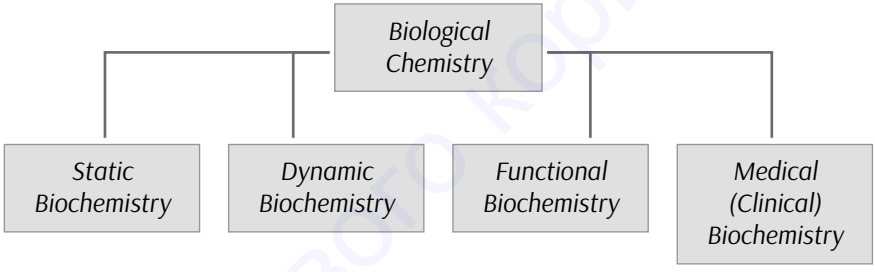
Saying in other words, biochemistry describes in molecular terms the chemical architecture and chemical processes that underlie the structures and mechanisms taking place in all living organisms. Study of biochemistry provides the insight into basic organizing principles that characterize the life in all its diverse forms, the principles which are collectively referred to, after A. Lehninger, as the "molecular logic of life".

Hence, the principal tasks of biochemistry comprise the elucidation of:

- chemical (or molecular) design of life, that is *biomolecules* structure and their arrangement in living systems;
- chemical reactions, that is biomolecules transformations, which are collectively known as *metabolism*.
- biochemical (enzymatic) reactions that underlie the *physiological phenomena*e in living systems including human body, e.g. digestion, blood clotting, muscle contraction, nerve impulse conduction etc.;
- the molecular foundations which determine the *origin and development of various diseases*.

Categories in Biochemistry

- ***General (or Static) biochemistry*** (which is similar to Bioorganic Chemistry of natural compounds), that investigates the structure and functions of biomolecules
- ***Dynamic biochemistry*** that studies chemical transformations, or metabolic reactions inside living cells, catalyzed by array of various enzymes (biocatalysts with extremely high substrate specificity)
- ***Functional biochemistry*** or Physiological chemistry, which principal object is disentangling of molecular mechanisms underlying physiological processes in the body
- ***Medical and clinical biochemistry*** which principal task is to elucidate the molecular basis both of inborn (inherited) and acquired human diseases.



1.2. Biomolecules – major classes, representatives

The major classes of biomolecules are:

- Proteins, peptides and proteinogenic amino acids
- Nucleic acids (DNA, RNA) and nucleotides
- Carbohydrates (or *sugars*): monosaccharides, disaccharides and polysaccharides
- Lipids: fatty acids, simple lipids, complex lipids, sterols
- Vitamins: water soluble (coenzymic vitamins), lipid soluble vitamins
- Hormones, which are signaling substances synthesized in endocrine glands.
- Inorganic compounds (H_2O, ions: Na^+, K^+, Ca^{2+}, Cl^-, SO^{4-}).

Proteins and proteinogenic amino acids as their constituents

Proteins are biomolecules that play crucial roles in virtually all biological processes, among which of the greatest importance is their participation as biocatalysts called ***enzymes.***

From chemical viewpoint, proteins are biomacromolecules made up from one or more long polypeptide chains. Thus, polypeptides and proteins are condensation polymers of amino acids, consisting predominantly of 20 natural L-α-amino acids, which are named *proteinogenic amino acids.*

As distinct from short-length peptides and polypeptides, proteins are very large molecules, having values of M_r which range from near 5.000 to several million daltons (D), or thousand kD.

Proteins make about 15 % of human body weight. The structural characteristics of proteins and their specific biochemical functions will be considered in the proper chapters of the textbook.

Nucleic acids (DNA, RNA) and nucleotides as their constituents

The nucleic acids (deoxyribonucleic acids – DNAs and ribonucleic acids – RNAs) are macromolecules with the peculiarities of structure and fundamental biological functions that involve storage, flow and realization of genetic information.

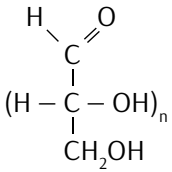
The monomer units of nucleic acids are nucleoside monophosphates (nucleotides), chemical nature of which as well as the structure of main classes of nucleic acids will be elucidated in Chapter 3.

Sugars and their derivatives

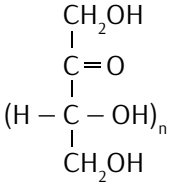
Carbohydrates (sugars) owe their name to the fact that all of them contain carbon, hydrogen and oxygen, and, furthermore, in most instances they have a general molecular formula $C_m(H_2O)_n$, which corresponds to the imaginary hydrate of carbon. Simple carbohydrates are also known as ***sugars*** or ***saccharides*** (from "sacharum" – sugar; *lat.*)

Chemically defined, carbohydrates are aldehyde and ketone derivatives of polyhydric alcohols. Being considered from this point of view, carbohy-

drates which structure responds to *polyhydroxy aldehydes*, are designated as ***aldoses,*** and those which are *polyhydroxyketones* are termed ***ketoses.*** The generalized representations of these types of sugars are:



Aldose
(Glucose, Ribose)



Ketose
(Fructose etc.)

Sugars are the most abundant organic molecules in biosphere. The vital importance for all living beings is *photosynthesis* – the process of carbohydrates production which takes place in the Earth in the leaves of green plants via the action of photosensitive pigment *chlorophyll:*

$$nCO_2 + n\,H_2O \rightarrow \underset{Starch}{(C_6H_{10}O_5)_n} + nO_2.$$

Animals and human beings consume starch and other carbohydrates as essential components of plant food and transform them into simple monosaccharides, predominantly *glucose.* Living cells utilize glucose and other monosaccharides as the paramount sources of chemical energy needed for their growth, movements, in particular muscle contraction, and other physiological and biochemical functions of the organism.

Carbohydrates are found in all cells of human body. They are essential to the very source of life (ex. pentoses ribose, deoxyribose in RNA and DNA) or sustaining life itself (ex. metabolic conversion of carbohydrates, predominantly glucose into usable biochemical energy, ATP). Another important role of carbohydrates is structural (ex. cellulose in plants, *glycosamines* as components of animal connective tissue *glycoproteins*).

Lipids

Biologically essential class of lipids constitute molecules of different chemical nature which are predominantly soluble in organic non-polar solvents and insoluble in water. The majority of lipids are complex esters of alcohols and long-chained carboxylic acids which are also named *fatty acids.*

The diverse class of lipids include *triacylglycerols*, *phospholipids*, *sphingolipids*, *glycolipids*, *steroids* etc., which are essential components of cellular membranes and participants in diverse physiological functions.

Vitamins

Vitamins, which are essential bioorganic compounds for many biological aspects, are required in very small quantities and have to be provided with the diet. According to generally accepted classification, the vitamins are divided into water-soluble vitamins (ascorbic acid, vitamins B_1, B_2, B_6, B_{12} etc.) and lipid-soluble vitamins (vitamins A, D, E, K). Most vitamins are precursors of *coenzymes t*hat are essential components of protein biocatalysts – enzymes.

Hormones

Hormones, which are chemical signaling substances synthesized by specialized cells in endocrine glands. After secreting into bloodstream hormones are transported to the target organs and hormone-sensitive cells, where, after being linked with the specific protein receptors, they exert there specific physiological and biochemical effects.

Inorganic biologically essential compounds

Inorganic elements, otherwise *minerals*, constitute a very heterogenic group of chemicals, essential for structure and biological functions of every living cell.

Minerals can be divided into *macroelements* and *microelements* which are found in the body in the trace quantities (*trace elements*).

- The daily requirement of macroelements for humans is > 100 mg. They include sodium (Na), potassium (K), calcium (Ca), magnesium (Mg), chlorine (Cl), phosphorus (P) and sulfur (S).
- The essential microelements, which daily requirement is < 100 mg, include iron (Fe), zinc (Zn), manganese (Mn), copper (Cu), cobalt (Co), selenium (Se), iodine (I), fluoride (F).

Water

Life in the Earth has evolved in water and still depends on unique physico-chemical properties of the latter as solvent for many essential biomolecules and participant in biochemical reactions of metabolism. Moreover, water, through essential buffer systems, provides the maintenance of pH values essential for functioning of biochemical processes inside cells and in extracellular spaces.

Water makes about 55–60 % of human body weight and its daily requirement for adults is about 1,5–2 l.

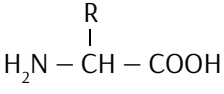
Chapter 2.

PROTEINS. AMINO ACIDS. PEPTIDES

Proteins are biopolymers which contain residues of specific set of 20 α-amino acid *(canonical set)*, linked by amide-, or *peptide* bond.

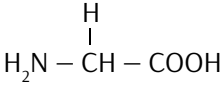
2.1. Amino acids: structure, properties

- **Amino acids** or amino carboxylic acids are derivatives of carboxylic acids, in which at least one hydrogen atom is replaced by an amine group.
- So, as their name implies, amino acid molecules are heterofunctional compounds which include α two functional groups, an amine group, $–NH_2$, and a carboxyl group, –COOH.
- Now about 200 various amino acids have been identified in different natural species. Some of them (about 20 different amino acids) are the compounds which are the building blocks of proteins and peptides. Because these amino acids appear as the products of proteins and peptides hydrolysis, they are called *proteinogenic amino acids*.
- Most of the amino acids thus far isolated from the various proteins have an amine group attached to the same carbon atom that is linked to the carboxyl group (2-nd carbon atom of the chain). So the proteinogenic amino acids are **α-amino acids**, or 2-aminocarboxylic acids.
- Thus, the general formula of **α-amino acid** is:

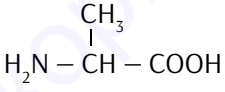


- The nature of amino acid side chain R varies considerably. Its structure defines significantly the chemical properties of individual α-amino acids and affects essentially biological characteristics of the proteins in which they are found.
- Some of the proteinogenic α-amino acids have additional amine groups in other (β- or γ-) positions of carbon chain (diaminomonocarboxylic acids – see below). There are also non-proteinogenic amino acids, which have amine groups attached in the positions distinctive from α-carbon (β-alanine, γ-aminobutyric acid). These can be found in living cells (animal, plant or bacterial) as free molecules or as constituents of other bioorganic compounds.

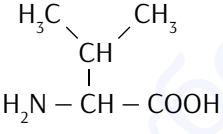
Proteinogenic amino acids are the constituents of natural proteins and peptides which chemically are polypeptides made of 20 α-amino acids ("Bioorganic Chemistry", 2009).



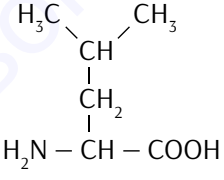
Glycine
(α-aminoacetic acid)



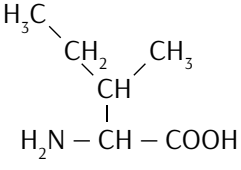
Alanine
(α-aminopropionic acid)



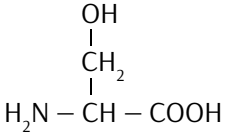
Valine
(α-aminoisovaleric acid)



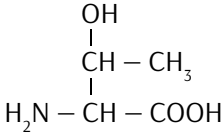
Leucine
(α-aminoisocaproic acid)



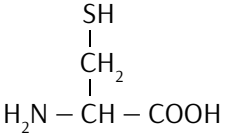
Isoleucine
(α-amino-β-methylvaleric acid)



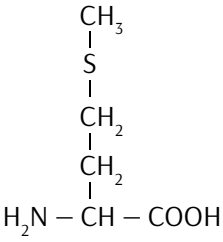
Serine
(α-amino-β-hydroxypropionic acid)



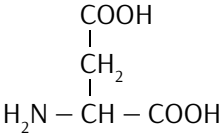
Threonine
(α-amino-β-hydroxybutyrie acid)



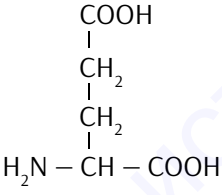
Cysteine
(α-amino-β-thiopropionic acid)



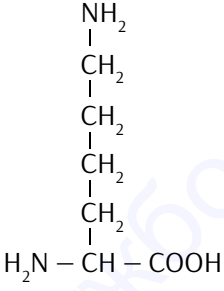
Methionine
β-amino-γ-methylthiobutyric acid)



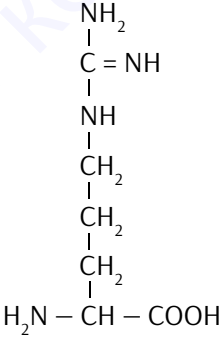
Aspartic acid (aspartate;
aminosuccinic acid)



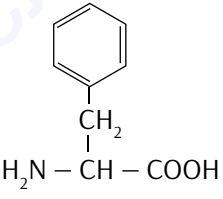
Glutamic acid (glutamate;
α-aminoglutaric acid)



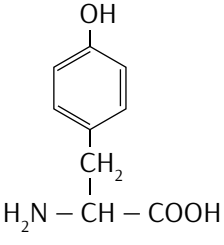
Lysine (α, ε-diaminocaproic acid)



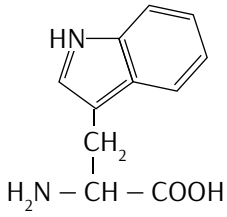
Arginine (α-amino-δ-guanidinovaleric acid)



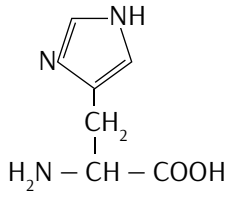
Phenylalanine
(α-amino-β-phenylprop ionic acid



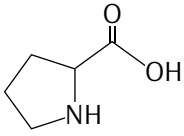
Tyrosine
(α-amino-β-hydroxyphenylpropionic acid)



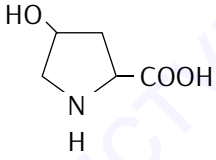
Tryptophan
(α-amino-β-indolylpropionic acid)



Histidine-amino-P-imidazolyl-
propionic acid)



Proline
(pyrrolidine-α-carboxylic acid



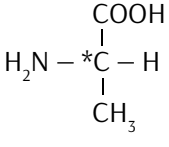
4-Hydroxyproline

Stereoisomerism and optical activity of α-amino acids

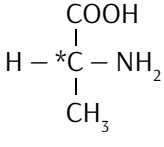
As is discussed in the course of Bioorganic chemistry, the carbon atom, which is bonded to four different groups, is called ***asymmetric*** carbon or a ***chiral*** carbon.

In the structure of proteinogenic amino acids, with the exception of glycine, the α-carbon atom, that is the carbon attached to carboxyl group, is an asymmetric carbon. So, **the amino acids are chiral molecules.**

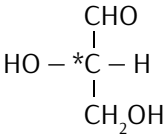
In compliance with the general principles of stereoisomers classification, that is the correspondence of a spatial arrangement of a molecule to the configuration of L- or D-glyceraldehyde, two kinds of amino acids stereoisomers exist which are called L-isomers and D-isomers. These stereoisomers, that is L- and D-amino acids, make up pairs of *enantiomers* or mirror images that cannot be superimposed.



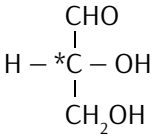
L-Alanine



D-Alanine

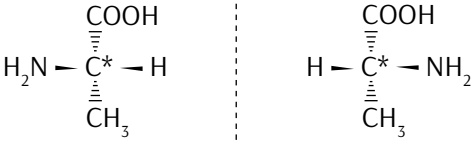


L-Glyceraldehyde



D-Glyceraldehyde

For example, the configurations of two enantiomers (non-superimposable mirror images) of α-alanine are as follows:



L-Alanine

D-Alanine

imaginary mirror

The amino acids that are incorporated into natural peptides and proteins are of the L-configuration. Only L-amino acids are intermediates in metabolic reactions in animal and human tissues, which is due to the particular chirality of biological catalysts *enzymes*.

According to the aforesaid structural properties, α-amino acids have optical activity. Two enantiomers are ***optical isomers***, which rotate the plane of polarized light to the *right* (clockwise) or to the *left* (counterclockwise). They are designated as (+)isomers and (-)isomers correspondingly.

Either geometrical objects in general, that lack the plane of symmetry and proceeding from this can make up two non-superimposable mirror images, are called *enantiomorphes*. Supposedly, enantiomorphes were funny twin brothers, Tweedledum and Tweedledee, whom L.Carroll`s Alice has come across in her marvelous adventures beyond the looking glass. On the whole, living beings, whose proteins are hypothetically built up of D-amino acids, can constitute the imaginary anti-world queerly enough predicted in science fiction.

2.2. Peptide bonds, polypeptides

Peptide bond formation

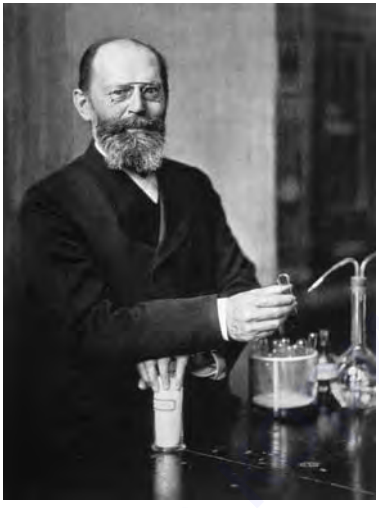
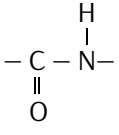


Figure 2.1. Fischer, Emil Herman (1852–1919). Scientific researches in the chemistry of peptides, sugars, purines/Nobel Prize, 1902

The ***peptide bond*** **(—CO—NH—)** is the bond formed between the α-carboxyl group of one amino acid and the α-amino group of another. This bond is also called an *amide bond,* and it is formed by the removal of the elements of water from two amino acid that form a ***peptide.***

Four atoms **(C, O, N, H)** make up a ***peptide group:***



Peptide group

The compounds which are made up by the considered mechanism are called ***peptides (dipeptides, tripeptides, tetrapeptides*** etc... ***polypeptides).***

An amino acid unit in a polypeptide is called *a residue*. The generalized scheme of dipeptide formation is shown in Figure 2.2:

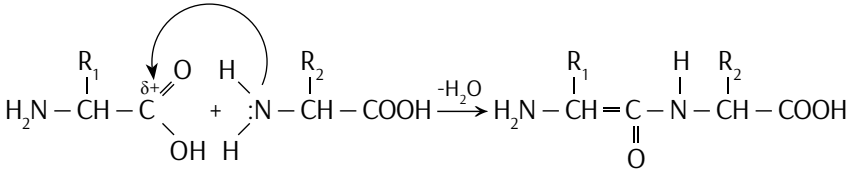


Figure 2.2. The scheme of peptide bond and dipeptide formation

Naming peptides. Abbreviations of amino acids

A polypeptide chain has the *direction* because its building blocks have different ends – namely, the α-amino and the α-carboxyl group. By convention, **the amino end is taken to be the beginning of a polypeptide chain,** and so the sequence of amino acids in a polypeptide chain is written and is read starting with the amino-terminal residues.

According to the IUPAC rules, to name peptides the names of acyl groups ending in ***"yl"*** are used. The C-terminal residue is represented by the name of the amino acid, and this ends the name of the whole peptide.

Thus, if the amino acids *glycine* and alanine condense so that glycine acylates alanine, the dipeptide formed is named *glycyl-alanine*, or *glycyl-L-alanine:*

$$NH_2 - CH_2 - COOH + NH_2 - CH(CH_3) - COOH \xrightarrow{-H_2O}$$

Glycine Alanine

$$\longrightarrow NH_2 - CH_2 - CO - NH - CH(CH_3) - COOH$$

Glycyl-L-alanine

Amino acids alanine and valine yield dipeptide alanyl-valine (Figure 2.3).

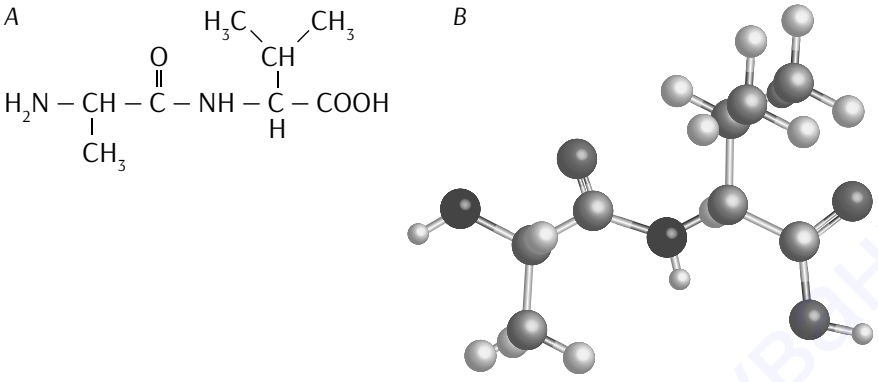
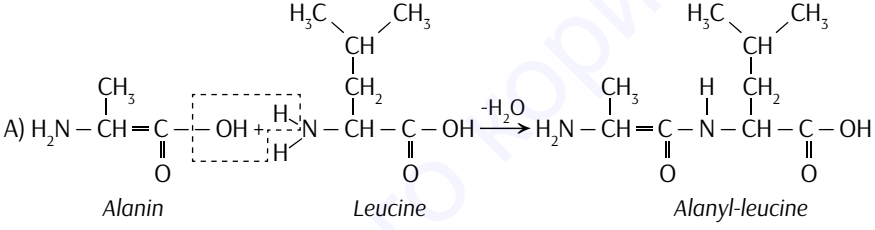
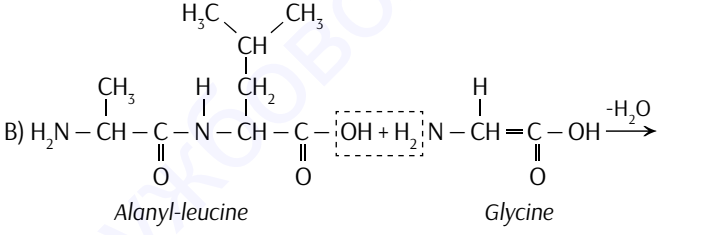


Figure 2.3. Dipeptide alanyl-valine: structural formula (A) and ball-and-stick model (B)

Higher peptides are named similarly, e.g. tripeptide *alanyl-leucyl-glycine:*





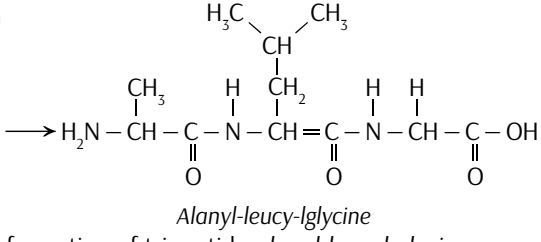


Figure 2.4. The formation of tripeptide *alanyl-leucyl-glycine*

Abbreviations. To save the space in writing structures of polypeptides

and proteins, the special symbols for amino acid units are employed. The symbols by which amino acids are designated are either three-letter abbreviations or one-letter conventional signs (Table 1.1).

Table 1.1. Abbreviations for amino acids

Amino acid	Three-letter abbreviation	One-letter abbreviation
Alanine	Ala	A
Arginine	Arg	R
Asparagine	Asn	N
Aspartic acid	Asp	D
Asparagine or aspartic acid	Asx	B
Cysteine	Cys	C
Glutamine	Gln	Q
Glutamic acid	Glu	E
Glutamine or glutamic acid	Glx	Z
Glycine	Gly	G
Histidine	His	H
Isoleucine	Ile	I
Leucine	Leu	L
Lysine	Lys	K
Methionine	Met	M
Phenylalanine	Phe	F
Proline	Pro	P
Serine	Ser	S
Threonine	Thr	T
Tryptophan	Trp	W
Tyrosine	Tyr	Y
Valine	Val	V

For example, presented above tripeptide *alanyl-leucyl-glycine* can be named Ala-Leu-Gly or simply A-L-G.

Attend that in the tripeptide designated as A-L-G *alanine* is the amino-terminal residue and *glycine* is the carboxy-terminal residue, whereas in the tripeptide G-L-A the direction of amino acids consequence is contrariwise.

Disulfide bonds

Some proteins contain ***disulfide bonds***. They are cross-links between polypeptide chains or between parts of a single chain which are formed by the oxidation of two *cysteine* residues (Figure 2.5). The resulting amino acid residue that forms "disulfide bridge" is called *cystine*.

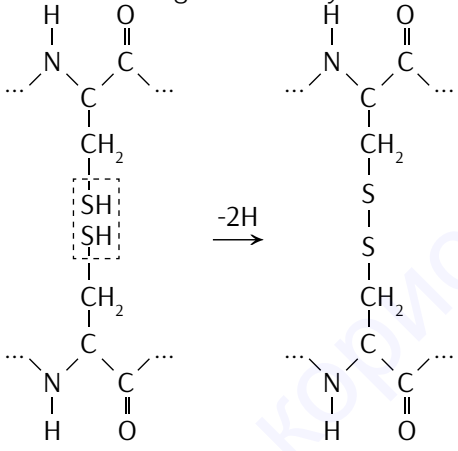


Figure 2.5. A disulfide bridge (–S–S–) between two chains is formed from the sulfhydryl groups (–SH) of two cysteine residues

2.3. Proteins. Levels of protein structure

Proteins are made up of one or more long polypeptide chains. Thus, polypeptides and proteins are condensation polymers of amino acids. As distinct from peptides, proteins are very large molecules, having values of M_r which range from 5.000 to several millions *daltons* (units of atomic mass; **D**) or thousand *kilodaltons* **(kD).**

Proteins make up about 15 per cent of our body weight. They are crucial biomolecules which are essential for realization of the majority of vital biological functions of living organisms. Proteins are envolved in enzymatic catalysis, the formation of cellular structure and extracellular architecture, control of genetic expression, hormonal regulation, muscle contraction, immune defense etc.

Levels of structure in protein architecture

Four levels of structure are commonly cited in discussions of protein architecture. They are named ***primary, secondary, tertiary*** and ***quaternary structures of protein.***

Primary structure

Primary structure is the amino acid sequence in polypeptide chain that forms a protein (Figure 2.6).

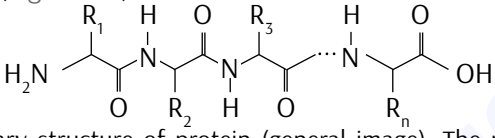


Figure 2.6. Primary structure of protein (general image). The polypeptide chain consists of n amino acid residues

Although there are only 20 amino acids which usually make up natural proteins, the number of ways in which they are combined to form the linear, unbranched polymers, is enormous. Take, for example, the protein *lysozyme,* an enzyme with antimicrobial activities, which occurs in human tears, salive and other secretions. The single polypeptide chain, that consti-tutes lysozyme molecule, has 129 amino acid residues, disposed in linear sequence. The number of different conceivable arrangements of the amino acids in this chain is 20^{129}, which is estimated to be more than twice the number of atoms in our galaxy.

It is known that the amino acids sequences of proteins are determined genetically. The sequence of nucleotides in DNA, the molecule of heredity, specifies the sequence of nucleotides in RNA, which in turn specifies the amino acid sequence of a definite protein.

Most natural polypeptide chains contain between 50 and about 2.000 amino acid residues. In 1953, Frederick Sanger determined the amino acid sequence of *insulin*, a protein hormone, which is constituted from two polypeptide chains, consisting of 21 and 30 amino acid residues (A-chain and B-chain correspondingly) – Figure 2.7.

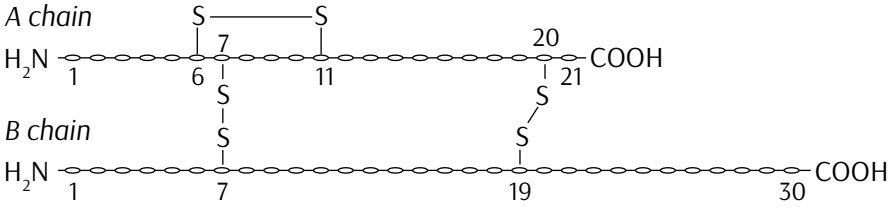


Figure 2.7. The scheme of insulin molecular architecture. Two polypeptide chains linked by S-S-bonds are shown

The fundamental investigation of F. Sanger showed for the first time that a protein has a precisely defined amino acid sequence. Since that, the complete amino acid sequences of more than 10.000 peptides and proteins were established. The examples of primary structure of three short-chain peptides from human body are presented:

Arg-Pro-Pro-Phe-Ser-Pro-Phe-Arg

Bradykinin
(octapeptide that displays powerful blood vessels dilatating activity)

Tyr-Gly-Gly-Phe-Met
Tyr-Gly-Gly-Phe-Leu

Endorphines
(met-encephaline and leu-encephaline which are human brain neurotransmitter peptides)

Secondary structure

Secondary structure refers to the spatial arrangement of amino acid residues that are near one another in the linear sequence. Some of these steric relationships are of a regular kind, giving rise to the periodic structures. The **α-helix** (Figure 2.8) and **β-strand (β-pleated sheet)** (Figure 2.9) are two distinctive designs of protein secondary structure.

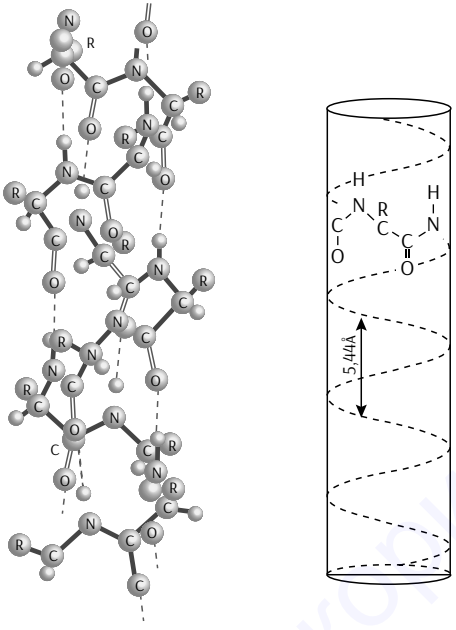


Figure 2.8. The secondary structure of a protein in the form of α-helix configuration (after L. Pauling and R. Corey). The dashed lines represent H-bonds

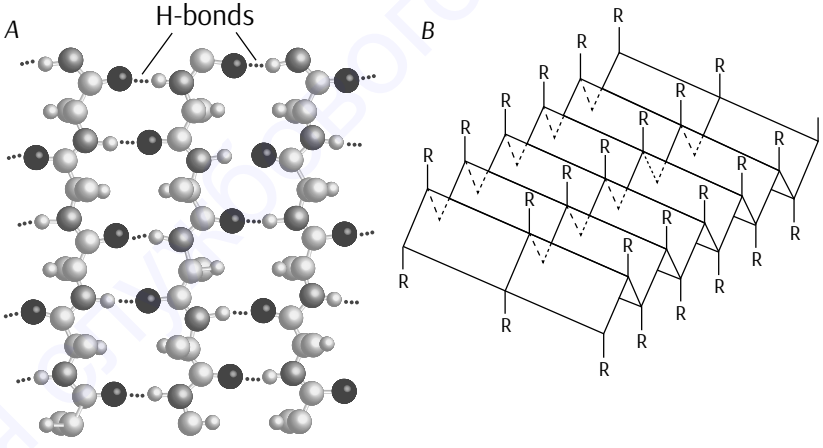


Figure 2.9. The secondary structure of protein in the form of β-configuration. The individual polypeptides are linked by multiple H-bonds (A) to form a planar, sheet-like network of chains – β-pleated sheet (B)

Figure 2.10. Linus Carl Pauling (1901–1994). The outstanding American scientist in the fields of general, bioorganic chemistry and molecular biology. Pacific activist. Nobel Prize in Chemistry (1954). Nobel Peace Prize (1962)

The α-helix configuration and β-pleated sheet are stabilized by hydrogen bonds (H-bonds).

The hydrogen bonds are formed by a hydrogen atom which is shared between two other atoms – the *hydrogen donor* and the *hydrogen acceptor*. Hydrogen donor and hydrogen acceptors markedly differ in their *electronegativity*, so that the acceptor has a partial negative charge which attracts the hydrogen atom.

Inside protein molecules the hydrogen bonds are made up predominantly between the NH and CO groups of polypeptide (within the same chain or between different chains) – Figure 2.11.

So, as it can be seen from the images presented, in the α-helix H-bonds form between the single peptide bonds in the same chain, whereas in β-pleated sheet H-bonds are made up between different polypeptides.

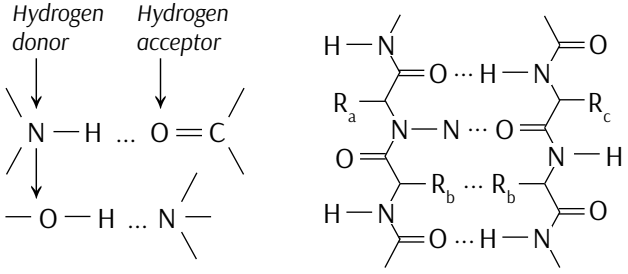


Figure 2.11. The mechanism of hydrogen bonds formation between NH- and CO-groups of polypeptide chains

Tertiary structure

Tertiary structure refers to the spatial arrangement of amino acids residues that are far apart in the linear sequence. In another words, tertiary structure of protein is the way of packing of polypeptide chain in determined volume in three-dimensional space.

In most cases of protein spatial architecture, the secondary ordered polypeptide chains tend to fold into globular structures with the hydrophobic side chains in the interior of the globule and the hydrophilic side chains outside in contact with water. This is the case for muscle myoglobin (Figure 1.12) and such soluble proteins as enzymes, hormones, blood plasma proteins etc.

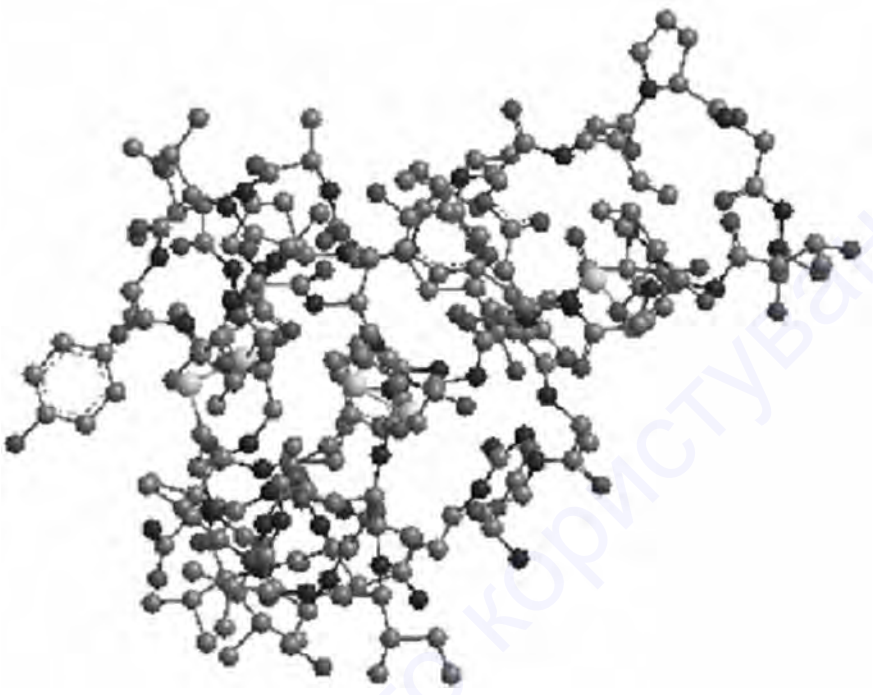


Figure 2.12. General image of protein molecule (ball-and-stick model; ChemOffice 3D-presentation)

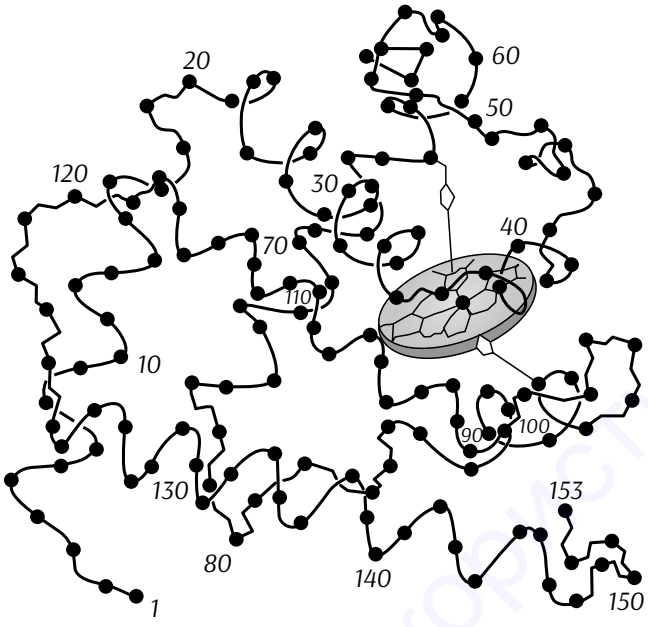


Figure 2.13. The spatial arrangement of muscle myoglobin as an example of globular tertiary structure

The types of the tertiary structure are ***globular*** proteins and ***fibrillar*** proteins (Figures 2.13–2.14).

Globular protein – myoglobin of muscle

Fragment of collagen fiber

Figure 2.14. Globular and fibrillar proteins as representative of tertiary protein structure

In most cases of protein spatial architecture, the secondary ordered polypeptide chains tend to fold into globular structures with the hydrophobic side chains in the interior of the globule and the hydrophilic side chains outside in contact with water. This is the case for hemoglobin, muscle myoglobin and such soluble proteins as many enzymes, hormones, blood plasma proteins etc.

The stability of protein tertiary structure is ensured by different kinds of noncovalent bonds, viz. *hydrogen bonds*, *electrostatic* (or *ionic*) *bonds* (also called *salt bridges*), *Van der Waals bonds* (or *dipole-dipole interactions*) and in some way by *hydrophobic interactions*.

Quaternary structure

Proteins containing several polypeptide chains exhibit an additional level of structural organization. Each polypeptide chain in such a protein is called a ***subunit*** which forms its own primary, secondary and tertiary levels of structure.

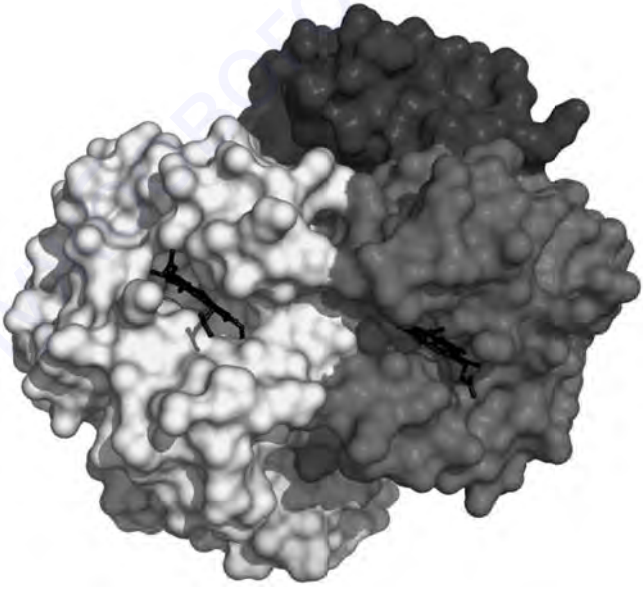


Figure 2.15. The spatial arrangement of hemoglobin subunits as an example of pro-

tein quaternary structure

Quaternary structure of a protein refers to the spatial arrangement of subunits and the nature of their contact. The example of a protein which has a quaternary structure is ***hemoglobin*** of erythrocytes. The hemoglobin which serves as an oxygen-transporting protein of human blood, consists of four subunits (polypeptide chains) – two α- and two β-chains, each of them includes 141 (α-chain) and 146 (β-chain) amino acid residues (Figure 2.15).

Chapter 3.

NUCLEIC ACIDS. NUCLEOTIDES. DNA. RNA

3.1. Nucleic acids and the flow of genetic information

Nucleic acids are polymers of ***nucleotides*** (or ***mononucleotides***). In accordance with their structure and properties nucleic acids are divided into two classes: ***deoxyribonucleic acids (DNA)*** and ***ribonucleic acids (RNA).***

The fundamental biological significance of nucleic acids is the storage, transmission and expression of genetic information.

The nucleic acids realize the flow of genetic information in every living cell and namely:

DNA as the chemical basis of genes specifies the kinds of proteins which are made by the cells and hereby all the structural and functional properties of living beings

RNA serve the templates for protein synthesis and thus are a part of protein-synthesizing machinery.

The transfer of genetic information from DNA to messenger RNA is called ***transcription*** and the synthesis of proteins according to instructions given by mRNA templates – ***translation***. Thus, the flow of genetic information in living cells can represented in this way:

$$\text{DNA} \xrightarrow{\textit{transcription}} \text{RNA} \xrightarrow{\textit{translation}} \text{Proteins}$$

3.2. Nucleotides: structure, biological functions

Nucleotides

Each monomeric unit of nucleic acid, that is **nucleotide,** consists of a ***nitrogenous base*** which is a certain heterocycle of *purine* or *pyrimidine* class, a ***pentose*** sugar and a ***phosphate residue.***

The coupling of nitrogenous base to sugar yields a ***nucleoside,*** which becomes a nucleotide when being phosphorylated.

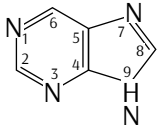
So: Nucleotide = nitrogenous base + pentose + phosphate;
Nucleoside = nitrogenous base + pentose, and
Nucleotide = nucleoside + phosphate.

Figure 3.1. Friedrich J. Miescher (1844–1895). The discovery of nucleic acids mankind owes to the pioneer work of Friedrich Johannes Miescher, Swiss physician and investigator. In 1869 F.Miescher provided the first evidence of the presence in human cells nuclei of acid substances which contained phosphate residues. From thence the epoch of nucleic acids study began

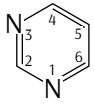
Nitrogenous bases of nucleotides

Nitrogenous bases, the key components of nucleotides and, to proceed from this of nucleic acids, are heterocyclic amines, derivatives of ***purines*** and ***pyrimidines.***

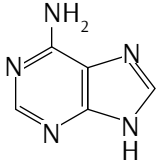
The set of five nitrogenous bases that are usually included into nucleotides comprises five major compounds of purine and pyrimidine nature.



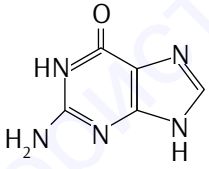
Purine



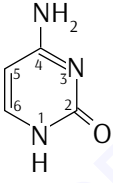
Pyrimidine



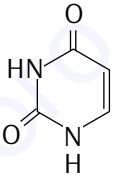
Adenime



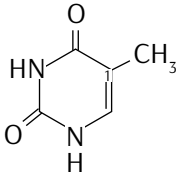
Guanine



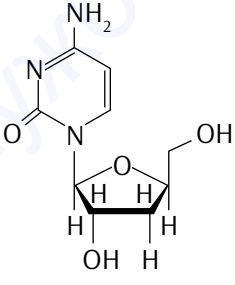
Cytosine



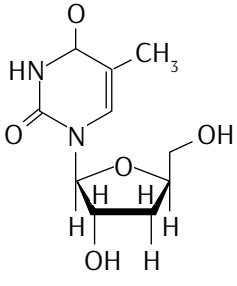
Uracil



Thymine



Deoxycytidine



Deoxythymidine

Purine and pyrimidine nucleobases

Being linked within *nucleotide* units of nucleic acids, the oxiderivatives

of nitrogenous bases exist normally in *keto* (*lactam*) forms which is preferential for realization of specific base pairing that is an essential structural attribute of DNA and RNA (see below).

Nucleosides

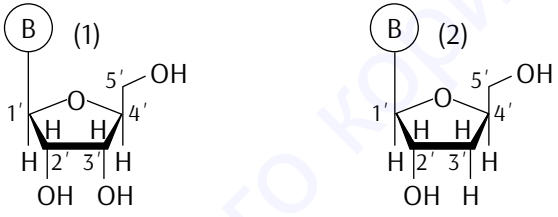
A ***nucleoside*** consists of a purine or pyrimidine base bonded to a sugar.

Chemically nucleosides are N-glycosides of ribose or 2`-deoxyribose and a certain nitrogenous base.

They are ***ribonucleosides*** and ***deoxyribonucleosides.***

In *ribonucleosides* N-9 of a purine or N-1 of a pyrimidine is attached to C-1 of ribose.

In *deoxyribonucleosides* N-9 of a purine or N-1 of a pyrimidine is attached to C-1 of 2`-deoxyribose (deoxyribose).



General formulae of ribonucleotides (1) and deoxyribonucleotides (2).
B – abbreviation for nitrogenous bases

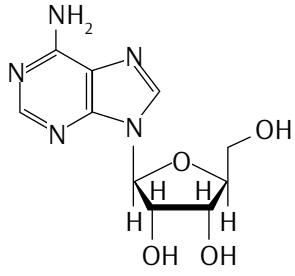
Distinctions in nitrogenous bases content of nucleic acids

Both *ribonucleosides* and *deoxyribonucleosides* (and nucleic acids RNA and DNA accordingly) include ***purines*** *adenine* and *guanine* as nitrogenous bases.

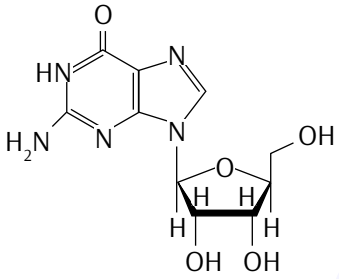
But two kinds of nucleosides (and nucleic acids formed from the corresponding nucleotides) differ in the ***pyrimidines*** that they incorporate. To be exact, *ribonucleosides* include *cytosine* and *uracil* whereas *deoxyribonucleosides* include *cytosine* and *thymine*.

Ribonucleosides

The names for purine ribonucleosides are ***adenosine*** and ***guanosine.***

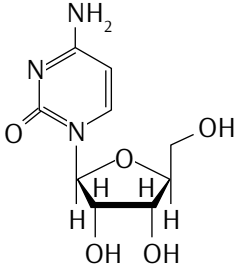


Adenosine

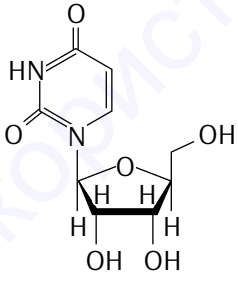


Guanosine

The names for pyrimidine ribonucleosides are ***cytidine*** and ***uridine.***



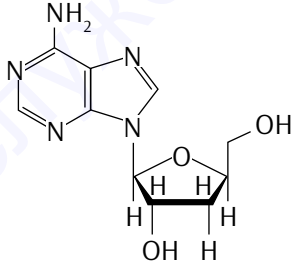
Cytidine



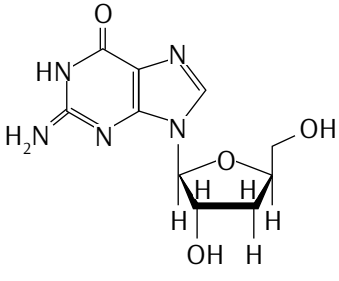
Uridine

Deoxyribonucleosides

The names for purine deoxyribonucleosides are *deoxyadenosine* and *deoxyguanosine*.



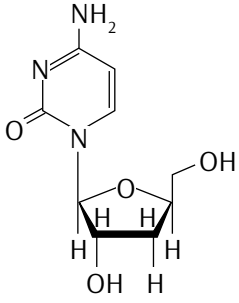
Deoxyadenosine



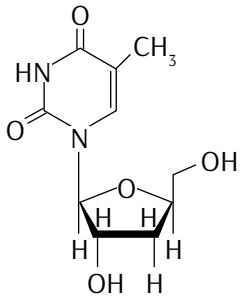
Deoxyguanosine

The names for pyrimidine deoxyribonucleosides are *deoxycytidine* and

deoxythymidine (or *thymidine*).



Deoxycytidine

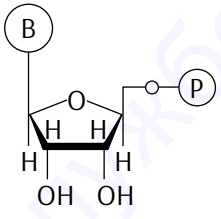


Deoxythymidine

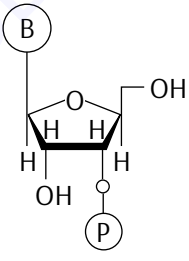
Nucleotides as components of nucleic acids

A ***nucleotide*** is a phosphate ester of a nucleoside. The most common site of esterification in naturally occurring nucleotides which constitute nucleic acids and are released under DNA and RNA hydrolysis is the hydroxyl group attached to C-5 of the sugar. Such a compound is called a *nucleoside 5`-phosphate* or a *5`-nucleotide*.

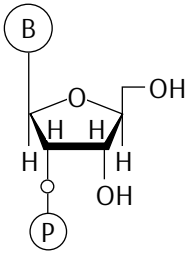
A primed number in the name of nucleotide denotes an atom of the sugar which is phosphorylated. It is noteworthy that nucleotides phosphorylated in other positions of the sugar (*3`-nucleotides* and *2`-nucleotides)* are normal intermediates of human metabolism.



Nucleoside 5'-phosphate



Nucleoside 3'-phosphate



Nucleoside 2'-phosphate

Nucleotides as metabolites and coenzymes

Besides being structural units for nucleic acids building, the nucleotides are significant chemicals for a wide range of other biochemical processes.

In particular, several purine and pyrimidine nucleotides (NAD, NADP, FAD, FMN) are the participants of vital reactions coupled with the metabolic energy generation and storage. Some of them play key roles as the components of complex enzyme systems involved in metabolism of carbohydrates, lipids and amino acids. The universal currency of free energy in biological systems is nucleotide *adenosine triphosphate (ATP).*

3.3. Nucleic acids as polynucleotides: DNA. RNA

Nucleic acids DNAs and RNAs are **polynucleotides** – ***polydeoxyribonucleotides*** and ***polyribonucleotides***, correspondingly.

To make up the polynucleotides, nucleotide monomers are linked together by phosphodiester bonds between the 3`-hydroxyl on the sugar of one nucleotide and the 5`-phosphate on the sugar of another nucleotide. This is called *primary structure of nucleic acids* (Figure 3.2).

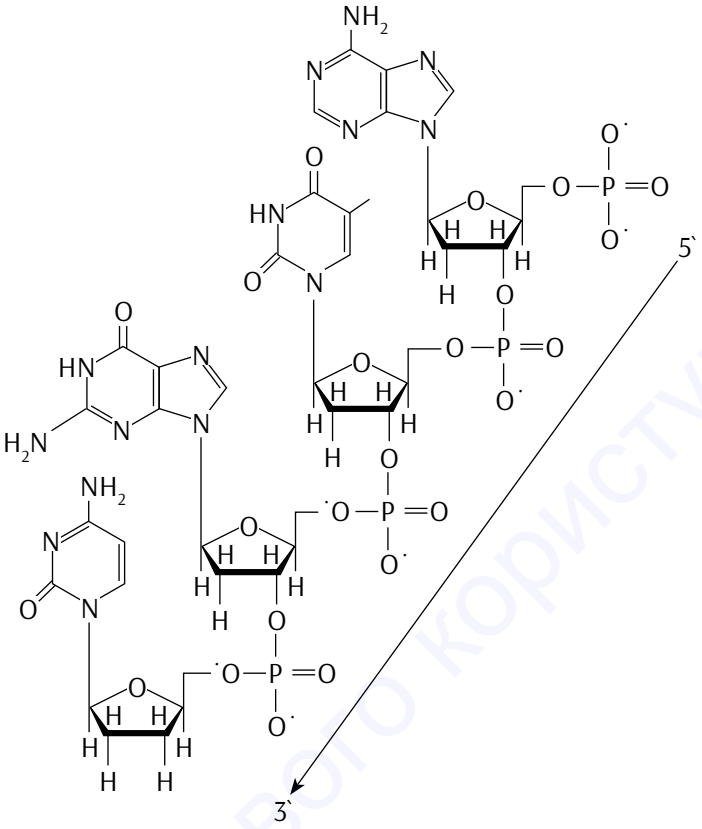


Figure 3.2. Fragment of DNA polynucleotide chain

The structure of a polynucleotide chain (DNA or RNA) can be concisely represented in a following way (Figure 3.3).

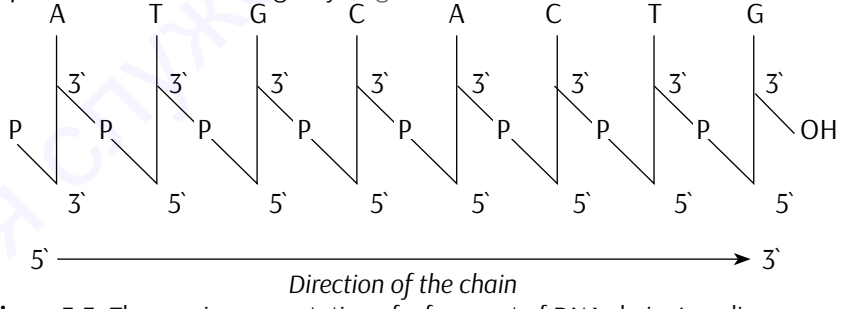


Figure 3.3. The concise presentation of a fragment of DNA chain. In a diagram presented the vertical lines refer to the sugar, whereas A, G, C, T represent the bases. The (P) within the diagonal line denotes a phosphodiester bond which links the 3`-

OH group of one sugar with the 5`-OH group of another

The abbreviation of purines and pyrimidines used for naming polynucleotides are: **A** (adenine), **G** (guanine), **C** (cytosine), **U** (uracil), **T** (thymine).

Thus, an even more abbreviated notation for the chain presented above is:

pApTpGpCpApCpTpG *or* pATGCACTG.

Polarity of polynucleotides

The polynucleotide chains have ***polarity*.** It means that one end of the molecule is different from the other and namely: one end of the chain has a 5`-OH group and the other a 3`-OH group, neither of which is linked to another nucleotide. In naturally occurring polynucleotides the 5`-OH group ("left") is usually phosphorylated and the 3`-OH group ("right") is not.

By convention, the sequence of bases in polynucleotide chains is written in the 5`→3` direction similarly as the amino sequence in proteins is written in the *amino → carboxyl* direction.

DNA and RNA: characteristic properties and biological functions DNA: double-helical structure and significance

In 1953, James Watson and Francis Crick deduced the three-dimensional structure of DNA that has become known as the **double helix.** Using this structure, Watson and Crick immediately proposed a mechanism for the replication of DNA that is still accurate today.

The characteristic properties of DNA structure as proposed by Watson and Crick ("double helix" model) are as follows:

(1) DNA molecule consists of two antiparallel polydeoxyribonucleotide chains. These chains are wound around each other with the purine and pyrimidine bases on the inside of the helix and the deoxyribose and phosphates on the outside (Figure 3.4).

(2) The two polydeoxyribonucleotide chains are held together by hydrogen bonds between pairs of bases (purines and pyrimidines). To say it precisely, *adenine* is always paired with *thymine*; *guanine* is always paired with *cytosine*. The corresponding bases are known as

complementary.

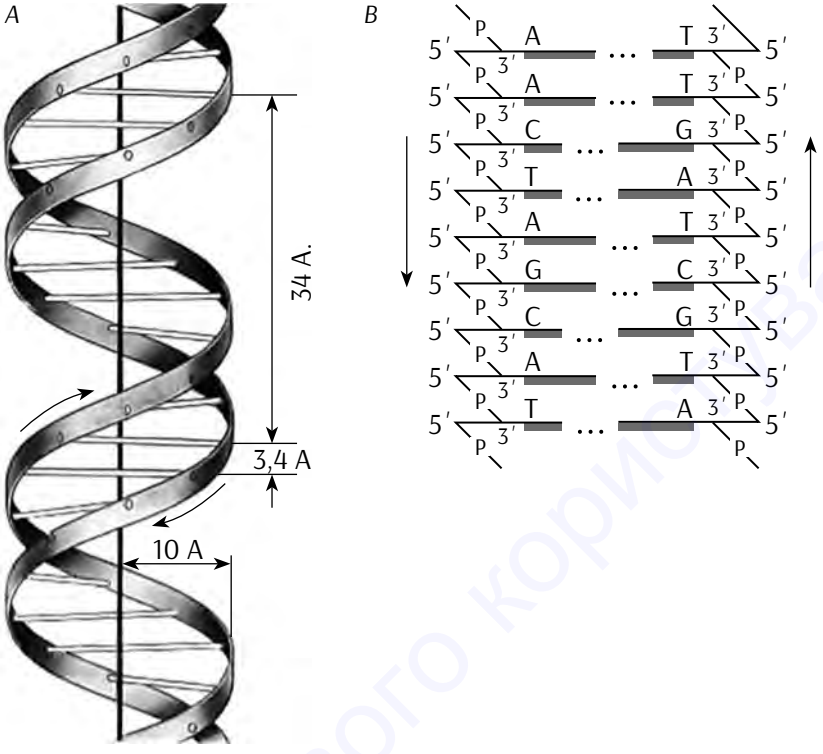


Figure 3.4. DNA molécule présentation as two antiparallel polydeoxyribonucleotide chai us (A) in which the purin e and pyriinidine bases are on the inside of the hélix and the deoxyribose and phosphates on the outside (B)

According to this, in each DNA molecule the content of *adenine* equals the content of *thymine*, and the content of *guanine* equals the content of *cytosine*, which was long before known as ***Chargaff `s rules:***

$$A = T;\ G = C.$$

Recall that in 1950, **Erwin Chargaff** found that the ratios of adenine to thymine and of guanine to cytosine equaled 1,0 in all species of DNA studied ("the rule of equivalence"). The discovery of Watson and Crick explained these experimental findings. Moreover, it led the way to the understanding of gene function in molecular terms. Now, at the beginning of XXI century we may ascertain that a brilliant accomplishment of James Watson and

Francis Crick ranks as one of the most significant in the history of biology and medicine.

The outline of double-helical DNA molecule as proposed by Watson and Crick (the so-called B-form of DNA) is presented in Figure 3.5.

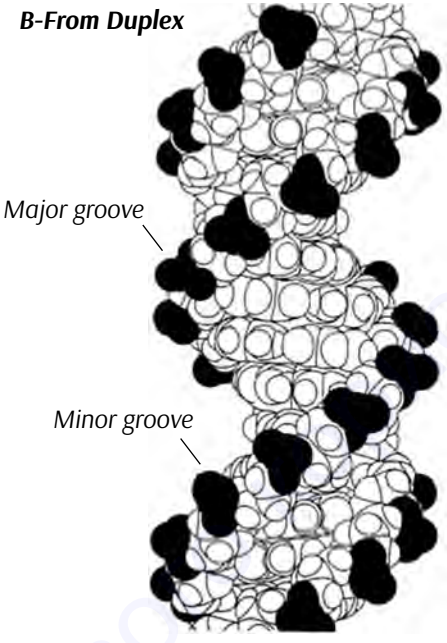


Figure 3.5. Conformations of double strand DNA molecule. B-form of DNA after Watson and Crick

(3) The sequence of nitrogenous bases along the polydeoxyribo-nucleotide chain of DNA carries the genetic information.

As was shown shortly afterwards, **the primary structure of DNA molecule determines the sequence of amino acids in a protein, which is synthesized in a living cell according to the information included in DNA.**

This notion brought the investigators to the problem of the **genetic code,** i.e. the relationship between the sequence of nitrogenous bases in DNA and the sequence of amino acids in a protein. The genetic and biochemical experiments performed showed that the inclusion of a specific amino acid into the protein synthesized is determined ('encoded') by a group of three nucleobases. This group of bases (or corresponding nucleotides) was called a **codon** (Chapter 19).

Chapter 4.

CARBOHYDRATES. SUGARS AND THEIR DERIVATIVES

- **Carbohydrates** (or ***sugars***): monosacharides and polysacharides (mainly glycogen and glycosaminoglycans) are essential metabolic fuel and structural components of animal and human organisms.

In accordance with their ability to hydrolysis, carbohydrates can be divided into the following classes:

1) ***monosaccharides*** which are simple sugars that cannot be broken down into smaller molecules by hydrolysis;
2) ***disaccharides*** which can be hydrolyzed to give two monosaccharides;
3) ***oligosaccharides*** which are made of two to ten monosaccharide units;
4) ***polysaccharides*** which are polymers consisting of many (hundreds and thousands) monosaccharide units.

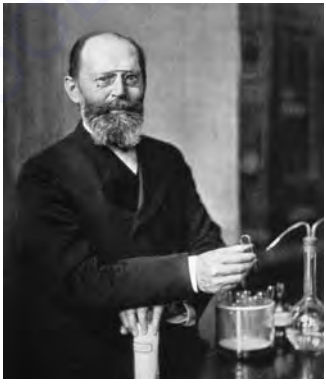
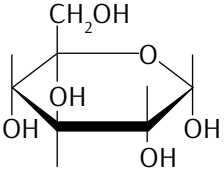


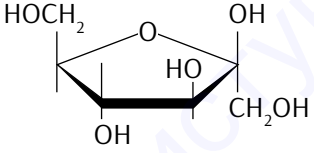
Figure 4.1. Hermann Emil Fischer (1852–1919). Outstanding German chemist. Nobel prize in chemistry (1902) for pioneer investigations in sugar and purine syntheses

4.1. Monosaccharides. Hexoses. Pentoses. Amino sugars

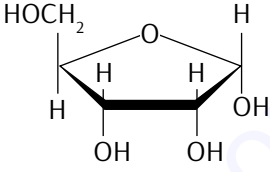
The majority of natural monosacharides present in human body belongs to the stereochemical D-family: D-ribose, D-glucose, D-galactose, D-fructose etc. These are examples of the most widespread monosacharides hexoses **(D-glucose** and **D-fructose)** and pentoses **(D-ribose** and **D-2-Deoxyribose):**



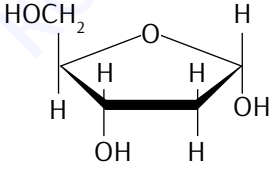
D-Glucose



D-Fructose



D-Ribose



D-2-Deoxyribose

D-Glucose is the aldohexose which has the greatest abundance and biological significance in plants and animals.

D-Glucose is found in most sweet fruits, especially in ripe grapes. The monosaccharide is also the building block for several other carbohydrates including plant *starch*, *sucrose*, *maltose*, *cellulose* as well as animal *glycogen* and *lactose*. It is also present as free molecule inside human cells, blood stream and in tissue fluids. The normal concentration of D-glucose in human blood plasma equals 3,33–5,55 mmoles per liter.

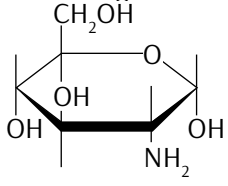
D-Glucose, which is consumed by high animals, including humans, predominantly in the form of food starch, serves as the principal source of chemical energy for the majority of physiological functions and biochemical processes in the body. In the living cells D-glucose forms the phosphoric acid esters, namely D-glucoso 6-phosphate and D-glucoso 1-phosphate. Chemi-

cal energy essential for animal and human cells bioenergetics is released via oxidation of glucose molecules that takes place through the consequence of enzymes catalyzed reaction (Chapter 11):

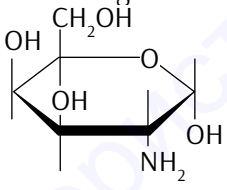
$$\underset{\text{Glucose}}{C_6H_{12}O_6} + 6O_2 \rightarrow 6CO_2 + 6H_2O.$$

Amino sugars

Amino sugars are derivatives of monosaccharides in which a hydroxyl group is replaced by an amine or an acetylamine group. The common representatives of amino sugars are *D-glucosamine* and *D-galactosamine.*



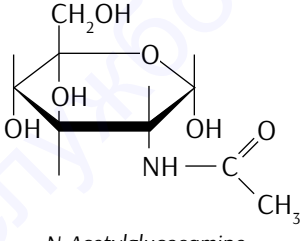
D-Glucosamine



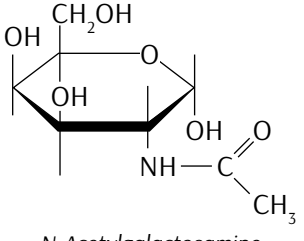
D-Galactosamine

Glucosamine is the product of the hydrolysis of chitin, the major polysaccharide of the shells of insects and crustaceans. *Galactosamine* is found in the polysaccharides of animal connective tissue and cartilage.

The components of human tissues heteropolysaccharides glycosamino-glycans are acetylaminosugars, that is ***N-acetylglucosamine*** and ***N-acetyl galactosamine.***

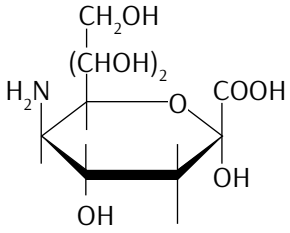


N-Acetylglucosamine

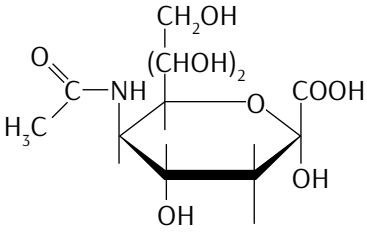


N-Acetylgalactosamine

The compound of special biological significance is *N-acetylneuraminic acid (sialic acid),* the substance which combines properties both of amino sugar and sugar acids and is a characteristic constituent of some animal and human and human connective tissue oligosaccharides.



N-Acetylglucosamine



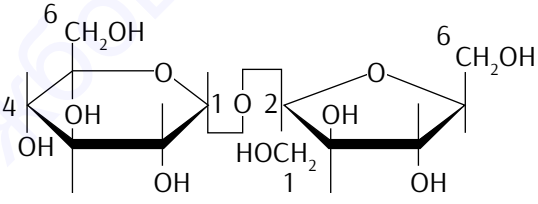
N-Acetylgalactosamine

4.2. Oligosaccharides. Homopolysaccarides

The biologically significant and widely distributed class of oligosaccharides constitute ***disaccharides*** that can be hydrolyzed to yield two molecules of simple sugars (monosaccharides). Three abundant in living nature disacharides are *sucrose (saccharose), lactose* and *maltose.*

Sucrose (Cane sugar, Beet sugar)

In the molecule of sucrose the anomeric carbon atoms of glucose unit and fructose unit are joined together. The configuration of glycosidic linkage is α- for glucose and β- for fructose:



Sucrose
(α -D-Glucopyranosyl-(1-2)-β-D-fmctofLiranoside)

To make designations of sugar linear chains in oligosaccharides molecules more convenient, the special abbreviations for monosaccharides and their derivatives are used:

Glc – Glucose
Gal – Galactose
GlcNAc – N-Acetylglucosamine.
GalNAc – N-Acetylgalactosamine.

Man – Mannose
Fru – Fructose
Fuc – Fucose
Rib – Ribose
Ara – Arabinose
ManNAc – N-Acetylmannosamine.
GlcA – Glucuronic acid.
GalA – Galacturonic acid.
NeuNAc – N-Acetylneuraminic acid.
Sia – Sialic acid.

According to this designation system the abbreviation denoting sucrose appears as:

$$\alpha - D - Glc - (1 \rightarrow 2) - \beta - D - Fru$$

Sucrose is obtained commercially from sugar cane or beet. The disaccharide is the major component of common table sugar.

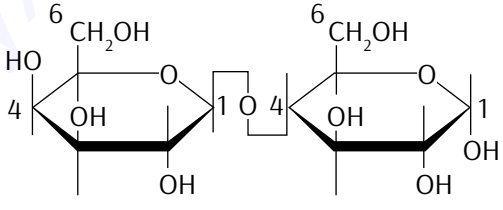
In human intestines the hydrolysis of sucrose catalyzed by the enzyme *sucrase* takes place. It gives molecules of glucose and fructose which are absorbed by epithelial cells lining the small intestine and ultimately penetrate into blood:

$$\text{Sucrose} + H_2O \rightarrow \text{D-Glucose} + \text{D-Fructose}.$$

Lactose (Milk sugar)

Lactose occurs normally in the milk of animals. Cow milk contains 4 to 6 % lactose; human milk has 5 to 8 %. Commercially, the disaccharide is obtained as a by-product in the manufacturing of cheese.

Lactose consists of D-galactose joined to D-glucose by a β-1,4-glycosidic linkage:



Lactose
(β-D-Galactopyranosyl-(1-4)-α-D-glucopyranose)

The abbreviated designation of lactose is:

$$\beta - D - Gal - (1 \rightarrow 4) - \alpha - D - Glc.$$

The hydrolysis of lactose in human intestines to give galactose and glucose is catalyzed by the enzyme *lactase:*

$$\text{Lactose} + H_2O \rightarrow \text{D-Galactose} + \text{D-Glucose}.$$

Owing to the presence of lactase, nearly all infants and children are able to digest lactose. In contrast, **a majority of the adults in the human population of the world are deficient in lactase,** which makes them intolerant of milk. In a lactase-deficient human body, lactose accumulates in the lumen of the small intestine after ingestion of milk because there is no biochemical mechanism for the uptake of this disaccharide.

The clinical symptoms of lactose intolerance are abdominal distension, nausea, pain and diarrhea. **Lactase deficiency** is an inherited disease resulted from the lack in some adult men and women of the expression of specific gene responsible for enzyme protein biosynthesis. The prevalence of lactase deficiency in human populations varies greatly. For example, 3 % of Danes are deficient in the enzyme, compared with 97 % of Thais (L.Stryer, 1995).

Homopolysaccharides. Three the most widespread in living nature poly-saccharides are *starch, glycogen, cellulose* and *dextran*. All of them are homo-polymers of D-glucose and differ in peculiarities of molecular arrangement.

Starch is the principal carbohydrate storage form of plant cells. Serving as the major carbohydrate component of human foodstuff, it is also the primary source of glucose for our body.

Starch is a mixture of polysaccharides that are distinctive in the pattern of glycosidic links. One of these homopolysaccharides is called ***amylose*** (a linear unbranched polymer of α-D-glucose units) and the other ***amylopectin*** (a branched polymer of α-D-glucose with α-1,4-glycosidic linkages and with α-1,6-branching points). The common starch isolated from beetroot or sugar-cane involves 10–20 % of amylose and 80–90 % of amylopectin.

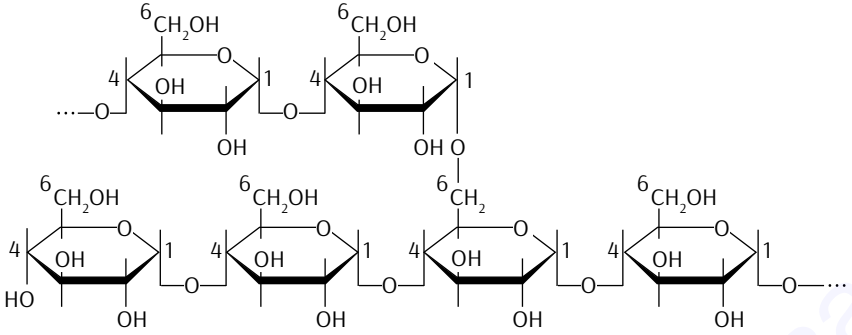


Figure 4.2. Fragment of starch molecule structure (amylopectin fraction)

Step-by-step enzymatic hydrolysis to maltose and finally to glucose is the fate of starch in human digestive tract.

Glycogen is a homopolysaccharide which serves as the major storage form of carbohydrate in animal tissues, predominantly in muscles and liver (Chapter 12).

Glycogen has a molecular structure very much like that of amylopectin fraction of starch ("animal starch"). However in glycogen the glycosidic chains are a lot more branched. In glycogen α-1,6-branching points occur every six to eight to ten D-glucose residues. The polysaccharide has a very high molecular weight (about 100 million kD) which favors the formation of intracellular granules that function as the tissue reserve of carbohydrate. The glycogen granules in hepatocytes cytosole range in diameter from 20 to 40 nm. They contain the enzymes that catalyze the synthesis and degradation of polysaccharide.

When additional expenditures in chemical energy are needed the intracellular glycogen storages are gradually decomposed, and glucose molecule are liberated into blood to meet demands of different tissues in metabolic fuel for diverse physiological activities.

Cellulose is a structural polysaccharide of plant cells. It is composed of chains of D-glucose units joined by β-1,4-glycosidic linkages. The chains are exclusively linear (i.e. unbranched).

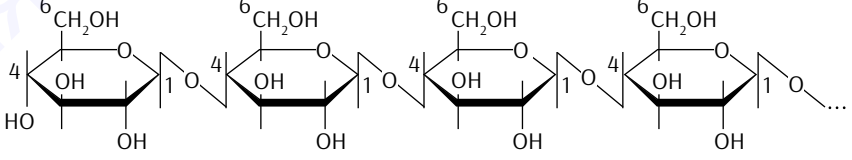


Figure 4.3. Fragment of cellulose molecule

Though a cellulose forms part of the human diet (i.e., in vegetables and fruit), it is not hydrolyzed by human intestines enzyme systems.

4.3. Heteropolysaccharides. Glycoproteins

Biologically essential heteropolysaccharides are branched high molecular weight glycans chains (*glycosaminoglycans*) consisting of disaccharide repeating units.

Animal tissues heteropolysaccharides commonly contains residues of N-acetylated amino sugars (*D-glucosamine*, *D-galactosamine* and their N-acetyl derivatives) as well as sugar acids, usually *D-glucuronic* or *L-iduronic acid*. At least one of the sugars in the repeating disaccharide unit of the glycosaminoglycan chain has a negatively charged carboxylate or sulfate group, which renders to glycosaminoglycans anionic properties.

The major localization of glycosaminoglycans in human and animal body is the extracellular space, predominantly the matrix of connective tissues. They constitute the basis of cartilages, tendons, skin, blood vessel walls, mucous coverings of internal organs, which accounts for the out of date name of glycosaminoglycans ***mucopolysaccharides.***

Specific types of animal and human tissue glycosaminoglycans are *hyaluronic acid, chondroitin sulfate, keratane sulfate, heparin.*

Hyaluronic acid (hyaluronate), is a polysaccharide, which has disac-charide units constituted from the residues of D-glucuronic acid and N-acetyl-glucosamine joined by β-1,3-glycosidic linkages. The individual disaccharide fragments are connected together by β-1,4-glycosidic bonds (Figure 6.3).

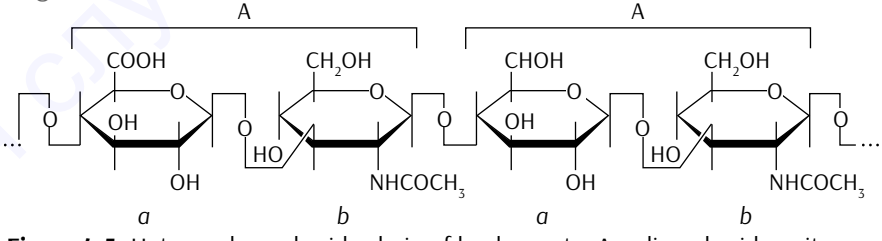


Figure 4.5. Heteropolysaccharide chain of hyaluronate: A – disaccharide units; a – D-glucuronic acid; b – N-acetylglucosamine

The disaccharide units of hyaluronate form long chains of rigid molecules, whose numerous anionic groups bind great number of water molecules.

Chondroitin sulfate. The other common glycosaminoglycans – chondroitin-4-sulfate and chondroitin-6-sulfate include from 50 to 1000 sulfated disaccharide units.

The disaccharide units of chondroitin sulfates are in their turn made of D-glucuronic acid and sulfated N-acetylglucosamine residues joined by β-1,3-glycosidic linkages. Negatively charged sulfate groups are attached to C-4 or C-6 of N-acetylglucosamine. The individual disaccharide fragments are connected together by α-1,4-glycosidic bonds (Figure 4.6).

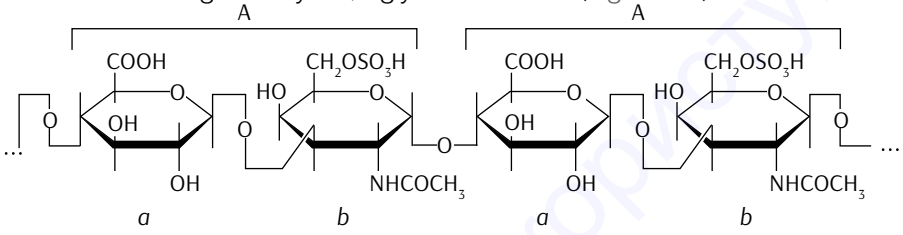


Figure 4.6. Heteropolysaccharide chain of chondroitin-6-sulfate: A – disaccharide units; a – D- glucuronic acid; b – N-acetyl-6-sulfogalactosamine

Heparin. In contrast to other glycosaminoglycans, heparin is not a structural constituent of connective tissue or mucous secretions. Heparin functions as the tissue anticoagulant, that is it inhibits intravessel blood clotting. It is widely used in medicine for treating tromboses accompanying severe diseases of cardiovascular system.

Disaccharide units, making up heparin polysaccharide chains, include N- or O-sulfated D-glucosamine (or N-acetylglucosamine) residues, which are joined by α-1,4-glycosidic bonds to L-iduronic (mainly) or D-glucuronic acid residues. The individual disaccharides are connected together by β-1,4-glycosidic bonds (Figure 4.7).

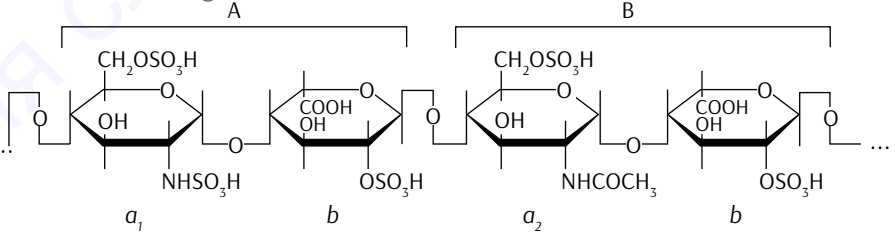


Figure 4.7. Heteropolysaccharide chain of heparin. A, B – disaccharide units that contain: a_1 – N- or O-sulfated D- glucosamine; a_2 – O-sulfated N-acetylglucosamine; b – O-sulfated L-iduronic acid

Proteoglycans and glycoproteins

Glycosaminoglycans covalently linked to polyleptide chains of some proteins form *proteoglycans* and *glycoproteins*.

Proteoglycans are structural proteins of connective tissue that include one or more covalently linked glycosaminoglycan chains. The best characterized members of this diverse class are the proteoglycans of the extracellular matrix of cartilage which contain several chondroitin sulfate and keratane sulfate chains covalently attached to a polypeptide backbone called the *core protein*.

Glycoproteins. Glycoproteins represent a class of membrane proteins and secreted proteins which contain very complex and diverse oligosaccharide units covalently bonded to polypeptide chains.

Carbohydrates are attached to either the side-chain nitrogen atom of *asparagine* residues by N-glycosidic linkages or to the side-chain oxygen of *serine* or *threonine* residues by O-glycosidic linkages. The distinctive feature of mostly wide-spread glycoproteins is a diverse combination of sugars derivatives, especially of mannose residues that makes up a great variety of oligosaccharide patterns found in glycoproteins (Figure 4.8).

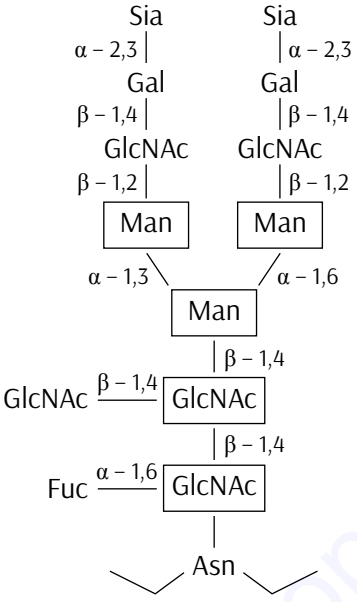


Figure 4.8. N-linked oligosaccharide unit in glycoprotein contains a common core of three mannose and two N-acetylglucosamine residues. Additional sugars are added to form many different patterns of glycoprotein

Chapter 5.

LIPIDS. FATTY ACIDS. BIOMEMBRANES

5.1. General characteristics of lipids. Biological functions

Lipids are a highly heterogenous class of bioorganic molecules. They have a distinctive feature of being soluble in the non-polar organic solvents but insoluble or only poorly soluble in water. The water-hating (*hydrophobic*) nature of lipids is due to the predominance of insoluble hydrocarbon chains ($R{-}CH_2{-}CH_2{-}CH_2{-}$) in their molecules.

From the chemical viewpoint, most of lipids are esters of alcohols and long-chain carboxylic acids, the so-called *fatty acids*. The individual classes of lipids differ in the nature of an alcohol (mainly glycerol, cholesterol, sphingosine) and fatty acids they are made up from (see: Yu. Gubskyi. Bioorganic Chemistry, 2009).

The Greek word "Lipos" means "Fat". And really, lipids make up the principal part of "fat", that is the special matter which is accumulated in human and animal body as a result of food consumption. In mammals, the major sites, where *fats* (or *lipids*) are found, are *fat cells* (or *adipose cells, adipocytes*) of adipose tissue. These cells are specialized for the synthesis and storage of *triacylglycerols* (*triglycerides*), the fraction which constitutes the predominant part of the tissue so-called "neutral lipids" as highly concentrated stores of metabolic energy. Other classes of lipids, different from triacyl-glycerols, play the roles of intrinsic components of cellular membranes, serve as signal molecules, take part in enzyme activity modification.

There are the following major classes of lipids:

- **Simple lipids** (predominantly ***triacylglycerols (triglycerides)***). Mix-

tures of triglycerides derived from animal tissues are commonly called ***fats*** (solids), and those from plants – ***oils*** (liquids).

- **Complex lipids *(glycerophospholipids, sphingophospholipids, glycolipids)*** are a great aggregation of lipids, that yields on hydrolysis alcohols, fatty acids and some additional substances (choline, ethanolamine, phosphoric acid, sugars).
- **Steroids** (cholesterol, bile acids, steroid hormones).

Biological functions of lipids

The following biological functions of lipids have been identified.

1. They serve as the storage and transport forms of metabolic fuel (mainly triacylglycerols).
2. Complex amphiphylic lipids are primary structural components of biomembranes (glycerophospholipids, sphingolipids, glycolipids).
3. Some of them serve as hormones and signal molecules of vital physiological importance (steroids, inositol phosphates, eicosanoids).

5.2. Fatty acids: structure and properties

Fatty acids are water-insoluble long-chain carboxylic acids. Lipids derived from animal and human tissues include fatty acids residues which contain usually from 12 to 24 carbon atoms. At that, the majority of natural fatty acids have hydrocarbon chains with even number of carbons, predominantly C_{16}–C_{24}.

Fatty acids are called mainly by their common (or trivial) names. Their systematic names as the representatives of carboxylic acids contain the number of carbon atoms with the suffix **– *anoic*** appended.

Saturated fatty acids

Saturated fatty acids have no double bonds in the chain.

Their general formula is CH_3–$(CH_2)_n$–COOH (or $C_nH_{2n+1}COOH$) where *n* specifies the number of methylene groups between the methyl and carboxyl carbons. The prevalent representatives of natural saturated fatty acids are:

- *palmitic* (*hexadecanoic*) *acid* – $C_{15}H_{31}COOH$ ($C_{16:0}$);

- *stearic* (*octadecanoic*) *acid* – $C_{17}H_{35}COOH$ ($C_{18:0}$);
- *arachidic* (*eicosanoic*) *acid* – $C_{19}H_{39}COOH$ ($C_{20:0}$).

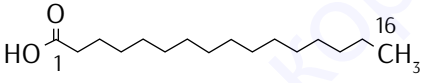
Unsaturated fatty acids

Unsaturated fatty acids have one or more double bonds in the chain.

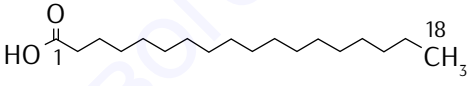
The representatives of unsaturated fatty acids are:

- *palmitoleic* (*cis-*Δ^9*-hexadecenoic*) *acid* – $C_{15}H_{29}COOH$ ($C_{16:1}$);
- *oleic* (*cis-*Δ^9*-octadecenoic*) *acid* – $C_{17}H_{33}COOH$ ($C_{18:1}$);
- *linoleic* (*cis-*Δ^9, Δ^{12}*-octadecadienoic*) *acid* – $C_{17}H_{31}COOH$ ($C_{18:2}$);
- *linolenic* (*cis-*Δ^9, Δ^{12}, Δ^{15}*-octadecatrienoic*) *acid* – $C_{17}H_{29}COOH$ ($C_{18:3}$);
- *arachidonic* (*cis-*Δ^5, Δ^8, Δ^{11}, Δ^{14}*-eicosatetraenoic*) *acid* – $C_{19}H_{31}COOH$.

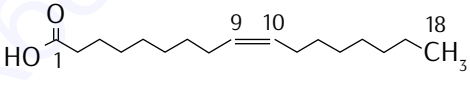
See the examples of fatty acids molecular structure using *skeletal formulae* of compounds which show the hydrocarbon skeletons shape more clearly:



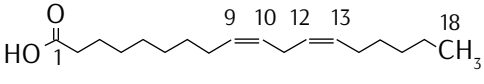
Palmitic acid



Stearic acid

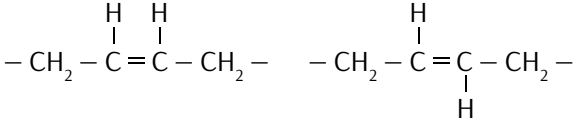


Oleic acid

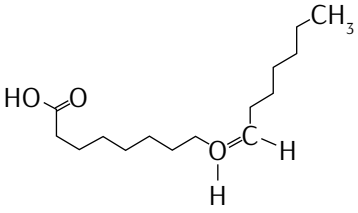


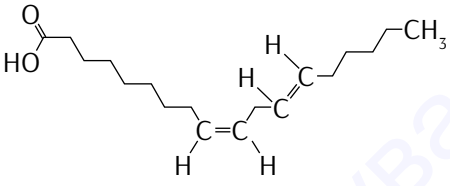
Linoleic acid

It is noteworthy that double bonds in naturally occurring fatty acids are always in a *cis-* as opposed to a *trans-* configuration:



Cis *Trans*





cis-Δ⁹-hexadecenoic acid *cis-Δ⁹, Δ¹²-octadecadienoic acid*

The properties of fatty acids and of lipids derived from them are markedly dependent on their chain length and on their degree of saturation. Unsaturated fatty acids have a lower melting point than saturated fatty acids of the same length (Table 5.1).

Table 5.1. Predominant fatty acids of mammalian tissues and their melting points

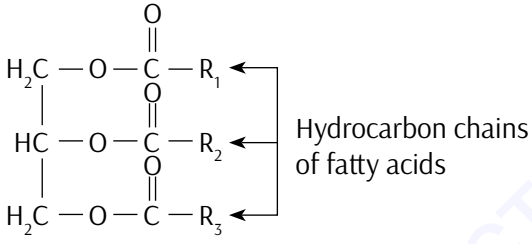
Fattyacid	No.CarbonAtoms	No.DoubleBonds	MeltingPoint(°C)
Lauric	12	0	43.5
Myristic	14	0	54.4
Palmitic	16	0	62.8
Stearic	18	0	69.6
Palmitoleic	16	1	1.0
Oleic	18	1	13.0
Linoleic	18	2	–11.0
Linolenic	18	3	–11.2
Arachidonic	20	4	–49.5

5.3. Structure and properties of special classes of lipids

Simple lipids

The hydrolysis of simple lipids yields fatty acids and alcohols (predominantly glycerol and some higher alcohols). This class includes *triacylglycerols* (also called sometimes "neutral lipids"), that are of great essence as energetic substrates in biological systems.

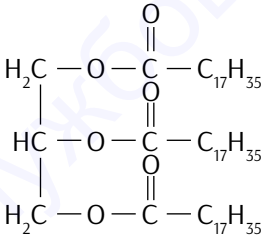
Triacylglycerols (usually known under the name of **triglycerides**) are esters formed between glycerol (propan-1,2,3-triol) and fatty acids.



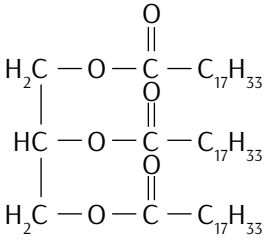
Triacylglycerol.
R represent long-chain alkyl groups

Triglycerides constitute the major part of *neutral fats* of animal adipose tissue and include predominantly the residues of saturated fatty acids. Compounds similar to *fats* are named *oils*. Chemically oils are tryacylglycerols which contain esterified residues of unsaturated fatty acids:

Oils are substances derived predominantly from plant material (corn oil, olive oil, peanut oil, etc.) whereas fats are usually derived from animal tissues. Under room temperature neutral fats are mainly solid substances, whereas oils are liquids.



Tristearoylglycerol (tristearin)
(Melting point = +71°C)



Trioleylglycerol (triolein)
(Melting point = –17°C)

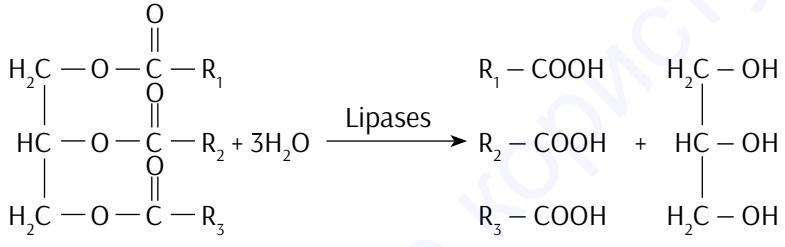
On the whole, the hardness and melting point of a fat depends upon the relative proportions of single fatty acids, namely saturated and unsaturated specimen. Specifically, fat derived from beef or human body contains a greater part of the *oleic acid* relatively to mutton fat, whereas in fluid olive

oil *oleic acid* prevails.

Hydrolysis of triacylglycerols

In human and animal tissues triacylglycerols are formed from glycerol and fatty acids, which are obtained from the diet or are synthesized within the cells from glucose. After normal overnight fasting triacylglycerols stored in the cells of adipose tissue (adipocytes) are subjected to hydrolysis (or ***lipolysis***) to yield glycerol and free fatty acids which, in turn, are oxidized to give metabolic energy in the form of ATP (Chapter 13).

Similarly, hydrolysis of triacylglycerols, which are consumed as food components, takes place in the digestive tract of human beings and animals:



Triglyceride — *Three different fatty acids* — *Glycerol*

Thus, the degree of unsaturation of lipids determines their physicochemical characteristics, in particular the melting point and the level of fluidity (fats or oils).

According to the modern medical conceptions, saturated fats are thought to be the essential cause of developing of ***atherosclerosis,*** a dangerous disease, associated with the thickening of the arteries walls. Hence, it is now recogni-zed that unsaturated and, in particular, polyunsaturated fats (those containing the polyunsaturated fatty acid residues) are most favorable for human health.

Complex lipids

The complex lipids yield on hydrolysis fatty acids, alcohols and other substances, particularly nitrogenous bases, phosphate and carbohydrates.

The complex lipids include **phospholipids** (*glycerophospholipids*,

sphin-gophospholipids) and **glycolipids.** These are the lipids which are biological membranes constituents. The special class of phospholipids, ***phosphoinosi-tides,*** have biological effects of signal molecules which take part in the transfer of chemical information inside the cells.

Phospholipids

Phospholipids are derived from either *glycerol*, a three carbon alcohol, or *sphingosine*, a more complex amino alcohol.

Owing to peculiar amphiphilic structure of their molecular architecture, and namely presence of hydroplylic "heads" and hydrophobic "tails" of the molecule, phospholipids are the major class of biomembranes matrix constituting lipids.

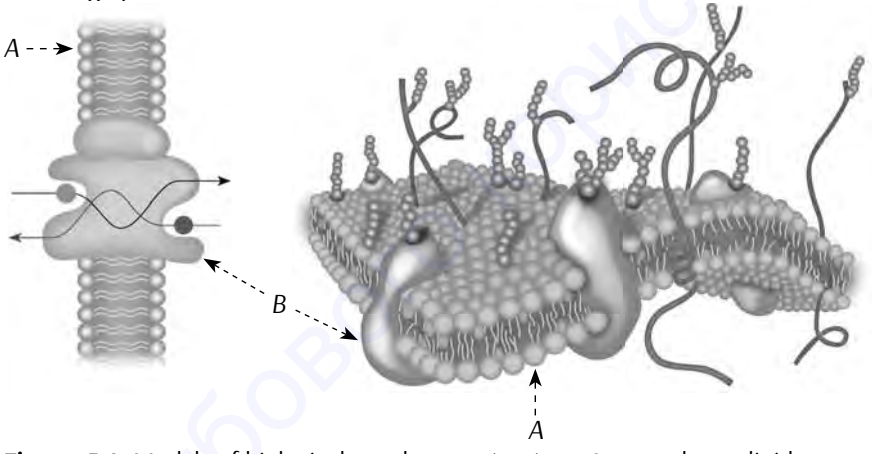
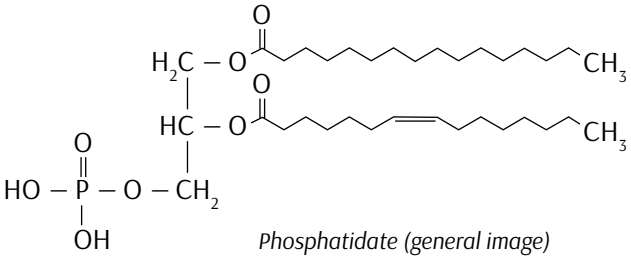


Figure. 5.1. Models of biological membranes structure. A – membrane lipids: B – membrane integral proteins

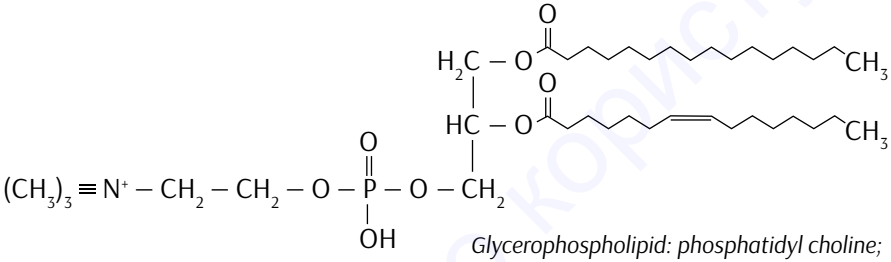
Glycerophospholipids (phosphoglycerides)

In glycerophospholipids the hydroxyl groups at C-1 and C-2 of glycerol are esterified to the carboxyl groups of two fatty chains. The C-3 hydroxyl group of the glycerol backbone is esterified to phosphoric acid. The resulting compound, named ***phosphatidic acid*** *(phosphatidate),* is phosphoglyceride which is the precursor of all glycerophospholipids.



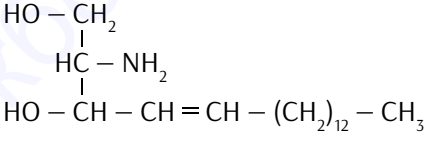
Phosphatidate (general image)

To make glycerophospholipids the phosphate group of phosphatidate becomes esterified to the hydroxyl group of one of several alcohols. The common alcohol moieties of phosphoglycerides are *choline, ethanolamine, serine* and inositol.



Glycerophospholipid: phosphatidyl choline; model presentation)

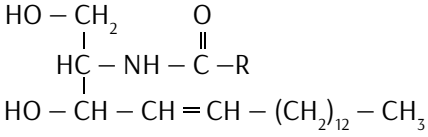
Sphingophospholipids. The backbone of *sphingophospholipids* (*sphingolipids*, or *sphingomyelines*) constitutes ***sphingosine,*** an amino alcohol which contains a long unsaturated hydrocarbon chain.



Sphingosine

Sphingosine is also the alcohol moiety of the special subclass of glycolipids, named ***glycosphingolipids*** (p. 2.2.2). That is why the common name for sphingosine containing lipids is ***sphingolipids.*** In human body the greatest concentration of sphingolipids is found in the central nervous system, particularly in white matter, although nearly all other tissues contain some. The core molecular structure of the latter is N-acylsphingosine which is called ***ceramide:***

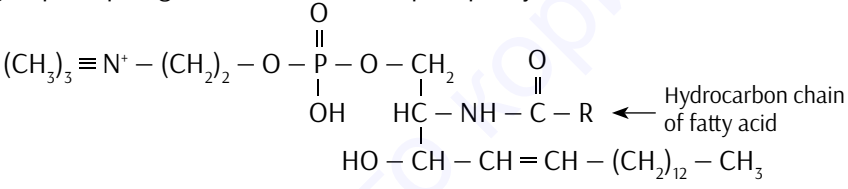
As can be seen from the formula presented, ***ceramides*** are formed as a result of N-acylation of sphingosine moiety with fatty acid residue, in particular C_{22}.



N-Acylsphingosine (ceramide)

Depending on the particular characteristics of molecular structure, sphingolipids can be divided into ***sphingophospholipids*** and ***glyco-sphingolipids*** (the latter see in the section 2.2.2).

In the ***sphingophospholipids*** the amino group of sphingosine backbone is linked to a fatty acid by an amide bond; in addition, the primary hydroxyl group of sphingosine is esterified to phosphoryl choline.



Sphingophospholipid (sphingomyelin)

Phospholipids (both *glycerophospholipids* and *sphingophospholipids*) are amphipatic substances, which possess both hydrophilic and hydrophobic groups. The hydrophilic (polar) are phosphates and amine groups, hydrophobic are hydrocarbon chains of fatty acids residues. Owing to these molecular structure features, phospholipids can inhabit transition regions between aqueous and non-aqueous phases.

That is why **phospholipids are abundant in all types of biological membranes**. They comprise about 40 % of the lipids in the erythrocyte membrane and over 95 % in the inner mitochondrial membrane.

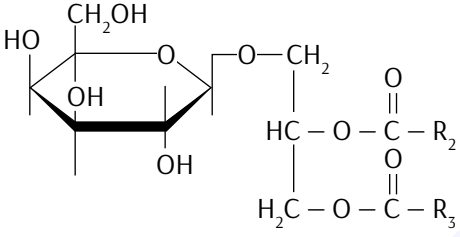
Glycolipids

Glycolipids, as their name implies, are sugar containing lipids. In glycolipids the lipid moiety is covalently bonded to a sugar, which is monosaccharide or oligosaccharide.

Depending on the nature of alcohol included (glycerol or sphingosine), glycolipids are divided into *glycosylglycerides* and *glycosphingolipids*.

Glycosylglycerides

Glycosylglycerides are O-glycosides of simple sugars and diacylglycerole, for example:

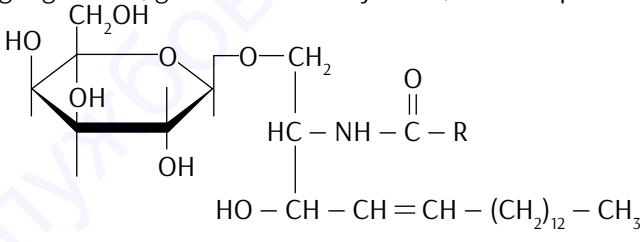


Glycosylglyceride (monogalactosyldiacylglycerole)

Glycosphingolipids

Similarly to sphingophospholipids, *glycosphingolipids* are formed on the basis of *ceramide*. To make up glycosphingolipid the ceramide is attached to a sugar (monosaccharide or oligosaccharide).

At least four classes of glycosphingolipids have been distinguished: *cerebrosides*, *gangliosides*, *globosides* and *sufatides*, for example:

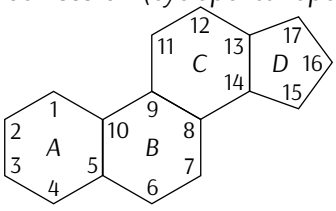


Cerebroside (galactocerebroside)

The most complicated glycosphingolipids, gangliosides, contain a branched oligosaccharide chain of as many as seven sugar residues. The structure, metabolism and clinical chemistry of these compounds are to be studied in the course of biochemistry.

5.4. Steroids: cholesterol, bile acids, steroid hormones

Steroids are lipids that contain four carbon rings joined to form the nucleus of cyclic hydrocarbon ***steran*** *(cyclopentanoperhydrophenanthrene)*.

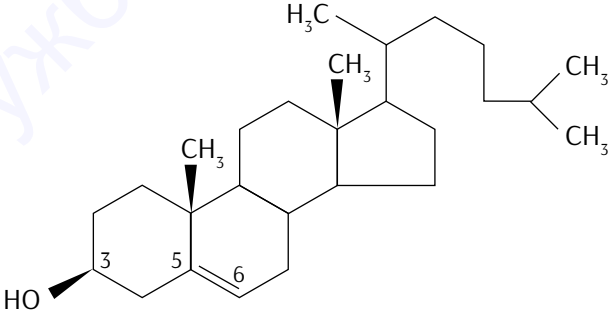


Cyclopentanoperhydrophenanthrene.
A, B ,C – cyclohexane rings; D-cyclopentang

The derivatives of *steran* are called ***steroids.*** Steroids comprise a wide class of chemicals, including animal and plant steroids, and namely *cholesterol* (*cholesterin*), *phytosterins*, *bile acids*, *steroid hormones* and *vitamin D*.

Sterols. Cholesterol

Sterols are a class of steroids which are characterized by the presence of a hydroxyl group at C-3 and an aliphatic chain of at least eight carbons at C-17. The most common representative of animal sterols is **cholesterol *(cholesterine):***



Cholesterol (cholesteu-5-ol-3β)

Esterification of cholesterol

The bulk of cholesterol in human tissues and about 65 % of blood plasma cholesterol is esterified to long-chain fatty acids to give *cholesterides*.

Biological functions of cholesterol

(1) The plasma membranes of eucaryotic cells are very rich with ***cholesterol*** which represents its key role in regulating the membranes structure properties. To say more definitely, the molecules of cholesterol being inserted into membranes, execute the control of lipid bilayers fluidity, membrane-bound enzyme activities and membrane permeability.

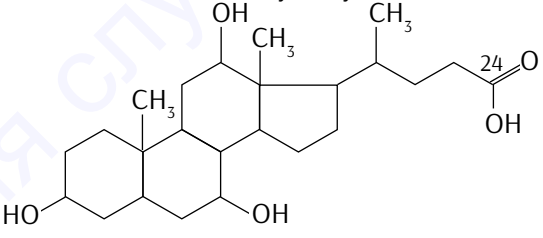
(2) Cholesterol is the precursor of a variety of steroid molecules which have significant physiological activities. The cholesterol molecule backbone is used for the biosynthesis of bile acids, vitamin D_3 and several major classes of steroid hormones including adrenal cortex steroids and gonadal steroids.

Cholesterol as the precursor of biomolecules

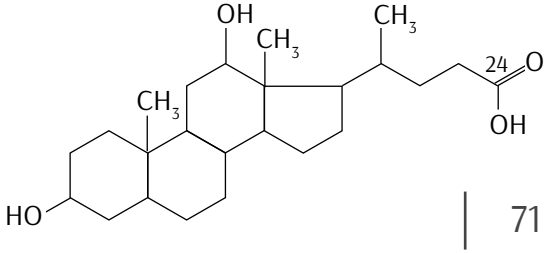
Bile acids. Bile acids are a group of steroid molecules which are synthesized in liver and stored in the gallbladder as components of the bile.

Chemically the bile acids are C-24 steroids which differ in the number of hydroxyl groups attached to steroid nucleus. According to the degree of hydroxylation, the following bile acids are distinguished:

- ***cholic acid*** hydroxylated at C-3, C-7 and C-12;
- ***deoxycholic acid*** hydroxylated at C-3 and C-12;
- ***chenodeoxycholic acid*** hydroxylated at C-3 and C-7;
- ***lithocholic acid*** hydroxylated at C-3.



Cholic acid



Deoxycholic acid

After the release of bile from gallbladder, bile acids enter the intestine, and namely duodenum. Due to their amphipathic structures, the bile acid salts act as the detergents thus aiding to the effects of duodenum lipases which hydrolyze complex lipids of the foodstuff.

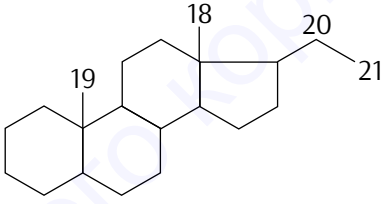
Steroid hormones

Steroid hormones derived from cholesterol are:

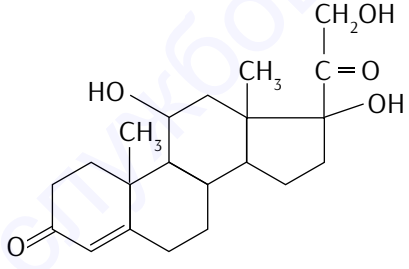
1) ***adrenocortical hormones,*** which include ***glucocorticoids*** and ***mineralocorticoids***.

The representative of glucocorticoids is **cortisol** produced by the cells of *zona fasciculata* of the adrenal cortex. The representative of mineralo-corticoids is **aldosterone** produced in the adrenal *zona glomerulosa*.

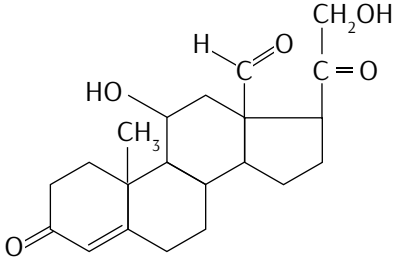
Chemically, adrenocortical hormones are derivatives of the cyclic hydro-carbon **pregnane** (C-21):



Pregnane



Cortisol

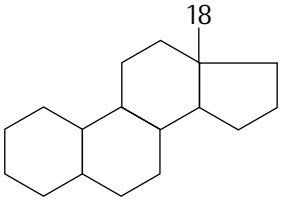


Aldosterone

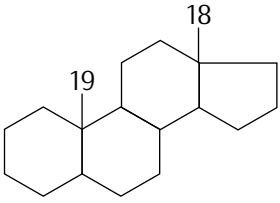
2) ***gonadal hormones*** including *testicular hormones* produced in males ***(androgens)*** and *ovarian hormones* produced in females ***(progesterone*** and ***estrogens).***

The principal representative of male gonadal hormones is ***testosterone,***

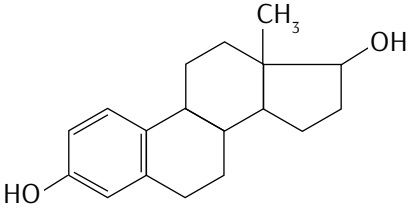
which is the derivative of cyclic hydrocarbon *androstane* (C-19). The female hormones are: ***progesterone*** (derivatives of C-21 hydrocarbon *pregnane* – see above) and ***estrogens,*** particularly ***estradiol*** – derivatives of C-18 hydrocarbon *estrane*.



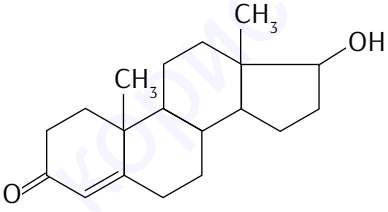
Estiane



Audrostane



Estradiol



Testosterone

More elaborately the structure and biological functions of steroid hormones will be considered in the Chapter 22.

Chapter 6.

CELLS. METABOLISM: GENERAL ASPECTS

6.1. Metabolism: overall conception, metabolic pathways

A. Overview. Living organisms maintain their complex order in a dynamic steady state by importing food and energy from their surroundings. They have the machinery needed to liberate and store chemical energy from foods and to create complex molecules from simpler ones for the building of new structure. These processes collectively are spoken of as ***metabolism*** and involve the transformation by enzyme-catalyzed reactions of both matter and energy.

B. Two aspects of metabolism: catabolism and anabolism.

1. **Catabolism** encompasses the degradative processes whereby complex molecules are broken down into simpler ones.
   - One important function of catabolism is to transform the molecules derived from ingested food materials into simpler "building block" compounds.
   - In catabolic processes, there can be an attendant release of the free energy of the complex molecules.
   - Some of this free energy can be conserved by coupled enzymatic reactions of **oxidative phosphorylation** and stored as **adenosine triphosphate (ATP).**
2. **Anabolism** encompasses the biosynthetic aspects of metabolism, which are concerned with combining building block compounds into the complex macro-molecules required by the organism. Anabolic processes require energy inputs, which can be supplied in two ways:
   - By ATP produced from the complex oxidation-reduction reactions of catabolic pathways.

- In some cases, by high-energy hydrogen in the form of reduced *nicotinamide adenine dinucleotide phosphate* (NADP).

6.2. Cells. Compartmentalization of metabolic pathways

The majority of metabolic pathways in natural biological systems organism are taking place inside cells. According to the essential peculiarities of the cell nucleus and genetic material organization, all the living organisms are divided into two major classes, and namely ***prokaryotes*** and ***eukaryotes*** *(procariota; lat. – eucariota)*.

Prokaryotes

The prokaryotes comprise mainly bacteria. The prokaryotic cells do not have morphologically organized nucleus. Their genetic material is presented by the *nucleoid* which constitutes the double-helical DNA that makes up distinctive circular structures (Figures 6.1–6.2).

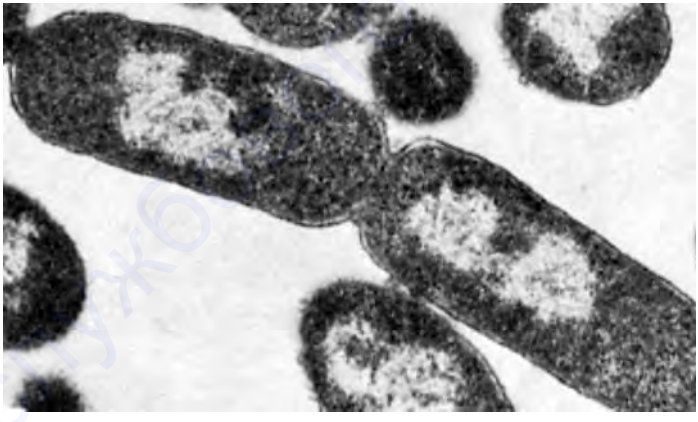


Figure 6.1. Prokaryotic cell *(Escherichia coli)* in the process of division – two individual cells are formed *(Albert L. Lehninger, 1982)*

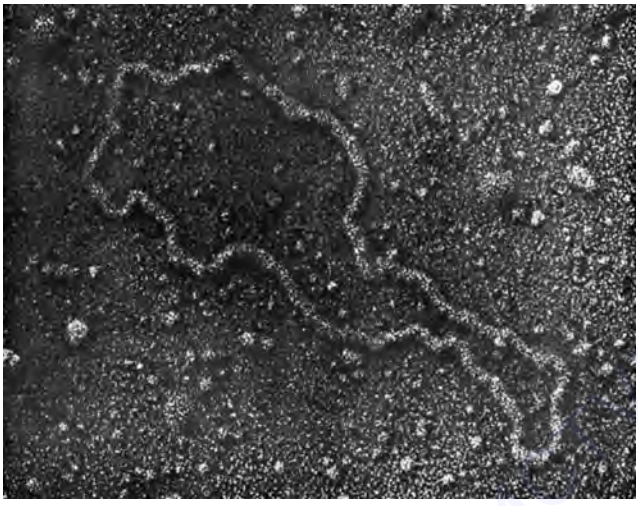


Figure 6.2. Circular DNA molecule in E.coli cell – plasmid pSC 101. (Stanley Cohen, 1976)

Eukaryotic cell structure

Eukaryotic cells are the cells that possess nucleus with the definite array of chromosomes. They comprise animal (Figures 6.3–6.4) and higher plant cells.

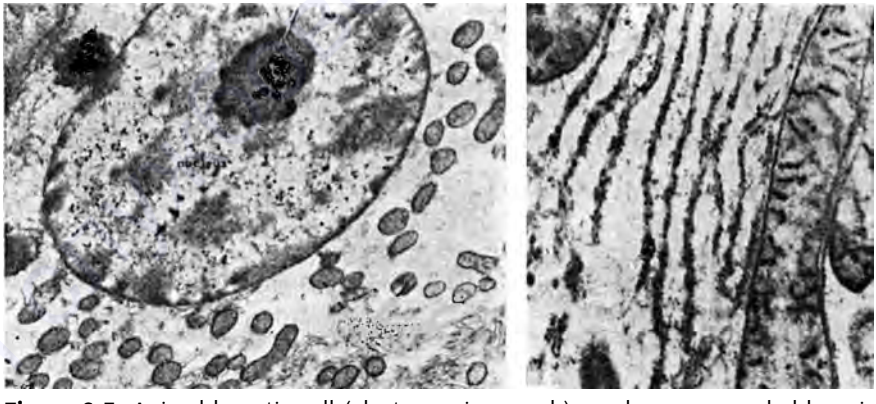


Figure 6.3. Animal hepatic cell (electron micrograph): nucleus surrounded by mitochondria (left photo); membranes of endoplasmic reticulum with mitochondrion nearby (right photo)

The cardinal biochemical reactions, which constitute the essence of me-

tabolism in human organisms, take place in living cells, and namely – inside cytoplasm and particular subcellular organelles – nucleus, mitochondria, endoplasmic reticulum, lisosomes, peroxysomes, Golgi apparatus.

Every subcellular structure is characterized with specific array of substrate-specific enzymes and, subsequently – strictly determined consequences of metabolic reactions. The latter property of any cell is known as the ***compartmentalization of metabolism.***

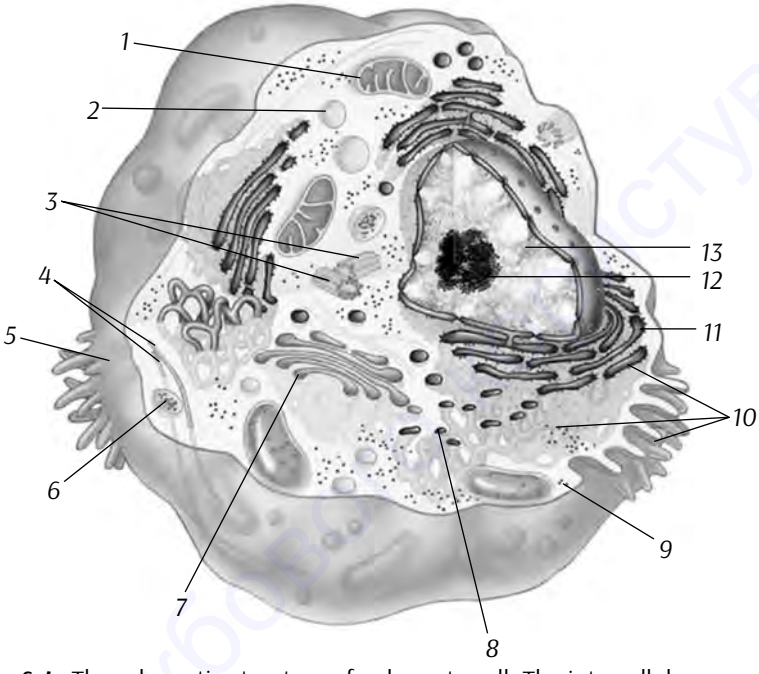


Figure 6.4. The schematic structure of eukaryote cell. The intracellular organelles: 1 – mitochondrion, 2 – lysosome, 3 – centrosome, 4 – microtubules and microfilaments, 5 – microvilli, 6 – lyposome, 7 – Golgi apparatus, 8 – smooth endoplasmic reticulum, 9 – cytosole with inclusions of glycogen granules, 10 – ribosomes, 11 – granular endoplasmic reticulum, 12 – nucleolus, 13 – nucleus

Compartmentalization of metabolic pathways in the cell

The enzymes catalyzing the definite metabolic pathways are usually localized inside specific intracellular organelles, or cellular *compartments*.

This is named ***compartmentation of metabolism.***

For example:

- The entire sequence of enzymes involved in the conversion of glucose to *pyruvate* or *lactate* (the *glycolytic pathway*) is found in the soluble part of the cell that is called *cytosole.*
- On the other hand, the subsequent reactions of *pyruvate degradation* to give CO_2 and H_2O are taking place inside *mitochondria*. On the whole, the inner membrane of mitochondria constrains all the host of biochemical reactions that are concerned with the final oxidation of various biochemical substrates.
- The intricate processes of cell genetic material reduplication, that is *DNA replication*, are happening inside *nucleus*.
- The *lysosomes* contain hydrolytic enzymes for the catabolism of cellular macromolecules e.g. *proteolysis*.
- The vesicles and cisternae of *Golgi apparatus* contain enzymes which are concerned with the manufacturing, sorting and outside cell exporting of biologically significant proteins.
- The special set of enzymes, bound to the *membranes of endoplasmic reticulum,* presumably of hepatic cells, carry out the *oxidation of foreign compounds*, that is toxins and drugs entering our body, the so called *xenobiotics*. This is named "microsomal oxidation" and, thus, a vitally important detoxification function of human liver is realized.

All this is Biochemistry!

Part 2

ENZYMES. GENERAL METABOLIC PATHWAYS

Chapter 7.

METABOLISM. ENZYMATIC REACTIONS

7.1. Metabolism: general conceptions, metabolic pathways

The fundamental processes which compose the basis of every living system functioning are carried out by a highly integrated network of chemical reactions. There are thousands of chemical reactions occurring in even as simple an organism as the bacterium *Escherichia coli*. The total combination of these reactions is designated as **metabolism** and the compounds involved are **metabolites.**

Traditionally, the whole metabolism, which encompasses a great number of enzyme catalyzed biochemical reaction, is considered as constituting two aspects of biomolecules transformations, and namely ***anabolism*** and ***catabolism.*** According to this viewpoint, the sum total of biochemical reactions taking place in living organisms, may be divided into anabolic and catabolic pathways.

Anabolism includes *anabolic pathways,* that are involved in the synthesis of biochemical compounds inside cells and multicellular body (human, animal, plant) as a whole.

Anabolic processes are essentially the making of more complex molecular structures from simpler compounds and, as such, they are *endergonic*, that is require expenditure of chemical energy.

Catabolism (*catabolic pathways*) are the enzymatic reactions which produce breaking down of biochemical nutrients (proteins, carbohydrates, lipids) and preexisting biomolecules of the body to give smaller bioorganic compounds.

Catabolic processes are *exergonic*; they include oxidative reactions taking place in mitochondria which produce chemical energy mainly in the form

of adenosine triphosphate (ATP) molecules.

The molecular machines, which catalyze multiple biochemical reactions of biomolecules synthesis, degradation and interconversion, are called **enzymes.**

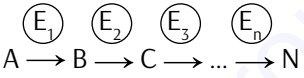
Metabolic pathways

The intracellular and extracellular transformations of biomolecules often involve a multistep sequence of reactions that are catalyzed by a number of enzymes **(E):**

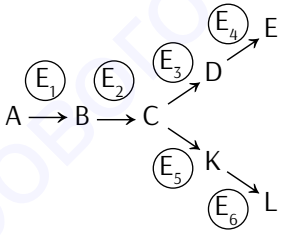
$$A \xrightarrow{E_1} B \xrightarrow{E_2} C \xrightarrow{E_3} \ldots \xrightarrow{E_n} K$$

This sequence of enzymatic reactions collectively constitutes a metabolic pathway which can be linear, branched or cyclic.

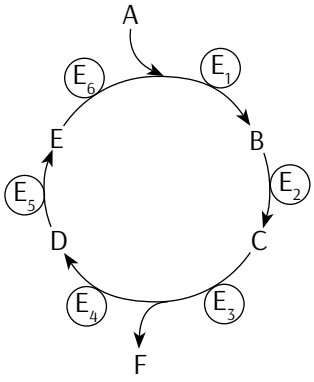
Linear metabolic pathway:



Branched metabolic pathway:



Cyclic metabolic pathway:



In such sequences of biochemical reactions, the product of one enzyme reaction becomes the substrate for the next reaction in the sequence, the successive products of the reactions being known as ***metabolites,*** or metabolic ***intermediates.***

The examples of metabolic pathways are glycolysis, β-oxidation of fatty acids (linear and branched pathways), tricarboxylic acid cycle (cyclic metabolic pathway) etc.

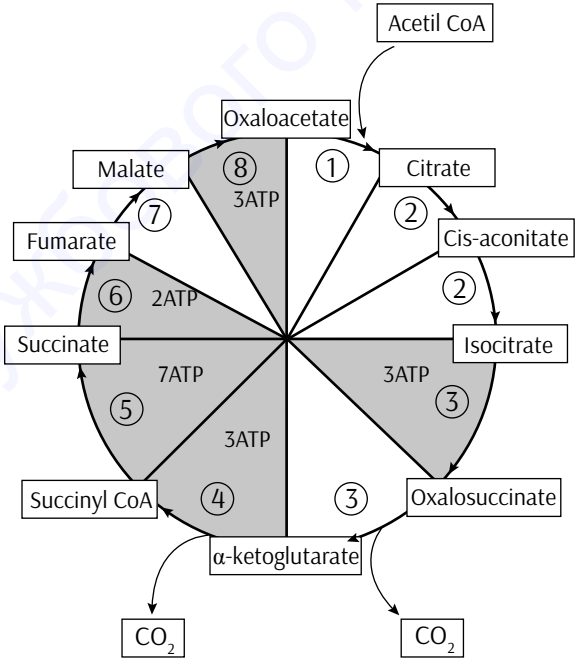


Figure 7.1. An example of cyclic consequence of enzymic reactions is the so-called TCA-cycle *(Tricarboxylic Acid Cycle, or Krebs cycle)* as shown here

The overall network that involves many billions of biochemical reactions that constantly take place in the whole human organism or in the isolated animal or bacterial cell, is generally named ***intermediary metabolism.***

7.2. Enzymes as the principal molecular devices of metabolism

7.2.1. Enzymes: general definitions

Enzymes are biological catalysts, which determine the rate and pattern of chemical transformations in biological systems that underlie the metabolic pathways. According to their chemical nature, all the enzymes are proteins.

Shortly speaking, ***enzymes*** are designated as ***biological catalysts of a protein nature.***

Under the catalytic action of enzymes biomolecules ***(substrates)*** are transformed into the ***products*** of biochemical reactions:

$$S \xrightarrow{E} P,$$

where: S – substrate, P – product, E – enzyme.

The enzymes not only accelerate chemical reactions that take place in living organisms ***(biochemical reactions)*** but also provide the temporal and spatial coordination of many thousands of intracellular and extracellular biochemical reactions.

The orderly progression of metabolic pathways would be impossible if it were not for the genetically determined suite of enzymes found inside every living cell and multicellular organism (bacterium, plant or human).

7.2.2. Enzymes properties

There are some characteristics of enzymes which markedly distinguish

them from chemical catalysts. They are:

- enzymes are invariably proteins (simple or conjugated);
- enzymes have immense catalytic power;
- enzymes are highly specific catalysts;
- catalytic activity of enzymes is expressed in a narrow range of physicochemical microenvironment characteristics;
- catalytic activities of many enzymes are regulated.

Consider the above stated characteristics of enzymes more thoroughly.

1. ***Chemical nature of enzymes.***

As was already stated above, **all the enzymes are proteins:**

- Some of them are ***simple proteins*** that is their molecules consist from one or more polypeptide chains and have no attached groups of different chemical nature. The examples of such are enzymes which catalyze the decomposition of proteins in gastrointestinal tract of human beings and animals – the so-called ***proteases***. The representatives of gastrointestinal proteases are enzymes ***pepsin, trypsin, chymotrypsin.***
- Other enzymes are complex (or *conjugated*) proteins:
  - If the enzyme is a simple protein, only the native conformation of the protein moiety is required for realization of its activity.
  - If the enzyme is a ***conjugated protein***, enzymatic activity will depend upon the presence of a protein moiety of the molecule and the availability of additional chemical essence which is called **coenzyme** or sometimes – **cofactor.**

In the last case the entire molecular complex is called ***holoenzyme*** and the protein part – ***apoenzyme:***

$$\text{Apoenzyme} + \text{Coenzyme} = \text{Holoenzyme}$$

The enzymes – conjugated proteins are exemplified by such biological catalysts as ***oxidoreductases*** and ***transferases,*** that are enzymes which catalyze the reactions of intracellular oxidation of biomolecules and intermolecular transfer of different chemical groups.

2. ***Catalytic power of enzymes.***

Enzymes do not cause biochemical reactions to take place. **They speed up reactions that would ordinary proceed, but at a much slower rate, in**

their absence. Virtually, most reactions in biological systems do not occur at perceptible rates in the absence of enzymes.

The catalytic power of enzymes is very high, they are extremely effective catalysts. Some enzymes increase the rate of the reaction they catalyze by a factor of 10^{12} or more.

Measures of enzyme activity

A measure of enzyme catalytic action is designated as the ***activity of an enzyme.***

In its turn, the quantitative measure of ***enzyme activity*** is obtained by determining the extent of the *increase in reaction rate* under defined conditions, i.e. the difference between the rates of the catalyzed and the uncatalyzed reactions. Normally, **reaction rates are expressed as the change in concentrations of substrate per unit of time,** and thence follow the **units** of enzyme activity:

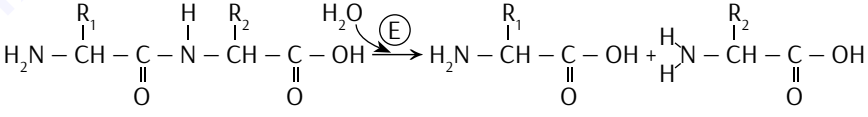
- the **katal (kat)** is the unit of enzyme activity that defines the turnover of 1 mol of substrate per second ($mol \times s^{-1}$); the katal is the unit of enzyme activity recommended by the Commission on Biochemical Nomenclature in accordance with the demands of the IUPAC;
- the international unit (U) is the customarily used unit of enzyme activity which corresponds to the amount of enzyme causing transformation of 1 μmol of substrate per minute;
- the **specific enzyme activity** is the number of units of enzyme activity per milligram of enzyme protein, that is **kat × mg^{-1}** or **U × mg^{-1}**; this unit is most widely used in practical biochemistry.

3. ***Reaction and substrate specificity of enzymes.***

The action of most enzymes is highly specific both in the **reaction** catalyzed and in their choice of reactants, that is **substrates:**

- **Reaction specificity.** An enzyme usually catalyzes a single chemical reaction or a set of closely related reactions.

For example, ***proteolytic enzymes (pepsin, trypsin)*** catalyze the hydrolysis of a peptide bond, that is the reaction of ***proteolysis:***



- **Substrate specificity** means that an enzyme catalyzes the transformation of a definite substrate ***(absolute specificity)*** or a group of chemically related substrates ***(relative specificity).***

4. ***Physicochemical factors affecting enzyme activity.***

Major physicochemical factors affecting activity of enzymes and, hence the rate of enzyme-catalyzed biochemical reactions, are temperature and pH.

Effect of temperature on enzyme activity

Temperature affects markedly on enzymes activity and, consequently, on the rate of enzyme-catalyzed reactions. Most enzymes are heat-sensitive molecular devices (Figure 7.2).

It can be seen from the curve submitted that:

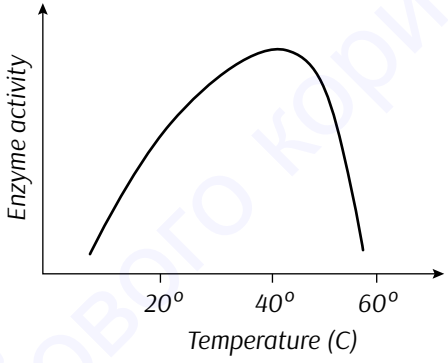


Figure 7.2. Effect of temperature on the activity of an enzyme

(a) the rate of an enzyme-catalyzed reaction usually increases with increasing temperature up to an optimum point (close to 40 °C);

(b) the apparent ***temperature optimum point*** for most enzymes, which is shown in Figure 1.1, is near 40 °C. It is the result of an initial increase in reaction rate due to a rise in temperature, followed by a decrease in enzymatic activity as the temperature continues upward. The temperature rise brings to the increasing thermal denaturation of the protein part enzyme molecule;

(c) the rate by which the velocity of a reaction changes with a 10 °C rise in temperature is known as the $\mathbf{Q_{10}}$ or **temperature coefficient.**

Effect of pH on enzyme activity

A change in pH can alter the rates of enzyme-catalyzed reactions with many enzymes exhibiting a bell-shaped curve when enzyme activity is plotted against pH (Figure 7.3).

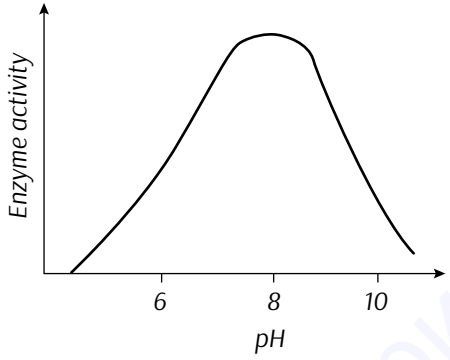


Figure 7.3. A typical pH – activity plot of enzyme catalyzed reaction

Each enzyme has its own pH optimum and this value is generally in a narrow range of pH scale, usually between 7.0 and 7.5. But there are some exceptions. **Pepsin,** which is the principal proteolytic enzyme of human gastric juice, operates in extremely acid medium that is in pH 1.5–2,5. Contrariwise, **trypsin,** whose proteolytic action is limited to the small intestine lumen, has pH optimum in alkaline medium, in the pH range of 7.5–8.0.

7.3. Enzyme nomenclature and classification

Today, approximately 2000 different enzymes are known.

Enzyme nomenclature

There are several ways of naming enzymes.

A. Trivial or common names.
These are usually names of substrates with the suffix -***ase*** added.
For example:

- **malt*ase*** – this is the name of an enzyme which catalyzes the transformation in human intestine of a disaccharide *maltose*;
- **glucose 6-phosphat*ase*** – this is the name of an enzyme under which catalytic action the hydrolysis of a phosphorylated glucose in human liver takes place.

Sometimes, trivial names of enzymes have historical roots that designate neither chemical name of substrate nor the kind of reaction it is subjected to.

For example:

- ***pepsin, trypsin*** – these are the common names of definite enzymes which catalyze the hydrolysis of proteins in human gastro-intestinal tract;
- ***thrombin*** – this is the name of enzyme which takes part in blood clotting to make *thrombuses* inside blood vessels.

B. Systematic names

Systematic names of enzymes take into account both the chemical name of a substrate and the kind of chemical reaction catalyzed, that is both the ***substrate specificity*** and the ***reaction specificity.***

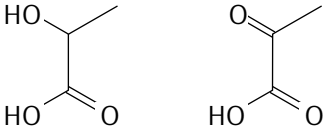
Systematic names of enzymes are included to the **Enzyme Catalogue**, published by the **Nomenclature Committee of the International Union of Biochemistry** (examples see below).

According to the international enzyme nomenclature and the Enzyme Catalogue, every enzyme has the **EC number**, a four-digit number.

In the EC number of an enzyme, the ***first digit*** indicates membership of one of the 6 major ***classes***, the ***next two*** indicate ***subclasses*** and ***sub-subclasses*** and the ***last digit*** indicates where the enzyme belongs in the sub-subclass.

Let us consider the formation of the **EC number** of the enzyme ***lactate dehydrogenase***. The reaction equation which an enzyme catalyzes is:

$$\text{Lactate} + NAD^+ \longrightarrow \text{Pyruvate} + NADH + H^+$$



Lactate *Pyruvate*

EC number of ***lactate dehydrogenase*** is 1.1.1.27, which means:

1. – 1st class – oxidoreductases;

1.1. – **subclass** of oxidoreductases that use a **–CH_2 –OH** group as the donor of electrons;

1.1.1. – **sub-subclass** – enzymes which use **NAD(P)**$^+$ as the electron acceptor;

1.1.1.1. – the **number** of an enzyme in the sub-subclass.

Enzyme classes

There are six major enzyme classes.

1. ***Oxidoreductases.***
2. ***Transferases.***
3. ***Hydrolases.***
4. ***Lyases.***
5. ***Isomerases.***
6. ***Ligases (synthetases).***

Each class contain enzymes with the same reaction specificity, and namely:

Class 1. ***Oxidoreductases*** – are involved in oxidation and reduction, that is they catalyze the transfer of reducing equivalents from one redox system to another.

Reaction type:

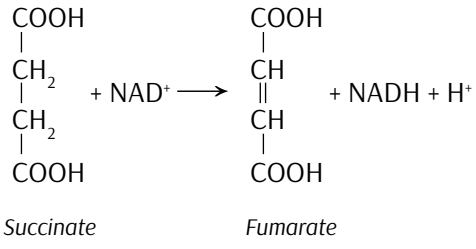
$$A_{red} + B_{ox} \leftrightarrow A_{ox} + B_{red}$$

Important subclasses:

1. ***Dehydrogenases.***
2. ***Oxidases, peroxidases.***
3. ***Reductases.***
4. ***Monooxygenases, dioxygenases.***

For example:

- Succinate + NAD^+ ⟶ Fumarate + NADH + H^+



Enzyme – ***succinate dehydrogenase;*** EC 1.3.5.1

- $Glutathione_{ox}$ + NADPH + H^+ ⟶ $Glutathione_{red}$ + $NADP^+$

Enzyme – ***glutathione reductase;*** EC 1.6.4.2

Class 2. Transferases – catalyze the transfer of other functional groups (e.g. amino or phosphate groups) from one substrate (donor) to the other (acceptor).

Reaction type:

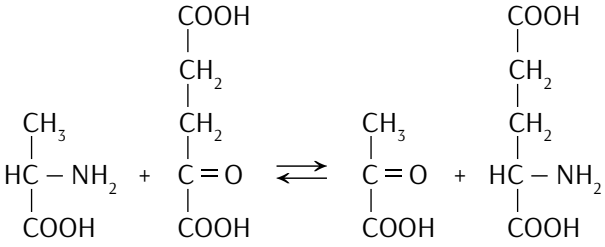
$$A - B + C \leftrightarrow A + B - C$$

Important subclasses:

1. ***Methyltransferases.***
2. ***Acyltransferases.***
3. ***Glycosyltransferases.***
4. ***Aminotransferases.***
5. ***Phosphotransferases.***

For example:

- transfer of amino group:



Alanine α-Ketoglutarate Pyruvate L-Glutamate

Enzyme – ***alanine aminotransferase*** (EC 2.6.1.2)

- transfer of phosphorus-containing group (this type of enzymes is called ***kinases***):

D-Glucose + ATP ⟶ D-Glucose 6-phosphate + ADP

Enzyme – ***ATP : D-hexose 6-phosphotransferase; hexokinase*** (EC 2.7.1.1)

Most oxidoreductases and transferases require coenzymes **NAD^+, $NADP^+$, FAD, FMN, thiamine phosphate, pyridoxal phosphate, tetrahydrofolate** etc.

Class 3. Hydrolases – enzymes which are also involved in group transfer, but the acceptor is always a water molecule, that is they catalyze the hydrolysis of a substrate.

Reaction type:

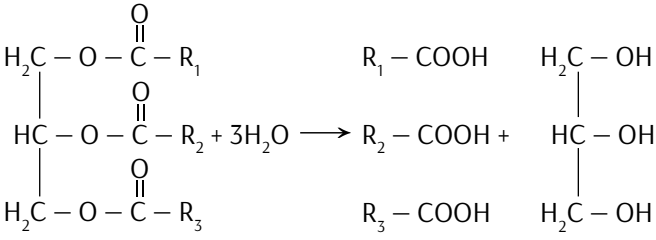
$$A - B + H_2O \longrightarrow A - H + B - OH$$

Important subclasses:

1. ***Esterases.***
2. ***Glycosidases.***
3. ***Peptidases.***
4. ***Amidases.***

For example:

- Reaction of triacylglycerols hydrolysis:



Triacylglycerol | *Three different fatty acids* | *Glycerol*

This is esterase enzyme which hydrolyses lipids and namely ***lipase*** (***triacylglycerol lipase;*** EC 3.1.1.3)

- Reaction of disaccharide sucrose hydrolysis:

$$\underset{\text{Sucrose}}{C_{12}H_{22}O_{11}} + H_2O \rightarrow \underset{\text{Glucose}}{C_6H_{12}O_6} + \underset{\text{Fructose}}{C_6H_{12}O_6}$$

Enzyme is ***sucrase*** of human intestines (***sucrose-α-glucosidase***; EC 3.2.1.26)

- Reaction of peptide hydrolysis:

$$\text{Peptide} + H_2O \rightarrow (\text{Amino acid})_n$$

Enzyme is of ***peptidases*** type (***pepsin, trypsin*** etc).

Class 4. **Lyases** – enzymes which catalyze reactions involving either the breakage (non-hydrolytic) or formation of covalent bonds **C—C—, C—O—, C—N—** and **C—S—.**

Reaction type:

$$A + B \leftrightarrow A - B$$

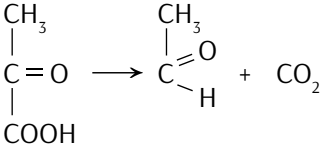
When the reverse reaction is more essential, the enzyme is called ***"synthase".***

Important subclasses:

1. ***C — C-lyases.***
2. ***C — O-lyases.***
3. ***C — N-lyases.***

4. ***C — S-lyases.***

For example (C-C-lyase):



Enzyme – ***pyruvate decarboxylase*** (EC 4.1.1.1)

Class 5. ***Isomerases*** – catalyze changes within one molecule to form isomers. They include *racemases* and *epimerases*, *cis-trans* isomerases, *intramolecular transferases*, and *intramolecular lyases.*

Reaction type:

$$A \leftrightarrow Iso - A$$

Important subclasses:

1. ***Racemases.***
2. ***Epimerases.***
3. ***cis-trans Isomerases.***
4. ***Intramolecular transferases.***

For example:

D-Glucose 6-phosphate ↔ D-Fructose 6-phosphate

Enzyme – ***phosphohexose isomerase*** (EC 5.3.1.8) of glycolytic pathway of glucose intracellular transformation.

Class 6. Ligases (synthetases) – enzymes which catalyze the ligation reactions. They join two molecules together and thence their action is energy-dependent. As such, this kind of enzymatic catalysis is coupled to the hydrolysis of *macroergic* nucleoside triphosphate, predominantly ***adenosine triphosphate (ATP).***

Reaction type:

$$A + B + ATP \longrightarrow A - B + ADP$$

Important subclasses:

1. ***C — C-lygases.***
2. ***C — O-lygases.***
3. ***C — N-lygases.***
4. ***C — S-lyases.***

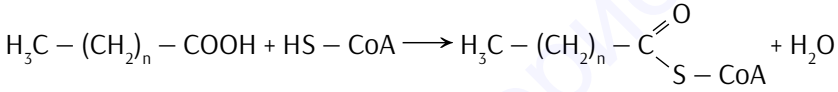
For example:

- C-O-bond formation:

$$\text{Amino acid} + \text{tRNA} \rightarrow \text{Aminoacyl-tRNA}$$

Enzyme – ***aminoacyl-tRNA synthetase*** (EC 6.1.1.n)

- C-S-bond formation:



Fatty acid *Coenzyme A* *Acyl-CoA*

Enzyme – ***fatty acid-CoA ligase*** (EC 6.2.1.3)

- C-N-bond formation:

$$\text{L-Glutamate} + NH_3 \rightarrow \text{Glutamine} + H_2O$$

Enzyme – ***glutamate-NH_3 ligase*** (***glutamine synthetase***; EC 6.3.1.2).

7.4. Molecular mechanisms of enzyme catalysis

1. Energy of activation

Any chemical reaction occurs when a certain proportion of the substrate molecules are sufficiently energized to reach a so-called **transition state.** In the transition state, there is a high probability for a chemical bond to be made or broken to form the product. The **initial state** is the free energy of

the substrate at the start of the reaction.

Hence the energy of activation is equal to the difference in free energy between the transition state and the initial state (Figure 1.3).

2. Free-energy changes

Figure 7.4 illustrates the free-energy changes that occur during a chemical reaction when it is catalyzed (lower curve) and uncatalyzed (upper curve). As can be seen from the curves presented, **the effect of catalysts (enzymes) is to decrease the energy of activation.**

3. Active sites

Formation of an ***enzyme-substrate complex*** **(ES)** is the key step in enzymatic catalyzis:

$$E + S \rightarrow ES \rightarrow E + P.$$

The **active site** of an enzyme is the region that binds the substrates and contains the ***functional groups*** which directly participate in the making and breaking of covalent bonds in substrate molecules in the course of reaction.

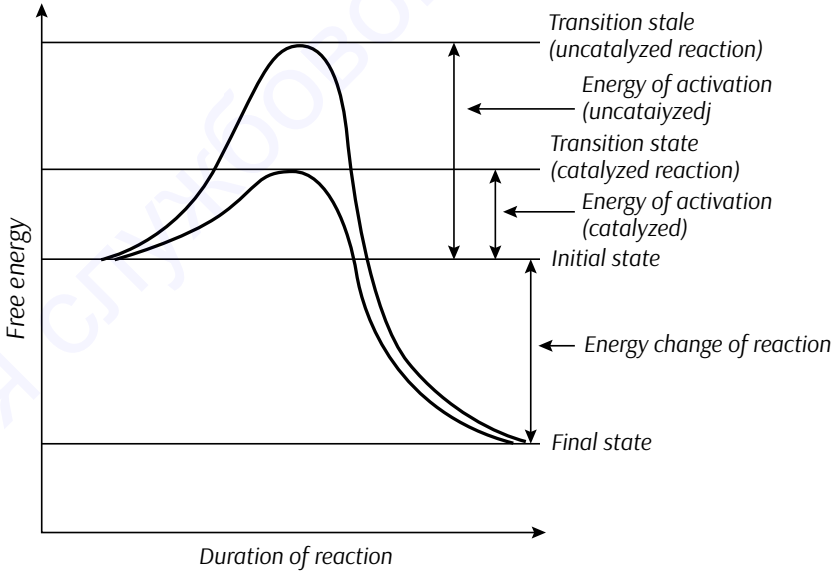


Figure 7.4. Diagrammatic representation of the free energy of activation of a chemical reaction

From the viewpoint of protein chemistry, the functional groups are the ***amino acids residues*** of apoenzyme molecule that form reversible noncovalent interactions between enzyme and substrate.

The **noncovalent interactions** which participate in making enzyme-substrate complexes are mediated predominantly by:

- *electrostatic (ionic) bonds;*
- *hydrogen bonds;*
- *van der Waals forces;*
- *hydrophobic interactions.*

As an example the formation of electrostatic bonds between charged residues of two amino acids can be shown (Figure 7.5).

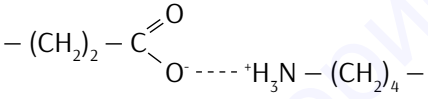


Figure 7.5. Electrostatic bond *(salt bridge)* between negatively charged group of enzyme, *(amino acid glutamate residue)* and amino acid lysine side chain

Active sites and the specificity of enzymes

The specificity of an enzyme is determined by:

1) the functional groups of the substrate (or product);
2) the functional groups of the enzyme and its cofactors;
3) the physical proximity of these various functional groups.

Two theories have been proposed to explain the specificity of enzyme action.

(1) The lock and key theory. The active site of the enzyme is complementary in conformation to the substrate, so that enzyme and substrate "recognize" one another.

(2) The induced-fit theory. The enzyme changes shape upon binding the substrate, so that the conformation of substrate and enzyme protein become complementary in the course of binding step.

Chapter 8.

COENZYMES. CONTROL OF ENZYMATIC REACTIONS

8.1. Coenzymes: classification, structure. Vitamins as coenzymes precursors

Coenzymes: general definitions

Many enzyme-catalyzed reactions involve the transfer of electrons or groups of atoms from one substrate to another. Such reactions always involve "helper molecules", which act as temporary acceptors of the group being transferred. Helper molecules of this type are called **coenzymes.**

The principal significance of **coenzymes** molecules is that **they define the type of chemical reaction being catalyzed** but not the substrate specificity of a definite enzyme. The latter depends on the protein part of enzyme molecule which is called *apoenzyme* (Lecture 1). According to this, the same coenzyme cooperates, as a rule, with many different enzymes of different substrate specificity. For example, NAD^+ molecule (see below) is a coenzyme of tens of different dehydrogenases enzymes.

Coenzymes molecules differ in their chemical nature; the major group of coenzymes is represented by derivatives of **vitamins.** Most water-soluble vitamins, known as the "vitamin B complex", are components of coenzymes.

The vitamins which serve as coenzyme precursors are:

- Vitamin B_1 (Thiamine);
- Vitamin B_2 (Riboflavine);

- Vitamin PP (Nicotinamide);
- Vitamin B_6 (Pyridoxine);
- Vitamin B_{12} (Cobalamin);
- Vitamin B_c (Folic acid);
- Vitamin H (Biotin);
- Pantothenic acid.

Besides, a distinction is made between ***soluble coenzymes*** and ***prosthetic groups,*** based on the nature of their interaction with the enzyme and namely:

(a) ***soluble coenzymes*** – during a reaction they are bond with an apoprotein moieties like substrates, undergo a chemical change, and are then released. Coenzymes of this kind can be exemplified by such compounds as nicotinamide adenine dinucleotide (NAD) or nicotinamide adenine dinucleotide phosphate (NADP);

(b) ***prosthetic groups*** – these coenzymes are tightly bound to the enzyme protein, making a conjugated protein (Lecture 1) as in the case of flavin adenine dinucleotide (FAD).

In accordance with the type of reaction they help in catalyzing, there are the following major classes of coenzymes:

- redox coenzymes;
- group-transferring coenzymes;
- rearrangement coenzymes.

8.1.1. Redox Coenzymes

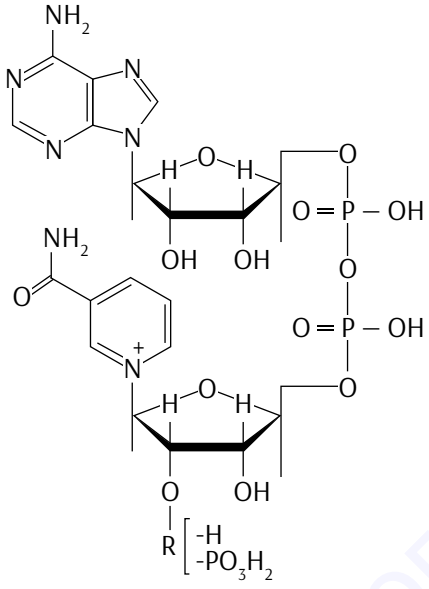
Redox reactions involve the transfer of one or two protons, together with the electrons, from one molecule to another. In some reactions only the transfer of electrons occurs. Together, protons and electrons are referred to as *reducing equivalents,* and the intermolecular transfer of these is carried out by ***redox coenzymes.***

The principal representatives of redox coenzymes are:

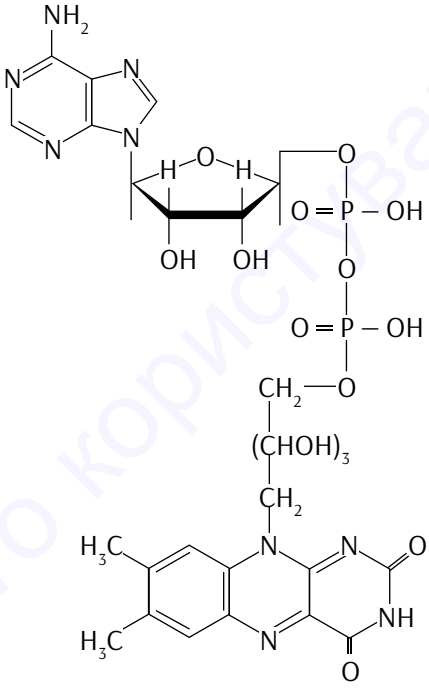
- **Pyridine nucleotides,** and namely:
  - nicotinamide adenine dinucleotide (NAD);
  - nicotinamide adenine dinucleotide phosphate (NADP);
- **Flavin nucleotides,** and namely:

- flavin adenine dinucleotide (FAD);
- flavinmononucleotide (FMN).

- **Ubiquinone (coenzyme Q);**
- **Heme coenzymes.**



Nicotinamide adenine diuucleotide (NAD^+) and nicotinamide adenine diuucleotide phosphate ($NADP^+$)



Flavin adenine dinucleotide (FAD)

Biochemical functions of redox coenzymes

(A) ***Both NAD^+ and FAD are coenzymes for the dehydrogenases enzymes*** involved in the oxidation of fuel molecules, that is carbohydrates, fatty acids and amino acids metabolites:

- in dehydrogenases, using FAD as the electron acceptor, the coenzyme is firmly bound to protein (apoenzyme) moiety ("conjugated proteins");
- NAD is not tightly bound to dehydrogenase protein and interacts with the latter only for the period of catalytic action.

(B) ***NADH and $FADH_2$ as electron carriers.***

Reduced NAD (designated as **NADH**) and **FAD ($FADH_2$)** molecules serve as specialized electron carriers which are the components of mitochondrial electron transport systems that accomplish the reduction of molecular oxygen. In aerobic organisms these complicated biochemical systems provide the oxidation of fuel molecules and fundamental processes of chemical energy release and accumulation.

Reduced NADP molecules (designated **NADPH**) serve as the donors of hydrogen and electrons in ***reductive syntheses,*** where the precursors are more oxidized than the biosyntheses products.

To realize a redox reaction, redox coenzymes must undergo a chemical change and, to be exact, the addition of reducing equivalents, for example:

(1) When the oxidation-reduction reaction includes **$NAD(P)^+$** as the reducing equivalents acceptor, the equation of the reaction is as follows:

$$NAD(P) + SH_2 \rightarrow NAD(P)H + H^+$$

The subsequent oxidation of reduced pyridine nucleotide restores the initial form of molecule, that is oxidized form of coenzyme **($NAD(P)^+$)**.

(2) When the oxidation-reduction process includes **FAD,** the reaction equation is as follows:

$$E - FAD + SH_2 \rightarrow E - FADH_2 + R$$

A second, independent reaction is required for the regeneration of the original form of the coenzyme, which is achieved as a result of **$FADH_2$** interaction with the additional acceptor of reducing equivalents (A):

$$E - FADH_2 + A \rightarrow E - FAD + AH_2$$

(C) ***Reduced ubiquinone (QH_2) and heme coenzymes*** which function as the intermediate electron carriers in mitocho-ndrial electron transport chains.

8.1.2. Group-Transferring Coenzymes

The group-transferring coenzymes are involved in biochemical reactions of intermolecular transfer of different groups including one-carbon (C_1) radicals of different oxidation state, of acyl groups, of active aldehyde groups, of amino groups etc.

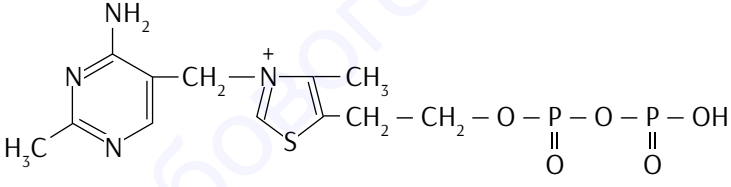
These are derivatives of vitamin B_1 (thiamine diphosphate), vitamin B_6 (pyridoxal phosphate and pyridoxamine phosphate), vitamin B_c (tetra-hydrofolate), vitamin H (carboxybiotin), pantothenic acid (coenzyme A).

8.1.3. Rearrangement Coenzymes

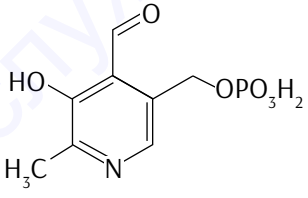
Rearrangement reactions are accomplished with the help of vitamin B_{12} derivatives. Vitamin B_{12}, or cobalamin, is one of the most complex biomolecules in nature that contains a complicated ring system, called *corrine*, with cobalt (CO^{2+}) as the central atom.

In metabolism, derivative of Vitamin B_{12}, 5`-deoxyadenosylcobalamine, acts as coenzyme in intramolecular rearrangements, involved in amino acids transformations.

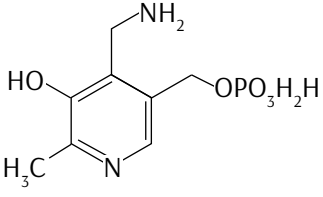
Group-transferring and rearrangement coenzymes



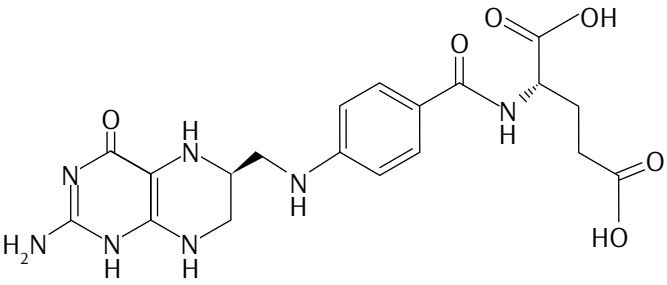
Thiamine diphosphate (TDP)



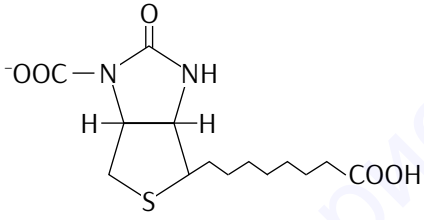
Pyridoxal phosphate



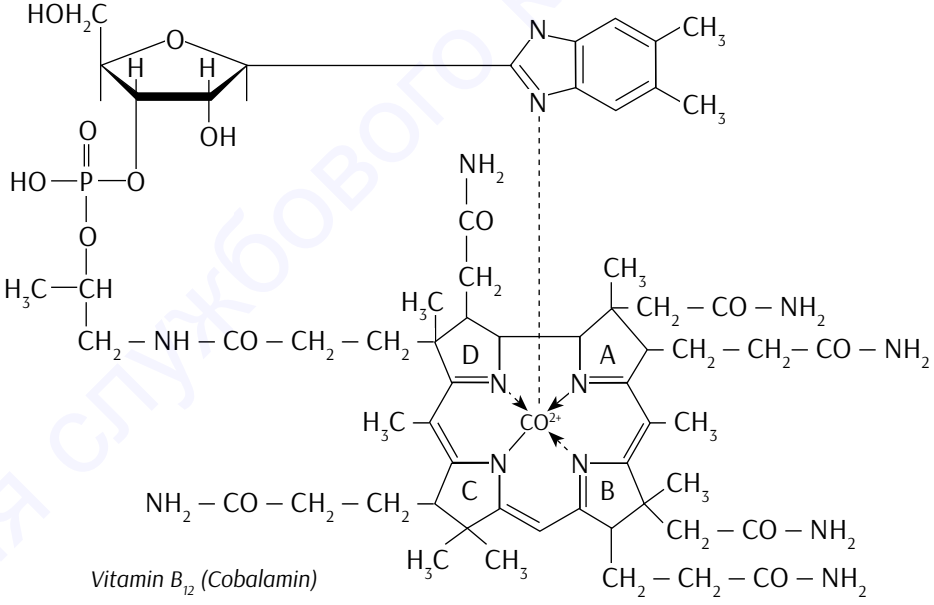
Pyridoxamine phosphate



Tetrahydrofolate



Carboxybiotin



Vitamin B_{12} (Cobalamin)

8.2. Kinetics of enzyme catalysis. Michaelis-Menten theory

The enzyme kinetics studies the action of the enzyme and substrate concentration on the rate of enzyme-catalyzed biochemical reactions. Besides, enzymes kinetics deals with the dependence of enzymatic reactions rates on the presence and concentration of some other molecules which can specifically modulate the properties of enzymes as enzyme inhibitors and enzyme activators.

8.2.1. Effect of enzyme concentration on reaction velocity

If the substrate concentration is held constant, the velocity of enzyme catalyzed reaction is proportional to the enzyme concentration (Figure 8.1).

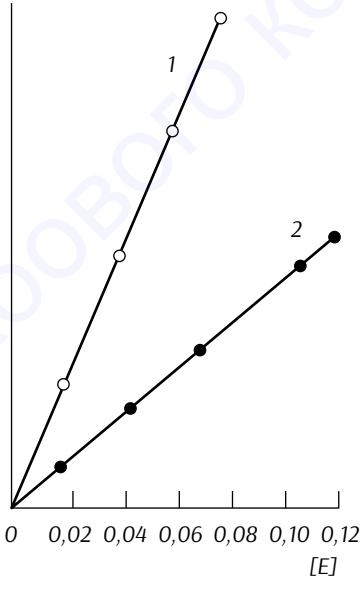


Figure 8.1. Effect of enzyme concentration (enzymes 1 and 2) on the reaction velocity (v)

8.2.2. Effect of substrate concentration on reaction velocity

Figure 8.2 illustrates the effect of the substrate concentration on the rea ction velocity for a typical enzyme catalyzed reaction.

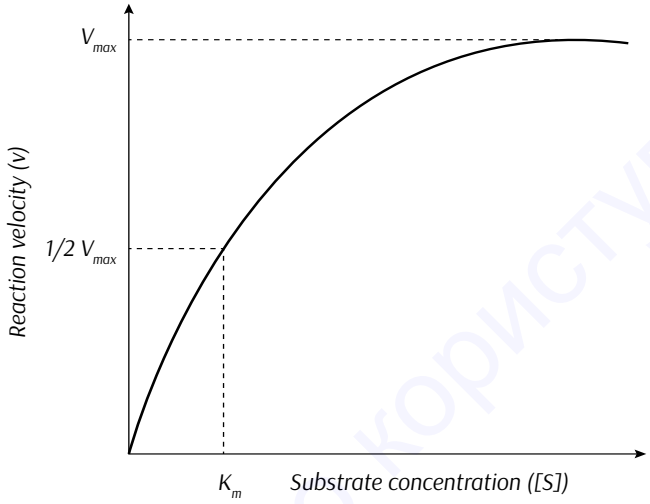


Figure 8.2. The rectangular hyperbola obtained by plotting reaction velocity **(v)** against substrate concentration **([S])** for an enzyme catalyzed reaction. K_m is the substrate concentration at $\frac{1}{2}$ V_{max} (the Michaelis constant)

It can be seen from the figure that:

- when the substrate concentration is low, the reaction is first-order with **v ~ [S];**
- at mid-**[S],** the proportionality is changing;
- at a high substrate concentration, the reaction is zero order, and **v** is independent of **[S].**

Michaelis-Menten kinetic theory

In 1913, Leonor Michaelis and Maud Menten proposed a simple chemical model to account for the experimentally observed relationship of substrate concentration to reaction velocity.

The investigators proposed that an enzyme-catalyzed reaction involved the reversible formation of an enzyme-substrate complex, which then broke

down to form free enzyme and one or more products.

Their postulate can be depicted in this way:

$$E + S \underset{k_2}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_3} E + P$$

where **E** – the free enzyme, **S** – the substrate, **ES** – the enzyme-substrate complex, **P** – the product, $\mathbf{k_1}$ – the rate constant for the formation of **ES**, $\mathbf{k_2}$ – the rate constant for the dissociation of **ES** to **E** and **S**, and $\mathbf{k_3}$ – the rate constant for the transformation of **ES** to **E** and **P.**

Because $\mathbf{k_1}$, $\mathbf{k_2}$, and $\mathbf{k_3}$ are constants, we can define a new constant, $\mathbf{K_m}$, which is expressed as:

$$K_m = \frac{k_2 + k_3}{k_1}$$

and called the ***Michaelis constant.***

To arrive at the Michaelis-Menten equation, we have to introduce a new term and that is the maximum velocity $\mathbf{V_{max}}$. $\mathbf{V_{max}}$ is the rate of the reaction which is attained when the concentration of **ES** is maximal. This will be the case when all of the enzyme is bound to the enzyme-substrate complex.

Thus, the relationship of substrate concentration to reaction velocity may be written as:

$$V = \frac{V_{max} + [S]}{K_m + [S]}$$

which is known as the **Michaelis-Menten equation.**

The Michaelis-Menten equation accounts for the kinetic data presented in Figure 2.2. Using the equation, we can conclude that the **Michaelis constant $\mathbf{K_m}$** corresponds to the substrate concentration at which v is half of the maximum velocity, that is:

$$\text{when } v = \tfrac{1}{2} V_{max},$$
$$K_m = [S].$$

The significance of $\mathbf{K_m}$ for biochemical analysis is that an enzyme with a high affinity for its substrate has a low $\mathbf{K_m}$ value and *vice versa.*

The values of kinetic constants $\mathbf{K_m}$ and $\mathbf{V_{max}}$ can be estimated from the hyperbola which represents the effect of the substrate concentration on the

reaction velocity (Figure 2.2). But it is much more convenient to determine $\mathbf{K_m}$ and $\mathbf{V_{max}}$ from the linear transformation of the Michaelis-Menten equation which gives a straight-line plot. It can be done by taking the reciprocal of both sides of the equation that gives:

$$\frac{1}{v} = \frac{1}{V_{max}} + \frac{K_m}{V_{max}} \times \frac{1}{[S]}$$

A plot of **1/v** versus **1/[S]** is called **a Lineweaver-Burk plot,** and it constitutes a straight line with an intercept of **1/Vmax** and a slope of $\mathbf{K_m/V_{max}}$ (Figure 8.3).

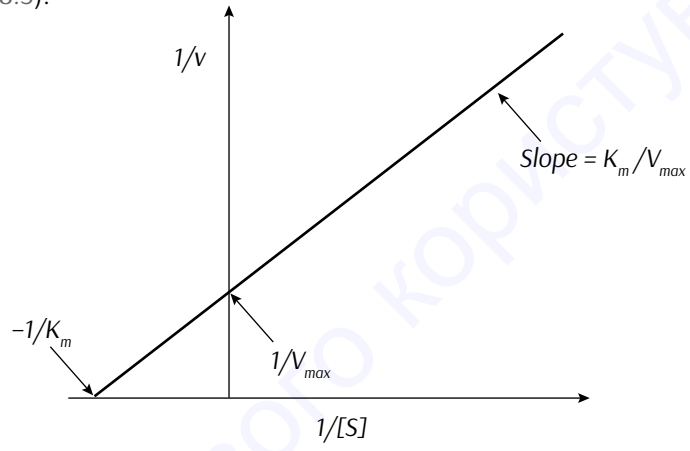


Figure 8.3. A plot of the 1/[v] against 1/[S] for an enzyme-catalyzed reaction. This straight-line representation is the Lineweaver-Burk transformation of the Michaelis-Menten equation

8.3. Inhibition of enzymes. Kinds of inhibitors

Generals views

There are numerous substances that affect metabolic processes by modulating the activity of specific enzymes. The chemicals which specifically slow down the rate of a definite enzyme catalyzed reaction are known as ***enzyme inhibitors.***

Chemically, inhibitors are small molecules and ions which bind selectively with an enzyme to hamper its catalytic activity. Many drugs and toxic agents, either natural or synthetic in origin, act as enzyme inhibitors. Moreover, the inhibition of enzymatic activity by certain biomolecules and metabolites is important because it serves as a major control mechanism in biological systems (see point 2.4).

Kinds of enzyme inhibitors

Some enzyme inhibitors act ***reversibly,*** i.e. their action is characterized by a rapid dissociation of the enzyme-inhibitor complex. In contradistinction to that, under ***irreversible*** inhibition the inhibitor dissociates very slowly, if does on the whole, from the target enzyme molecule, and this is characteristic of many toxic substances which affect human organism.

Besides, depending on the chemical similarity between substrate and inhibitor, it is possible to distinguish ***competitive inhibitors*** from ***non-competitive inhibitors***. And this distinction is of great importance in medicine because some competitive inhibitors are used as pharmaceutical drugs which can specifically impede the course of definite biochemical processes.

A. Competitive inhibitors

In competitive inhibition the substrate and an inhibitor compete for binding to the same active site of the enzyme.

The competitive inhibitors resemble substrates in their chemical nature, and operate in the interaction with the enzyme as the ***substrate analogs.*** Therefore the enzyme can bind substrate (S) or inhibitor (I) alternatively, forming ES- or EI-complexes.

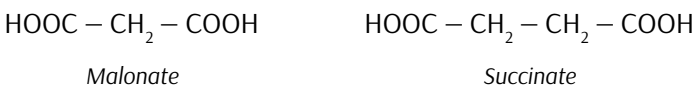
$$E + S \rightarrow ES$$
$$E + I \rightarrow EI.$$

Thus, **under competitive inhibition, the substrate is prevented from binding to the active center of enzyme, which is occupied by inhibitor!**

As the result of inhibition, substrates cannot be converted to the products. Thus, they reversibly block a certain fraction of the enzyme molecules present.

A classic example of competitive inhibition is the action of toxic sub-

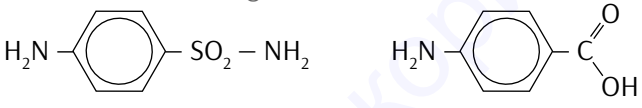
stance, *malonate* on *succinate dehydrogenase*, an enzyme that removes two hydrogen atoms from succinate.



Malonate *Succinate*

Malonat***e*** structurally resembles succinate but differs from the latter in having one rather than two methylene groups and thence, after binding with the same active site in *succinate dehydrogenase,* the inhibitor hinder the interaction of an enzyme with its veritable substrate.

A great many competitive inhibitors, which are structural analogs of essential biomolecules, such as amino acids, vitamins, nucleosides etc. are used in medicine as pharmaceutical drugs, e.g. antibacterial and anticancer agents. The example of the competitive inhibitor applied in antimicrobial chemotherapy is the use of medicines ***sulfonamides,*** which block the bacterial synthesis of folic acid; (Figure 8.4).



Sulfanilamide (Streptocyde) *Para-Aminobenzoic acid*

Figure 8.4. Sulfonamides are derivatives of sulfanilamide. They inhibit the Operation of enzymes which incorporate p-aminobenzoic acid into folic acid that is necessary factor for nucleic acids biosynthesis inside bacterial cells. Resulting from that, sulfonamides supress the growth of microorganisms

The competitive inhibition is reversed by increasing the substrate concentration relative to that of the inhibitor. As can be stated from the kinetics of enzyme catalyzed reactions occurring in the presence of competitive inhibitors, the latter change the K_m of the enzyme, but not the V_{max} (see below).

B. Non-competitive reversible inhibitors

In non-competitive inhibition, an inhibitor interacts with a functional group that is essential for enzyme activity, but does not affect binding of the substrate to the enzyme molecule:

$$E + I \rightarrow EI.$$
$$ES + S \rightarrow ESI$$

However, the binding of the ihhibitor to the enzyme molecule blocks its catalytic activity, that is an ability to transform the substrate into products of reaction. Kinetics analysis shows that in case of non-competitive reversible inhibition, the V_{max} of reaction is lowered but the K_m which reflects the affinity of enzyme to substrate is not affected (Figure 8.5).

This type of inhibition can be reversed, but not by adding more substrate. For example, the inhibitor of enzymes which contain Mg^{2+} as the part of their active sites, is ethylenediaminetetraacetate (EDTA), a chelator of Mg^{2+} ions. In this case, the inhibition can be reversed by adding excess Mg^{2+} that restores the catalytic activity of an enzyme.

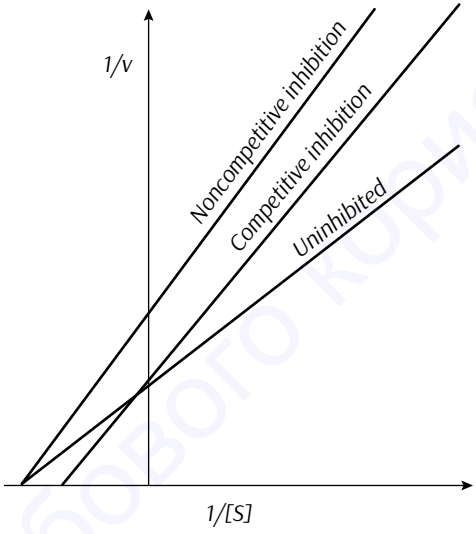


Figure 8.5. Lineweaver-Burk plot showing the effect of competitive and non-competitive inhibitors on kinetics of enzyme-catalyzed reactions

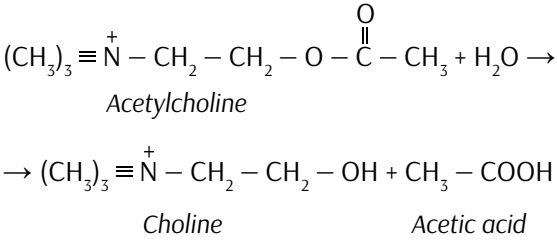
C. Non-competitive irreversible inhibitors

In non-competitive irreversible inhibition, the inhibitor binds irreversibly to the enzyme and effectively removes the latter from the reaction. In this type of inhibition, the V_{max} of reaction is lowered, but the K_m of enzyme is unchanged.

Non-competitive irreversible inhibitors modify functional groups of the target enzymes. Sometimes, they virtually bring about irreversible changes in the chemical architecture of the active sites of enzyme molecules.

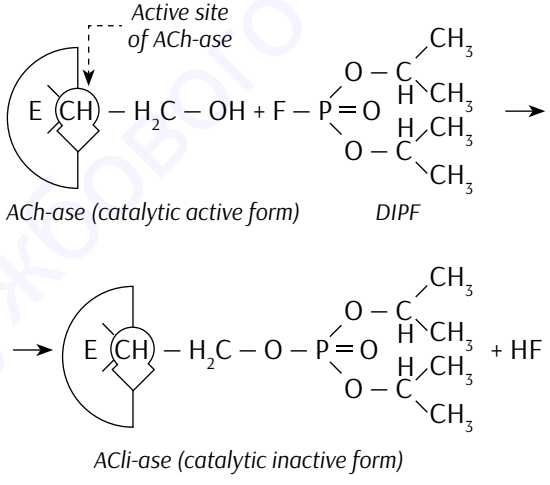
Inactivation of acetylcholinesterase. One of the major chemical me-

diators which transmit nerve impulses is **acetylcholine,** a quaternary ammonium ester. This neurotransmitter is effectively broken down by enzyme ***acetylcholinesterase,*** which hydrolyses the ester to give choline and acetic acid:



The catalytic activity of ***acetylcholinesterase*** is inhibited by toxic agents **organophosphates.** The latter react with the active site on the enzyme to form a phosphorylated protein that is catalytic inactive.

For example, an insecticide ***diisopropylphosphofluoridate (DIPF),*** one of these compounds, reacts with a critical serine residue at the ***acetylcholinesterase*** active site to form catalytic inactive enzyme derivative (Figure 8.6).



Fiaure 8.6. Inactivation of acetvlcholinesterase bv DIPF

Enzyme, catalytic activity of which is blocked by organophosphate, is incapable of acetylcholine hydrolysis. This results in neurotransmitter excessive accumulation and drastic disorders in the nervous system function.

8.4. Enzymic catalysis regulatory mechanisms

The velocity of every metabolic pathway is constantly monitored and adjusted so that the synthesis and the degradation of metabolites satisfy the prevailing physiological requirements. It can be well understood that the flux of any intermediate through a metabolic pathway is determined by the presence of definite enzymes and their catalytic activity.

On the other hand, for the purposes of metabolic regulation, it is sufficient to change the activity of the enzyme catalyzing the slowest step in the reaction chain. Such **key enzymes,** which are subject to various regulatory mechanisms, are found in most metabolic pathways. As a rule, the regulatory key enzyme is the one, which catalyzes the first step in a multi-stage biosynthetic pathway and it is usually inhibited by the ultimate product of a succession (Figure 8.7).

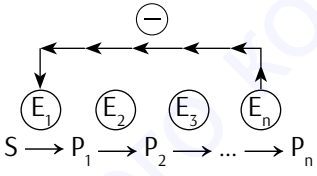


Figure 8.7. Feed-back inhibition of the first enzyme in a pathway by reversible binding of a ligand which is the final product of a succession

This type of metabolic control is called ***feed-back inhibition*** (examples see below).

Summarizing, we conclude that, in general, the control of the enzyme-catalyzed reactions rate can be accomplished by using one of the following biochemical mechanisms:

- regulation of the activity of metabolic pathways key enzymes;
- control of the amount of key enzymes which are present in a cell.

A. The precise regulation of enzymes catalytic activity. This, in turn, is realized in four principal ways:

1) Allosteric regulation.
2) Reversible covalent modification.
3) Proteolytic activation.

4) Stimulation and inhibition by control proteins.

1. ***Allosteric regulation*** of enzymes is realized by specific ***effectors (modulators)*** which bind to a regulatory enzyme and modulate its catalytic activity. Depending on the direction of effector-induced enzyme activity change, there are **allosteric activators** and **allosteric inhibitors.**

This kind of regulation is achieved by the binding of the ***effector*** to the enzyme at a **regulatory site**, which is distinct from the catalytic site. Allosteric enzymes exist in two forms, a less active ***T form*** ("tense") and a more active ***R form*** ("relaxed") which differ in their spatial conformation. The binding of allosteric effector (inhibitor or activator) shifts the equilibrium between two catalytic distinctive conformations to give the predominance of ***T conformation*** or ***R conformation*** and this increases the fraction of less or more active enzyme molecules (Figure 8.8):

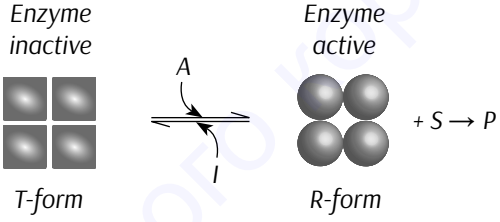


Figure 8.8. The allosteric regulation of enzyme activity. The enzyme, which is oligomer, in the absence of effector exists in two isomeric forms: T *(catalytic inactive* and *R /catalytic active)*. The binding of an effector *(A – activator or I – inhibitory)* displaces the equilibrium to sive the predominance of a certain conformation: T or R.

The **allosteric inhibition** is a wide-spread form of key enzymes activity control as in the case of feed-back inhibition of numerous biosynthetic processes. The classic example of allosteric enzyme regulation is the metabolic control of *aspartate carbamoyltransferase (ACTase),* a key enzyme of pyrimidine biosynthesis (Figure 8.9).

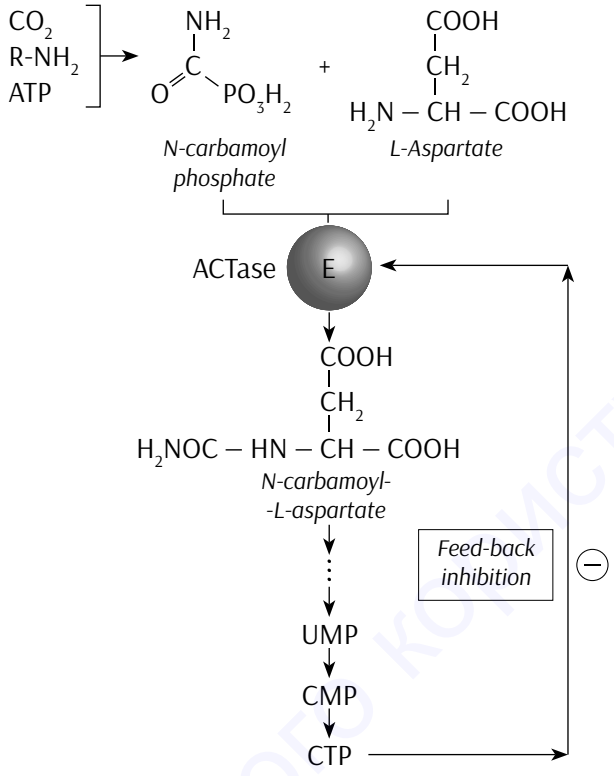


Figure 8.9. ACTase as a key enzyme in feed-back control of nucleotides biosynthesis

ACTase catalyzes the transfer of a carbamoyl residue from carbamoyl phosphate to the amino group of L-aspartate. The N-carbamoyl-L-aspartate thus formed is a precursor of nucleoside phosphates which are components of nucleic acids RNA and DNA. The enzyme is feed-back inhibited by cytidine triphosphate (CTP), the final product of this biosynthetic pathway. This assures that N-carbamoyl-L-aspartate and subsequent intermediates are not needlessly forms when pyrimidines are abundant.

2. *Reversible covalent modification.* This is the form of metabolic regulation which is achieved by the conversion of an enzyme into molecular form that is distinct in its catalytic activity as compared to the unmodified protein molecule.

The most wide-spread way of many enzymes activity regulation is the

phosphorylation of their protein moieties.

The covalent attachment of a phosphoryl group markedly alters the catalytic properties of many enzymes. For example, ***glycogen phosphorylase*** (the enzyme that releases monosaccharide units from polysaccharide glycogen) is activated by phosphorylation of a specific serine residue. ATP serves as the phosphoryl donor in these reactions, which are catalyzed by enzymes ***protein kinases:***

$$R - OH + ATP \longrightarrow R - O - PO_3H_2 + ADP$$

Protein kinases are, in their turn, stimulated by the cyclic nucleotide **cAMP** (3`,5`-cyclic adenosine monophosphate), which serves as a *signal molecule*, whose intracellular concentration is controlled by many hormones and neurotransmitters.

Enzymes ***protein phosphatases*** reverse the effect of ***protein kinases*** by catalyzing the hydrolysis of phosphoryl groups attached to proteins:

$$R - O - PO_3H_2 + H_2O \longrightarrow R - OH + H_3PO_4\ (P_i).$$

The conversion of inactive enzyme into catalytically active form takes place when the physiological need in stimulation of definite metabolic route arises, and this usually occurs in response to a hormonal signal. In addition to the regulation of enzyme activity, reversible phosphorylation-dephosphorylation is the essential biochemical mechanism in controlling the function of membrane channels and some other target proteins.

3. Proteolytic activation. Many enzymes are activated by specific proteolytic cleavage. This is due the fact that there occur enzymes which are synthesized in the form of ***catalytic inactive precursors*** **(proenzymes,** or **zymogens).**

In this case the regulatory mechanism consists in the cleavage of one or a few specific peptide bonds in proenzyme molecule to form an active substance. Activation of enzymes by specific proteolysis recurs frequently in biological systems and namely:

- The ***digestive enzymes*** that hydrolyze food proteins are initially synthesized and secreted in the stomach and pancreas as inactive zymogens (Table 8.1, Figure 8.10).

Table 8.1. Proenzymes and enzymes of human digestive tract

Zymogen	Active enzyme
Pepsinogen	Pepsin
Chymotrypsinogen	Chymotrypsin
Trypsinogen	Trypsinogen
Procarboxypeptidase	Carboxypeptidase
Proelastase	Elastase

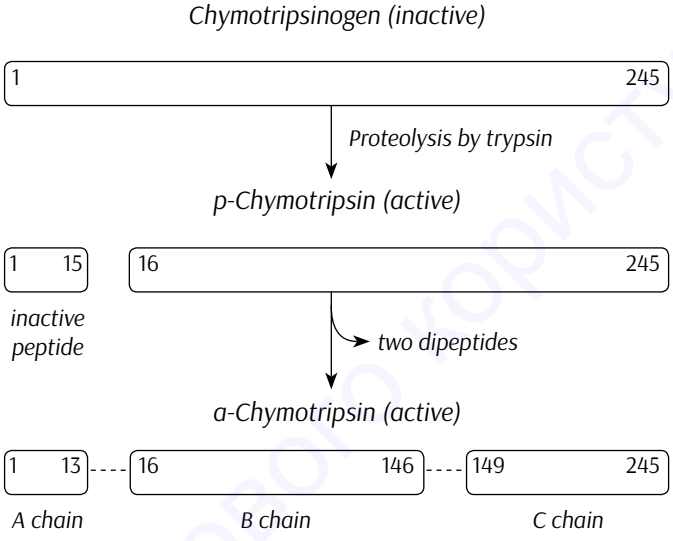


Figure 8.10. Proteolytic activation of Chymotripsinogen in human small intestines

- Enzymes that mediate ***blood clotting.*** Blood clotting, or ***coagulation,*** is a system of proteins and other substances which constitute a cascade of proteolytic reactions stimulated as a response to blood vessel trauma. The activation of blood clotting proteases occurs as a result of proteolytic cleavage of certain zymogens to form finally a **thrombus** which stops hemorrhage.

4. Stimulation and inhibition by control proteins. The catalytic activity of some enzymes is also regulated by special stimulatory or inhibitory proteins.

The example is *calmodulin*, a protein that activates multiple enzymes

when the intracellular concentration of Ca^{2+} rises. Calmodulin serves as a ubiquitous calcium sensor in eukaryotic cells, which calcium-bound form stimulates a wide array of enzymes, membrane pumps and other target proteins.

B. The control of key enzymes synthesis. Induction and repression

As can be seen from the enzyme kinetics characteristics examined above, the quantity of enzyme molecules present in the cell is the major factor which determines the velocity of any biochemical reaction.

Thus, the **genetic expression of enzyme proteins is the first level of any metabolic pathway regulation.** In its turn, the rate of enzyme protein synthesis is the object of the strict ***transcriptional control*,** that is depends on the transcription of the particular gene. The amount of enzyme synthesized can be increased or decreased according to the physiological requirements of an organism, and it is the biochemical manifestation of ***adaptation processes.***

There can be distinguished:

- **Enzyme induction,** which is the stimulation of enzyme synthesis. The example of this can be a rise of the level of several enzymes involved in carbohydrate metabolism as the result of the increase of carbohydrates consumed as food nutrients.
- **Enzyme repression,** that means the inhibition of certain enzymes synthesis. This is usually a consequence of the diminishing of enzyme substrates cellular concentration which can occur when the suitable substrate compound is absent from food or its penetration into tissue and cellular compartments is hampered.

Chapter 9.

BIOENERGETICS-1. CATABOLIC PATHWAYS. TRICARBOXYLIC ACID CYCLE

9.1. Bioenergetics. ATP and other high-energy compounds

Bioenergetics is a subsection of general biochemistry which studies the molecular and cellular events associated with a release of free chemical energy inside living organism and the mechanisms of energy accumulation via synthesis of high energy bonds of adenosine triphosphate molecules (ATP). Major bioenergetic processes take place inside mitochondria and include the participation of membranes embedded enzymatic complexes and coenzymes that realize consequences of oxidation-reduction reactions of catabolism.

The central role of ATP in energy *exchanges* (*transformations*) in biological systems was perceived in 1941 by Fritz Lipmann and Herman Kalckar.

ATP is a nucleotide consisting of an adenine, a ribose, and a triphosphate unit linked to the 5`-hydroxyl group of adenosine (Figure 9.1).

The phosphate residues in ATP molecule are designated as α, β, and γ. The α phosphate **(Pα)** is bond to ribose by a phosphoric acid diester bond. The other two phosphate linkages, on the other hand, involve much more labile *phosphoric acid anhydride bonds.*

It is noteworthy that **there are two** "high energy" phosphate bonds **in ATP molecule, and namely phosphoric acid anhydride bonds between α and β and β and γ phosphate residues.** It means that **when the stated bonds are hydrolyzed, much free energy is released.**

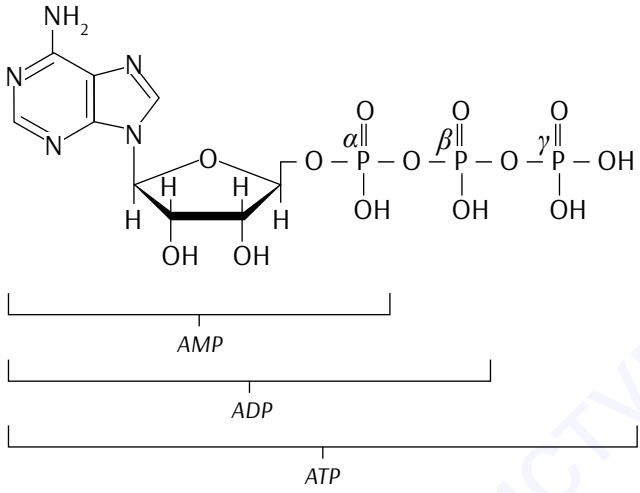


Figure 9.1. Structure of nucleosidephosphates ATP *(adenosine triphosphate)*, ADP *(adenosine diphosphate)* and AMP *(adenosine monophosphate)*

In particular, when ATP is hydrolyzed to ADP and orthophosphate (P_i) (Lecture 4), the free energy change of the reaction (ΔG^0) amounts to –7.3 kcal/mol. Because of this property **ATP serves as the storage form and the principal immediate donor of free energy in the majority of biological systems.**

According to F.Lipmann, "high energy" phosphate bonds ("macroergic bonds") in ATP molecules are designated ~P (squiggle P):

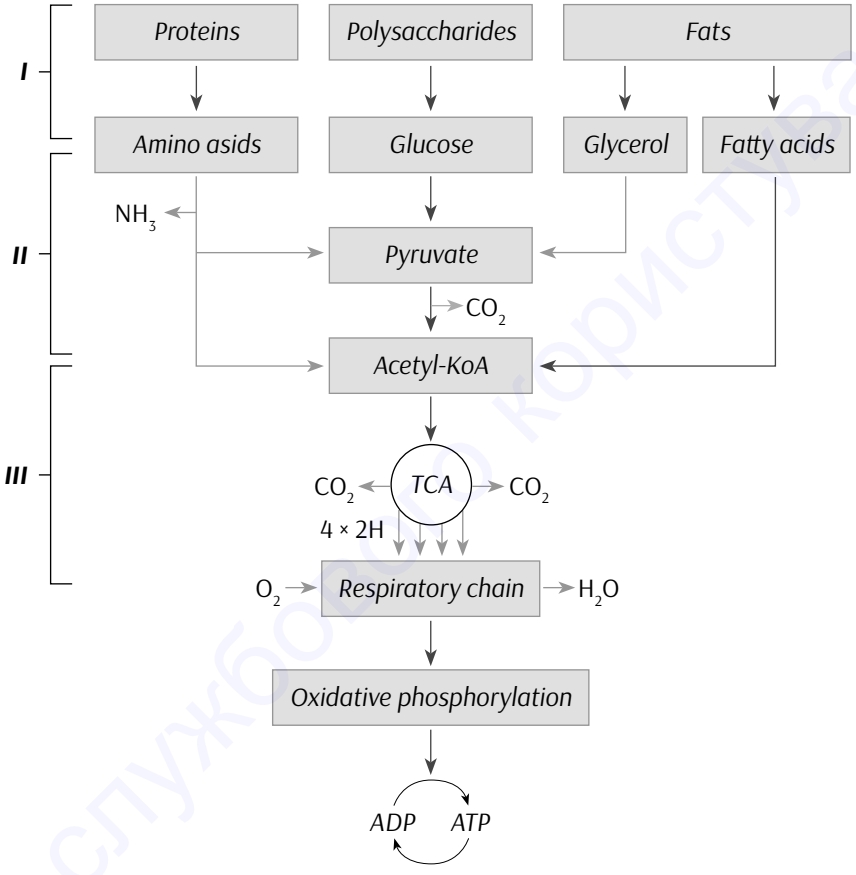
$$\text{Adenine} - \text{ribose} - P\alpha \sim P\beta \sim P\gamma$$

In addition to ATP there occur some other compounds with high-energy phosphate bonds and they are *creatine phosphate*, *acetyl phosphate*, *carbamoyl phosphate* and *phosphoenolpyruvate*. The macroergic biomolecule of especially great importance is creatine phosphate, which serves as a reservoir of easily accessible chemical energy in the form of ~P used in muscle contraction.

9.2. Overview of catabolic pathways steps

There are following steps of biomolecules catabolism in human body (Figure 9.2):

Step I. Hydrolysis of bioorganic substances, which are food constituents (nutrients) in gastrointestinal tract. Carbohydrates are hydrolyzed to mono-saccharides (predominantly glucose), fats to fatty acids and glycerol, proteins and peptides to amino acids.



Step II. The conversion of numerous metabolites of Step 2 to give a single two carbon intermediate – **acetyl-Coenzyme A** (acetyl-CoA). The reactions of Step 2 occur intracellularly.

Step III. The final oxidation of acetyl-CoA to give CO_2 and H_2O. The overall process includes the so-called ***tricarboxylic acid cycle*** (TCA – see below) – the metabolic pathway in which CO_2 and reducing equivalents (H^+

and e^-) are produced. The close functional and structural relation of TCA to the mitochondrial enzymatic systems of electron transport and oxidative phosphorylation yields the production of ATP, which is the major intracellular value of chemical energy.

9.3. Tricarboxylic acid cycle: overview

1. The tricarboxylic acid cycle (Figure 9.2), also known as the **citric acid cycle** or the **Krebs cycle,** provides the degradation of two-carbon acetyl residues derived from carbohydrates (glucose), fatty acids and amino acids.
2. **TCA is a cyclic series of enzymatically catalyzed reactions carried out by a multienzyme system located in mitochondria.**
3. **TCA** oxidizes the acetyl group of acetyl coenzyme A (acetyl-CoA) to carbon dioxide (CO_2), reduced nicotinamide adenine dinucleotide (NADH), hydrogen ion (H^+), and reduced flavin adenine dinucleotide ($FADH_2$), using oxidized nicotinamide adenine dinucleotide (NAD^+) and oxidized flavin adenine dinucleotide (FAD) as electron acceptors.

The summary reaction catalyzed by the cycle in a rough sketch is:

$$CH_3COOH + 2H_2O \rightarrow 2CO_2 + 8H^+.$$

As can be seen from the outline presented in Figure 3.3, the cycle starts from the condensation of a two-carbon **(C2)** ***acetyl unit*** with a four-carbon **(C4)** compound which is ***oxaloacetate***. The resulting six-carbon **(C6)** compound ***(citrate)*** is objected to multi-stage transformations to yield finally the initial substrate, that is ***oxaloacetate***. Two reactions of decarboxylation and four of dehydrogenation accompany the overall process (Figure 9.3).

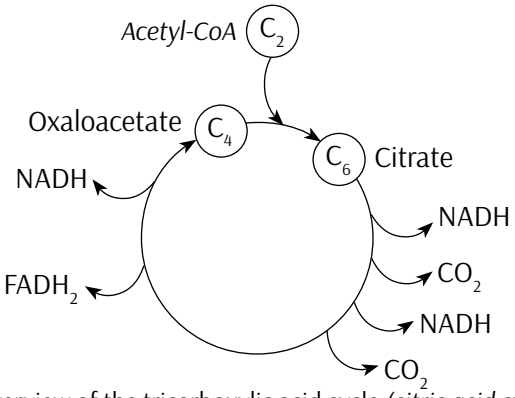


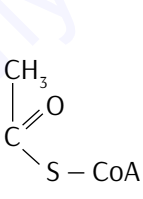
Figure 9.3. An overview of the tricarboxylic acid cycle *(citric acid cycle; Krebs cycle)*

In the course of TCA-coupled dehydrogenations three reduced NAD^+ molecules (NADH) and a reduced FAD molecule ($FADH_2$) are also produced. Hence, four pairs of protons (and four, of electrons) are transferred into mitochondrial electron transport chain to provide ATP generation as a result of oxidative phosphorylation.

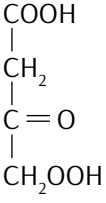
9.4. Tricarboxylic acid cycle: reactions and enzymes

1. The TCA cycle proper begins with the formation of citrate via the condensation of a two-carbon unit, the acetyl group of **acetyl-CoA,** with a four-carbon unit, **oxaloacetate,** which is dicarboxylic acid. The product of reaction is the tricarboxylic acid **citrate:**

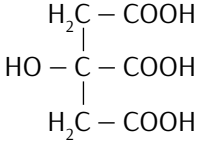
$$\text{Acetyl-KoA} + \text{Oxaloacetate} + H_2O \rightarrow \text{Citrate} + \text{CoA-SH}$$



Acetyl-CoA



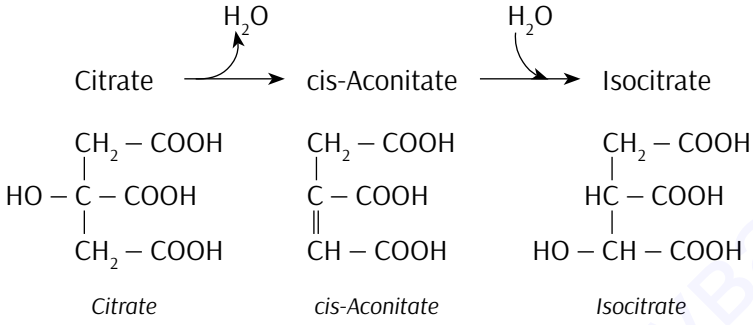
Oxaloacetate



Citrate

The reaction, which is an aldol condensation followed by a hydrolysis, is catalyzed by ***citrate synthase.***

2. Citrate is converted to **isocitrate** via cis-aconitate in the following reaction:

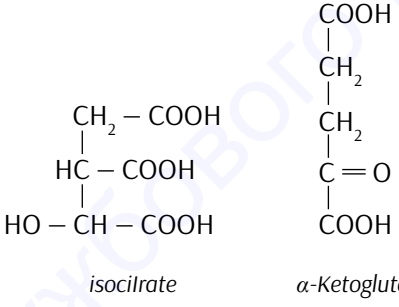


The enzyme catalyzing both steps of the reaction is called ***aconitase.***

3. The next step of TCA is *oxidative decarboxylation* (that is oxidation and decarboxylation) of isocitrate to yield **α-ketoglutarate.**

This is the first of four oxidation-reduction reactions in the TCA and the first of two carbons are lost as CO_2 and the first of three NADH molecules are produced.

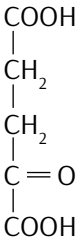
$$\text{Isocitrate} + NAD^+ \longrightarrow \text{a-Ketoglutarate} + NADH + H^+ + CO_2$$



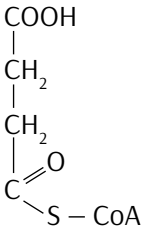
This multistep reaction is catalyzed by the NAD-linked enzyme ***isocitrate dehydrogenase.***

4. The second *oxidative decarboxylation* reaction in TCA is the conversion of α-ketoglutarate into succinyl-CoA. The reaction includes the liberation of the second of two carbon atoms in the form of CO_2 and the generation of the second of three reduced NAD^+ (NADH) molecules:

a-Ketoglutarate + NAD^+ + CoA-SH → Succinyl-CoA + NADH + H^+ + CO_2



a-Ketoglutarate



Succinyl-CoA

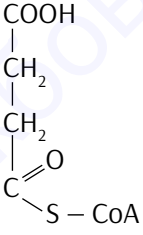
This reaction is catalyzed by the ***α-ketoglutarate dehydrogenase complex,*** which is an organized protein assembly consisting of three kinds of enzymes.

The mechanism of the reaction which is catalyzed by ***α-ketoglutarate dehydrogenase*** is very similar to that of the conversion of pyruvate into acetyl-CoA (see Lecture 5).

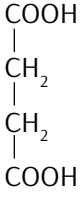
5. The succinyl thioester of Coenzyme A has an energy-rich bond (-8 kcal/mol), which is comparable with that of ATP.

Proceeding from this, the cleavage of thioester bond of succinyl-CoA is coupled to the phosphorylation of nucleotide **guanosine diphosphate (GDP):**

Succinyl-CoA + GDP + P_i → Succinate + GTP + CoA-SH



Succinyl-CoA



a-Ketoglutarate

The reaction is catalyzed by ***succinyl-CoA synthetase.***

GTP itself has a high-energy phosphate bond and is used as a phosphoryl donor. The γ-phosphoryl group of GTP can be readily transferred to adenosine diphosphate (ADP) to form ATP:

GTP + ADP → GDP + ATP

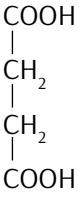
The latter reaction is catalyzed by ***nucleoside diphosphokinase.***

This is called ***substrate phosphorylation*** in contradistinction to the ***oxidative phosphorylation*** (see Lecture 4).

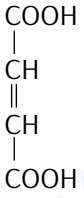
The final stage of the TCA constitute the reactions of four-carbon compounds, in which succinate is converted into oxaloacetate in three steps: an oxidation, a hydration, and a second oxidation reaction.

6. The oxidation of succinate to **fumarate:**

$$\text{Succinate} + \text{FAD} \rightarrow \text{Fumarate} + \text{FADH}_2$$



Succinate



Fumarate

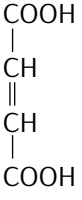
The reaction is catalyzed by ***succinate dehydrogenase.***

The hydrogen acceptor in the reaction is FAD rather than NAD^+, which is used the other three oxidation reactions of the cycle.

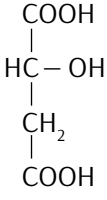
Both kinds of reduced coenzymes, that is ($NADH + H^+$) and $FADH_2$ path their high potential electrons to the electron transport chain of mitochondria, which is the crucial molecular event in deriving and accumulating chemical energy.

7. The hydration of fumarate to form **L-malate:**

$$\text{Succinate} + \text{FAD} \rightarrow \text{Fumarate} + \text{FADH}_2$$



Fumarate

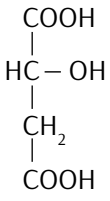


Malate

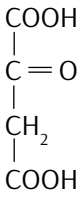
The reaction is catalyzed by ***fumarate hydratase.***

8. Finally, malate is oxidized to form **oxaloacetate**. This reaction is catalyzed by ***malate dehydrogenase,*** and NAD^+ is again the hydrogen acceptor:

$$\text{Malate} + NAD^+ \rightarrow \text{Oxaloacetate} + NAD^+ + H^+$$



Malate



Oxaloacetate

Stoichiometry of the TCA reactions

The net reaction of the tricarboxylic acid cycle is:

$$\text{Acetyl-CoA} + 3NAD^+ + FAD + GDP + P_i + 2H_2O \rightarrow$$
$$2CO_2 + 3NADH + FADH_2 + GTP + 2H^+ + CoA.$$

The metabolic chart of the cycle is given in Figure 9.4.

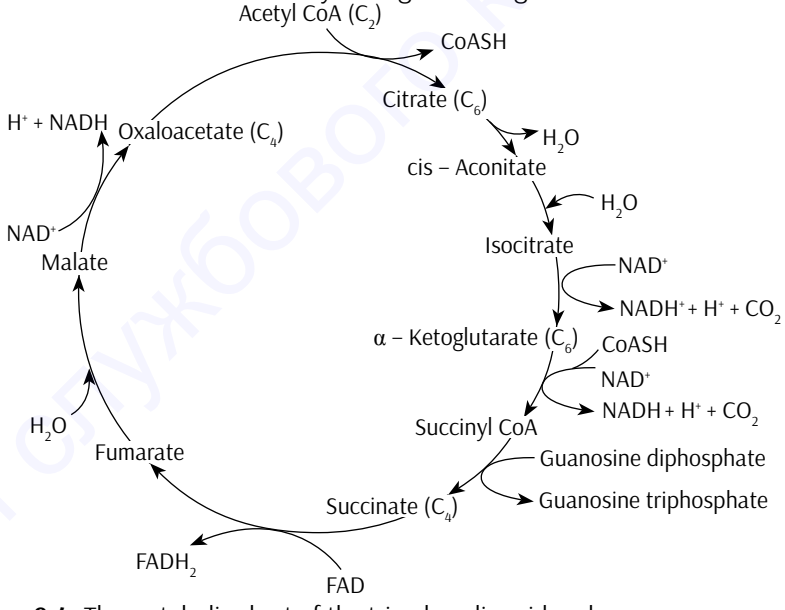


Figure 9.4. The metabolic chart of the tricarboxylic acid cycle

Make a recapitulation of the key reactions which form the equation ap-

pointed:

1. Two carbon atoms enter the cycle in the condensation of an acetyl unit (from acetyl-CoA) with oxaloacetate.
2. Two carbon atoms leave the cycle in the form of CO_2 as the result of two successive decarboxylations catalyzed by *isocitratedehydrogenase* and *α-ketoglutarate dehydrogenase.*
3. Four pairs of hydrogen atoms leave the cycle in the course of four oxidation reactions:
   - two NAD^+ molecules are reduced in the oxidative decarboxylations of isocitrate and α-ketoglutarate;
   - one FAD molecule is reduced in the oxidation of succinate;
   - and one NAD^+ molecule is reduced in the oxidation of malate.
4. One high energy phosphate bond (in the form of GTP) is generated from the energy-riched thioester linkage in succinyl-CoA molecule.
5. Two water molecules are consumed: one in the synthesis of citrate, the other in the hydration of fumarate.

Chapter 10.

BIOENERGETICS-2. MITOCHONDRIAL ELECTRON TRANSPORT. OXIDATIVE PHOSPHORYLATION

10.1. General notions of free energy transfer in biochemical systems

Every living thing requires a continual input of free energy for three major purposes:

- The performance of mechanical work in muscle contraction and other cellular movements.
- The active transport of molecules and ions across the plane of biomembranes.
- The synthesis of macromolecules and other biomolecules from simple precursors.

Autotrophs and heterotrophs

The free energy used in the stated biological processes is derived from the environment. According to the way, in which the needs in energy are satisfied, organisms can be divided into two groups – *autotrophic* and *heterotrophic*.

Autotrophic organisms (autotrophs, or ***phototrophs)***, which comprise plants, algae and certain bacteria, obtain energy by trapping solar energy in the process of *photosynthesis*:

$$H_2O^* + CO_2 \xrightarrow{Light} \underset{Carbohydrates}{(CH_2O)n} + O_2^*.$$

Photosynthesis in the leaves of green plants takes place in subcellular particles called *chloroplasts* with the participation of a magnesium-porphyrin containing protein ***chlorophy ll.***

Heterotrophic organisms (heterotrophs, also called ***chemotrophs),*** which comprise animals and humans, **obtain energy needed for their life by oxidation of nutrients provided in foodstuffs**. That is why animal and human beings are dependent on a constant supply of organic compounds in their diet.

Essential part of the free energy derived from the oxidation of principal nutrients, especially carbohydrates, lipids and proteins, before it is used for motion, active transport, and biosynthesis, is subjected to the transfor-mation into a highly accessible molecular form. And this the most important storage form of chemical energy in all cells is **adenosine triphosphate (ATP).**

Thus, the ATP molecule serves as the free-energy donor in the majority of energy-requiring **(endergonic)** biological processes.

This is due to the fact that the cleavage of ATP to give ADP and phosphate is strongly **exergonic** reaction. When ATP molecule is hydro-lysed to ADP and P_i, the free energy liberation equals to 7,3 kcal/mol:

$$ATP + H_2O \rightarrow ADP + P_i$$
$$\Delta\ Go = -\ 7{,}3\ kcal/mol$$

The free energy liberated in the above reaction is harnessed to drive reactions that require the consumption of chemical energy. **Hence, by the process of energetic coupling, the exergonic hydrolysis of ATP can be used to drive energy-dependent (endergonic) processes.**

In turn, ATP is formed from ADP and P_i when fuel molecules are broken down in the oxidative step of catabolism, and this is designated as **oxidative phosphorylation** that occurs in mitochondria.

This ATP-ADP cycle is the fundamental mode of energy exchange in biological systems (Figure 10.1).

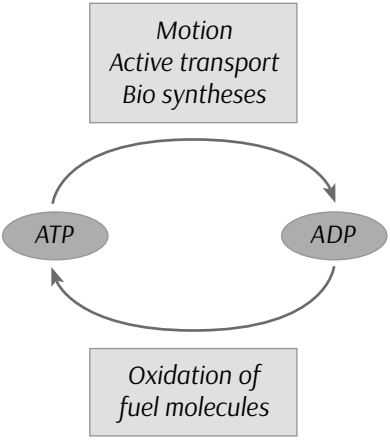


Figure 10.1. The ATP-ADP cycle is the fundamental mode of energy exchange in biological systems

The synthesis of the major part of ATP in aerobic cells, that is oxidative phosphorylation, is coupled with the functioning of complex oxidation-reduction systems of mitochondria which compose the ***electron transport chain.***

10.2. Electron transport chain in mitochondria

The principal portion of chemical energy needed for heterotrophs vital activity, is derived from the oxidation of such fuel molecules as glucose and fatty acids. However, electrons obtained from the substrates oxidized, are not directly transferred from fuel molecules and their breakdown products (e.g. TCA metabolites) to O_2. Instead, these substrates transfer electrons to special molecule carriers which are chiefly coenzymes NAD^+ and FAD. The reduced forms of these carriers then transfer their high-potential electrons to O_2 by means of an electron transport chain located in the inner membrane of mitochondria.

I. General characteristics of electron transport chain

The electron transport chain (or the respiratory chain) is the final common pathway by which in aerobic cells electrons derived from various sub-

strates are transferred to molecular oxygen (O_2).

1. The electron transport chain is a series of highly organized oxidation-reduction enzymes whose reactions can be represented by:

Reduced A + oxidized B ⟶ oxidized A + reduced B

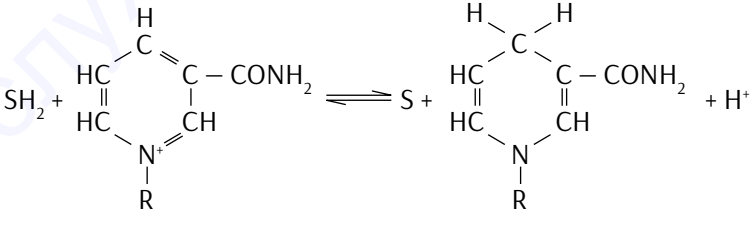
2. It is possible for a variety of substrates to utilize a common pathway because many of them are oxidized by enzymes that use as electron acceptors (or carriers) **oxidized nicotinamide adenine dinucleotide (NAD^+)** and **oxidized flavin adenine dinucleotide (FAD),** which operate in these reactions as redox coenzymes.
   - Enzymes which include NAD^+ are called ***NAD-linked dehydrogenases.***

They comprise:

- the ***isocitrate***-, ***α-ketoglutarate***- and ***malate dehydrogenases*** of the TCA cycle;
- pyruvate dehydrogenase;
- ***L-3-hydroxylacyl coenzyme A dehydrogenase*** of fatty acid oxidation;
- miscellaneous ***NAD-linked dehydrogenases.***

The active portion of NAD^+ and $NADP^+$ molecules is ***nicotinamide ring,*** which accepts a proton and two electrons (equivalent to a hydride ion, H^-) from the substrate.

Hence, the oxidation of biological substrates by ***NAD-linked dehydrogenases*** can be represented by the following equation:



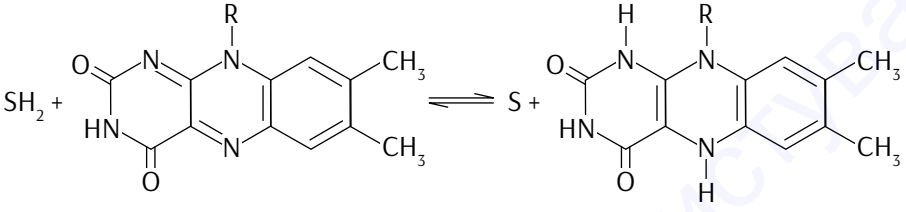
- Enzymes which include FAD are called ***FAD-linked dehydrogenases.***

They comprise:

- ***succinate dehydrogenase*** of the TCA cycle;

- ***acyl-coenzyme A dehydrogenase*** of fatty acid oxidation;
- miscellaneous ***FAD-linked dehydrogenases.***

The active portion of FAD (or FMN) molecule is the ***iso-alloxazine ring,*** which accepts two protons and two electrons from the substrate SH_2. Thus, the oxidation of biological substrates by ***FAD-linked dehydrogenases*** can be represented as:



II. Localization of the electron transport chains

1. The electron transport chains are located in ***mitochondria*** of aerobic cells.
2. Mitochondrial structure is illustrated in Figure 10.2.

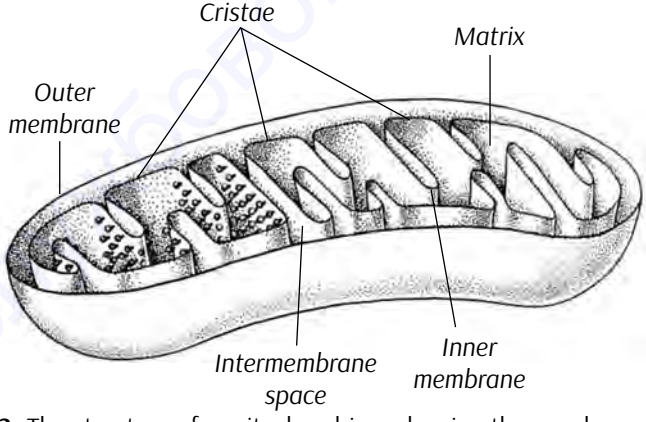


Figure 10.2. The structure of a mitochondrion, showing the membrane and intermembrane compartments. The infoldings of the inner membrane are called cristae

3. **Components of mitochondria.**
   - The **outer membrane,** which is permeable to most substrate mol-

ecules which are metabolized inside mitochondria.

- The **intermembrane space** which also presents no barrier to the passage of intermediates (i.e., substances entering or leaving the mitochondrial matrix).
- The **inner membrane** which has the components of electron transport chain and ATP synthesis embedded into its lipoprotein structure.
- The **matrix** which is bounded by the inner membrane and contains the enzymes of the tricarboxylic acid cycle, of fatty acids β-oxidation system and miscellaneous other enzymes.

III. Organization of the electron transport chain

The electron transport chain is the enzyme consequence which catalyzes the transport of reducing equivalents, that is of protons and electrons, from NADH or $FADH_2$ to molecular oxygen.

Most of the chemical energy released as the result of electron transport via the respiratory chain is used for the synthesis of ATP which is called the **oxidative phosphorylation**.

Components of electron transport chain

The electron transport chain consists of four protein complexes ***(complexes I, II, II*** and ***IV),*** which function as electron carriers, integrated in the inner mitochondrial membrane. Moreover, the chain includes two mobile carrier molecules ***ubiquinone*** (coenzyme Q) and ***cytochrome c*** (Figure 10.3).

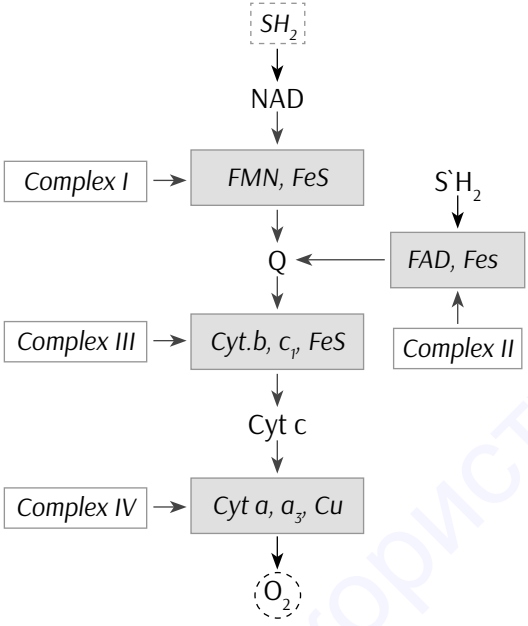


Figure 10.3. Sequence of electron carriers in mitochondrial respiratory chain

Complex I (*NADH-dehydrogenase*; *NADH: ubiquinone-oxidoreductase; NADH-coenzyme Q reductase*). This enzyme complex includes flavin mononucleotide (FMN), FeS-containing proteins and catalyzes the transfer of reducing equivalents to ubiquinone (Figure 10.4).

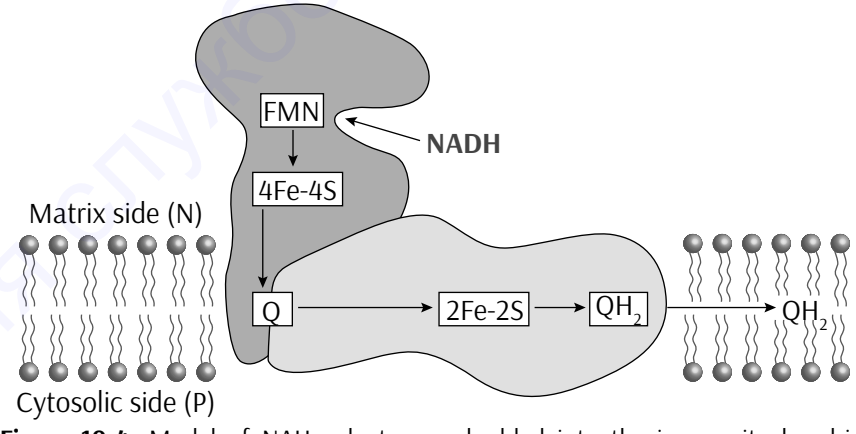
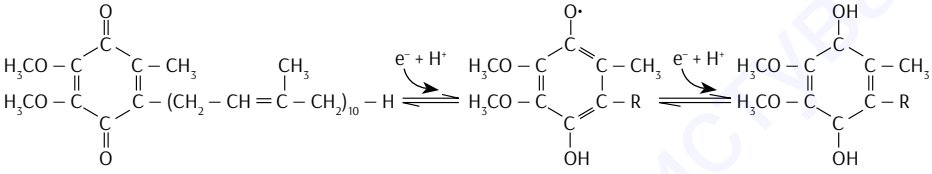


Figure 10.4. Model of NAH-reductase embedded into the inner mitochondrial

membrane

Complex II *(Succinate dehydrogenase; succinate: ubiquinone-oxidoreductase).* *Succinate dehydrogenase* actually belongs to the citric acid cycle, but it also can be considered as the part of the respiratory chain. By means of this complex the reducing equivalents derived from the oxidation of succinate, acyl-CoA and some other substrates are transferred to ubiquinone too (Figure 10.5). The complex includes FAD and FeS-containing center.



Oxidized form of coenzyme Q (Q, ubiquinone] — *Semiquinone intermediate ($Q^{\cdot -}$)* — *Reduced form of coenzyme Q (QH_2, ubiquinol)*

Figure 10.5. Coenzyme Q as proton carrier

Complex III *(Ubiquinol: cytochrome c-oxidoreductase; cytochrome c reductase).* The reduced ubiquinone ***(ubiquinol; QH_2)*** transfers electrons to complex III, which in turn delivers them to the soluble heme protein ***cytochrome c***. The complex includes cytochromes b, c_1 and FeS-protein center.

Complex IV *(Cytochrome oxidase).* Electrons supplied by the reduced cytochrome c are passed to complex IV. *Cytochrome oxidase*, in its turn, catalyzes the final transfer of the electrons to oxygen to give O^{2-}. The complex contains subunits designated as *cytochromes a, a_3* and two copper ions.

The strongly basic O^{2-} anion, produced by means of oxygen reduction with *cytochrome oxidase*, immediately binds two protons and is thereby converted to water:

$$O^{2-} + 2H^+ \rightarrow H_2O$$

10.3. Oxidative phosphorylation. ATP synthase

Oxidative phosphorylation is the process in which ATP is formed as a

result of the transfer of electrons from NADH or $FADH_2$ to O_2 by a series of carriers constituting electron transport chains in mitochondria.

The modern notion as to the mechanism of mitochondrial oxidation and ADP phosphorylation coupling was proposed by Peter Mitchell (Figure 10.6) in 1961 in the form of the so-called ***chemiosmotic hypothesis.***

Figure 10.6. Peter Mitchell (born 1920), biochemist (Great Britain). Nobel Prize (1978)

According to chemiosmotic coupling hypothesis, oxidative phosphorylation is conceptually simple, but its molecular mechanisms proved to be rather complicated.

- The flow of electrons from NADH or $FADH_2$ to O_2 through protein complexes located in the inner membrane of mitochondria leads to the pumping of protons out of the mitochondrial matrix (Figures 10.7–10.8).

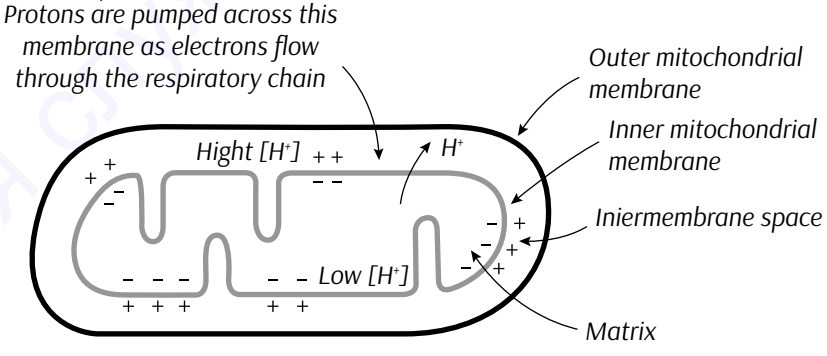


Figure 10.7. Pumping of protons out of the mitochondrial matrix coupled to electron transport in the respiratory chain

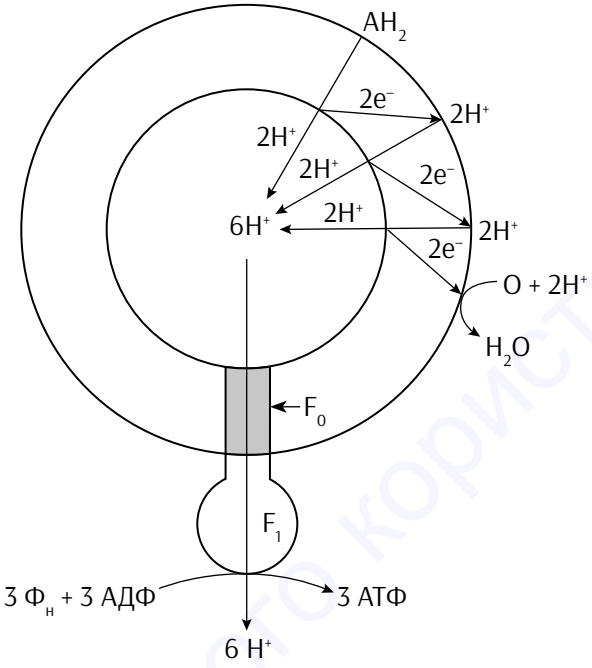


Figure 10.8. Schematic representation of chemiosmotic coupling of electron transport, proton gradient generation and ATP synthesis in mitochondria *(E. Racker, 1976)*

- Virtually, complexes I, III and IV function as **proton pumps,** which generate the formation of **proton gradient** directed across the plane of inner mitochondrial membrane. In other words, the respiratory chain consists of three electron-driven proton pumps ***(NADH-coenzyme Q reductase, cytochrome c reductase, and cytochrome oxidase)*** linked by two mobile electron carriers (coenzyme Q and cytochrome c) – Figure 10.9.

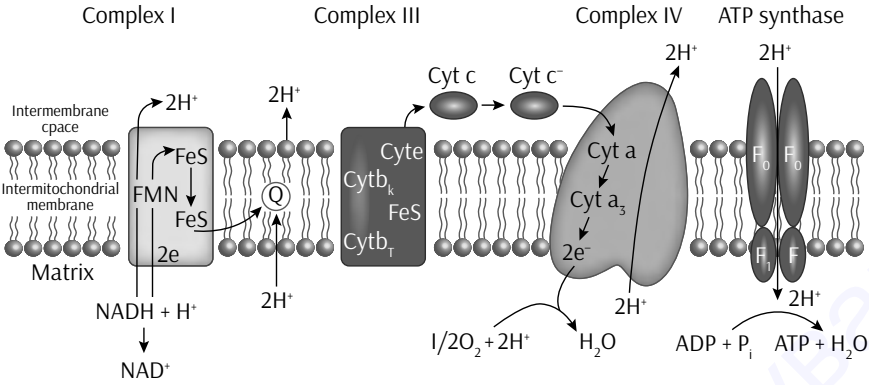


Figure 10.9. An outline of the mitochondrial electron- transport chain indicating the pathway of electron transfer and proton pumping (O. O. Mardashko, N. Ye. Yasinenko, 2003)

- The energy of transmembrane proton gradient (that is pH gradient and a transmembrane electric potential) is a driven force for the synthesis of ATP. And this event is realized by the enzyme complex **ATP synthase** located in the inner mitochondrial membrane according the equation:

$$ADP^{3-} + P_i^{2-} + H^+ \longrightarrow ATP^{4-} + H_2O$$

- The synthesis of ATP by *ATP synthase* is mediated by the reverse flux of protons across the inner mitochondrial membrane (from the intermembrane space into the matrix).
- In accordance, ATP-synthase is the H^+-transporting device. Virtually, the enzyme complex consists of two parts – a membrane-integrated *proton channel* (Fo) made up of at least 13 subunits, and a proper ATP-synthesizing *catalytic subunit* (F1) (Figure 10.10).

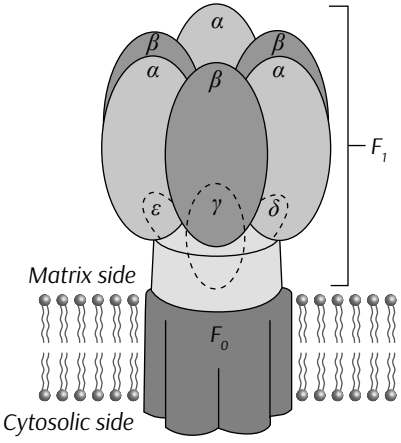


Figure 10.10. Schematic diagram of ATP-synthase showing the proton-conducting (F_0) and the ATP-synthesizing (F_1) units

The subunit composition of mitochondrial *ATP-synthase* is presented in Table 10.1.

Table 10.1. Components of the mitochondrial ATP-synthesizing complex (L. Stryer, 1995)

Subunit Subunits F,	Mass (kd)	Role Contains catalytic site	Location Spherical headpiece
F_1	378	Contains catalytic site for ATP synthesis	Spherical head piece on matrix side
α	56		
β	52		
γ	34		
δ	14		
ε	6		
Fo	25	Contains proton	Trans membrane
	21	channel	
	12		

Subunit Subunits F,	Mass (kd)	Role Contains catalytic site	Location Spherical headpiece
	8		
F_1 inhibitor	10	Regulates proton flow and ATP synthesis	Stalk between F_0 and F_1

Uncouplers of oxidative phosphorylation

There is a group of synthetic and natural substances which damage oxidative phosphorylation in mitochondria through uncoupling of biochemical relation of electron transport with ATP synthesis. Uncouplers are such chemicals as 2,4-dinitrophenol, thyroid hormone thyroxine and other amphiphilic substances.

Uncoupling agents increase the permeability of the inner mitochondrial membrane to protons and thus induce leakage of H^+ across the membrane, which results in reducing or fully collapsing of the electrochemical proton gradient across the membrane which is the driving force for the synthesis of ATP from ADP and P_i.

In the presence of uncouplers, the mitochondrial oxidation of biomolecules proceeds without concomitant ADP phosphorylation which leads to the profound pathology of the cells affected and the organism as a whole that is the case in the patients suffering from thyrotoxicosis, or *Graves' disease*.

Part 3

METABOLISM OF MAJOR CLASSES BIOMOLECULES

Chapter 11.

CARBOHYDRATE METABOLISM-1. PATHWAYS OF GLUCOSE OXIDATION

11.1. Major routes of carbohydrate metabolism

As was ascertained in the previous lectures, carbohydrates and lipids are the two principal sources of metabolic fuel for human and animal organisms.

Among different representatives of carbohydrates (monosaccharides, olygo- and polysaccharides) which are presented as food constituents in human diet, the principal immediate source of cellular chemical energy is **glucose.** The other sugars (glycogen, disaccharides, hexoses fructose and galactose) produce chemical energy in the form of ATP after converting to glucose molecules.

Most animal cells obtain glucose from the blood. Sugars are metabolized mainly as phosphate esters. So, once inside the cell, glucose molecule is phosphorylated to **glucose 6-phosphate** which gives way for the central route of glucose catabolism called **glycolysis.**

Under aerobic conditions the final product of glycolysis, which is **pyruvate,** enters mitochondria and, after conversion to C_2-metabolite, is subjected to oxidation via **citric acid cycle** (TCA cycle) to yield **ATP** in the course of oxidative phosphorylation.

Besides, glucose 6-phosphate is an intermediate of pivotal importance: it is not only a metabolite of glycolysis but also serves as a precursor for several other carbohydrates metabolic pathways that produce glycogen and phosphorylated pentoses – substances of vital biological importance.

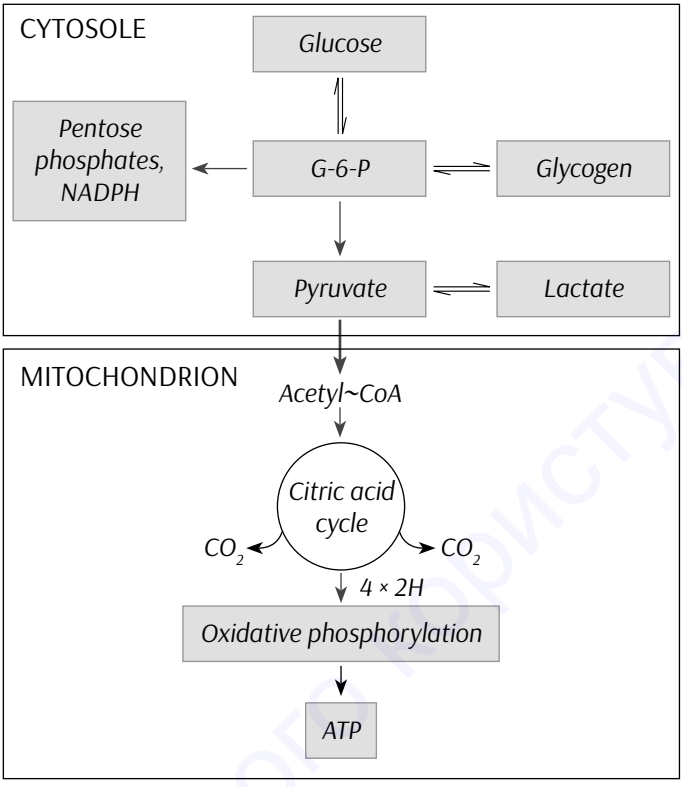


Figure 11.1. Pathways of glucose metabolism

Thus, the major routes of glucose metabolism in human and animal cells are presented in Figure 11.1.

- **Glycolysis,** that is **the sequence of enzymic reactions of glucose splitting into two molecules of pyruvate** with the concomitant production of two ATP molecules.

As was mention earlier, in aerobic cells pyruvate penetrates into mitochondria, where it is transformed to acetyl-CoA and completely oxidized to CO_2 and H_2O.

Thus, under conditions of active cellular respiration, glycolysis is the prelude to the citric acid cycle and the oxidative phosphorylation which, coupled together, harvest most of chemical energy contained in glucose molecules.

- **Glycogen synthesis,** that is the metabolic pathway which leads to the production of polysacharide which is the main storage form of glucose

in humans and animals.

- **Pentose phosphate pathway** which is the alternative route of oxidative metabolism of glucose. This consequence of reactions converts phosphorylated glucose into five-carbon sugars generating concomitantly NADPH. Ribose-5-phosphate molecules produced in the course of pentose phosphate pathway are the precursors of nucleotides needed for DNA and RNA biosynthesis. And the molecules of NADPH are the readily available currency of reducing power in most synthetic processes.

11.2. Aerobic and anaerobic oxidation of glucose. Glycolysis

Glycolysis is a catabolic pathway of glucose occuring in the cytoplasm of most eucaryotic cells.

Glycolysis has a simple balance: for every glucose molecule degraded, two molecules of pyruvate (or lactate) are formed:

$$\underset{\textit{Glucose}}{C_6H_{12}O_6} \rightarrow 2\ \underset{\textit{Pyruvate}}{C_3H_4O_3}\ (2\ \underset{\textit{Lactate}}{C_3H_6O_3}).$$

Depending on the presence of oxygen, the two kinds of glycolysis take place in human and animal cells and namely:

(a) aerobic glycolysis, which occurs under aerobic conditions that is under sufficient oxygen provision. The additional products of aerobic glycolysis are two molecules of reduced NAD^+ (NADH) and two ATP molecules (see below):

$$\underset{\textit{Glucose}}{C_6H_{12}O_6} + 2NAD^+ \rightarrow 2\underset{\textit{Pyruvate}}{C_3H_4O_3} + 2(NADH + H^+)$$

(b) anaerobic glycolysis which takes place under anaerobic conditi-ons, that is in the absence of oxygen in the cell, to yield two molecules of lactate:

$$\underset{\textit{Glucose}}{C_6H_{12}O_6} \rightarrow 2\underset{\textit{Lactate}}{C_3H_6O_3}.$$

The majority of reactions of aerobic and anaerobic glycolysis coinside till

the stage of pyruvate generation.

Stages of glycolysis

Two stages of glycolysis can be distinguished and they are:

(1) The conversion of glucose to triose phosphates. This stage involves a series of reactions that requires the expenditure of two molecules of ATP for each molecule of hexose that is split:

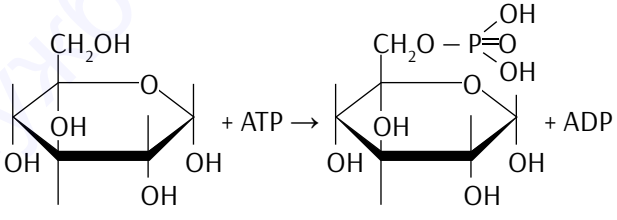
$$\underset{(6C)}{\text{Glucose}} \longrightarrow 2(\underset{(3C)}{\text{Glyceraldehyde 3-phosphate}})$$

(2) The conversion of triose phosphate to pyruvate (or lactate, in anaerobic conditions). During this stage, two molecules of ATP are produced for each molecule of triose phosphate converted to pyruvate, or four molecules of ATP per molecule of hexose used.

11.3. Enzymatic reactions of glycolysis. Regulation of glycolysis

(1) Phosphorylation of glucose molecule in ATP-dependent reaction to give *glucose 6-phosphate* (G6P):

$$\alpha\text{-D-Glucose} + \text{ATP} \longrightarrow \text{D-Glucose 6-phosphate} + \text{ADP}$$



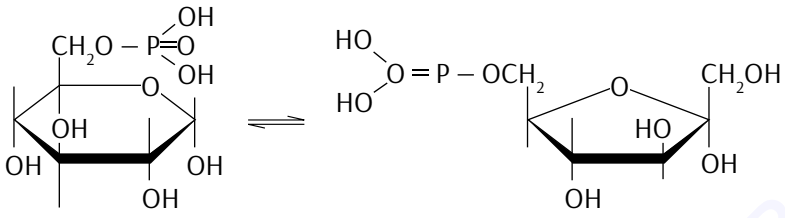
α-D-Glucose *Glucose 6-phosphate*

The reaction is catalyzed by enzyme designated as *hexokinase*. An alternative enzyme that catalyzes the phosphorylation of glucose is *glucokinase*.

(2) The second reaction of glycolysis is the isomerization of glucose

6-phosphate into *fructose 6-phosphate* (F6P):

D-Glucose 6-phosphate ↔ D-Fructose 6-phosphate;

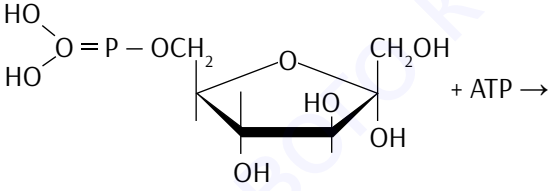


D-Glucose 6-phosphate *D-Fructose 6-phosphate*

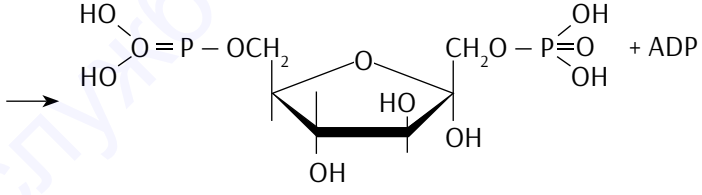
The reaction is catalyzed by the enzyme *phosphoglucose isomerase.*

(3) The third reaction of glycolysis is another phosphorylation step which leads to *fructose 1,6-bisphosphate (F1,6BP):*

D-Fructose 6-phosphate + ATP ⟶ Fructose 1,6-bisphosphate + ADP



D-Fructose 6-phosphate

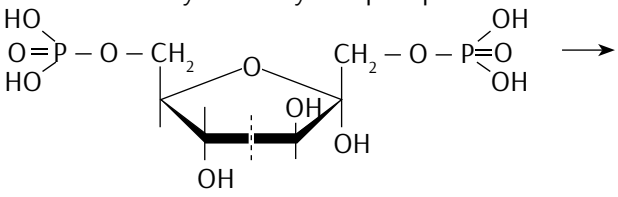


D-Fructose 6-phosphate

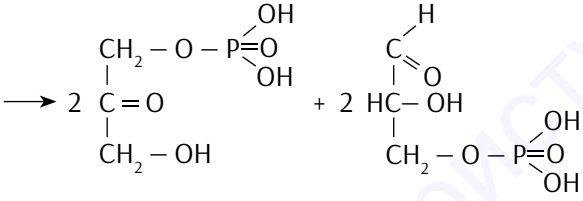
The enzyme that catalyzes the reaction is *phosphofructokinase.*

(4) The next glycolytic reaction is the splitting of fructose 1,6- bisphosphate to give two phosphotrioses, and namely: *dihydroxyacetone phosphate (DHAP)* and *glyceraldehyde 3-phosphate (G3P):*

Fructose 1,6-bisphosphate → Dihydroxyacetone phosphate +
+ Glyceraldehyde 3-phosphate



D-Fructose 1,6-bisphosphate



Dihydroxyacetone phosphate *Glyceraldehyde 3-phosphate*

The reaction is catalyzed by the enzyme *aldolase*.

As can be seen from the reaction equation presented, the mechanism of transformation includes the splitting of covalent bond between C_3 and C_4 in fructose 1,6-bisphosphate molecule.

Upon the step of two phosphotrioses formation the first stage of glycolysis is terminated.

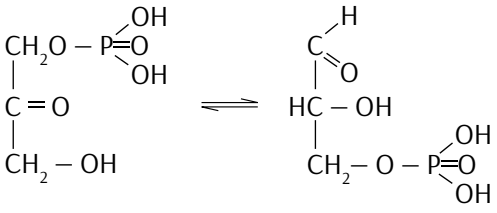
The following enzymatic reactions of glycolytic pathway comprise the steps which lead to the production of two pyruvate molecules and include the oxidation of glyceraldehyde 3-phosphate and chemical energy conservation.

5) **Glyceraldehyde 3-phosphate** is on the direct pathway of glycolysis. and dihydroxyacetone phosphate is not. But **dihydroxyacetone phosphate** can be readily converted into glyceraldehyde 3-phosphate because these compounds are isomers: they are ketose and aldose correspondingly.

So, the next reaction of glycolysis is isomerization and it is catalyzed by ***triose phosphate isomerase***.

Because the efficient removal of glyceraldehyde-3-phosphate in subsequent reactions takes place, the equilibrium is shifted from ketose to aldose:

Dihydroxyacetone phosphate → Glyceraldehyde 3-phosphate



Dihydroxy acetone phosphate *Glyceraldehyde 3-phosphate*

Thus, by the sequential action of *aldolase* and *triose phosphate isomerase* two molecules of glyceraldehyde 3-phosphate are formed from one molecule of fructose 1,6-bisphosphate:

Fructose 1,6-bisphosphate → 2 glyceraldehyde 3-phosphate.

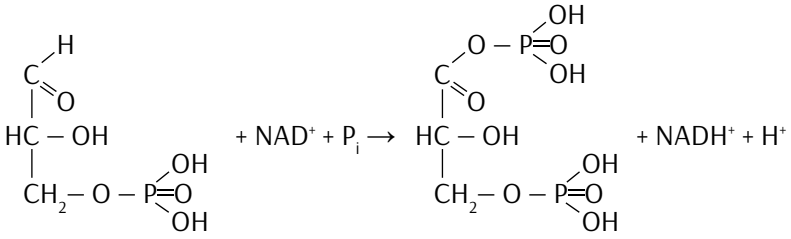
Summarily, *the preceding reactions resulted in transformation of one glucose molecule into two molecules of glyceraldehyde 3-phosphate* – the first stage of glycolysis (see above). No available chemical energy has yet been obtained.

The next reactions of glycolysis harvest some of energy contained in two molecules of glyceraldehyde 3-phosphate. The first step in this sequence is transformation of glyceraldehyde 3-phosphate to 3-phosphoglycerate. This complex process comprise reactions **(6)** and **(7)** that follow.

6) Transformation of glyceraldehyde 3-phosphate to **1,3-bisphosphoglycerate (1,3-BPG)** that occurs in the oxidation-reduction reaction**.** During this reaction oxidation and phosphorylation are coupled and a high-potential phosphorylated compound is generated:

2 Glyceraldehyde 3-phosphate + 2 NAD^+ + 2 P_i →

→ 2 1,3-Bisphosphoglycerate + 2 NADH + $2H^+$



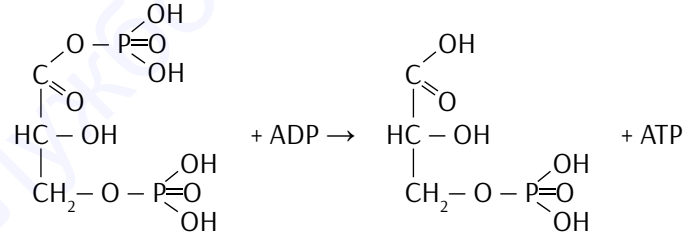
Glyceraldehyde 3-phosphate *1,3-Bisphosphoglycerate*

The reaction is catalyzed by **glyceraldehyde 3-phosphate dehydrogenase.**

The reducing equivalents, that is 2(NADH + H^+), formed in this reaction, are transferred to mitochondria to give **3 molecules of ATP** generated per molecule of NADH oxidized in electron transport chain.

7) Transformation of 1,3-bisphosphoglycerate to **3-phosphoglycerate (3-PG)**. An ATP molecule is formed. This is the first step in glycolysis that generates ATP and it is called ***substrate-level oxidative phosphorylation*** (in contradistinction to ***oxidative phosphorylation coupled to mitochondrial electron transport*** – Lecture 4).

2 1,3-Bisphosphoglycerate + 2 ADP ⟶ 2 3-Phosphoglycerate + 2 ATP



1,3-Bisphosphoglycerate *3-Phosphoglycerate*

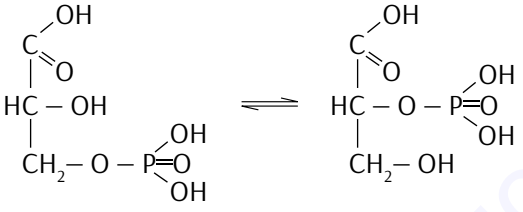
The enzyme which catalyzes the reaction is called ***phosphoglycerate kinase.***

Pay attention that in the prior glycolytic steps two molecules of 1,3-DPG were formed from each molecule of glucose. **Therefore, owing to this re-**

action, two ATP molecules are now formed per original molecule of glucose.

8) The rearrangement of **3-phosphoglycerate** to yield **2-phosphoglycerate (2-PG):**

2 3-Phosphoglycerate ↔ 2 2-Phosphoglycerate

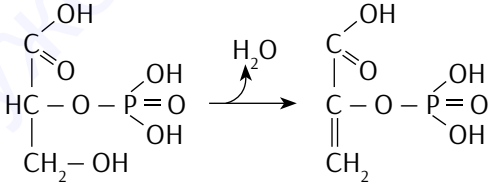


3-Phosphoglycerate *2-Phosphoglycerate*

The reaction is reversible. It is catalyzed by ***phosphoglycerate mutase***. In general, ***mutases*** are enzymes that catalyze the intramolecular shift of a chemical group, such as a phosphoryl group.

9) In this reaction an enol isomer of phosphopyruvate is formed as a result of the dehydration of 2-phosphoglycerate. The reaction is catalyzed by ***enolase*** and the product is **phosphoenolpyruvate (PEP).**

2 2-Phosphoglycerate → 2 Phosphoenolpyruvate + 2 H_2O

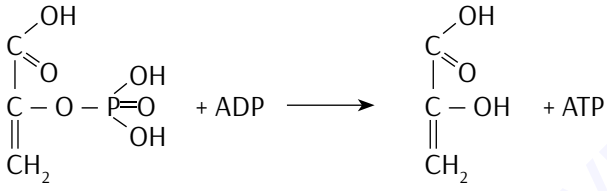


2-Phosphoglycerate *Phosphoenolpyruvate*

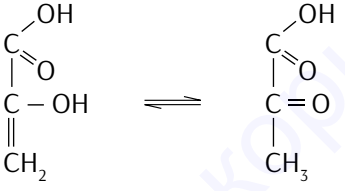
An enolphosphate has a high phosphoryl transfer potential, and therefore a phosphoryl group can be readily donated to ADP which takes place in the next step of glycolysis.

10) This is the final reaction of aerobic glycolysis**.** In the reaction, **pyruvate** is formed with ATP molecule concomitantly generating, and this is another example of ***substrate-level oxidative phosphorylation.***

2 Phosphoenolpyruvate + 2 ADP → 2 Pyruvate + 2 ATP



Phosphoenolpyruvate *Pyruvate*



Pyruvate (enol-form) *Pyruvate (enol-form)*

The reaction is catalyzed by ***pyruvate kinase.***

As far as two molecules of pyruvate are formed from each molecule of glucose that enters glycolytic route, **two molecules of ATP** are produced in this step.

This reaction concludes the multistep pathway of glycolysis that occurs in aerobic conditions.

Energy balance of glycolysis

A summary of biochemical steps of glycolysis in which ATP is consumed or generated is given in Table 11.1.

Table 11.1. Consumption and generation of ATP in glycolysis

Reaction	ATP change per glucose

Glucose → glucose 6-phosphate Fructose 6-phosphate → fructose 1,6-bisphosphate (2) 1,3-Bisphosphoglycerate → (2) 3-phosphoglycerate (2) Phosphoenolpyruvate → (2) Pyruvate	–1 –1 +2 +2
Glucose → (2) Pyruvate	∑ = + 2

Basing on this, the net reaction in the transformation of glucose into pyruvate is:

$$C_6H_{12}O_6 + 2NAD^+ + 2(ADP + P_i) \rightarrow 2C_3H_4O_3 + 2(NADH + H^+) + 2ATP$$

Summarizing, we conclude that **two molecules of ATP are generated in the course of aerobic glycolysis.** Additional chemical energy is concentrated inside two molecules of pyruvate, and it can be liberated as a result of the further triose oxidation which makes up a subsequent stage of the aerobic oxidation of glucose (see below).

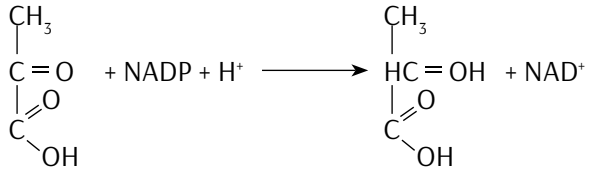
Anaerobic glycolysis

In aerobic conditions, reducing equivalents generated during the glycolysis ($2NADH + 2H^+$ per glucose molecule) enter the mitochondrial matrix where are subjected to oxidation by ***NADH-dehydrogenase*** of electron transport chain.

But in the absence of oxygen, i.e. under **anaerobic conditions,** the final electron acceptor for mitochondrial respiratory chain, that is O_2, is missing.

In this metabolic situation, in order to continue the degradation of glucose by glycolysis and to generate ATP, the reduced NAD^+ (so-called "glycolytic NAD^+") has to be continuously reoxidized. Since this can no longer occur in mitochondria, the majority of animal cells dispose of these reducing equivalents by reducing pyruvate to lactate:

$$Pyruvate + NADH + H^+ \leftrightarrow Lactate + NAD^+$$



Pyruvate *Lactate*

The reaction is catalyzed by ***lactate dehydrogenase.***

This reaction is an essential step in metabolic pathway which is summarily called **anaerobic glycolysis:**

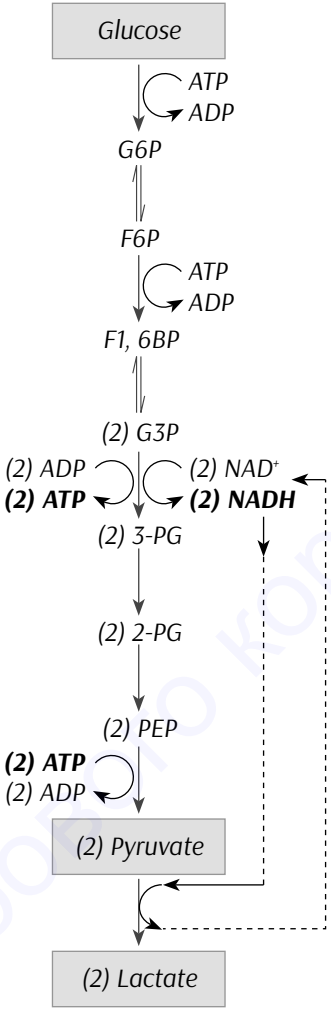
$$C_6H_{12}O_6 \rightarrow 2C_3H_6O_3$$

The anaerobic glycolysis is characteristic, in particular, for skeletal muscle during the hard physical activity. In these metabolic conditions, the lactate molecules are accumulated in muscle tissue. At the period of relaxation, lactate is released from muscle cells and is transferred by blood into liver, where it is used for glycogen synthesis.

Metabolic Chart of Glycolysis

The successive enzymatic reactions of glucose transformation to give pyruvate or lactate constitute the metabolic chart of glycolytic pathway.

The metabolic chart of glycolysis is presented in Figure 5.2.



Fioure 11.2. Metabolic Chart of Glvcoksis

Enzymatic control of glycolysis

Three regulatory enzymes take part in the control of glycolysis. They are:

- **Hexokinase.**
- **Phosphofructokinase.**
- **Pyruvate kinase.**
  - ***Hexokinase.*** An enzyme catalyzes the key reaction of glucose met-

abolic activation and constitutes the first control site of glycolysis rate regulation. Hexokinase in allosteric way is inhibited by glucose 6-phosphate. Thus, the intracellular accumulation of G6P limits utilization of glucose which is the manifestation of vital metabolites economy.

- ***Phosphofructokinase.*** This is the major regulatory enzyme of glycolysis. The allosteric **inhibitors** of an enzyme are **ATP** and **citrate**, one of the principal metabolites of TAC. Because of this control mechanism, the decrease of ATP and citrate stimulates glycolysis. On the other hand, glucose splitting is activated when the intracellular energy charge falls.
- ***Pyruvate kinase*** is **inhibited** by **ATP, acetyl-CoA** and **fatty acids**. Similarly to the motif observed in phosphofructokinase this kind of metabolic control is directed to the restriction of glucose and glycolytic intermediates consuming when ATP and other high-energy metabolites are abundant.

11.4. Aerobic oxidation of glucose. Oxidative decarboxylation of pyruvate

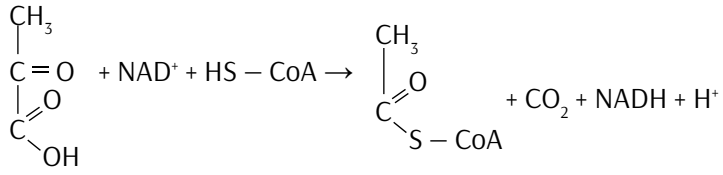
Aerobic oxidation of glucose includes three stages, and namely:

I – Glycolytic stage, which constitutes the conversioin of glucose into two molecules of ***pyruvate:***

$$\text{Glucose} \longrightarrow 2 \text{ moles Pyruvate}$$

II – Oxidative decarboxylation of pyruvate. The products of reaction are acetyl (C_2) residues attached by enrgy-rich thioester bond to SH-group of coenzyme A – ***acetyl-CoA:***

$$\text{Pyruvate} + \text{CoA-SH} + NAD^+ \longrightarrow \text{Acetyl-CoA} + CO_2 + \text{NADH} + H^+$$



Pyruvate *Acetyl-CoA*

This irreversible funneling of pyruvate, the glycolytic product, into the citric acid cycle is catalyzed by ***pyruvate dehydrogenase complex* (PDH).**

PDH is very large multienzyme complex which consists of three different enzymes catalyzing partial steps of overall complicated process. They are:

- ***pyruvate dehydrogenase*** properly;
- ***dihydrolipoamide acetyltransferase*;**
- ***dihydrolipoamide dehydrogenase.***

The PDH complex of the bacterium Escherichia Coli, that is particularly well characterized, consists of a total of 60 polypeptides. It has a molecular mass of 5.3×10^6 and a diameter of more than 30 nm, making it larger than a ribosome.

The five different coenzymes are associated in different ways with the various protein components of the complex. These coenzymes are: **thiamine diphosphate**, **lipoamide, FAD, NAD^+** and **CoA.**

III – Citric acid cycle serves as the concluding stage of aerobic glucose oxidation. In a citric acid cycle, **acetyl-CoA,** derived from pyruvate, as also from fatty acids or amino acids catabolism, is completely oxidized to CO_2. The reducing equivalents detached are transported into mitochondrial respiratory chain to yield H_2O and generate ATP as the result of oxidative phosphorylation.

The three stages appointed constitute the overall process of glucose aerobic oxidation which can be represented by a following total equation:

$$C_6H_{12}O_6 + 6O_2 + 38\ ADP + 38\ P_i \rightarrow 6CO_2 + 6H_2O + 38\ ATP$$

11.5. Pentose phosphate pathway

General characteristics

Pentose phosphate pathway is the alternative metabolic route of glucose oxidation which supplies cells with reduced $NADP^+$ **(NADPH)** and **ribose 5-phosphate** required for many biosynthetic processes.

Pentose phosphate pathway is also known as the hexose monophosphate pathway (HMP), or the pentose shunt. Like glycolysis, HMP begins with glucose 6-phosphate and its enzymes and metabolites are located in the cytoplasm.

The pathway is most active in the liver, adipose tissue, the mammary glands and the adrenal cortex. In the tissues appointed, HMP provides precursors, that are needed for an array of vital biosyntheses:

- **Reduced nicotinamide adenine dinucleotide phosphate (NADPH + H^+)** serves as hydride ion donor for reductive biosyntheses, particularly for fatty acids formation as the lipogenesis step.
- **Ribose 5-phosphate** is a necessary precursor in nucleic acids (DNA, RNA) and certain nucleotide coenzymes formation.

Reactions of pentose phosphate pathway

The HMP metabolic route is a rather complicated collection of enzymatic reactions, which expression differs depending on the tissue and the prevailing requirements of cell metabolism in NADPH or ribose 5-phosphate. In general, there are two branches of HMP:

- the ***oxidative branch*,** which converts glucose 6-phosphate to ribulose 5-phosphate, yielding two molecules of NADPH;
- the ***regenerative branch*,** or ***branch of isomerizations*,** which produces three-, four-, five-, six- and seven-carbon sugars in a series of nonoxidative reactions.

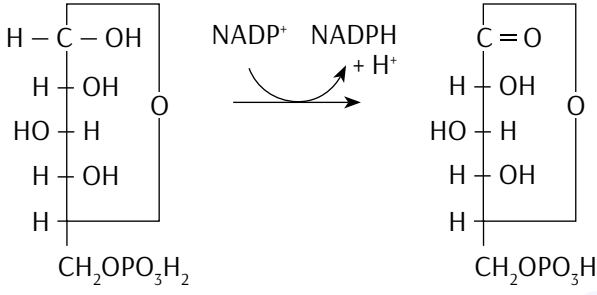
Oxidative branch of HMP

This segment of HMP uses glucose 6-phosphate as a substrate and includes two oxidations catalyzed by *NADP-linked dehydrogenases*.

(1) The oxidation of **glucose 6-phosphate (G6P)** to give **6-phosphogluconate.** The reaction is catalyzed by ***glucose 6-phosphate dehydrogenase*** and comprises two steps:

- the dehydrogenation of **G6P** at C-1**,** the product is **6-phosphoglucono-δ-lactone:**

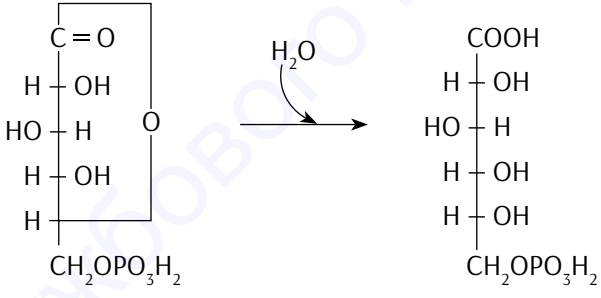
Glucose 6-phosphate + $NADP^+$ ⟶ 6-phosphoglucono-δ-lactone + NADPH + H^+



Glucose 6-phosphate *6-Phosphoglucono-δ-lactone*

- the hydrolysis of **6-phosphoglucono-δ-lactone** to **6-phosphogluco-nate:**

6-Phosphoglucono-δ-lactone + H_2O ⟶ 6-phosphogluconate

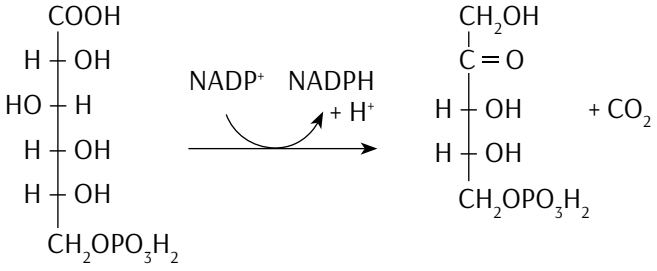


6-Phosphoglucono-δ-lactone *6-Phosphogluconate*

This step requires additional enzyme, ***lactonase.***

(2) The oxidative decarboxylation of **6-phosphogluconate** to yield **ribuloso 5-phosphate:**

6-Phosphogluconate + $NADP^+$ ⟶ ribuloso 5-phosphate + CO_2 + NADPH + H^+



6-Phosphogluconate *Ribuloso 5-phosphate*

To summarize reactions of HMP oxidative branch gives:

$$\text{Glucose 6-phosphate} + 2NADP^+ + H_2O \rightarrow \text{ribuloso 5-phosphate} + 2(NADPH + H^+) + CO_2$$

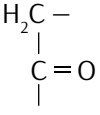
Regenerative (isomerization) branch of HMP

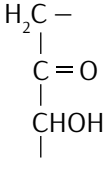
This part of HMP includes the interconversions of the various sugar phosphates. The intermediates of the branch are:

- five-carbon sugars **ribose-5-phosphate** and **xylulose 5-phosphate;**
- four-carbon sugar **erythrose 4-phosphate**;
- three-carbon sugar **glyceraldehyde 3-phosphate,** which can be supplied by glycolysis;
- seven-carbon sugar **sedoheptulose 7-phosphate;**
- six-carbon sugar **fructose 6-phosphate,** which constitutes the linkage of HMP with glycolysis.

There are two enzymes essential for HMP, and namely:

- ***transketolase*** (TDP-dependent enzyme), that transfers two-carbon units;
- ***transaldolase*,** that transfers three-carbon units.





Trans ketolase-transported group *Trans aldolase-transported group*

The reactions catalyzed by these special enzymes of HMP can be pre-

sented as:

$$C_5 + C_5 \xrightleftharpoons{\text{Transketolase } (C_2)} C_3 + C_7$$

$$C_7 + C_3 \xrightleftharpoons{\text{Transketolase } (C_3)} C_4 + C_6$$

$$C_5 + C_4 \xrightleftharpoons{\text{Transketolase } (C_2)} C_3 + C_6$$

The over-simplified representation of the two branches of pentose phosphate pathway ant its relation to glycolysis can be given as follows (Figure 11.3).

Glucose 6-phosphate dehydrogenase (G6PD) deficiency

Red blood cells (erythrocytes) depend upon the pentose phosphate pathway for the formation of NADPH, which the cells require to maintain glutathione in a reduced state. The reduced glutathione is involved in maintaining the integrity of the red cell membrane.

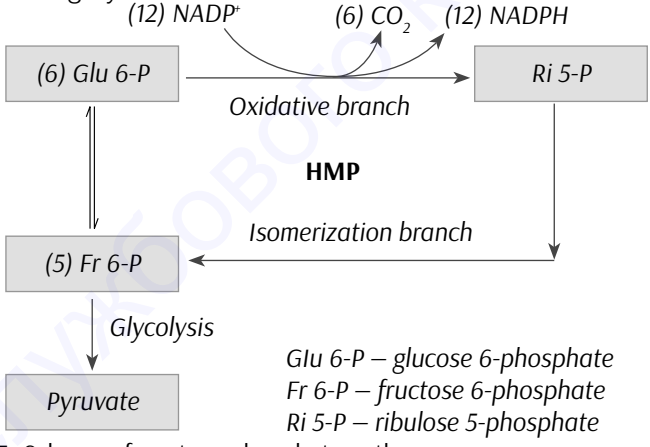


Figure 11.3. Scheme of pentose phosphate pathway

G6PD activity may be genetically absent or may be present as a partially active variant. In affected individuals, many oxidizing substances (e.g., the antimalarial drug primaquine, aspirin, sulfonamides, nitrofurans, phenacetin, and, in some persons, Fava beans – Vicia faba) will cause hemolysis of red cells, leading to a hemolytic anemia.

Chapter 12.

CARBOHYDRATE METABOLISM-2. GLYCOGEN METABOLISM. GLUCONEOGENESIS

12.1. Glycogen metabolism. Glycogen-storage diseases

Glycogen: structure and properties

Glycogen is a very large, highly branched polymer, formed of glucose residues.

Inside main, unbranched lines of glycogen, monosaccharide units are linked by α-1,4-glycosidic bonds. Branches arise by α-1,6-glycosidic bonds at about every tenth residue.

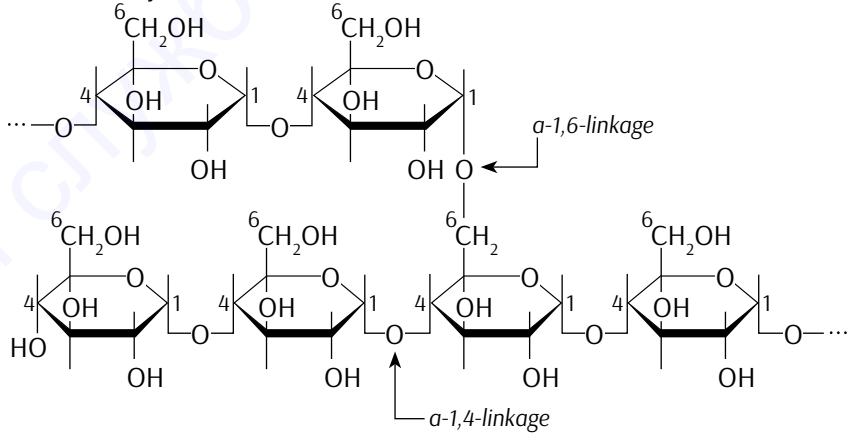


Figure 12.1. Fragment of glycogen molecule

The whole glycogen molecule includes up to 50,000 glucose residues with molecular mass of up to 10^7 Da.

In animals, glycogen serves as a carbohydrate reserve, from which glucose phosphates and glucose itself can be released as a response to physiological requirements.

The presence of glycogen in human and animal tissues provides the storage form of metabolic fuel readily available between meals and especially needed during muscular activity. Moreover, glucose is virtually the only fuel used by the brain.

The human body can store up to 500 g of glycogen, predominantly in the muscle tissue and in the liver. Glycogen is present in the cytosol of hepatocytes and myocytes in the form of granules ranging in diameter from 10 to 40 nm. In addition to glycogen these granules contain the enzymes necessary for synthesis and degradation of the polysaccharide as well as the regulatory proteins.

The concentration of glycogen is higher in liver than in muscles, but more glycogen is stored in skeletal muscle because of its total much greater mass.

The glucose in the body fluids of an average 70-kg man has an energy content of only 40 kcal, whereas the total body glycogen has an energy content of more than 600 kcal.

Glycogen degradation (glycogenolysis)

1. Phosphorolysis of glycogen

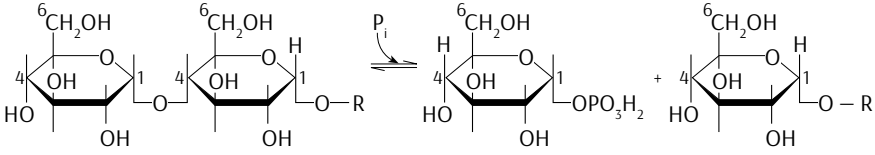
(1) Phosphorolytic cleavage of terminal α-1,4-glycosidic bonds.

The rate-limiting step in glycogen catabolism is the phosphorolysis of glycogen by ***glycogen phosphorylase.*** The enzyme has a molecular mass of 97 kD and exists in two interconvertible forms: an active ***phosphorylase a*** and an inactive ***phosphorylase b.***

The active form of enzyme cleaves **glycogen molecules** with the help of orthophosphate **(H_3PO_4)** to yield a phosphorylated sugar **glucose 1-phosphate (G1P):**

$$(C_6H_{10}O_5)_n + H_3PO_4\ (P_i) \rightarrow (C_6H_{10}O_5)_{n-1} + G1P$$

Glycogen$_{n\ residues}$ *Glycogen$_{(n-1)\ residues}$*



Glycogen (n residues) *Glucose 1-phosphate* *Glycogen (n-1 residues)*

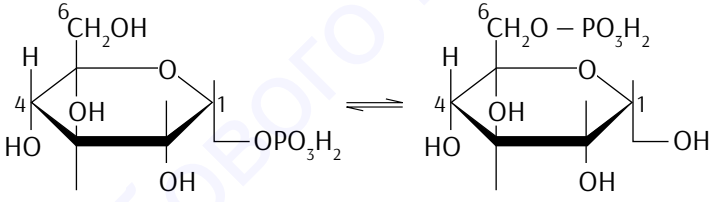
It can be seen that, under ***phosphorylase*** catalysis, orthophosphate splits the glycosidic linkage between C-1 of the terminal glucose residue and C-4 of the adjacent one. **Glucose 1-phosphate** residues release, one after another, from the non-reducing ends of glycogen molecule.

The phosphorolysis results in a gradually shrinking of the linear polysaccharide chain.

(2) Conversion of glucose 1-phosphate into glucose 6-phosphate.

Glucose 1-phosphate released from glycogen can readily be isomerized to give **glucose 6-phosphate,** an important metabolic intermediate:

Glucose 1-phosphate ↔ glucose 6-phosphate



Glucose 1-phosphate *Glucose 6-phosphate*

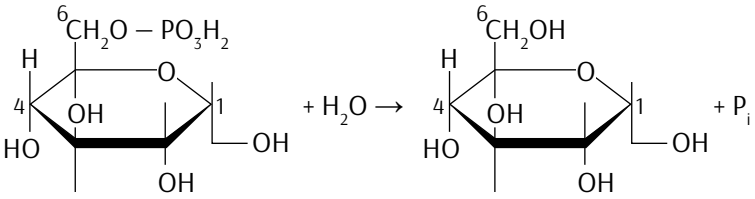
The reaction is catalyzed by ***phosphoglucomutase,*** enzyme which makes intramolecular shift in hexose phosphate.

(3) Hydrolysis of glucose 6-phosphate.

A major function of the liver is to maintain a relatively constant level of glucose in the blood. This is accomplished owing to the presence in hepatocytes of a hydrolytic enzyme, ***glucose 6-phosphatase.***

Glucose 6-phosphatase catalyzes a reaction:

Glucose 6-phosphate + H_2O ⟶ glucose + H_3PO_4 (P_i)



Glucose 6-phosphate *Glucose*

Phosphorylated glucose cannot penetrate cellular membranes and diffuse out of cells. But glucose proper is readily released into blood plasma. It is taken up primarily by the brain and by skeletal muscle and used as principal metabolic fuel.

2. Removal of branches in glycogen molecule

Glycogen is degraded by ***phosphorylase*** alone to a limited extent. The α-1,6-glycosidic bonds at the branched points are not succeptible to *phosphorylase*. Therefore, cleavage by *phosphorylase* stops when this enzyme approaches the branched section of polysaccharide chain.

At this stage another biochemical mechanism is needed, and it is brought about by additional enzyme. The reactions, that make up the removal of branches, are catalyzed by a special ***debranching enzyme,*** which has two distinct enzymatic activities (Figure 12.2):

- As a ***glucosyl transferase,*** it transfers a block of three glycosyl residues from the outer branch onto the unbranched chain terminus. A single glucosyl residue attached to C-6 is left.
- As an ***α-1,6-glycosidase,*** it removes the single residues on C-6 to yield free glucose molecules.

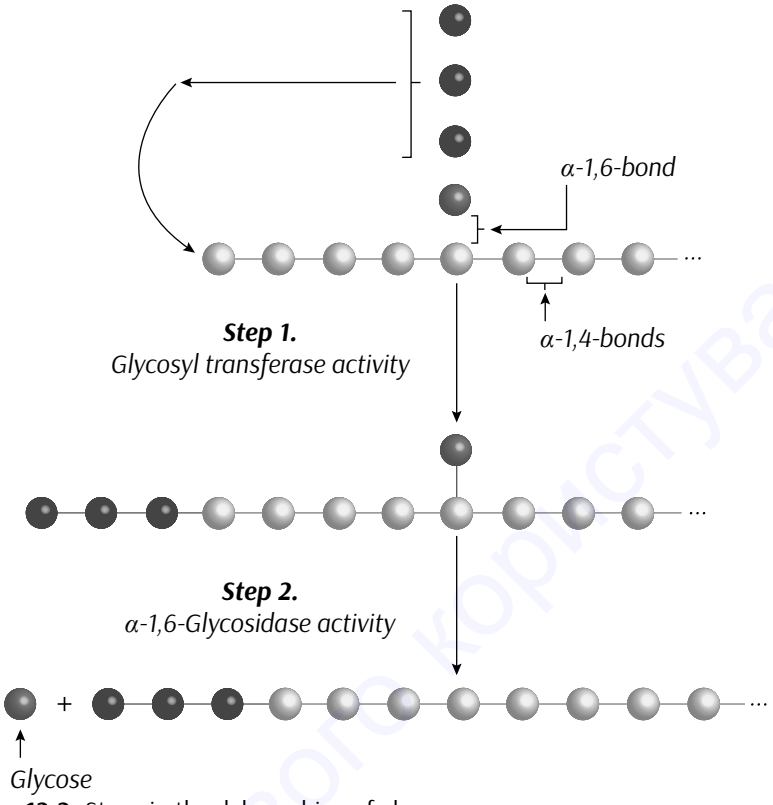


Figure 12.2. Steps in the debranching of glycogen

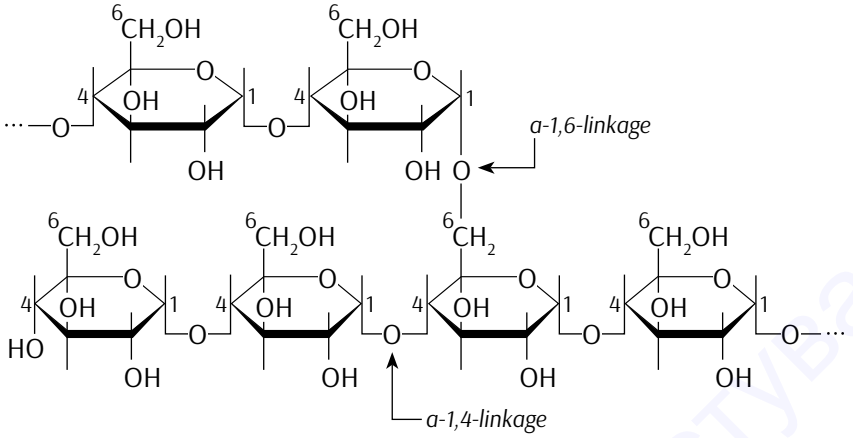
Glycogen synthesis (glycogenesis)

1. Synthesis of nucleotide precursor.

To use glucose molecules for glycogen synthesis, the cell need an activated form of the sugar which is its nucleotide derivative **UDP-glucose.**

This nucleotide precursor, the glucose donor in glycogen biosynthesis, is formed from **glucose 1-phosphate** and **uridine triphosphate (UTP):**

$$\text{Glucose 1-phosphate} + \text{UTP} \rightarrow \text{UDP-glucose} + PP_i$$



The reaction is catalyzed by an enzyme ***UDP-glucose pyrophosphorylase***.

As a result of the reaction, the C-1 atom of the glucose becomes activated because its hydroxyl group is esterified to the diphosphate moiety of UDP.

2. Synthesis of glycogen molecule.

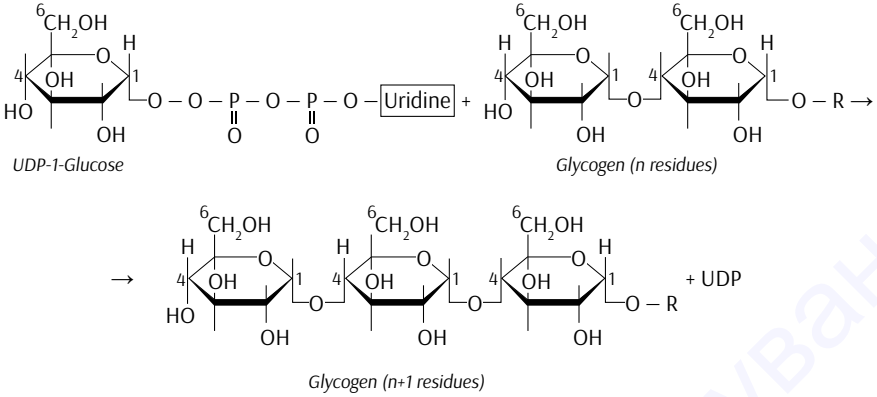
(1) Formation of unbranched (amylose) chains.

The synthesis of new glycogen molecule requires the presence of glucosyl residudes from UDP-glucose and already existing glycogen chains.

The glucosyl residues are successively transferred to a C-4 atom of the pre- existing non-reducing end of glycogen molecule. The new α-1,4-glycosidic linkages are formed and the chain is elongating:

$$\text{UDP-1-glucose} + (C_6H_{10}O_5)_n \rightarrow \text{UDP} + (C_6H_{10}O_5)_{n+1}$$

Glycogen$_{n\ residues}$ *Glycogen$_{n+1\ residues}$*



The reaction is catalyzed by ***glycogen synthase (UDP-glycogen transferase).***

(2) Formation of branched chains of glycogen.

Glycogen synthase catalyzes only the synthesis of α-1,4 linkages. To form the α-1,6-linkages that make glycogen a branched polymer, another enzyme is needed. It is special ***branching enzyme (glycosyl – 4 : 6-transferase).***

Branching occurs when the length of the unbranched chain constitutes at least eleven glucose residues. A branch is created by transferring the block of seven or so residues to C-6 hydroxyl group of a glucose residue that is located in a more interior syte of the chain (Figure 12.3).

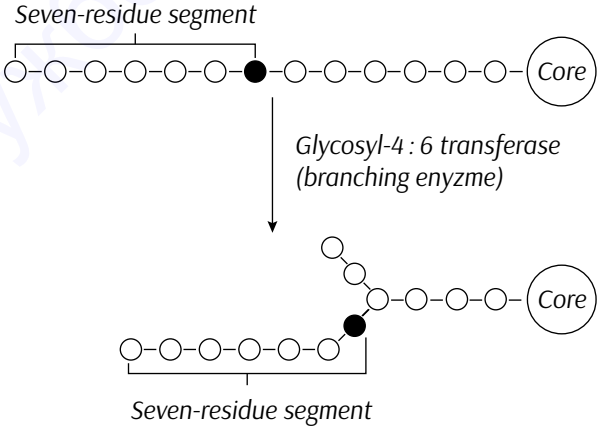


Figure 12.3. Formation of branches in glycogen molecule. A 7-unit segment is transferred onto the main chain at position C-6 of a residue that is at least 4 residues from the next branch

Regulation of glycogen metabolism

A. Regulation of glycogenolysis.

(1) ***Glycogen phosphorylase.*** The rate-limiting step in glycogen catabolism is the phosphorylation of glycogen by ***glycogen phosphorylase (phosphorylase).***

The enzyme is a dimer or tetramer which can be in catalytically inactive form ***(phosphorylase b)*** and an active form ***(phosphorylase a).***

- The ***b*** form is converted to the ***a*** form by phosphorylation of a specific serine group on each subunit:

Phosphorylase **b** + 2ATP → phosphorylase **a** + 2ADP

This covalent modification is catalyzed by enzyme ***phosphorylase kinase***.

- Cleavage of the phosphate from ***a*** form by ***protein phosphatase*** results in deactivation of enzyme that reconverts to the ***b*** form.

(2) ***Phosphorylase kinase***, that activates glycogen phosphorylase, is a regulatory enzyme in itself. It is a very large protein with a molecular mass 1.200 kD and the subunit composition $(\alpha\beta\gamma\delta)_4$. The enzyme also exists in inactive and active forms, which are interconverted by phosphorylation-dephosphorylation:
  - phosphorylation of the enzyme at the expence of ATP catalyzed by ***cyclic AMP (cAMP)***-dependent ***protein kinase A*** converts the ***phosphorylase kinase*** to the active form;
  - dephosphorylation of ***phosphorylase kinase*** by a ***phosphatase*** inactivates the enzyme.

(3) ***Protein kinase A*** is a tetramer made up of two catalytic subunits **(C)** and two regulatory subunits **(R),** that makes up a subunit structure of an enzyme $\mathbf{C_2R_2}$**.**

The binding of four molecules of cAMP by regulatory subunits $\mathbf{(R_2)}$ caus-

es these subunits to dissociate from catalytic subunits **(C_2):**

$$C_2R_2 + 4\ cAMP \rightarrow C_2 + R_2 - cAMP_4$$

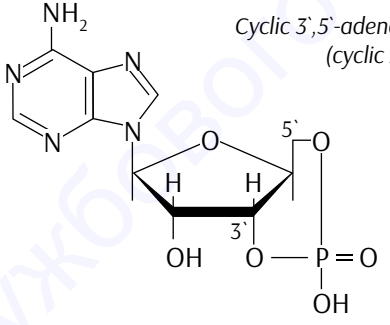
The free C subunits are catalytically active and can phosphorylate ***phosphorylase kinase.***

Phosphorylation cascade

Thus, the enzymes of glycogen catabolism undergo a sequential covalent modification by means of phosphorylation. This process provides a very large amplification of the initial biochemical stimulus. This type of amplification system is called an **enzyme cascade,** or a **signal transduction cascade.**

To switch the cascade, it needs to produce **cAMP** which is made by an enzyme ***adenylyl cyclase:***

$$ATP \rightarrow cAMP + PP_i$$



Cyclic 3`,5`-adenosine monophosphate (cyclic AMP; cAMP)

In its turn, ***adenylyl cyclase*** is activated by circulating hormones, especially **epinephrine** and **glucagon,** which stimulate the glycogen phosphorolysis (see below). Thus, cyclic AMP serves as the intracellular messenger ("second messenger") in mediating the physiologic action of many hormones (Figure 12.4).

The *adenylyl cyclase* is the protein complex, which is located in the plasmatic membranes of the cells, which are sensitive to hormones action, such as hepatocytes, myocytes, adipocytes (see Lecture 13). Thus, as can be seen

from the scheme presented, the interaction of hormone (epinephrine, glucagon etc.) with cellular membrane, switches on the phosphorylation cascade, that includes the subsequent activation of the catalytic activities of four enzymes, and namely *adenylyl cyclase, protein kinase A, phosphorylation kinase and glycogen phosphorylation.*

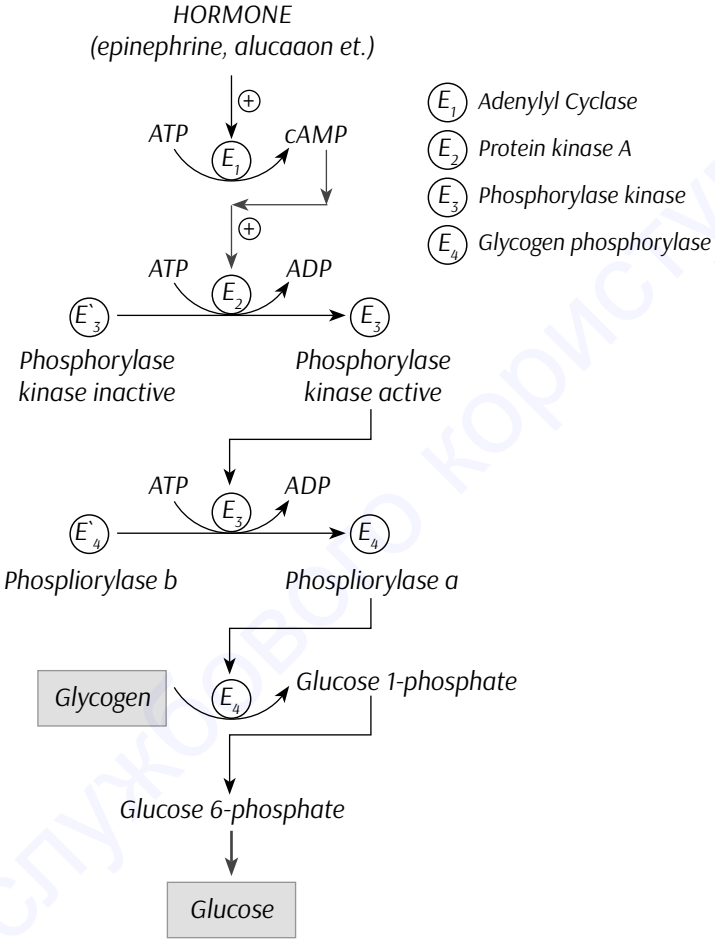
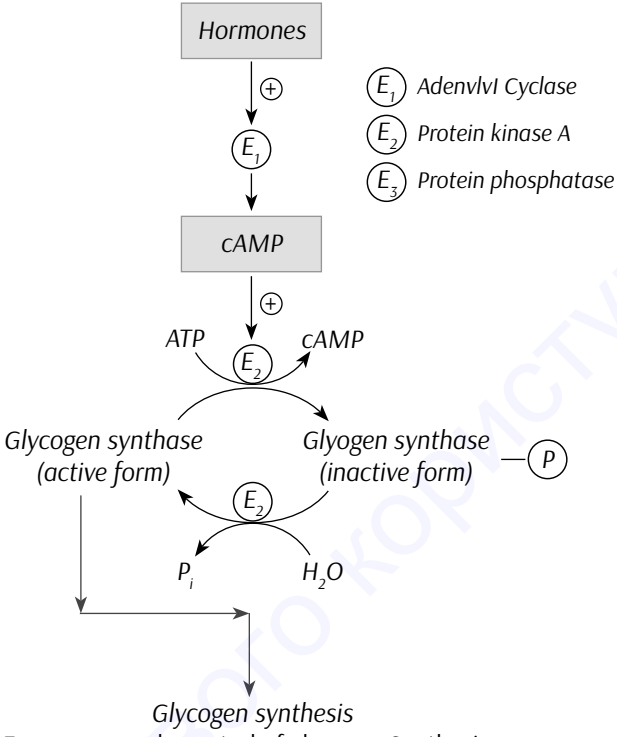


Figure 12.4. Enzyme cascade control of glycogen phosphorolysis

B. Control mechanisms of glycogen synthesis.

The synthesis of glycogen is closely coordinated with its degradation. The rate-limiting step in glycogen formation is the addition of activated glycosyl units to an existing glycogen chain by ***glycogen synthase***. The activity

of ***glycogen synthase,*** like that of ***phosphorylase,*** is regulated by covalent modification (Figure 12.5).



Fiaiire 12.5. Enzyme cascade control of glycogen Synthesis

As can be seen from the scheme presented in Figure 12.5, hormones **(epinephrine, glucagon)** inactivate *glycogen synthase* by stimulating cAMP production via ***adenylyl cyclase***. This is another case of enzyme cascade in controlling of metabolic pathway.

Thus, **cAMP-dependent phosphorylation has opposite effects on the enzymatic activities of glycogen phosphorylase and glycogen synthase.** *Glycogen phosphorylase* is active when *synthase* is inactive, and vice versa. Hence, **epinephrine** and **glucagon** stimulate glycogen breakdown and inhibit its synthesis by increasing the cytosolic level of cyclic AMP, which activates ***protein kinase A.***

Genetic defects in glycogen metabolism

A number of genetic disorders is known that impairs the orderly interrelation between glycogen synthesis and degradation. These biochemical abnormalities result from the inherited insufficiency of definite glycogen metabolism enzyme synthesis and are manifested clinically as **glycogen-storage diseases.**

The first glycogen-storage disease was described by Edgar von Gierke in 1929. An infant with this disease had a huge abdomen caused by a massive enlargement of the liver. He also suffered from a pronounced hypoglycemia between meals which resulted in severe convulsions because of the profound disfunction of the nervous system.

The enzymic defect in von Gierke`s disease was elucidated by Gerty Cori and Carl Cori in 1952, who found that it was ***glucose 6-phosphatase*** which was missing from the liver of the patient. The absence of glucose 6-phosphatase caused the low blood glucose level because the sugar could not be formed from glucose 6-phosphate.

So far seven other glycogen-storage diseases ***(glycogenoses)*** have been characterized which are associated with definite glycogen metabolism enzymes insufficiency. Some of them are shown in the Table 12.1.

Table 12.1. Glycogen-storage diseases

Type of glycogenose	Defective enzyme	Organs affected
I (Von Gierke`s disease)	Glucose 6-phosphatase	Liver and kidney
III (Cori`s disease)	Debranching enzyme (amylo-1,6-glucosidase)	Muscle and liver
IV (Andersen`s disease)	Branching enzyme (α-1,4 → α-1,6)	Liver and spleen
V (McArdle`s disease)	Phosphorylase	Muscle
VI (Hers` disease)	Phosphorylase	Liver

12.2. Gluconeogenesis: reactions, regulation

Gluconeogenesis is the synthesis of carbohydrates, and glucose specially, from the noncarbohydrate precursors. Gluconeogenesis is especially important in maintaining blood sugar concentration and glucose supply of the

brain during starvation or during periods of limited carbohydrates uptake.

The **precursors of gluconeogenesis** are lactate, pyruvate, glycerol and some amino acids, termed ***glucogenic amino acids***.

For the most part, gluconeogenesis is confined to liver, which is responsible for 85 to 95 % of glucose that is synthesized de novo. During starvation or during metabolic acidosis, the kidney is capable of making up to 50 % of the glucose formed, since, in these conditions, the amount, contributed by liver, is decreased considerably.

Gluconeogenic pathway.

From the biochemical viewpoint, the pathway of gluconeogenesis, that is the conversion of lactate or pyruvate to glucose, is the reversal of the glycolytic pathway (which converts glucose to lactate) except for three irreversible steps in glycolysis:

(a) the phosphorylation of glucose by ***glucokinase:***

α-D-Glucose + ATP ⟶ D-Glucose 6-phosphate + ADP;

(b) the conversion of fructose 6-phosphate to fructose 1,6-bisphosphate by ***phosphofructokinase:***

D-Fructose 6-phosphate + ATP ⟶ Fructose 1,6-bisphosphate + ADP;

(c) the conversion of phosphoenolpyruvate to pyruvate by ***pyruvate kinase:***

Phosphoenolpyruvate + ADP ⟶ Pyruvate + ATP.

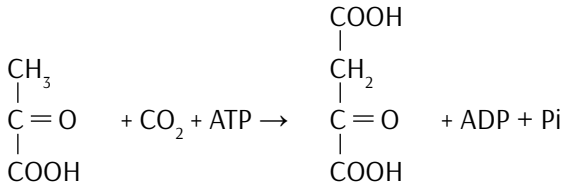
These irreversible glycolytic reactions must be bypassed, and this is made by the *special reactions of gluconeogenesis*.

Reactions of gluconeogenesis

(1) The formation of phosphoenolpyruvate from pyruvate by the way of oxaloacetate. This is achieved in two reactions:

1.a. Conversion of pyruvate to oxaloacetate.

$$\text{Pyruvate} + CO_2 + ATP \rightarrow \text{Oxaloacetate} + ADP + P_i$$

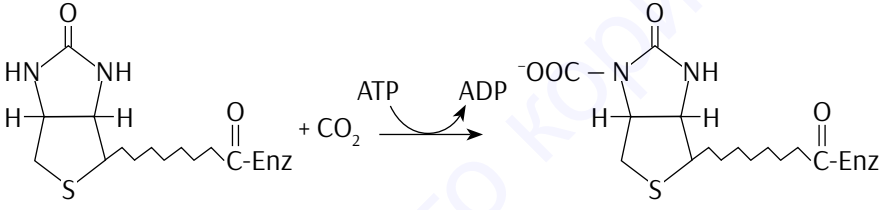


Pyruvate *Oxaloacetate*

The reaction is catalyzed by ***pyruvate carboxylase.***

The mobile carrier of activated CO_2 for the carboxylation reaction is enzyme-bound **biotin (vitamin H).**

Biotin reacts with CO_2 to yield **carboxybiotin,** in which the carboxyl group is bonded to the N-1 nitrogen atom of the biotin ring. The reaction requires the hydrolysis of ATP:

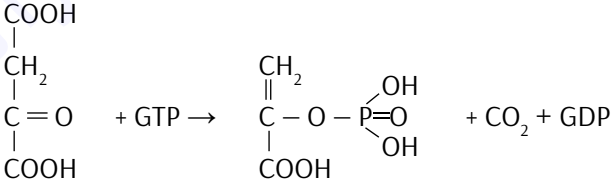


The activated carboxyl group is the transferred from carboxybiotin to pyruvate to form oxaloacetate.

1.b. The conversion of oxaloacetate to phosphoenolpyruvate.

Oxaloacetate is simultaneously decarboxylated and phosphorylated to give phosphoenolpyruvate:

$$\text{Oxaloacetate} + GTP \rightarrow \text{Phosphoenolpyruvate} + GDP + P_i$$



Oxaloacetate *Phosphoenolpyruvate*

The CO_2 that was added to pyruvate by ***pyruvate carboxylase*** comes

off in this step. The enzyme that catalyzes the reaction is called ***phosphoenolpyruvate carboxykinase.***

(2) The conversion of phosphoenolpyruvate to fructose 1,6-bisphosphate occurs by the way of the reverse action of the glycolytic enzymes that is:

Phosphoenolpyruvate → 2-phosphoglycerate;

2-Phosphoglycerate → 3-phosphoglycerate;

3-Phosphoglycerate → 1,3-bisphosphoglycerate;

1,3-Bisphosphoglycerate → glyceraldehyde 3-phosphate;

Glyceraldehyde 3-phosphate → dihydroxyacetone phosphate;

Glyceraldehyde 3-phosphate + dihydroxyacetone phosphate →
→ fructose 1,6-bisphosphate

(3) The conversion of fructose 1,6-bisphosphate to fructose 6-phosphate.

This conversion cannot be effected by glycolytic enzyme, ***phosphofructokinase,*** which action is irreversible. Cells of liver, the kidney and the intestinal epithelium have a special enzyme, ***fructose 1,6-bisphosphatase,*** which can cleave F1,6BP to fructose 6-phosphate:

Fructose 1,6-bisphosphate + H_2O → fructose 6-phosphate + P_i

(4) The conversion of fructose 6-phosphate into glucose 6-phosphate is realized by the simple conversion of glycolytic reaction which is catalyzed by ***phosphoglucose isomerase:***

Fructose 6-phosphate → glucose 6-phosphate

(5) The formation of free glucose from glucose 6-phosphate. This cannot occur by reversal of ***hexokinase*** reaction. The gluconeogenic tissues, and the liver for the most part, contain ***glucose 6-phosphatase,*** an enzyme which removes the phosphate from glucose 6-phosphate:

Glucose 6-phosphate + H_2O → glucose + P_i.

Enzymes of gluconeogenesis

Summarizing, we can recount the special enzymes that are needed for gluconeogenesis:

- Pyruvate carboxylase;
- Phosphoenolpyruvate carboxykinase;
- Fructose 1,6-bisphosphatase;
- Glucose 6-phosphatase.

The whole metabolic pathway of gluconeogenesis is presented in the Figure 12.6.

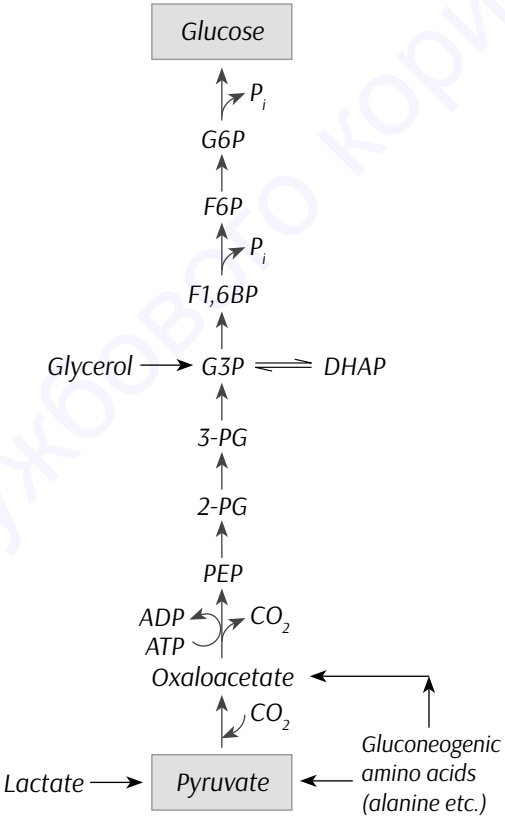


Figure 12.6. Metabolic pathway of gluconeogenesis

12.3. Regulation of carbohydrate metabolism

In higher organisms, the metabolism of the carbohydrates is the subject to complex regulatory mechanisms that involve hormones, metabolites and coenzymes. The most complicated control mechanisms apply to the liver, which is the central site of carbohydrate metabolism.

One of the most important physiological tasks of the liver in human and animal organism is to store excess glucose in the form of glycogen, and to release the monosaccharide from glycogen according to the metabolic requirement of the whole organism, especially the brain. When the glycogen reserves, accumulated inside hepatocytes, are exhausted, which takes place under starvation, the liver cells can provide glucose by *de novo* synthesis that is *gluconeogenesis*, predominantly from lactate and alanine, supplied by muscle.

The hormones that influence carbohydrate metabolism include ***insulin, glucagon, epinephrine, cortisol.***

- **Insulin is** the protein of molecular mass close to 6 kD, secreted by the ***β-cells*** of the ***islets of Langerhans*** in the pancreas. **Insulin** molecule consists of two polypeptide chains: A-chain that includes 21 amino acid residues and B chain that includes 30 amino acid residues. The two polypeptide chains in insulin molecule are linked together by two disulfide bonds (Figure 12.7).

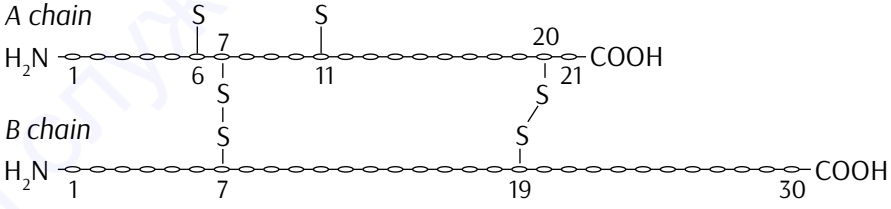


Figure 12.7. Amino acid sequence of insulin molecule. The amino acid sequence of insulin was firstly elucidated by Frederick Sanger in 1953, and this is an outstanding landmark in biochemistry development

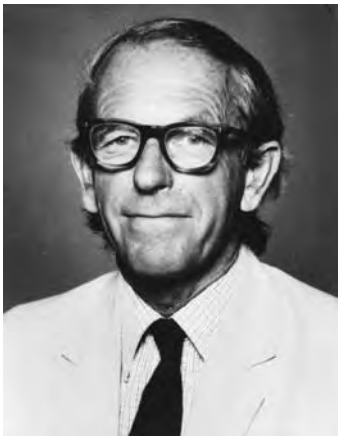


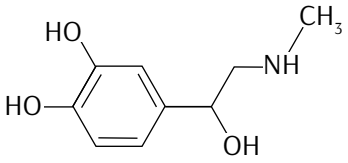
Figure 12.8. Frederick Sanger (born 1918). Nobel Prize, 1958

The principal metabolic effects of insulin relate to the metabolism of carbohydrates and lipids. Insulin **stimulates the expression of *glycogen synthase*** in both muscle and liver cells and **inhibits the synthesis of key enzymes of gluconeogenesis** by the liver. Moreover, the hormone also **accelerates the entry of glucose molecules into muscle and adipose cells** as well as **stimulates glycolysis** in the liver. The rise of glucose in adipose tissue results in the increase of lipogenesis that is the synthesis and storage of triacylglycerols in adipocytes.

- **Glucagon** is also a peptide with a molecular mass of 3.5 kD. Glucagon is produced by the pancreatic ***α-cells*** and, referring to the influence on carbohydrate metabolism, it is considered as the antagonist of insulin.

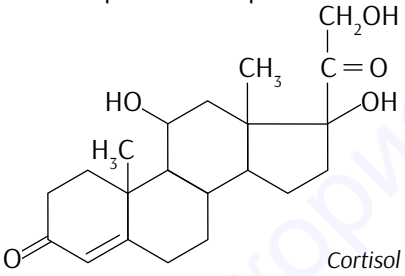
The glucagon production is stimulated in the fasting state, when the blood sugar level decreases**. The principal metabolic effect of glucagon is stimulating of glycogenolysis and inhibiting of glycogen synthesis.** The metabolic actions of glucagon are mediated by the intracellular rise of the second messenger cAMP, which activates ***protein kinases*** leading to the activation of ***glycogen phosphorylase.***

- **Epinephrine** is the catecholamine, that is synthesized predominantly by the cells of adrenal medulla. Metabolic effects of epinephrine are similar to those of glucagons. In distinction from glucagons, its action upon glycogen breakdown is more pronounced in muscle than in liver.



Epinephrine (adrenaline)

- **Cortisol** is the substance of steroid nature. The hormone is synthesized by the adrenal cortex, being the most significant ***glucocorticoid***. Cortisol is a potent inducer of key enzymes of gluconeogenesis, and it also contributes to the provision of precursors for glucose synthesis.



Cortisol

12.4. Control of blood plasma glucose. Diabetes mellitus

The blood glucose level in a typical person after an overnight fast is 80 mg/dl (80 mg/100 ml, or 4.4 mM). The blood glucose level during the day normally ranges from about 80 mg/dl (4.4. mM) to 120 mg/dl (6.7 mM).

The blood glucose level is controlled primarily by the liver, which can take up or release large amounts of glucose in response to hormonal signals and the level of glucose itself (Figure 12.9).

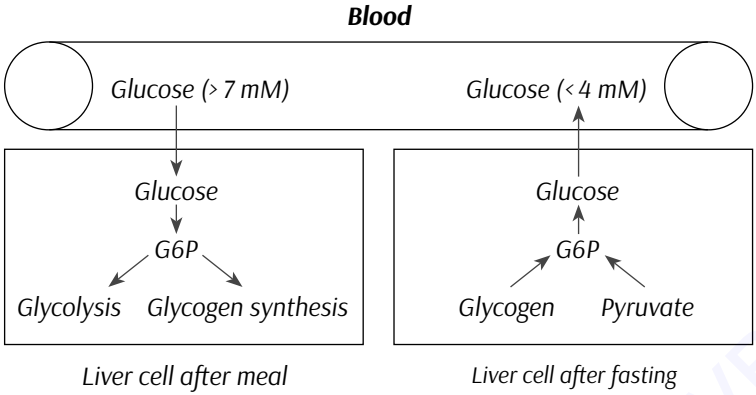


Figure 12.9. Control of the blood glucose level by the liver

Accordingly, **the principal hormonal regulators of blood glucose level are insulin and glucagon,** which action constitutes the basis of the biochemical adaptation of the whole organism to the supply of metabolic fuel.

- After a meal, the rise in the blood glucose level leads to increased secretion of insulin and decreased secretion of glucagon. Consequently, glucose entry into muscle and adipose cells is stimulated, and glycogen synthesis as well as glycolysis in liver and muscle increase. The stimulated entry of glucose into adipocytes provides glycerol 3-phosphate for the synthesis of triacylglycerols as the main storage form of lipids.
- Several hours after a meal, the blood glucose level begins to drop. This results in the decreased insulin secretion and increased glucagon secretion. The metabolic events just described are reversed. The activated degradation of glycogen and the stimulation of gluconeogenesis lead to the increase of liver glucose 6-phosphate which, after hydrolysis to glucose, releases the sugar into blood. The additional glucose is taken up by the brain and other tissues highly dependent on the monosaccharide oxidation.

Diabetes mellitus

Diabetes mellitus is a very common metabolic disease which is due to an absolute or a relative **deficiency of insulin.** The lack of metabolic effects normally produced by this hormone is accompanied by severe derangements of carbohydrates and lipid metabolism.

Diabetes mellitus occurs in two forms.

- In the case of diabetes **type I** (insulin-dependent diabetes mellitus, IDDM), the insulin-forming cells of the pancreas are destroyed already at a very young age by an autoimmune reaction.
- The less severe **type 2** diabetes (non-insulin-dependent form, NIDDM) usually first arises at an older age. It is due to decreased insulin secretion, or caused by a defect in insulin-receptor function.

A characteristic symptom of the disease is **the elevation of the glucose concentration in the blood** from 5 mM to 9 mM and above (**hyperglycemia**, elevated "blood sugar level"). Due to the impairment of insulin function, the entry of glucose into muscle and adipose cells is slown down. Hence, glycolysis in hepatocytes is inhibited, and glucose utilization in the liver is wholly reduced. At the same time, gluconeogenesis is stimulated, which increases the blood sugar level still further. When the capacity of the kidneys for the reabsorption of glucose is exceeded (at levels of 9mM or more), the glucose is excreted in the urine **(glucosuria).** Water accompanies the excreted glucose, and so an untreated diabetic patient in the acute phase of the disease suffers from thirsty and hungry.

As a result of lypolysis activation, the ketone bodies (acetone and acetoacetate) formation increases extremely, which leads to *ketonemia*, a characteristic metabolic feature of untreated diabetes mellitus.

Chapter 13.

LIPIDS METABOLISM-1. TRIACYLGLYCEROL CATABOLISM. FATTY ACIDS OXIDATION. KETOGENESIS

13.1. Lipids: general characteristics; biological functions

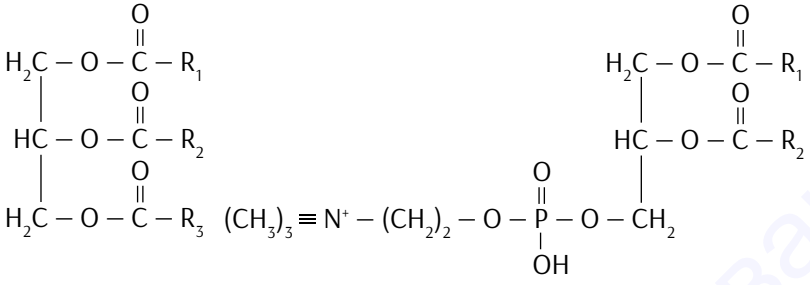
Lipids are a large group of substances of biological origin, which dissolve well inorganic solvents (chloroform, benzene etc.), but are, on the other hand, either insoluble or only sparingly soluble in water.

From the viewpoint of chemical structure, most of lipids are ester of special alcohols (commonly, glycerol, cholesterol, sphingosine) and long chain carboxylic acid, that are customarily designated as ***fatty acids*** (simple lipids, for example ***triacylglycerols (triglicerides),*** which are usually called fats, or neutral fats). Complex lipids ***(glycerolphospholipids***, ***sphingophospholipids, glycolipids)*** are a class of lipids, which yield on hydrolysis alcohols, fatty acids and some additional substances, mainly a phosphate residue, amino alcohols (choline, ethanolamine etc.), carbohydrates – Figure 13.1.

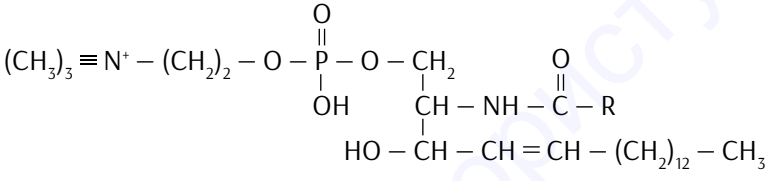
The principal biological functions of lipids.

1. Lipids (predominantly triacylglycerols, which are accumulated in specialized cells of adipose tissue) serve as the main storage of metabolic fuel of the body.
2. Amphipathic complex lipids are building blocks for cellular membranes (biomembranes). Typical membrane lipids are phospholipids (glycero-phospholipids, sphingophospholipids), glycolipids, and cholesterol.
3. Some lipids have adopted special roles in the human and animal body

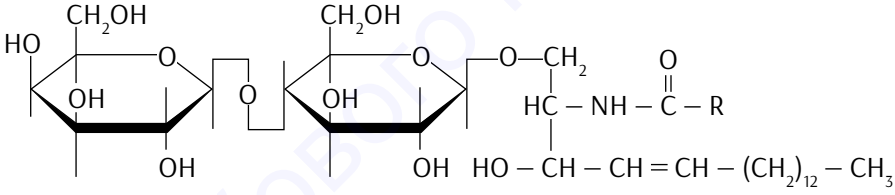
as signaling molecules (steroid hormones, eicosanoids), and antioxidants (vitamin E).



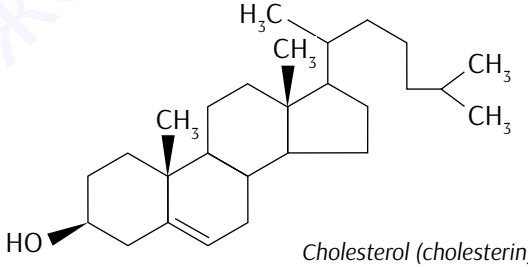
Triacylglycerol *Phosphatidyl choline (lecithi)*



Sphingophospholipid (sphingomyelin)



Glycolipid (globoside)



Cholesterol (cholesterin)

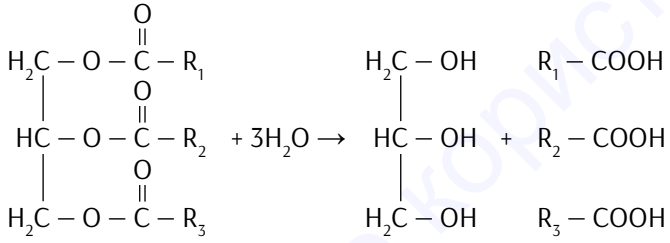
Fiaure 13.1. The prevalent linids of human tissues

13.2. Fat metabolism: overview; lipolysis

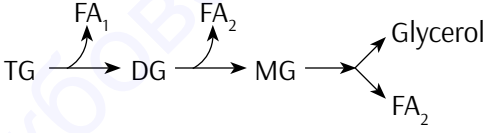
Body fats (triacylglycerols) are the most essential energy reserve in humans and animals. Fats are found in many body cells in the form of small droplets which are located in the cellular cytosol. But the major place of fats storage are the cells of the adipose tissue that are called ***adipocytes,*** and their storage ability meets the energy requirements of a human body for about two to three months of fasting.

Lipolysis

The initial event in the utilization of neutral fats as energy sources is the hydrolysis of triacylglycerols by enzymes ***lipases.*** And this is called **lypolysis:**



The overall hydrolysis of **triacylglycerols (TG)** to form glycerol and fatty acids **(FA)** is accomplished in three sequential steps which yield **diacylglycerols (DG)** and **2-monoacylglycerols (MG)** as intermediate products:



The fatty acids released by the adipose tissue are transported in the blood in the form of unesterified molecules, which are designated as **FFA** (free fatty acids). Because only the short-chain fatty acids are water soluble, the prevalent part of FFA are transported in circulation being bound to serum **albumin.**

(1) Glycerol, formed by lipolysis, is successively phosphorylated oxidized to give **dihydroxyacetone phosphate**. The latter, in turn, can be isomerized to **glyceraldehydes 3-phosphate** that enters the glycolytic pathway (Figure 13.2).

(2) Fatty acids, that are released under lipolysis, are subjected to oxidative degradation to acetyl-CoA through the multistep enzymatic

process that is designated as **β-oxidation** (see below). And the electrons removed from fatty acids during β-oxidation pass through the respiratory chain, driving ATP synthesis in oxidative phosphorylation.

The oxidation of long-chain fatty acids in mitochondria is a central energy-yielding pathway in many tissues. In particular, in mammalian heart and liver it provides as much as 80 % of the energetic needs of the organ.

Regulation of lipolysis

The first step in lipolytic process is catalyzed by a ***hormone-sensitive lipase.*** The enzyme is the subject to complex control by different hormones and neurotransmitters, the principals of which are **catecholamines *(epinephrine*, *norepinephrine),* glucagon** and **insulin.**

cAMP-dependent enzyme cascade in the control of lipolysis

The hormones that carry out the rapid promoting of lipolysis, and specifically **epinephrine** and **glucagon,** are secreted in response to low blood glucose levels. They activate the enzyme ***adenylyl cyclase*** in the adipocyte plasma membrane which is bound to the adipocytes plasma membrane and coupled to the lipolytic enzyme cascade complex.

The activation of the enzyme that converts ATP to **cAMP (3`,5`-AMP)** results in accumulation of cyclic nucleotide that serves as a signalling molecule in enzyme action control via the reversible protein phosphorylation. In the case under consideration, ***cAMP-dependent protein kinase*** phosphorylates a sensitive protein, **perilipin A,** which makes cytosolic ***hormone-sensitive lipase*** to attach to the lipid droplet surface and begin hydrolyzing triacylglycerols to free fatty acids and glycerol – Figure 13.3. It is noteworthy that adipocytes with defective perilipin genes do not show adequate response to increases in cAMP concentration because their hormone-sensitive lipase does not associate with lipid droplets.

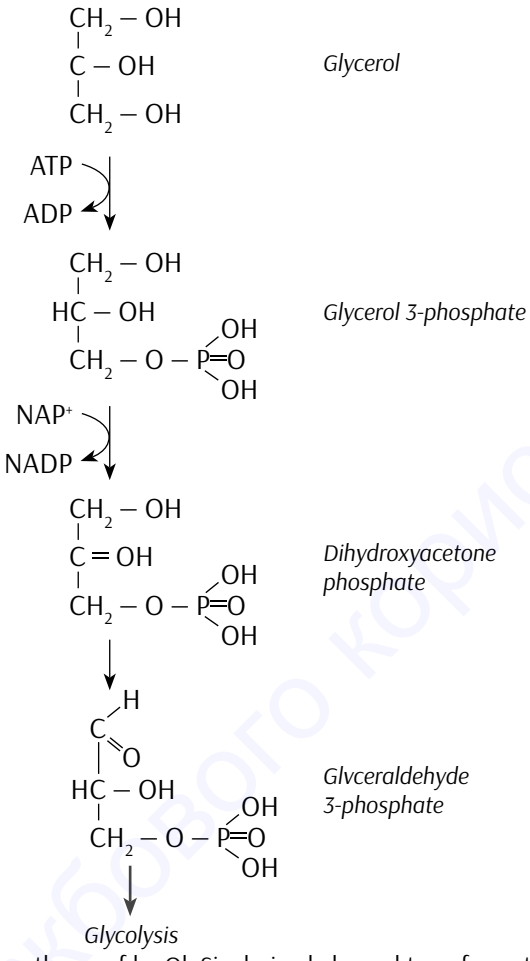


Figure 13.3. The pathway of lypOlySis derived glycerol transformation

The action of insulin

Pancreatic hormone insulin antagonizes effects of above-mentioned hormones as to the lipolysis. In contrast to catecholamines and glucagon, insulin inhibits the synthesis of cAMP at the ***adenylyl cyclase*** site and concomitantly stimulates the activity of ***phosphodiesterase*** which cleaves cAMP, that summarily counteracts the lipolysis stimulation.

Leptin, the recently discovered hormone that controls body weight and sense of hunger, acts through the stimulation of lipolysis and inhibition of lipogenesis. The hormones influences the metabolic pathways of fatty acids

degradation and synthesis.

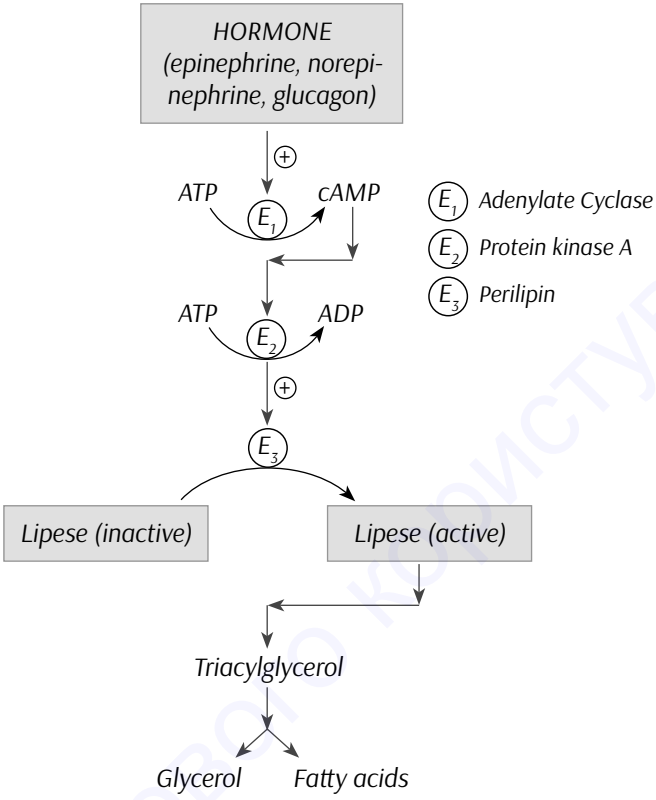


Figure 13.4. cAMP-dependent cascade of lipolysis hormone control

13.3. Degradation of fatty acids (β-oxidation)

The major fatty acids found in mammalian tissues are presented in Table 13.1. You can see that the number of carbon atoms in fatty acids, that are widespread in biological systems, is typically between 14 and 24. The unbranched, saturated and unsaturated, 16- and 18-carbon carboxylic acids are most common.

The configuration of the double bonds in most unsaturated fatty acids is cis-, and the double bonds in polyunsaturated fatty acids are separated at

least by one methylene (–CH_2–) group.

It needs to be also noticed that the principal fatty acids of human cells contain an even number of C-atom (12, 14, 16, 18, 20 etc.). And this structural feature of natural fatty acids is essential for the mechanism of their biochemical degradation which was designated as β-oxidation (Franz Knoop, 1904).

Table 13.1. Prevalent fatty acids in human adipose tissue triacylglycerols (Yu.Gubsky, 2004, modified)

Number of carbons	Common name	Formula	Average number, %
		Saturated fatty acids	
14	Myristate	$CH_3(CH_2)_{12}COOH$	3
16	Palmitate	$CH_3(CH_2)_{14}COOH$	20
18	Stearate	$CH_3(CH_2)_{16}COOH$	5
		Unsaturated fatty acids	
16:1	Palmitooleate	$CH_3(CH_2)_5CH{=}CH(CH_2)_7COOH$	5
18:1	Oleate	$CH_3(CH_2)_7CH{=}CH(CH_2)_7COOH$	55–60
18:2	cis-Linoleate	$CH_3(CH_2)_4(CH{=}CHCH_2)_2(CH_2)_6COOH$	10
18:4	Arachidonate	$CH_3(CH_2)_4(CH{=}CHCH_2)_4(CH_2)_2COOH$	0,2

β-Oxidation is the biochemical route for the catabolism of fatty acids which involves oxidation of the β-carbon of the long-chain carboxylic acid to form a β-keto acid and the subsequent shortening of the carbohydrate chain for two carbon atoms.

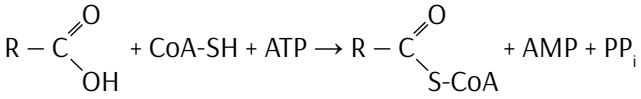
The total scheme of natural fatty acids β-oxidation can be represented by the way of the following equations:

a) $R-\overset{\beta}{C}H_2-\overset{\alpha}{C}H_2-C(=O)OH$ (C_n) $+ O_2 \rightarrow R-\overset{\beta}{C}(=O)-\overset{\alpha}{C}H_2-C(=O)OH + H_2O$

b) $R-\overset{\beta}{C}(=O)-\overset{\alpha}{C}H_2-C(=O)OH \rightarrow R-C(=O)-S\text{-CoA} + CH_3-C(=O)OH$ (C_{n-2})

Activation of fatty acids

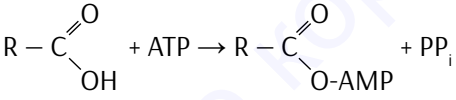
Free fatty acids are taken up from the circulation by cells in which they are oxidized,, and once inside the cell, fatty acid is converted into metabolic active product, that is acyl CoA:



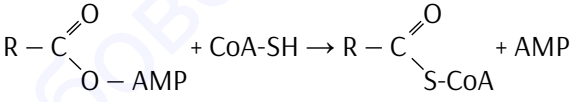
The enzyme that catalyzes the reaction is ***acyl CoA synthetase*** (also called ***fatty acid thiokinase***).

The activation of fatty acid actually occurs in two steps

Firstly, the fatty acid reacts with ATP. In the acyl adenylate that is formed, the carboxyl group of an acid is bonded to the phosphoryl group of AMP in the form of mixed anhydride:



Secondly, the sulfhydryl group of CoA attacks the acyl adenylate to yield acyl CoA and to release AMP:

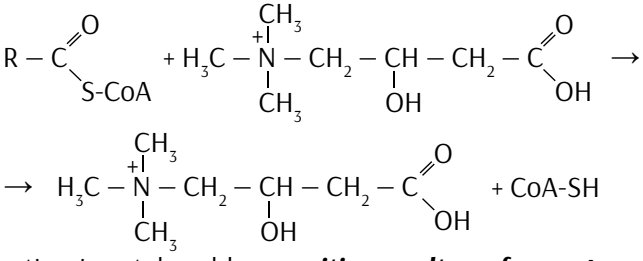


Role of carnitine in fatty acids oxidation

The activation of fatty acids takes place on the outer mitochondrial membrane, whereas the oxidation occurs inside mitochondrial matrix. Meanwhile, long-chained fatty acyl CoAs cannot freely diffuse across the inner mitochondrial membrane.

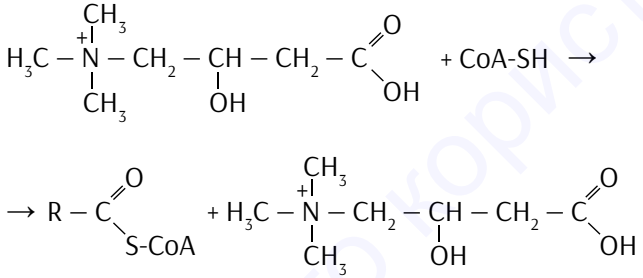
The problem is solved with the help of a mechanism which is granted by a presence in mitochondria of special transfer compound, **carnitine.**

(a) On the outer surface of the inner mitochondrial membrane, the carnitine takes up an acyl residue from acyl CoA to form **acyl carnitine:**



The reaction is catalyzed by ***carnitine acyltransferase I*,** and the acyl carnitine is freely shuttled across the inner mitochondrial membrane.

(b) On the matrix surface of the inner the mitochondrial matrix, the acyl residue is transferred back to CoA with the help of ***carnitine acyltransferase II:***



And the acyl CoA, which is released inside the mitochondrial matrix, can be subjected to β-oxidation by enzymatic system which is bound to the inner mitochondrial membrane (Figure 13.4).

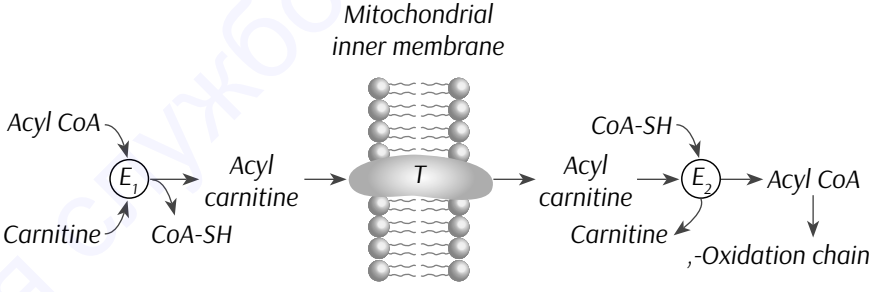
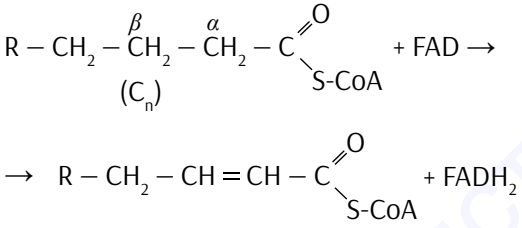


Figure 13.4. Mitochondrial carnitine transport System. E_1 – carnitine acyltransferase I; E_2 – carnitine acyltransferase II; T – translocase

Oxidation of saturated fatty acids: reactions

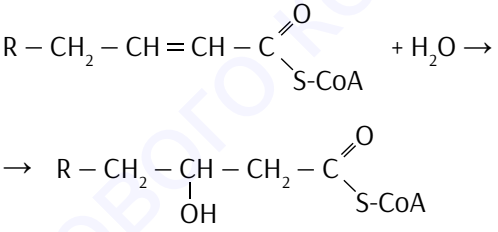
The overall process of saturated fatty acids mitochondrial β-oxidation constitutes a recurring sequence of four subsequent enzymatic reaction, which include two oxidations, catalyzed by FAD- and NAD-dependent dehydrogenases, a hydration, and a thiolysis with the participation of CoA.

(1) The first reaction among the aforementioned is the oxidation of acyl CoA to yield an unsaturated derivative of a substrate, that is enoyl CoA with a trans double bond between C-2 (α) and C-3 (β):



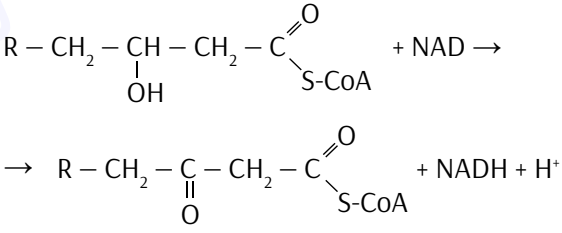
The reaction is catalyzed by FAD-requiring ***acyl CoA dehydrogenase.***

(2) The second reaction is the hydration of a double bond berween C-2 and C-3 to give the hydroxyacyl derivative of CoA thioester:



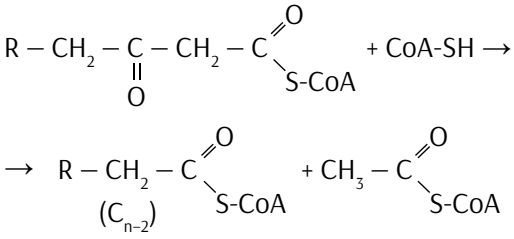
The enzyme that catalyzes the reaction is ***enoyl CoA hydratase.***

(3) The next reaction is the second oxidation that convert the hydroxyl group at C-3 of the substrate into a keto group, and the product is 3(β)-ketoacyl CoA:



The oxidizing enzyme is NAD-dependent ***3-hydroxyacyl CoA dehydrogenase.***

(4) And the final reaction in the β-oxidation round is the cleavage of 3-ketoacyl CoA, which yields a molecule of acetyl CoA and an acyl CoA shortened by two carbon atoms:



The reaction is catalyzed by ***β-ketothiolase.***

The shortened **acyl CoA (C_{n-2})** then undergoes the next round of β-oxidation, and the acetyl CoA **(C_2)** is oxidized to CO_2 and H_2O by the citric acid cycle. The four-step process is successively repeated, until the last two-carbon fragment is obtained.

Stoichiometry of β-oxidation

Thus, to accomplish the full cleavage of **n C-atoms** containing fatty acid into **two C-atoms** containing acetyl CoA, it need **(n/2–1)** rounds of β-oxidation. Hence, it follows that to obtain the complete transformation of a **palmitate** molecule (16 carbon atoms) into **8 acetyl CoA molecules** (**two carbon atoms** each), it takes **7 rounds of β-oxidation.**

(1) From this, the stoichiometric equation of palmitate β-oxidation is as follows:

$$\text{Palmitoyl CoA} + 7\text{FAD} + 7\text{NAD}^+ + 7\text{CoA} + 7\text{H}_2\text{O} \rightarrow 8\ \text{Acetyl CoA} + 7\text{FADH}_2 + 7\text{NADH} + 7\text{H}^+$$

And then, the oxidation of β-oxidation rounds generated $FADH_2$ and NADH in the mitochondrial respiratory chain follows:

(a) 7 molecules of $FADH_2$ yield 14 (7 × 2) molecules of ATP (through electron transport and oxidative phosphorylation);

(b) 7 molecules of NADH yield 21 (7 × 3) molecules of ATP (through electron transport and oxidative phosphorylation).

Therefore:

$$\text{Palmitoyl CoA} + 7\text{CoA} + 7O_2 + 35\ \text{ADP} + 35P_i \rightarrow 8\text{Acetyl CoA} + \\ + 35\ \text{ATP} + 42H_2O$$

(2) Recall that the oxidation of **acetyl CoA** by the citric cycle yields **12 ATP** molecules. And this is the stoichiometric equation of the full oxidation of **8 acetyl CoA** molecules derived from palmitate, when the generation of ATP through oxidative phosphorylation is taken into account:

$$8\text{Acetyl CoA} + 16O_2 + 96\text{ADP} + 96P_i \rightarrow 8\text{CoA-SH} + \\ + 96\text{ATP} + 16CO_2 + 104H_2O$$

Taking into account **(1)** and **(2),** the **equation of complete β-oxidation of palmitate CoA, combined with the coupled yield of ATP (35 + 96 – 1 = 130,** that is corrected by 1 ATP molecule consumed in the step of fatty acid activation), is this:

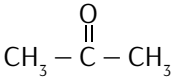
$$C_{15}H_{31}COOH + 23O_2 + 130\text{ADP} + 130P_i \rightarrow 16CO_2 + 16H_2O + 130\text{ATP}$$

It is much more than the bioenergetics yield of full aerobic oxidation of a glucose molecule that is 38 ATP (Lecture 5).

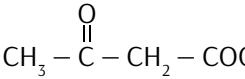
13.4. Ketone bodies. Ketogenesis in diabetes mellitus

Ketone bodies are three molecules, and specifically **acetone, acetoacetate** and **β-hydroxybutyrate,** that are used as additional metabolic sources of chemical energy.

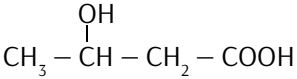
Ketone bodies are synthesized in liver mitochondria and released from that organ for utilization as metabolic fuel by other tissues. The liver itself cannot use ketone bodies as energy source.



Acetone



Acetoacetate



β-Hydroxybutyrate

Biosynthesis of ketone bodies

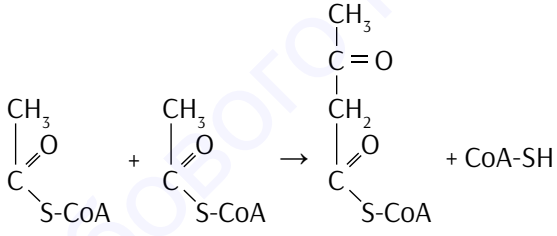
The stimulation of hepatic **ketogenesis,** and specifically the biosynyhesis of **acetoacetate,** is associated with the elevated rate of fatty acids oxidation, and (very important!) with the coupled decrease of citric acid cycle mediated acetyl CoA utilization.

In the denoted metabolic situation, the liver can produce extremely high quantities of **acetoacetate** and **3-hydroxybutyrate,** and the former can be readily decarboxylated to yield **acetone.** And the key biochemical event responsible for the activated ketogenesis is the accumulation in hepatic cells of the excess number of acetyl CoA molecules, which is the most commonly encountered under starvation and ***diabetes mellitus.***

Reactions of ketogenesis

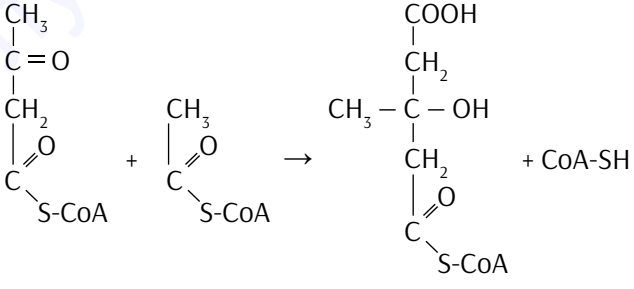
Enzymes responsible for ketone bodies formation are bound to mitochondria. The reactions are as follows.

1) Condensation of **two acetyl CoA molecules** to yield **acetoacetyl-CoA**:



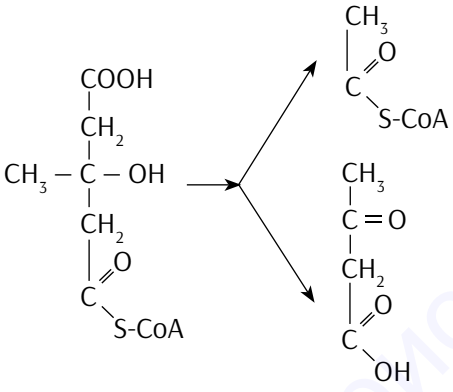
The reaction is catalyzed under the reverse action of ***thiolase.***

2) Condensation of **acetoacetyl-CoA** with another molecule of **acetyl CoA** to give **3(β)-hydroxy-3(β)-methylglutaryl-CoA (HMG-CoA).**



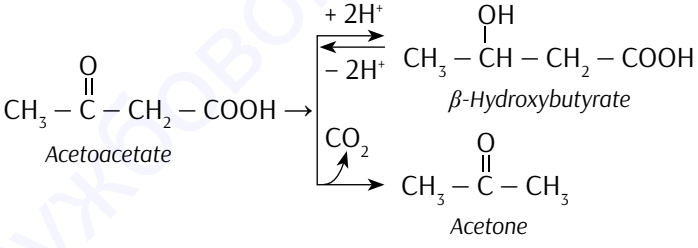
The enzyme is ***3(β)-hydroxy-3(β)-methylglutaryl-CoA synthase (HMG-CoA synthase).***

3) Then the splitting of **HMG-CoA** to **acetyl CoA** and free **acetoacetate** occurs.



The enzyme is *3(β)-hydroxy-3(β)-methylglutaryl-CoA lyase (HMG-CoA lyase).*

4) The reduction of acetoacetate **by NAD-dependent *3-hydroxybutyrate dehydrogenase*** (reversible reaction!) yields **3-hydroxybutyrate,** and the spontaneous decarboxylation of acetoacetate gives **acetone.**



Extrahepatic catabolism of acetoacetate

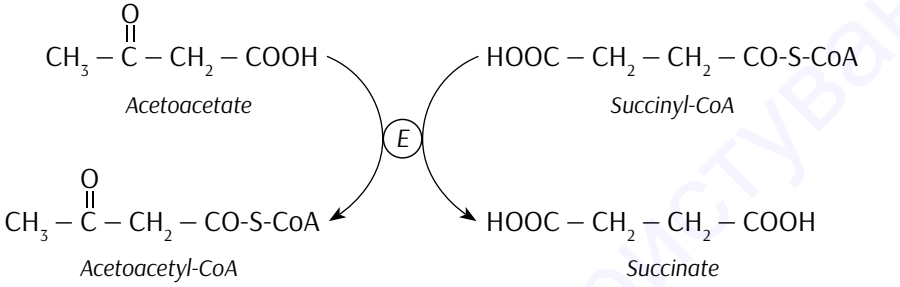
(1) The liver appears to be the only organ to add significant quantities of ketone bodies to the blood circulation.

(2) In extrahepatic tissues **acetoacetate** and **3-hydroxybutyrate**, which is readily oxidized to the former, constitute an essential metabolic fuel. The biochemical prerequisite for this is the ability of muscle and brain cells to transform free acetoacetate into acetoacetyl-CoA,

which can be further oxidized, and the enzymes necessary for this are lacking in hepatocytes.

Ketone bodies are most extensively used as sources for ATP generation when carbohydrate resources become exhausted.

(3) The key reaction essential for **acetoacetate activation** is the interaction of the latter with **succinyl-CoA,** which is catalyzed by **succinyl-CoA-acetoacetate CoA transferase.**



(4) Acetoacetyl-CoA, formed in the preceding reaction, is split into two **acetyl CoA** molecules which are oxidized in the citric acid cycle. The enzyme is ***thiolase***.

$$\text{Acetoacetyl-CoA} + \text{CoA-SH} \longrightarrow 2\ \text{Acetyl CoA}$$

Ketone bodies accumulation under human diseases

The activated hepatic ketogenesis results in the significant rise of ketone bodies concentration in blood plasma which is called ***ketonemia (hyperketonemia).*** And the excretion of the excess of ketone body via kidneys results in ***ketonuria.*** Acetone, which has no metabolic significance, is exhaled via lungs and sweat, and in the conditions of ketone body abnormal accumulation (see below) this brings to a characteristic acetone smell of the body.

In most cases, this is observed when the depletion of available carbohydrate reserves occurs. The metabolic situation is typical for **starvation** and **diabetes mellitus,** when the cellular glycogen stores are exhausted, which leads to the adipocytes lypolysis activation, and the release of fatty acids

into circulation ("the mobilization of free fatty acids").

Ketosis in diabetes mellitus

The most severe cases of ***ketonemia*** and ***ketonuria*** take place in **diabetes mellitus,** which is due to the decrease of **insulin/glucagon** ratio. Insulin insufficiency, that is the biochemical basis of the disease, leads to the decrease of glucose entry into cells, and hence, the glycolysis is inhibited, which, in turn, is associated with the low production of acetyl CoA from pyruvate. This bioenergetics disbalance results in the adipocytes lypolysis activation, and the free fatty acids are released into blood in large quantities to become (through β-oxidation) the sources of superfluous acetyl CoA accumulation.

Acetoacetate, which is formed in liver, can be partially utilized in hepatocytes as a precursor in cholesterol synthesis (Lecture 8). But under starvation and diabetes mellitus, for the reasons given above, the increased quantities of acetoacetate, which exceed the oxidizing abilities of extrahepatic organs, become greatly accumulated in tissues and blood plasma, and the overall condition is designated as **ketosis.** As far as acetoacetate and 3-hydroxybutyrate are rather strong acids, their continual overproduction depletes the alkali reserves of the blood and can markedly lower the plasma pH value, that is the **ketoacidosis** develops which is a life-threatening state for human beings.

Chapter 14.

LIPIDS METABOLISM-2. LIPOGENESIS. CHOLESTEROL METABOLISM. LIPID METABOLISM PATHOLOGY

14.1. Biosynthesis of fatty acids: reactions, enzymes

The tissues of human beings have the whole set of enzymes needed to the biosynthesis of saturated fatty acids which are used as immediate energy sources or utilized as components of different simple and complex lipids. This is called **lipogenesis.**

In most mammals, the primary substrate for lipogenesis is dietary **glucose,** and the appropriate enzyme system is present in liver, adipose tissue, kidneys, lungs, brain and mammary gland. On contrary, with a high-fat diet, fatty acid synthesis is limited.

The major site of fatty acid synthesis in humans is liver, the principal product is **palmitate ($C_{16:0}$; *hexadecanoic acid*),** and the immediate substrate for lipogenesis is acetyl CoA.

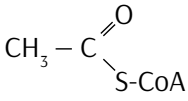
Reactions of fatty acids biosynthesis

Source of acetyl CoA.

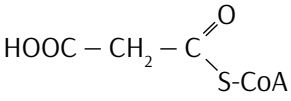
Enzymes that synthesize fatty acids are located in the cytosol, and the process develops by polymerizing two-carbon fragments supplied by **acetyl CoA, which in turn is derived from the oxidation of glucose via the glycolytic pathway.**

Production of malonyl-CoA.

In the whole sequence reactions of fatty acid synthesis the **acetyl CoA** is used as a primer which forms C-15 and C-16 atoms of palmitate. And the addition of all the subsequent C_2 units is accomplished through the elongation process which utilizes carbon atoms of **malonyl-CoA**.

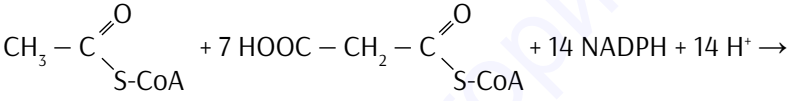


Acetyl CoA

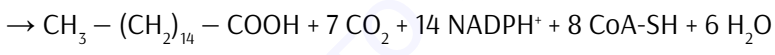


Malonyl-CoA

In other words, the assembly of two-carbon units **(C_2)** to form palmitic acid **(C_{16})** begins with acetyl CoA, followed by the sequential addition of malonyl-CoA residues with the concomitant loss of carbon dioxide. This can be represented by the generalized equation:



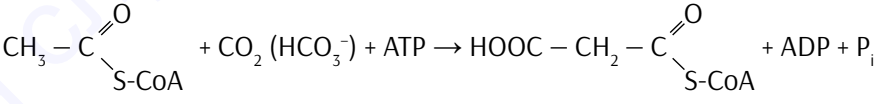
Acetyl-CoA *Malonyl-CoA*



Palmitoyl-CoA

As can be seen from the equation, the overall synthesis of palmitate requires the **expenditure of fourteen NADPH molecules,** each pair of which is utilized in the linking of two-carbon fragments. In human liver, most of the NADPH needed is provided by the pentose phosphate pathway of glucose oxidation.

The production of malonyl-CoA itself is carried out through the carboxylation of acetyl CoA which is the initial step in fatty acid synthesis:



Acetyl-CoA *Malonyl-CoA*

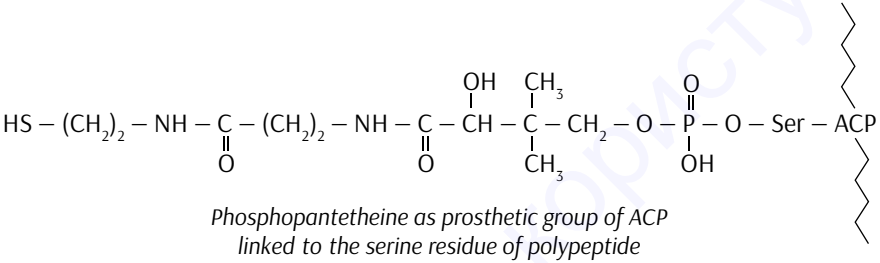
The reaction is catalyzed by ***acetyl CoA carboxylase***. The coenzyme (prosthetic group) of carboxylase is **biotin (vitamin H),** that reversibly converts into carboxybiotin, and the mechanism of the reaction as a whole is

similar to that for pyruvate carboxylation in gluconeogenesis (Lecture 6).

Elongation phase of fatty acid synthesis

The individual enzymes and subsidiary proteins occupied in the synthesis are assembled into the **fatty acid synthase complex** which has several distinctive catalytic activities.

The essential participant of a process is special acyl carrier protein (ACP). The reactive site of ACP contains a **phosphopantethene group** (**pantothenic acid** derivative), to which sulfhydryl terminus (-SH) the intermediates in the synthesis are being linked.



Phosphopantetheine as prosthetic group of ACP linked to the serine residue of polypeptide

In this way, the elongation reactions are preceded with a formation of **acetyl-ACP** and **malonyl-ACP** complexes:

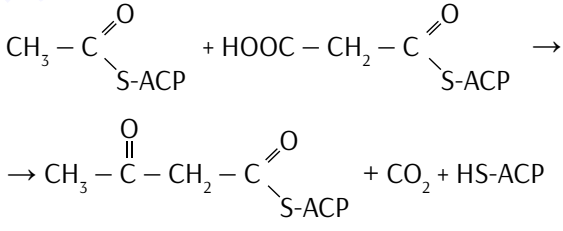
$$\text{Acetyl CoA} + \text{HS-ACP} \rightleftharpoons \text{Acetyl-S-ACP} + \text{CoA-SH}$$

$$\text{Malonyl-CoA} + \text{HS-ACP} \rightleftharpoons \text{Malonyl-S-ACP} + \text{CoA-SH}$$

Acetyl transacylase and ***malonyl transacylase*** catalyze the reactions.

Elongation reactions

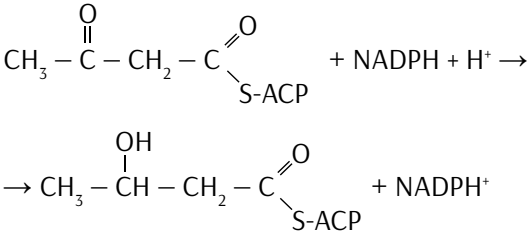
1. **Acetyl-ACP** and **malonyl-ACP** react to form **acetoacetyl-ACP.** Carbon dioxide is released.



This condensation reaction is catalyzed by the ***acyl-malonyl-ACP con-***

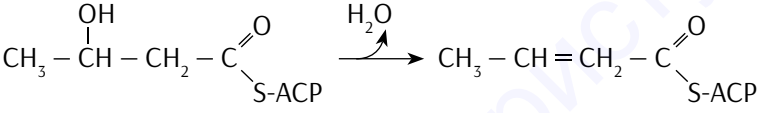
densing enzyme.

2. Reduction of **acetoacetyl-ACP** to yield **3-hydroxybutyryl-ACP** occurs.



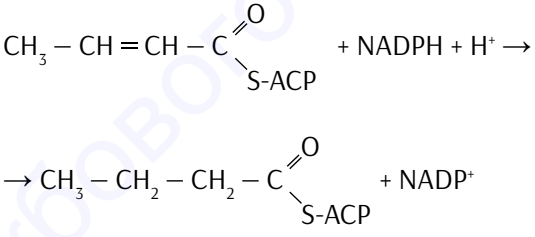
The enzyme is ***NADPH-requiring acetoacetyl-ACP reductase.***

3. Dehydration of **3-hydroxybutyryl-ACP** to yield **crotonyl-ACP.**



The reaction is catalyzed by specific **dehydratase.**

4. The second reduction in the cycle to give saturated product **– butyryl-ACP.**



The enzyme is *NADPH-requiring crotonyl-ACP reductase.*

The four-carbon (butyryl-) radical reacts with a new malonyl residue, and the elongation sequence is recommenced. After seven repeated analogous rounds the 16-carbon palmitate is formed (see equation above). The product (palmitic acid) is released under the action of **palmitoyl deacylase.**

14.2. Biosynthesis of acylglycerols: triglycerides, phosphoglycerides

Most of the fatty acids synthesized or ingested by an organism have one

of two metabolic fates: incorporation into triacylglycerols for the storage of metabolic energy or incorporation into the phospholipid components of membranes.

Biosynthetic pathways of triacylglycerols and glycerophospholipids (such as phosphatidylethanolamine) have identical precursors, namely fatty acyl-CoAs, L-glycerol 3-phosphate, phosphatidic acid, 1,2-diacylglycerol and several common biochemical steps.

Biosynthesis of triacylglycerols

Fatty acids obtained from diet or synthesized from glucose, can be converted to triacylglycerols, used for prolonged storage as metabolic fuel or transported to other tissues for immediate expenditure. It is noteworthy that humans can store only a few hundred grams of glycogen in liver and muscle, barely enough to supply the body's energy needs for 12 hours. In contrast, the total amount of stored triacylglycerol in a 70-kg man of average build is about 15 kg, enough to support basal energy needs for as long as three months. Moreover, triacylglycerols have the highest energy content of all stored nutrients—more than 38 kJ/g. Thus, whenever carbohydrates is ingested in excess of the organism's capacity to store glycogen, the excessive glucose is converted to triacylcylglycerols and stored in adipose tissue.

Steps in triacylglycerols biosynthesis.

(1) The **first stage** in the biosynthesis of triacylglycerols is the acylation of the two free hydroxyl groups of l-glycerol 3-phosphate by two molecules of fatty acyl-CoA to yield diacylglycerol 3-phosphate, more commonly called **phosphatidic acid** or **phosphatidate.**

Phosphatidic acid is present in only trace amounts in cells but is a central intermediate in lipid biosynthesis; it can be converted either to a triacylglycerol or to a glycerophospholipid.

(2) In the pathway to triacylglycerols, phosphatidic acid is hydrolyzed by phosphatidic acid phosphatase to form a 1,2-diacylglycerol.

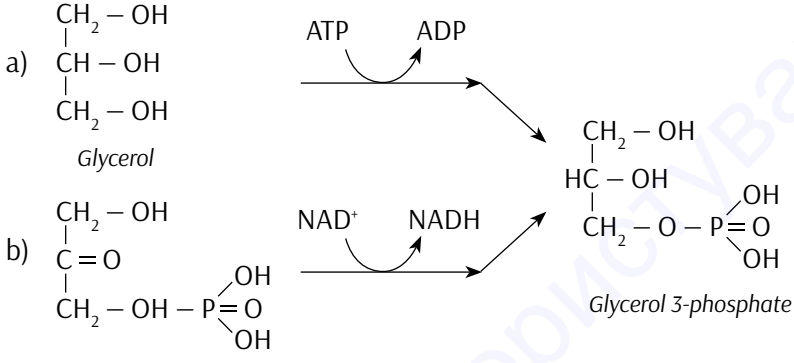
(3) Diacylglycerols are then converted to triacylglycerols by transesterification with a third fatty acyl-CoA.

Synthesis of phosphatidic acid

1. Production (formation) of glycerol 3-phosphate.

In human tissues, the vast majority of the **glycerol 3-phosphate** is derived from the glycolytic intermediate dihydroxyacetone phosphate (DHAP) by the action of the cytosolic NAD-linked ***glycerol 3-phosphate dehydrogenase.***

In liver and kidney, a small amount of glycerol 3-phosphate is also formed from glycerol by the action of ***glycerol klnase.***



2. Formation of acyl-CoAs.

The other precursors of triacylglycerols are fatty acyl-CoAs, formed from fatty acids by ***acyl-CoA synthetases***, the same enzymes responsible for the activation of fatty acids for β-oxidation (Lecture 7).

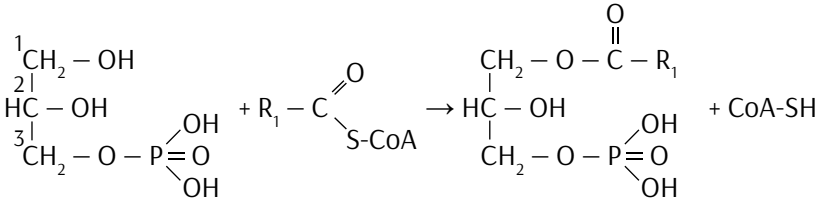
3. Conversion of glycerol 3-phosphate to phosphatidic acid.

Two molecules of **acyl-CoAs** combine with **glycerol 3-phosphate** to give **1,2-diacylglycerol 3-phosphate,** which is called **phosphatidic acid (phosphatidate).**

The process is divided into the two steps which are catalyzed by sucessive action of ***glycerol-3-phosphate acyltransferase*** and ***1-acylglycerol-3-phosphate acyltransferase.***

3.1. Production of lysophosphatidate.

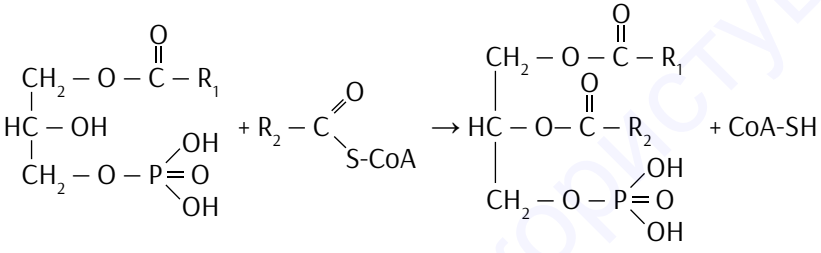
The esterification of **glycerol 3-phosphate** in a position C_1 yields **1-acyl-glycerol-3-phosphate (lysophosphatidate).**



Glycerol 3-phosphate

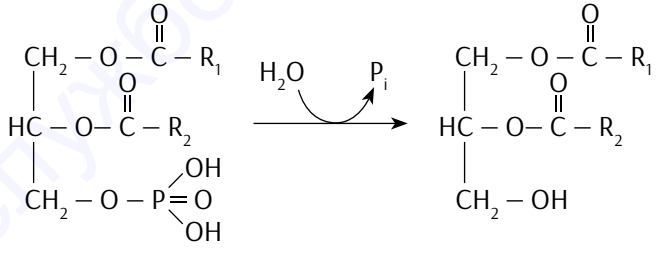
3.2. Production of phosphatidic acid (phosphatidate).

The esterification of **1-acylglycerol 3-phosphate** in a position C_2 yields **1,2-diacylglycerol-3-phosphate (phosphatidate).**



Synthesis of triacylglycerols (triglycerides)

(1) Cleavage of phosphate group from **phosphatide** occurs. The reaction is catalyzed by ***phosphatidate phosphohyrolase,*** and the product is **1,2-diacylglycerol.**



(2) 1,2-Diacylglycerol is esterified by the third molecule of acyl-CoA, and the **triacylglycerol** is formed. The enzyme is ***diacylglycerol acyltransferase.***

Synthesis of membrane glycerophospholipids (phosphoglycerides)

The first steps of glycerophospholipid synthesis are shared with the pathway to triacylglycerols, and namely the esterification of L-glycerol 3-phosphate in the positions C-l and C-2 by two fatty acyl groups to form **phosphatidic acid which is transformed into 1,2-**diacylglycerol. Commonly but not invariably, the fatty acid at C-l is saturated and that at C-2 is unsaturated.

Thus, the immediate precursor of triacylglycerols and phosphoglycerides is 1,2-diacylglycerol.

(1) In the biosynthesis of **phosphoglycerides (phosphatidylcholine, phospha-tidylethanolamine),** it needs for choline or ethanolamine to be activated by phosphorylation with ATP-granted phosphate:

$$HO-CH_2-CH_2-\overset{+}{N}\equiv(CH_3)_3 + ATP \rightarrow \textcircled{P}-O-CH_2-CH_2-\overset{+}{N}\equiv(CH_3)_3 + ADP$$

$$HO-CH_2-CH_2-NH_2 + ATP \rightarrow \textcircled{P}-O-CH_2-CH_2-NH_2 + ADP$$

(2) This is followed by the interaction of phosphocholine (phosphoethano-lamine) with **nucleoside triphosphate CTP** to yield **CDP-choline** (**CDP-ethanolamine**) which is the immediate substrate for introducing the amino alcohol into phosphoglyceride molecule:

$$\textcircled{P}-O-CH_2-CH_2-\overset{+}{N}\equiv(CH_3)_3 + CTP \rightarrow CDP-O-CH_2-CH_2-\overset{+}{N}\equiv(CH_3)_3 + PP_i$$

$$\textcircled{P}-O-CH_2-CH_2-NH_2 + CTP \rightarrow CDP-O-CH_2-CH_2-NH_2 + PP_i$$

(3) CDP-linked amino alcohol residues react with 1,2-diacylglycerol to yield **phosphatidylcholine** or **phosphatidylethanolamine,** respectively (Figure 14.1).

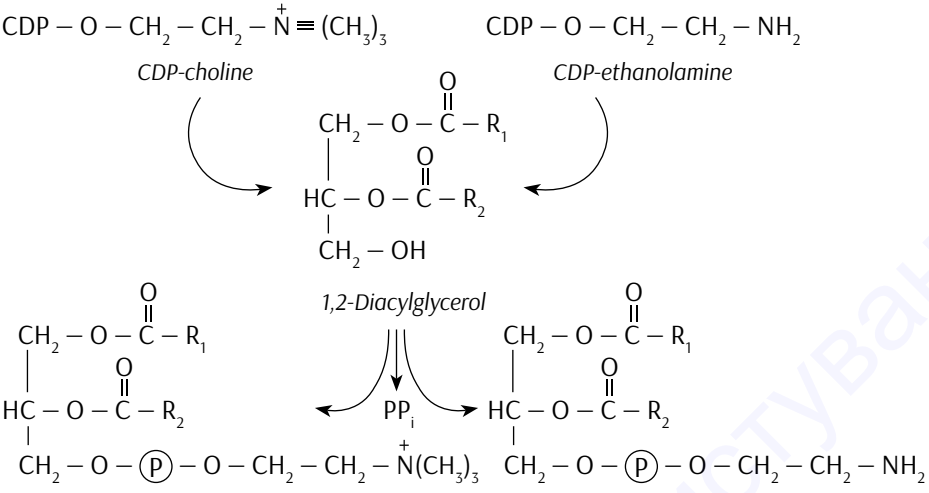


Figure 14.1. Formation of phosphatidylcholine and phosphatidylethanolainine from CDP-linked amino alcohols and 1,2-diacylglycerol

In liver cells, another pathways exists which enables **phosphatidyl-ethanolamine** to be transformed into **phosphatidylcholine by** means of direct methylation of etanolamine residue.

Control mechanisms

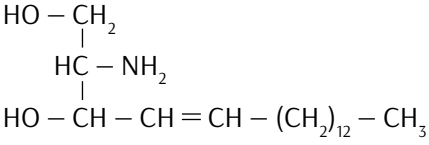
The availability of free fatty acids is a key factor in the regulation of triacylgly-cerols and phosphoglycerides biosynthesis. Provided that the immediate needs in fatty acids mitochondrial oxidation are satisfied, the additional Acyl-CoAs become preferentially converted to membrane phospholipids and are utilized forneutral fats synthesis.

14.3. Sphingolipids: representatives, metabolism

Sphingolipids are a class of lipids which are esters of **sphingosin**e. These complex lipids are found in every tissue, and exceptionally great quantities are located in nervous tissue.

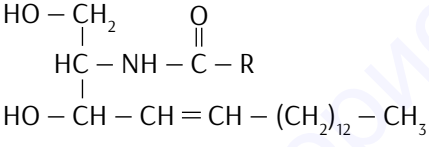
The mostly widespread representatives of human brain sphyngolipids

are **sphingomyelins.** On hydrolysis of **sphingomyelins**, fatty acids, phosphoric acid, choline, and unsatutated, long-chained amino alcohol, **sphingosine**, are released.

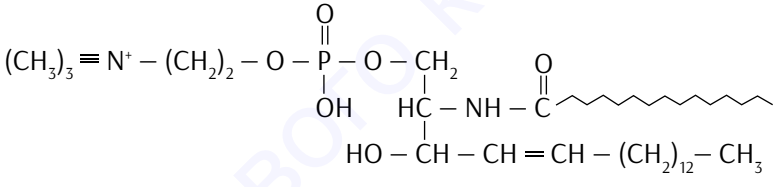


Sphingosine

The core structure of naturally occurring **sphingomyelins** is N-acylsphingosine that is known as **ceramide.** Ceramides are formed as a result of N-acylation of sphingosine moiety with fatty acid residue:



N-Acylsphingosine (ceramide)



Sphingomyelin

In **sphingomyelins** the amino group of the sphingosine backbone is linked to a fatty acid by an amide bond (see above); in addition, the primary hydroxyl group of sphingosine is esterified to phosphocholine.

Glycolipids are subclass of sphyngolipids which are present on the outer surface of the plasma membrane in all tissues. They are composed of sphingosine, a fatty acid and a monosaccharide or oligosaccharide residue, while the phosphate group, typical of phospholipids, is absent.

Biosynthesis of sphingolipids

(1) Sphingosine is produced in the membranes of endoplasmic reticulum. Its precursors are amino acid **serine** and activated **palmitoate**

(C_{16}) – Figure 14.2.

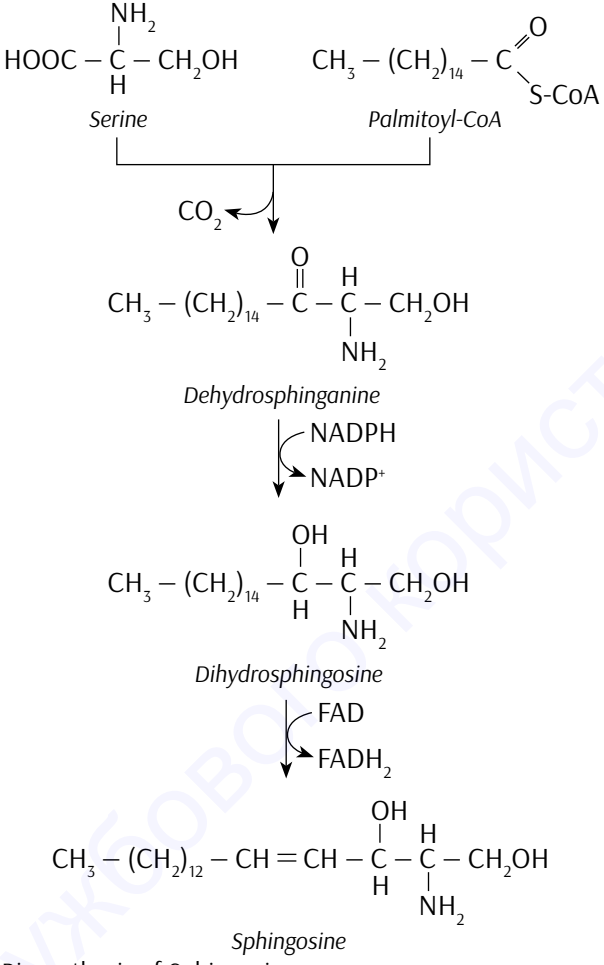


Figure 14.2. Biosynthesis of Sphingosine

(2) Ceramide which is the core structure of different sphyngolipids is formed by the acylation of **sphingosine.**

Ceramide by itself is a substance of great biological importance as an intracellular signalling molecule. It takes part in the control of cellular differ-ent-tiation, senescence and apoptosis that is programmed death of the cell.

(3) Sphingomyelins are produced by the addition of **phosphatidylcho-line** to **ceramide. Sphingomyelin** and **diacylglycerol** are the prod-ucts of the reaction (Figure 14.3). The synthesis of sphingomyelins

occurs in the Golgi apparatus and partly in the plasma membranes.

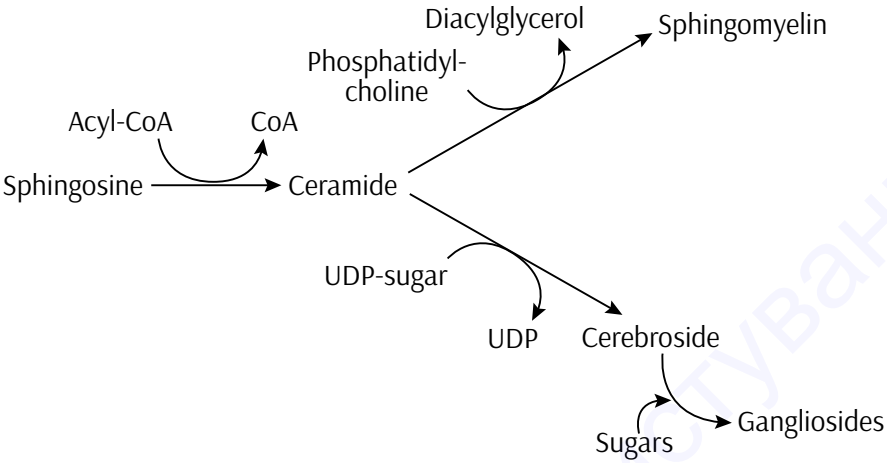
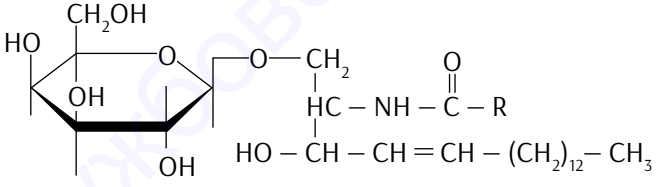


Figure 14.3. Metabolic ways of sphingolipids biosynthesis

(4) Glycosphingolipids are a combination of ceramide with one or more residues of the monosacharides or their derivatives.

Depending on the content of carbohydrate moiety of the molecule, there are such glycosphingolipids as **cerebrosides, gangliosides, globosides** and **sulfatides.**

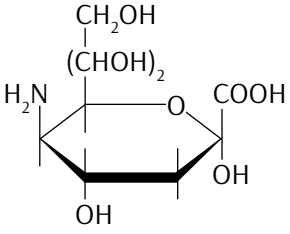
Glycosphingolipids contain one (cerebrosides, sulfatides) or more (gangliosides, globosides) sugar derivatives.



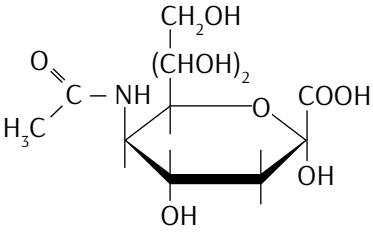
Cerebroside (galactosylceramide)

Galactosylceramide is a major glycosphingophospholipid ot human brain and other parts of nervous system. The characteristic structural feature of galactosylceramide is the presence of specific fatty acid C_{24}, cerebronic acid

Gangliosides are also present in nervous tissue of human beings in high concentrations, especially in ganglion cells. The distinctive feature of gangliosides is the presence in their oligosaccharide constituents of such sugar derivatives as **N-acetylgalactosamine** and sugar acids, namely **neuraminic acid** and **N-acetylneuraminic (sialic) acid.**



Neuraminic acid



N-Acervlneuraminic (sialic acid)

There is a shorthand nomenclature of gangliosides, in which the letter **G** denotes a ganglioside, and subscripts **M, D, T** or **Q** indicate, respectively, mono-, di-, tri- or quarto- content of sialic molecules.

GM_1: Gal – GalNAc – GAl – Glc – Cer
NeuAc

GM_2: GalNAc – GAl – Glc – Cer
NeuAc

GM_3: GAl – Glc – Cer
NeuAc

Gangliosides G_{M1}, G_{M2}, G_{M3},
Gal – galactose; Glc – glucose; GalNAc – N-acetylgalactosamine;
XeuAc- N-acetylneuraminic acid; Cer – ceramide

To produce **glycolipids,** monosaccharides, activated by uridine triphosphate in the form of **UDP-sugars,** are used (see Figure 8.3).

Inherited diseases of sphingolipid metabolism

There is a group of severe inherited diseases which are characterized by an abnormal accumulation of sphingolipids in nervous tissue, especially in brain. These metabolic abnormalities are called **sphingolipidoses.** They are due to the genetic injuries of certain enzymes that are responsible for the physiological degradation of sphingolipids and share part with the other **storage-diseases** of early chidhood.

The catabolism of sphingolipids in the normal organism occurs by the stepwise hydrolysis of the complex molecules with the sequential release of monosaccharide moieties. The enzymes responsible for the process are located in lysosomes. A number of lipidoses are known that result from the

inherited lack of gangliosides degrading lysosomal enzymes. The most common of these are **Tay-Sachs disease** and **G_{M1} gangliosidosis.**

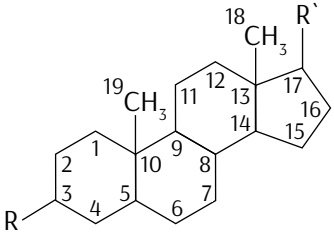
(a) Tay-Sachs disease (G_{M2} gangliosidosis) is due to a deficiency of lysosomal hexosaminidaseA, which hydrolyzes off the terminal N-galactosamine of G_{M2} ganglioside. The lack of enzyme ctivity leads to the abnormal accumulation of ganglioside in brain tissue. The injured infants suffer from the mental retardation, seizures, blindness, and macrocephaly.

(b) G_{M1} gangliosidosis. The disease results from the deficiency of ***β-galac-tosidase*** which cleaves the galactose residue from the ganglioside molecule. Its clinical symptoms are similar to those observed in Tay-Sachs disease.

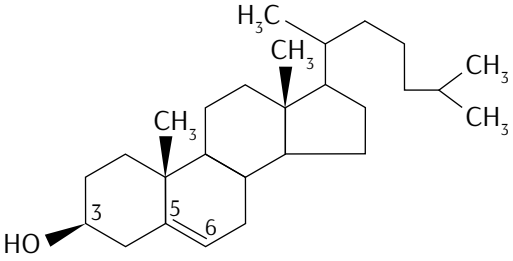
(c) Gaucher disease is a *genetic disorder* in which ***glucocerebroside*** (a *sphingolipid*, also known as **glucosylceramide**) accumulates in cells and certain organs. The disease is caused by a hereditary deficiency of the enzyme ***glucocerebrosidase*** (also known as glucosylceramidase), which acts on glucocerebroside. When the enzyme is defective, glucocerebroside accumulates, particularly in white blood cells and especially in macrophages (*mononuclear leukocytes*) and also in brain, liver, kidney, spleen etc.

14.4. Cholesterol synthesis and biotransformation. Atherosclerosis

Cholesterol (cholesterine) is a major representative of animal steroids. From the chemical viewpoint, cholesterol is a 27-carbon sterane (cyclopentanoperhydro-phenanthrene) derivative, which is characterized by the presence of a hydroxyl group at C-3 of sterane ring, a double bond at C-5 – C-6 at an aliphatic branched chain of eight carbon atoms at C-17.



The common structure of steroids



Cholesterol (cholesten-5-ol-3b)

Biological functions of cholesterol

1. Cholesterol is an essential structural component of biological membranes.

Especially rich with cholesterol are the plasma membranes of eucaryotic cells, that represents key role of steroid in regulating of the membranes structure. To say more definitely, the molecules of cholesterol can be inserted into membranes thus controlling the degree of lipid bilayers ***fluidity*.**

2. Cholesterol is an immediate precursor of physiologically essential steroids, the principals of which are **bile acids, vitamin D_3 (cholecalciferol)** and **steroid hormones** such as **progesterone, testosterone, estradiol, and cortisol.**

Cholesterol is present in tissues and in blood plasma either as a free molecule or as a storage form which is cholesteryl ester with long-chain fatty acids. Both chemical forms are transported in plasma in the form of complex lipoproteins.

The consumption of cholesterol through diet is realized via foods of animal origin, chiefly as a component of meat, liver, egg yolk, brain.

Biosynthesis of cholesterol

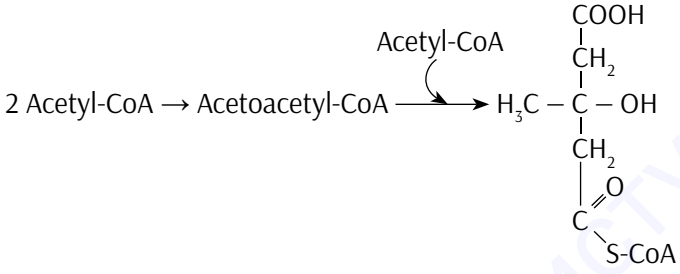
The major part of cholesterol *de novo* synthesis in human body occurs in liver and intestines. At the same time, near the half of the cholesterol requirements for cellular structure and physiological functions, is provided by the diet.

The main precursor in cholesterol biosynthesis is acetyl-CoA which

donates all carbon atoms in sterol molecule.

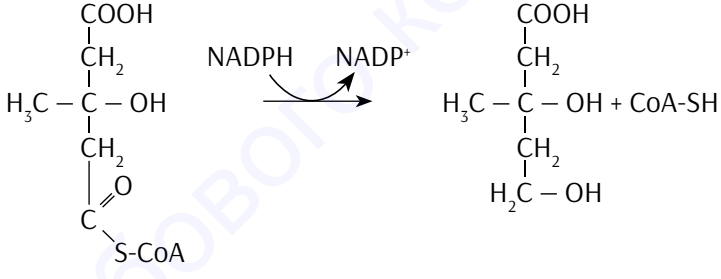
(1) The first stage in the synthesis of cholesterol is the formation of **3-hydrohy-3-methylglutaryl CoA (HMG-CoA)** from **acetyl CoA** and **acetoacetyl CoA.**

This set of reactions was discussed in regard to the formation of ketone bodies (Lecture 7).



3-Hydroxy-3-methylglutaryl-CoA

(2) The metabolic route of cholesterol synthesis, properly, begins from the reducing of HMG-CoA to **mevalonic acid (mevalonate).**



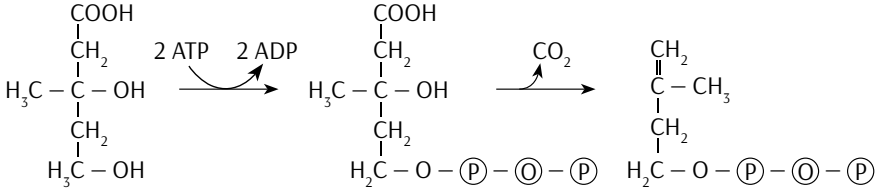
3-Hydroxy-3-methylglutaryl-CoA *Mevalonate*

The reaction is catalyzed by NADPH-dependent ***HMG-CoA reductase.*** The enzyme is feedback inhibited by cellular cholesterol, and this is an important control site in overall cholesterol synthesis.

There are two intracellular pools of HMG-CoA in liver cells, those of cytosol and of mitochondria. The mitochondrial pool of the intermediate is mainly a precursor of ketone body, whereas the cytoplasmic pool is a precursor for producing cholesterol.

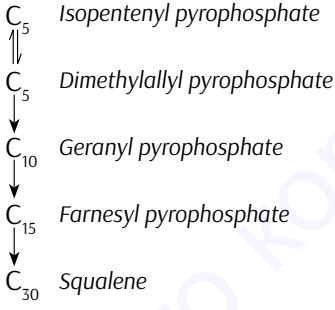
(3) The next step in the pathway is the conversion of **mevalonate** to activated isoprene units. The reactions include the phosphorylation of mevalonate at the expense of two ATP molecules to yield 5-pyrophosphomevalonate, and the subsequent isomerization associated

release of CO_2 that gives **isopentenyl pyrophosphate.**

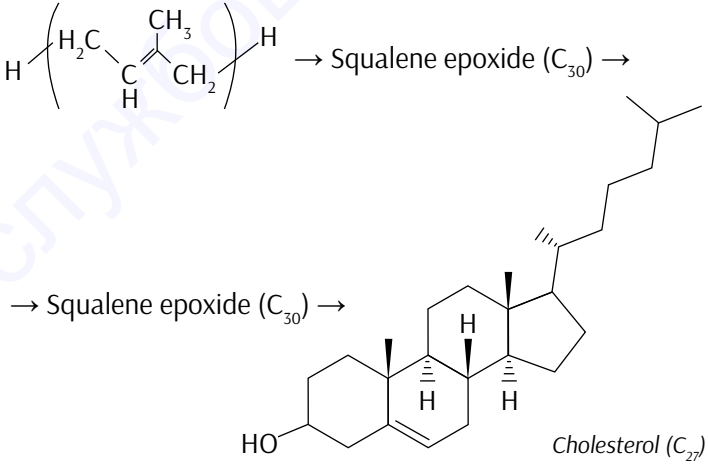


Mevalonate *5-Pyrophosphomevalonate* *Isopentenyl pyrophosphate*

(4) Then follows the polymerization of six 5-carbon isoprene units to form the 30-carbon linear unsaturated hydrocarbon **squalene.** The reaction sequence is as such:



(5) The **cyclization of squalene** to form the four rings of the steroid nucleus takes place, and a further series of biochemical changes (oxidations, removal or migration of methyl groups) produce **cholesterol.**

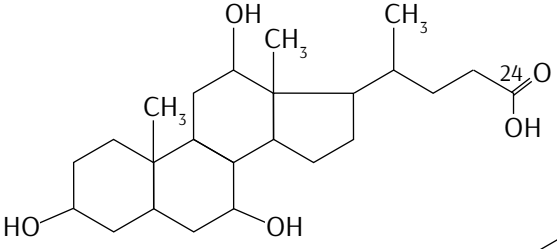


Cholesterol (C_{27})

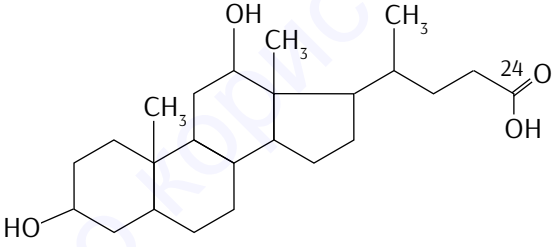
Biotransformation and excretion of cholesterol

Three major groups of biologically important biomolecules are derived from cholesterol, and they are: bile acids, steroid hormones, vitamin D.

(1) Bile acids are C_{24}-steroids, which are highly effective detergents required for intestinal fat digestion, that is due to the amphiphilic properties of their salts. The major representatives of bile acids are: **cholic acid, deoxycholic acid, chenodeoxycholic acid, lithocholic acid.**



Cholic acid



Deoxycholic acid

Being the products of cholesterol biotransformation in hepatocytes, bile acids are accumulated inside gall bladder, and after the release of bile from the latter, the bile acids enter the intestine, and specifically duodenum. Because of their detergent activities, the the sodium and potassium bile salts solubilize dietary lipids, which makes the neutral fats susceptible to the catalytic action of intestinal ***lipases*.**

Furthermore, bile acids are the major final products of cholesterol biotransformation which are excreted from the body (see below).

(2) Cholesterol is the precursor of the five major groups of **steroid hormones**, and namely:

- Progestagens – C_{21} (progesterone);
- Glucocorticoids – C_{21} (cortisol is the principal member of the class);
- Mineralocorticoids – C_{21} (primarily aldosterone);
- Sex hormones (androgens – C_{19} and estrogens – C_{18}).

(3) Cholesterol is also a precursor of **vitamin D_3,** which plays an essential role in the regulation of calcium and inorganic phosphates

metabolism ***(cholecalciferol).***

In humans, about 1 g of cholesterol is excreted daily from the body in the form of **bile acids** or as an unchanged molecule. This excretion is accomplished mainly by the bile which components are formed in liver and stored in gall-bladder. Bile acids that are quantitatively the principal products of cholesterol biotransformation are eliminated via the feces, and so does the superfluous cholesterol which is transformed into **coprostanol** by the intestinal bacteria enzymes.

Atherosclerosis

The principal and the most well-known role of cholesterol in human pathology is its significance in the pathogenesis of **atherosclerosis.**

The normal content of total plasma cholesterol in healthy humans is about 5,2 mmol/L, the concentration that is appreciably rising with age (R. K. Murray et al., 2003). As was mentioned above, cholesterol is transported between various tissue as a component of plasma lipoproteins, mainly in the form of **LDLs** (low-density lipoproteins) and **HDLs** (high-density lipoproteins). Whereas LDLs constitute the major carrier of cholesterol in blood and the vehicle form for uptake of plasma cholesterol by tissues, HDLs serve for removing the substance from tissues and transporting it into liver.

Michael Brown

Joseph Goldstein

Nobel Prize winners in physiology and medicine, 1985

The disbalance between the cellular consumption and plasma transport of cholesterol results in the deposition of the substance into arteries walls which forms the so-called ***atherosclerotic plaques.*** All this leads to the

narrowing of arteries and even to the blockade of the blood vessel lumen which is characteristic for atherosclerosis and is clinically manifested as heart attacks and and cerebral strokes.

It was shown recently by Michael Brown and Joseph Goldstein (Nobel prize winners, 1985) that the cellular uptake of cholesterol from plasma is mediated by the specialized tissue LDL receptors. The inherited deficiency of LDL receptors is accompanied by the marked elevation of the total concentration of cholesterol and LDL in blood plasma which is called familiar hypercholesterolemia and is accompanied by the abnormal deposition of cholesterol in most tissues.

Chapter 15.

AMINO ACID METABOLISM -1. AMINO ACIDS DEGRADATION: DEAMINATION, TRANSAMINATION, DECARBOXYLATION. UREA CYCLE

15.1. Protein turnover. Principal pathways of amino acid metabolism

Proteins are the most important group of biomolecules not on only from the viewpoint of their vital biological functions, but also in quantitative term. A person weighing 70 kg contains about 10 kg of protein.

Life span of proteins in the human organism

Approximately half of the body protein is associated with the skeletal muscles and supporting tissues, the major protein component of which is extracellular fibrous protein ***collagen.*** The metabolism of collagen is rather inactive, but the other half of the body proteins, which is mainly intracellular, turns over very fast. In other words, **most intracellular proteins, and especially enzymes, are short-lived biomolecules.**

The life span of biomolecules is customarily expressed as their ***half-life*** ($t_{1/2}$), that is the duration of time in the course of which their amount would become the half (50 %) of the initial number. The half-lives of most intracellular proteins, as well as the plasma proteins, average 2–8 days. Moreover, many key enzymes of intermediary metabolism have half-lives of just a few hours.

Owing to the dynamic state of body proteins, in order to replace

their constant degradation the uninterrupted protein synthesis is required!

Amino acid pool

The protein degradation, which constantly takes places in human organism, occurs via **proteolysis,** that is the breakdown of proteins to amino acids. And these free amino acids sum up to the molecules which come from the dietary protein. On the other hand, a great portion of free amino acids is reutilized in the synthesis of new proteins, and some are subjected to oxidative degradation, which makes an essential contribution to the generation of metabolic energy.

The common number of free amino acids, which are present in intracellular and extracellular compartments of the whole organism, makes up **amino acid pool** of the body (Figure 15.1).

As can be seen from the outline presented (Figure 15.1), the **equilibrium inside amino acid pool** is formed by the constant balance between *inputs to* and *outputs from,* which are:

- **Inputs to the amino acid pool** are from:
  - dietary protein;
  - proteolysis of cellular protein.
- **Outputs from the amino acid pool** are from:
  - protein synthesis, which is the main outflow of the pool;
  - amino acid degradation (catabolism);
  - utilization of amino acids skeletons to form other special biomolecules.

Essential and non-essential amino acids

As a rule, plants and microorganisms can synthesize from small precursors all amino acids needed for protein synthesis, that is the basic set of 20 α-amino acids. In contrast to these organisms, mammals, especially humans, have lost, during the course of evolution, the ability to synthesize approximately half of the 20 proteinogenic amino acids.

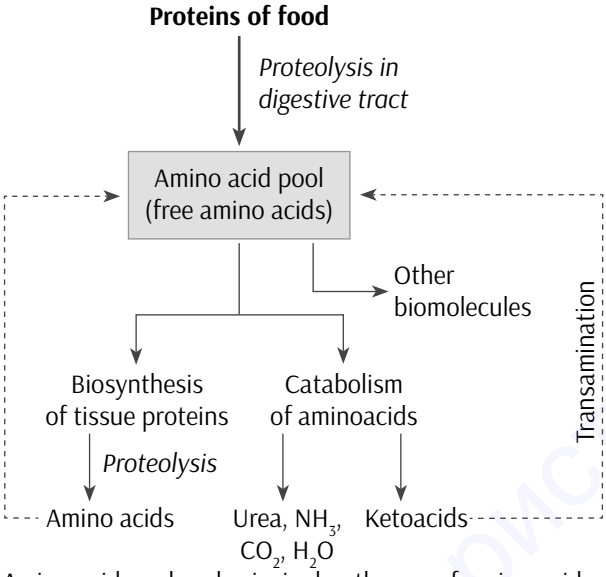


Figure 15.1. Amino acid pool and principal pathways of amino acid metabolism in human body

The α-amino acids, which are not synthesized in human (or other animal) body, must be constantly supplied in the diet. They are called **essential amino acids.** As opposed to these, amino acids which can be produced from other molecules, generally from citric acid cycle and glycolysis intermediates, are called **non-essential.** The simplest metabolic pathway that generates non-essential amino acids, is transamination from ketoacids, as, for example, generation of α-alanine from pyruvate.

Table 15.1. Basic set of proteinogenic amino acids (by L.Stryer, 1995)

Non-essential amino acids	Essential amino acids
Alanine Arginine Asparagine Aspartate Cysteine Glutamate Glutamine Glycine Proline Serine Tyrosine	Histidine Isoleucine Leucine Lysine Methionine Phenylalanine Threonine Tryptophan Valine

The nutritional value of food proteins critically depends on their content of essential amino acids. For example, many plant proteins are poor in *lysine* and *methionine*, whereas in animal proteins all amino acids are present in a balanced relationship.

Nitrogen balance

Because proteins (and amino acid residues as their constituents) make up the majority of living organisms *organic nitrogen*, the estimation of **nitrogen balance** produces valuable information as to the regular compliance between protein synthesis and degradation. If the daily body nitrogen losses (predominantly in the form of urea and ammonium ions) are greater, than dietary nitrogen intake, the subject is in **negative nitrogen balance**. This is the case in starvation or severe diseases involving tissues wasting.

The dietary deficiency of even one essential amino acid also results in a **negative nitrogen balance.** In this state, more protein is degraded inside body than synthesized, and so more nitrogen is excreted than is ingested.

Amino acid catabolism (overview)

In this and subsequent lectures the catabolism of amino acids will be studied. Under different metabolic conditions, amino acids lose their amino groups to form α-keto acids, the "carbon skeletons" of amino acids, and this occurs through the reactions of **transamination** and **deamination.**

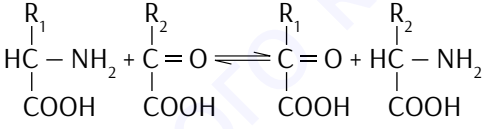
The α-keto acids, that are formed via amino group loss, undergo oxidation to CO_2 and H_2O or provide three- and four-carbon units that can be

converted by gluconeogenesis into glucose. The sugar, thus formed, is the essential fuel for brain, skeletal muscle, and other tissues. The fraction of metabolic energy obtained from amino acids, whether they are derived from dietary protein or from tissue protein, varies greatly with the type of organism and with metabolic conditions. These quantities vary in human being from 10 to 15 % of the body energy requirements, and this depends on the supply of other metabolic fuels, and primarily of carbohydrates and lipids.

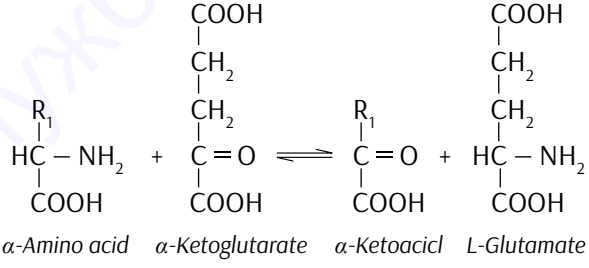
The physiologically important pathway of amino acid catabolism is **decarboxylation,** by means of which biomolecules with special properties, and predominantly hormones and neurotransmitters, are produced.

15.2. Transamination of amino acids: reactions; enzymes

This type of reaction involves the transfer of an α-amino group from an amino acid to an α-keto acid to form a new amino acid and a new α-keto acid:



The α-amino group of the most of amino acids is transferred to ***α-ketoglutarate*** to form ***L-glutamate,*** which is then oxidatively deaminated to yield $\mathbf{NH_4^+}$**:**



Aminotransferases

The enzymes that catalyze these reactions are called ***aminotransferas-***

***es*,** or ***transaminases*.**

Coenzyme in transamination reactions is ***pyridoxal phosphate***, that is phosphorylated derivative of vitamin B_6 ***(pyridoxine).*** Pyridoxal phosphate is tightly bound to protein moiety of enzyme similarly to other complex (conjugated) proteins prosthetic groups.

During catalytic act, pyridoxal phosphate is transiently converted into ***pyridoxamine phosphate*** (Figure 15.2).

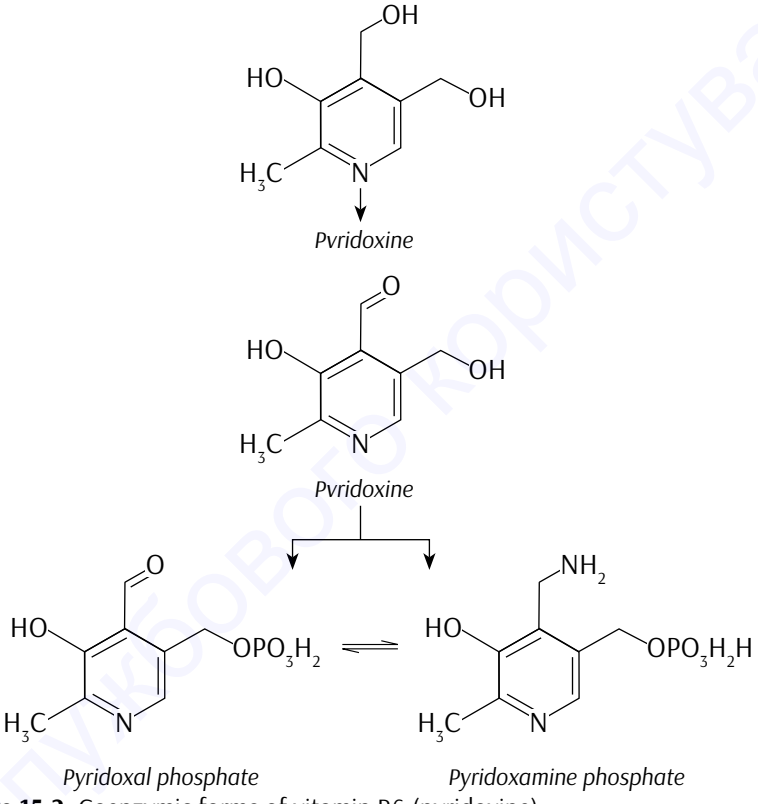


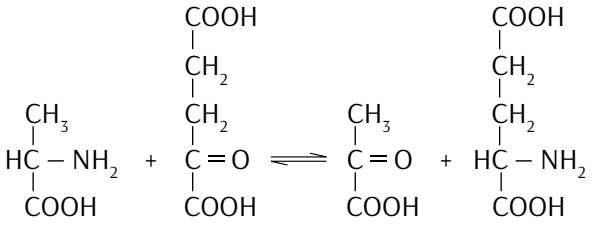
Figure 15.2. Coenzymic forms of vitamin B6 (pyridoxine)

The majority of biochemical reactions catalyzed by aminotransferases occurs in human liver. Some physiologically essential aminotransferases are:

(1) *Alanine aminotransferase (ALT),* also known as *glutamic-pyruvic transaminase (GPT);*

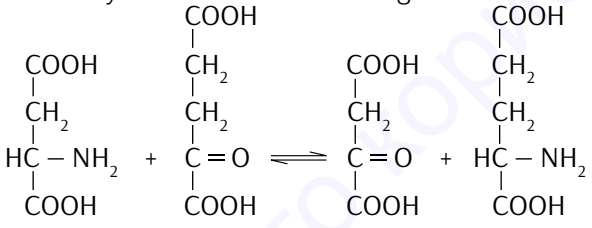
(2) *Aspartate aminotransferase (AST),* also known as *glutamic-oxaloacetic transaminase (GPT).*

Alanine aminotransferase, enzyme, that is the most widespread aminotransferase in mammalian tissues, catalyzes the transfer of the amino group of L-alanine to α-ketoglutarate:



L-Amino acid *α-Ketoglutarate* *α-Ketoacicl* *L-Glutamate*

Aspartate aminotransferase, an aminotransferase that is also prevalent in animal cells, catalyzes the transfer of the amino group of L-aspartate to α-ketoglutarate to yield oxaloacetate and L-glutamate:



L-Aspartate *α-Ketoglutarate* *Oxaloacetate* *L-Glutamate*

Biochemistry in Medicine. Enzyme assays for Tissue Damage

Analyses of certain enzyme activities in blood serum give valuable diagnostic information for a number of disease conditions.

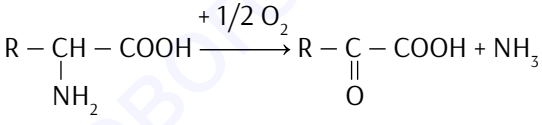
Alanine aminotransferase **(ALT;** also called glutamate-pyruvate transami-nase, **GPT**) and ***aspartate aminotransferase*** **(AST;** also called glutamate-oxaloacetate transaminase, **GOT)** are important in the diagnosis of heart and liver damage caused by heart attack, drug toxicity, or infection. After a heart attack, a variety of enzymes, including these aminotransferases, leak from the injured heart cells into the bloodstream. Measurements of the blood serum concentrations of the two aminotransferases by the **SGPT** and **SGOT** tests (**S** for *serum*)—and of another enzyme, ***creatine klnase,*** by the **SCK** test—can provide information about the severity of the damage.

Creatine kinase is the first heart enzyme to appear in the blood after a heart attack; it also disappears quickly from the blood. **GOT** is the next to appear, and **GPT** follows later. ***Lactate dehydrogenase*** also leaks from injured or anaerobic heart muscle. The **SGOT** and **SGPT** tests are also important in occupational medicine, to determine whether people exposed to carbon tetrachloride, chloroform, or other industrial solvents have suffered liver damage. Liver degeneration caused by these solvents is accompanied by leakage of various enzymes from injured hepatocytes into the blood. Aminotransferases are most useful in the monitoring of people exposed to these chemicals, because these enzyme activities are high in liver and can be detected in very small amounts.

15.3. Deamination of amino acids: reactions; enzymes

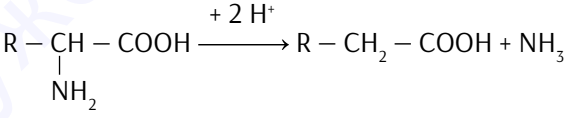
α-Amino group is able to be broken loose from amino acid molecule and converted into ammonia. This transformation is called **deamination of amino acids** and can proceed according to the following mechanisms:

a) oxidative deamination:



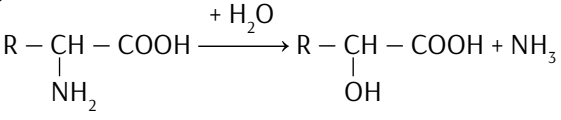
α-Amino acid *α-Keto acid*

b) reductive deamination:



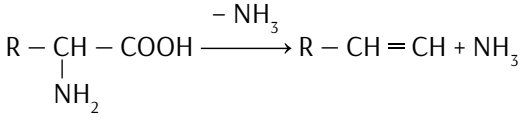
α-Amino acid *Carboxylic acid*

c) hydrolytic deamination:



α-Amino acid *Hydroxycarboxylic acid*

d) intramolecular deamination:

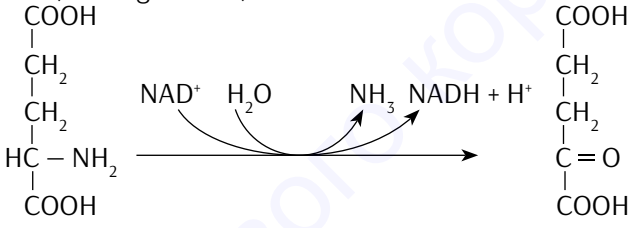


α-Amino acid *Unsaturated carboxylic acid*

Oxidative deamination of glutamate

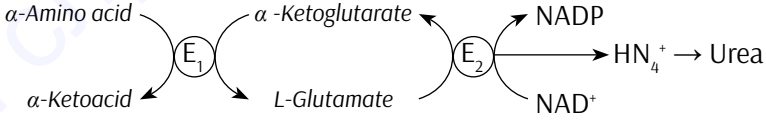
Of particular importance in human tissues is the reaction of oxidative deamination, and especially the oxidative deamination of L-glutamate. This is the kind of amino acids transformation which is prevalent in human tissues.

In the reaction, which is catalyzed by ***glutamate dehydrogenase*** of mitochondrial matrix, L-glutamic acid (L-glutamate) is converted to α-ketoglutaric acid (α-ketoglutarate):



L-Glutamate *α-Ketoglutarate*

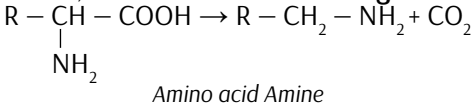
The associated activity of ***aminotransferases*** (E_1) which use α-ketoglutarate as amino group acceptor and ***glutamate dehydrogenase*** (E_2) constitutes **amino nitrogen flow from proteinogenic amino acids** (such as alanine) **via glutamate to produce ammonia that is finally converted into urea** (see below):



15.4. Decarboxylation of amino acids: reactions, biological significance

Biogenic amines

Decarboxylation of certain amino acids yields the formation of primary amines, or monoamines, which are also called **biogenic amines.**

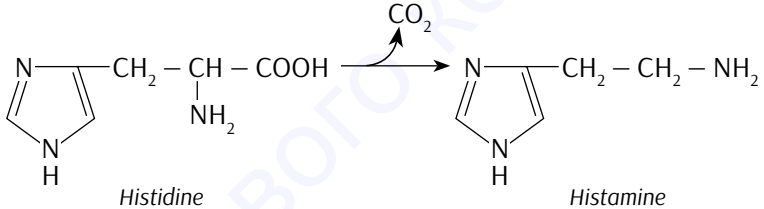


Amino acid Amine

Enzymes, that catalyze reactions of amino acids decarboxylation, are called ***decarboxylases.*** Similarly to aminotransferases, decarboxylases of amino acids also require **pyridoxal phosphate** as coenzyme.

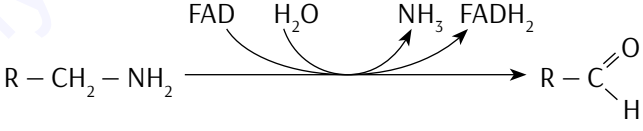
Many biogenic amines have special physiological activities as hormones and neurotransmitters. For example, this is the way of producing ***catecholamines*** (*epinephrine*, *norepinephrine*, *dopamine*) and ***serotonin***, which is a signalling substance with manifold kinds of action.

Decarboxylation of the amino acid **histidine** gives ***histamine,*** a potent vasodilatator which is accumulared in human tissues as a result of inflammation or allergic hypersensitivity



Histidine *Histamine*

Most of monoamines are inactivated under the action of enzymes ***monoamine oxidases,*** which are customarily abbreviated as **MAO.** The reaction involves simultaneous oxidation and deamination to aldehydes and requires **FAD** as transporter of reducing equivalents:



Certain pharmaceutical drugs, which inhibit MAO activity, play an important role as modulators of neurotransmitters metabolism, and hence – in regulation of human brain functions.

15.5. Amino nitrogen metabolism. Urea cycle: reactions, enzymes

As a result of deamination reactions the amino groups of amino acids are released as ammonia **(NH_3).** The additional amounts of NH_3 are produced during the deamination of free nucleotides derived purines, pyrimidines, and biogenic amines degradation.

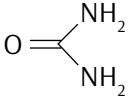
Together with amino acids derived ammonia, all this can bring to the intracellular accumulation of toxic **NH_3** molecules.

In water solutions the ammonia molecules can accept protons from water to form the ammonium ions which are rather strong bases:

$$NH_3 + H^+ \rightarrow NH_4^+$$

Ammonia is highly poisonous for living cells, especially for neurons of human brain. The molecular basis for this toxicity is not entirely understood. The terminal stages of ammonia intoxication in humans are characterized by onset of a comatose state accompanied by cerebral edema (an increase in the brain's water content) and rise of cranial pressure, so research and speculation on ammonia toxicity have focused on this tissue. Modern speculations center ammonia toxicity on a potential depletion of ATP in brain cells.

With regard to the high toxicity of ammonia, there are special biochemical mechanisms to its inactivation and excretion. In human body this occurs primarily through the formation of **urea.**



Urea

Chemically, urea is the diamide of carbonic acid **(H_2CO_3).** In contrast to ammonia, it is neutral and ***non-toxic***. Urea molecules are small, uncharged particles which can readily cross biomembranes by means of simple diffusion. For this reason and because of its high solubility in water, they can be easily transported by the blood plasma and excreted in the urine.

Urea cycle: reactions, enzymes

In nature, there are various different ways of inactivating and excreting ammonia.

- Aquatic animals, such as fishes, can excrete ammonia directly, through their gills, and they are called ***ammonotelic animals.***
- Birds and reptiles, which are called ***uricotelic animals,*** form uric acid as the final product of ammonia metabolism, and this is excreted predominantly as a solid in order to save water in their body.
- Terrestrial animals, including humans, excrete only small amounts of ammonia and even less of uric acid. Instead, in these organisms most of the ammonia is converted to urea prior to excretion, and they are designated as ***ureotelic animals.***

In human and high animals body the prevalent amount of urea is formed in specialized liver cells named ***hepatocytes***. This is done in a cyclic series of enzymatic reactions which constitute the **urea cycle.**

The urea cycle was discovered in 1932 by Hans Krebs (who later also discovered the citric acid cycle) and a medical student associate, Kurt Henseleit. Urea production occurs almost exclusively in the liver and is the fate of most of the ammonia channeled there. The urea passes into the bloodstream and thus to the kidneys and is excreted into the urine. The production of urea now becomes the focus of our discussion.

1. In the first step of urea cycle, ***ammonia*** molecules, generated in deamination reactions, are condensed with **carbon dioxide (CO_2)**, which is presented in water solutions as **hydrogen carbonate (HCO_3^-)**. The product of the reaction is **carbamoylphosphate**.

$$NH_4^+ + HCO_3^- + 2ATP + H_2O \rightarrow H_2N-\overset{\overset{O}{\|}}{C}-O-\overset{\overset{O}{\|}}{\underset{\underset{OH}{|}}{P}}-OH + 2ADP + P_i$$

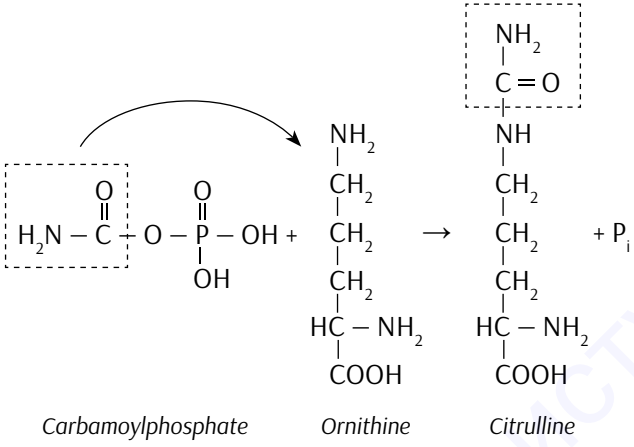
Carbamoylphosphate

The enzyme responsible for reaction catalysis is ***carbamoyl phosphate synthetase,*** that is located in the mitochondrial matrix.

Two ATP molecules are hydrolyzed in the course of reaction which makes it virtually irreversible. The reaction requires ***N-acetylglutamate*** as a positive allosteric effector.

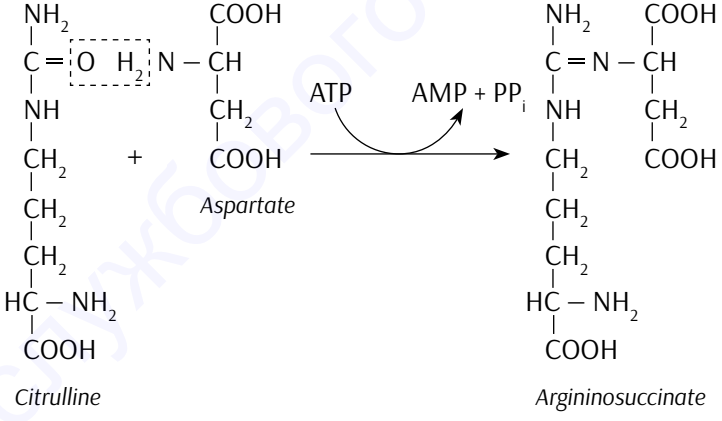
2. In the second step the ***carbamoyl group*** is transferred to δ-amino

group of the amino acid **ornithine. Citrulline** is formed in the reaction which also occurs in mitochondrial matrix.



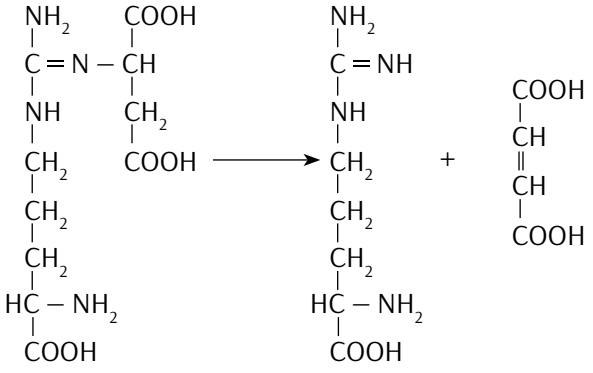
The enzyme is ***ornithine transcarbamoylase.***

3. ***Citrulline*** is then released from the mitochondria into the cytosol and its condensation with **aspartate** takes place. The product is **argininosuccinate,** and its synthesis is driven by **ATP** hydrolysis.



The catalyst for the reaction is enzyme ***argininosuccinate synthetase.***

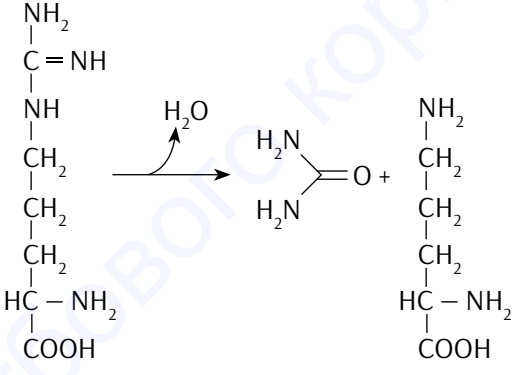
4. ***Argininosuccinate*** is cleaved to yield amino acid **arginine** and citric acid cycle intermediate **fumarate.**



Argininosuccinate *Arginine* *Fumarate*

The enzyme for reaction is ***argininosuccinate lyase.***

5. The fifth step of the process is the splitting of ***arginine*** to give **urea** and **ornithine,** which encloses the catalytic cycle (Figure 15.3).



Arginine *Urea* *Ornithine*

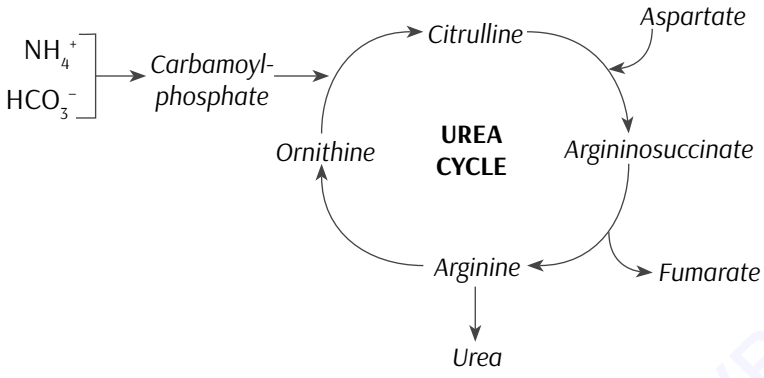


Figure 15.3. Urea cycle in hepatocytes

Genetic defects of urea cycle enzymes

A total or a partial lack of enzyme activities involved in urea synthesis has been recorded for each of the urea-cycle enzymes.

These abnormalities are due to the genetic defects concerning *carbamoyl phosphate* formation or any of the four subsequent steps of urea synthesis. ***Hyperammonemia*** is a symptom in most of these patients and its manifestation is the most severe under deficiency of *carbamoyl phosphate synthetase* and of *ornithine carbamoyl transferase*.

Generally, urea cycle enzymes insufficiency becomes evident a day or two after birth, when the afflicted infant shows symptoms of encephalopathy, such as vomiting, convulsions or lethargy. Most patients with a total absence of one of the urea cycle enzymes do not survive the neonatal period. The supplementing of a patient with a protein-restricted diet somewhat lessens hyperammonemia development.

Chapter 16.

AMINO ACID METABOLISM-2. AMINO ACID SPECIALIZED METABOLIC PATHWAYS. PORHYRINS METABOLISM

16.1. General pathways of amino acids carbon skeleton degradation

After removal of their amino groups, the carbon skeletons of amino acids undergo oxidation to compounds that can enter the citric acid cycle (TCA) for oxidation to CO_2 and H_2O.

The amino acid catabolism, taken together, normally accounts for only 10 % to 15 % of the human body's energy production. From the bioenergetics efficiency viewpoint, amino acid catabolism is not nearly as active as glycolysis and fatty acid oxidation. The availability of individual α-amino acid carbon skeleton to biological oxidation depends essentially on the balance between the requirements for protein biosynthesis and the supply of the principal metabolic fuels, above all of sugars and lipids. The utilization of α-amino acids as the fuel material markedly increases during periods of fasting, when tissue proteins, and primarily those of skeletal muscles become considerable sources of metabolic energy.

The pathways of amino acid catabolism are quite similar in most organisms. As in carbohydrate and fatty acid catabolism, the processes of amino acid degradation converge on the central cellular catabolic pathways. After enzymatic removal of nitrogen in the form of ammonium, the carbon skeletons of most amino acids find their way to the citric acid cycle.

The 20 individual α-amino acids catabolic pathways converge to form

only five major products, all of which enter the citric acid cycle (Figure 10.1).

The five intermediates that provide the entry of amino acids carbon skeletons into the citric acid cycle are: **acetyl-CoA, α-ketoglutarate, succinyl-CoA, fumarate,** and **oxaloacetate**. Some amino acids are degraded to **pyruvate** or **acetoacetyl-CoA,** which, in their turn, can be converted to either acetyl-CoA or oxaloacetate.

1. **Entry at acetyl-KoA:**
   - *isoleucine*, *leucine*, *tryptophan* and *threonine* are amino acids, which after deamination, form acetyl-CoA directly.
   - the amino acids producing pyruvate are: *alanine*, *cysteine*, *serine*, *threonine*, *glycine* and *tryptophan*.
   - *phenylalanine*, *tyrosine*, *leucine*, *lysine*, and *tryptophan* yield acetyl-CoA via acetoacetyl-CoA.
2. **Entry at α-ketoglutarate.** The carbon skeletons of five amino acids (*glutamate*, *glutamine*, *arginine*, *hystidine* and *proline*) enter the citric acid cycle as α-ketoglutarate.
3. **Entry at succinyl-CoA.** Four amino acids carbon skeletons are converted to succinyl-KoA. They are: *methionine*, *isoleucine*, *valine* and *threonine*.
4. **Entry at fumarate.** Two cyclic amino acids enter to TCA via the fumarate, and they are: *phenylalanine* and *tyrosine*.
5. **Entry at oxaloacetate.** Two amino acids, and namely *aspartate* and *asparagine*, are converted to oxaloacetate as TCA intermediate.

Thus, the individual paths for the 20 amino acids exist, each leading to a specific point of entry into the citric acid cycle. Via TCA, the carbon skeletons are completely oxidized to CO_2 and H_2O or are diverted to gluconeogenesis or ketogenesis.

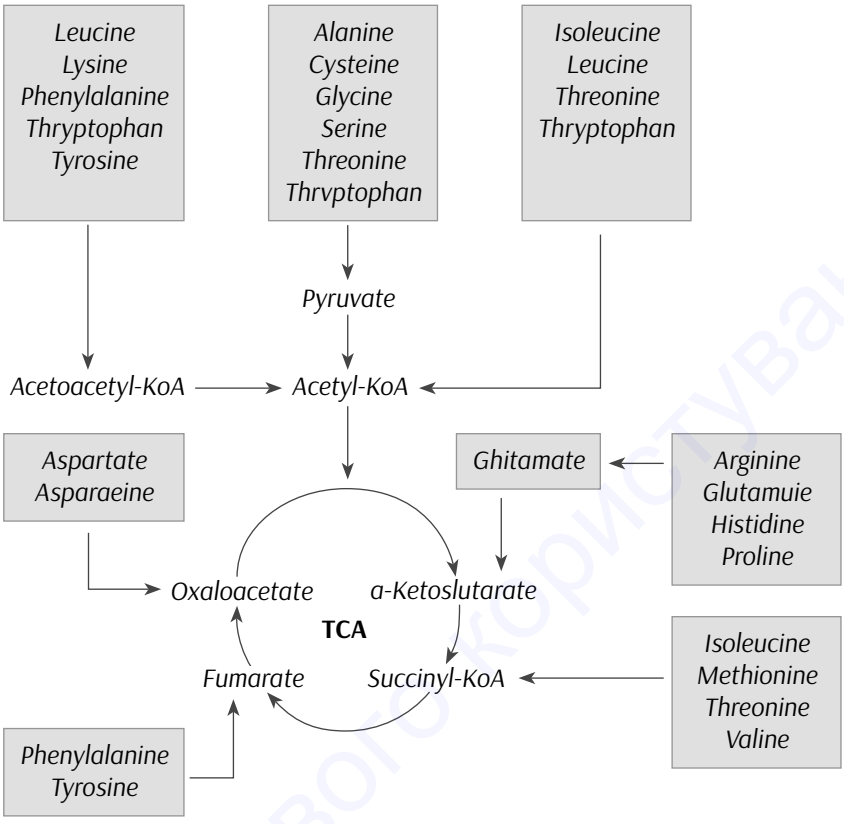


Figure 16.1. Summary of amino acid catabolism. Amino acids are grouped according to their major degradative end product. Some amino acids are listed more than once because different parts of their carbon skeletons are degraded to different end products

Glucogenic and ketogenic amino acids

Some amino acids are converted to glucose, others to ketone bodies.

1. **Ketogenic amino acids.** The seven amino acids that are degraded entirely or in part to ***acetoacetyl-CoA*** and/or ***acetyl-CoA***—*phenylalanine*, *tyrosine*, *isoleucine*, *leucine*, *tryptophan*, *threonine*, and *lysine* — can yield ***ketone bodies*** in the liver, where acetoacetyl-CoA is converted to acetoacetate and then to acetone and β-hydroxybutyrate. These are the **ketogenic amino acids.** Their ability to form ke-

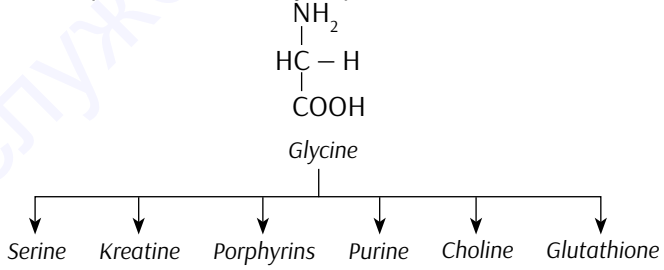
tone bodies is particularly evident in uncontrolled *diabetes mellitus*, in which the liver produces large amounts of ketone bodies from both fatty acids and the ketogenic amino acids.

2. **Glucogenic amino acids**. The amino acids that are degraded to ***pyruvate, α-ketoglutarate, succinyl-CoA, fumarate,*** and/or ***oxaloacetate*** can be converted to glucose and glycogen by *gluconeogenesis* pathway described in Lecture 9. They are the **glucogenic amino acids.** The division between ketogenic and glucogenic amino acids is not sharp; five amino acids – *tryptophan*, *phenylalanine*, *tyrosine*, *threonine*, and *isoleucine* – are both ketogenic and glucogenic. Catabolism of amino acids is particularly critical to the survival of animals with high-protein diets or during starvation. ***Leucine*** is an exclusively ketogenic amino acid that is very common in proteins. Its degradation makes a substantial contribution to ketosis under starvation conditions.

16.2. The specialized pathways of individual amino acids metabolism

16.2.1. Metabolism of glycine and serine. Concept of one-carbon transfers

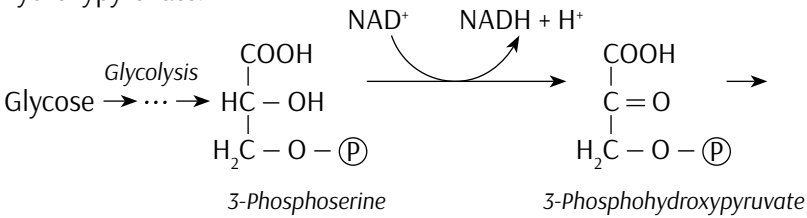
Glycine (α-amino acetic acid) is an intermediate of diverse biochemical processes and a precursor of vitally important biomolecules:

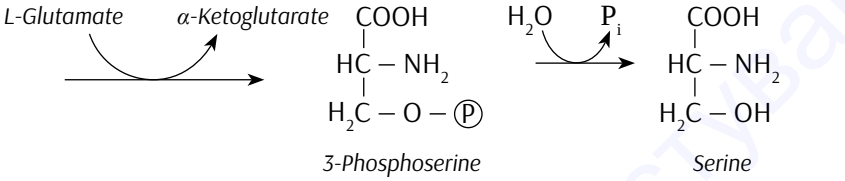


Synthesis of glycine

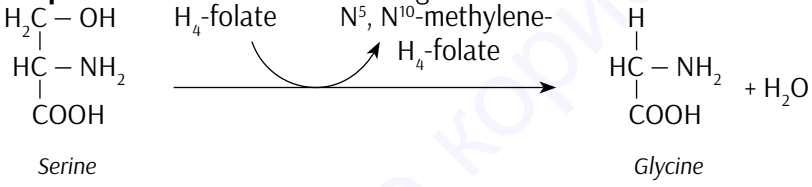
In human body the biochemical precursor of ***glycine*** is ***serine,*** which, in its turn, is synthesized from glucose via 3-phosphoglycerate and 3-phos-

pho-hydroxypyruvate.





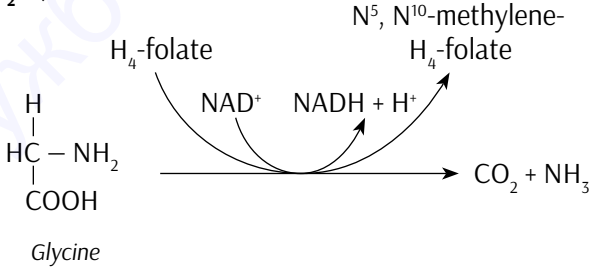
Step 1. Formation of L-serine from glucose



Step 2. Formation of glycine from L-serine

Catabolism of glycine

Glycine undergoes oxidative cleavage to **CO_2, NH_4^+**, and a methylene group (– **CH_2** –):

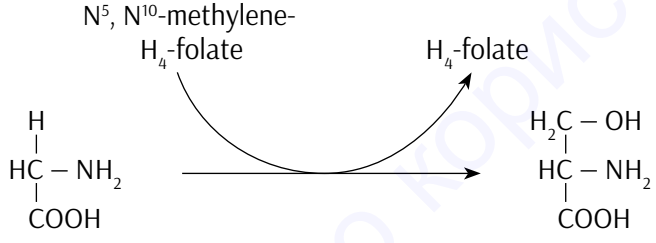


This reaction is catalyzed by ***glycine cleavage enzyme*** and requires as coenzyme **tetrahydrofolate (H_4-folate)**, which accepts the methylene group (see below). In this oxidative cleavage pathway the two carbon atoms of glycine do not enter the citric acid cycle. One carbon is lost as CO_2 and

the other becomes the methylene group of **N^5, N^{10}-methylenetetrahydrofolate,** which is an essential one-carbon group donor in certain biosynthetic pathways.

This pathway for glycine degradation appears to be critical in mammals. Humans with serious defects in glycine cleavage enzyme activity suffer from a condition known as **nonketotic hyperglycinemia**. The condition is character-rized by elevated serum levels of glycine, leading to severe mental deficiencies and death in very early childhood. At high levels, glycine is an inhibitory neurotransmitter, perhaps explaining the neurological effects of the disease.

Glycine can be reconverted to ***serine*** in the reaction that requires ***N^5,N^{10}-methylenetetrahydrofolate*** and ***pyridoxal phosphate*** as coenzymes:



This reaction is catalyzed by the enzyme ***serine hydroxymethyl transferase.***

Transfer of one-carbon groups

As can be seen from the reactions of glycine transformations, the important type of reaction in amino acid catabolism is one-carbon transfers, which usually involve one of three cofactors: **biotin, tetrahydrofolate,** or **5-adenosyl-methionine.** These cofactors transfer one-carbon groups in different oxidation states: ***biotin*** transfers carbon in its most oxidized state, CO_2; ***tetrahydrofolate*** transfers one-carbon groups in intermediate oxidation states and sometimes as methyl groups; and ***5-adenosylmethionine*** transfers methyl groups, the most reduced state of carbon. The latter two cofactors are especially important in amino acid and nucleotide metabolism.

Tetrahydrofolate (H_4-folate), which is synthesized in bacteria, consists of substituted *pterin* (6-methylpterin), *p-aminobenzoate*, and *glutamate*

moieties.

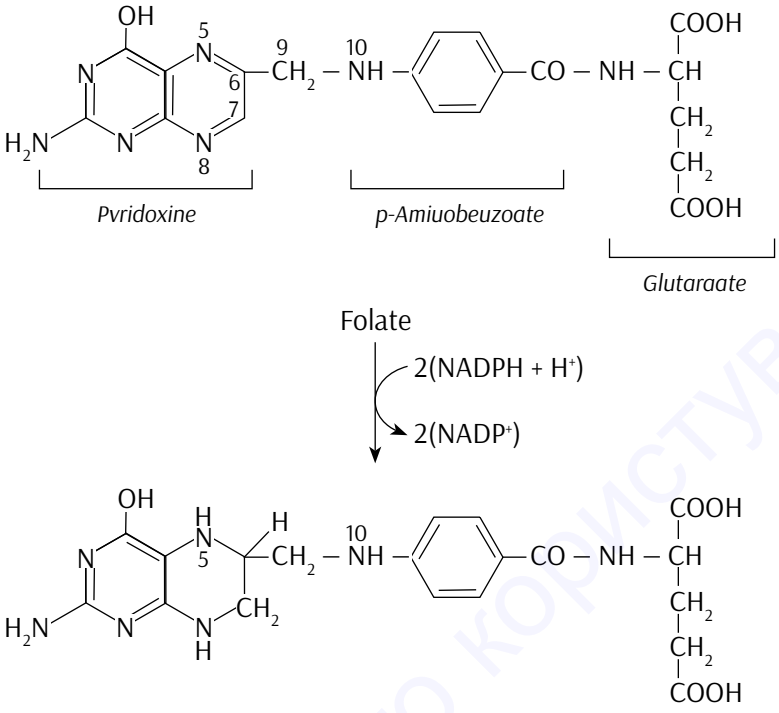
The oxidized form, ***folic acid (folate; pteroylglutamate),*** is a vitamin for mammals (vitamin B_c); it is converted in two steps to ***tetrahydrofolate (H_4-folate)*** by the enzyme ***dihydrofolate reductase*** (Figure 16.2).

The one-carbon group undergoing transfer, in any of three oxidation states, is bonded to **N-5** or **N-10** of tetrahydrofolate, or both.

The most reduced form of the cofactor carries a ***methyl group*** **(—CH_3)**, a more oxidized form carries a ***methylene group*** **(—CH_2—),** and the most oxidized forms carry a ***methenyl*** **(—CH=)**, ***formyl*** **(—CHO),** or ***formimino*** **(—CHNH)** group. Most forms of tetrahydrofolate are interconvertible and serve as donors of one-carbon units in a variety of metabolic reactions.

The examples of biosyntheses which are supplied with one-carbon groups transferred by tetrahydrofolate derivatives are as follows:

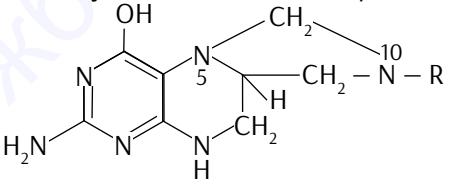
- Regeneration of ***methionine*** from *homocysteine* in an active-methyl cycle (see below).
- Synthesis of ***purine molecules rings,*** that utilizes some carbon atoms from the N^{10}-formyl derivatives of H_4-folate.
- The methyl group of DNA pyrimidine, ***thymine,*** comes from N^5,N^{10}-methylene-H_4-folate.



5,6,7,8-Tetrahydrofolate

Figure 16.2. Formation of 5,6,7,8-tetrahydrofolate from folate

The primary source of one-carbon units for tetrahydrofolate is the carbon removed in the conversion of serine to glycine, producing **N^5,N^{10}-methylene-tetrahydrofolate** (reaction equation see above).



N^5,$N1^0$-Methylenetetrahydrofolate

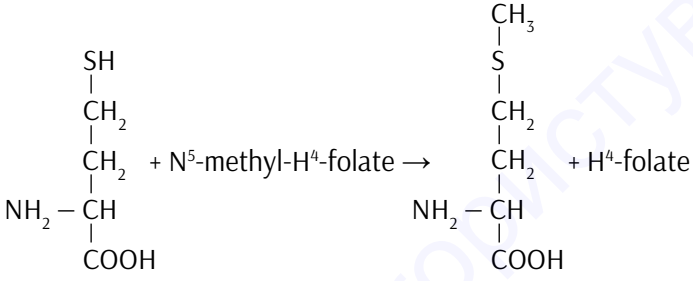
N^5,N^{10}-methylenetetrahydrofolate can be converted into **N^5-methyltetra-hydrofolate,** which is transporter of **methyl groups (–CH_3).** The enzymatic transfer of formyl groups, that occurs in purine synthesis and in the formation of formylmethionine is realized by **N^{10}-formyltetrahydrofolate,** which, in its turn, can be transformed into N^5,N^{10}-methenyltetrahydro-

folate by the expence of ATP chemical energy.

16.2.2. Methionine. S-Adenosylmethionine

The ability of N^5,N^{10}-methylene-H_4-folate for transporting **–CH_3** groups is rather limited. Thus, **the principal transporter of methyl groups is amino acid L-methionine.**

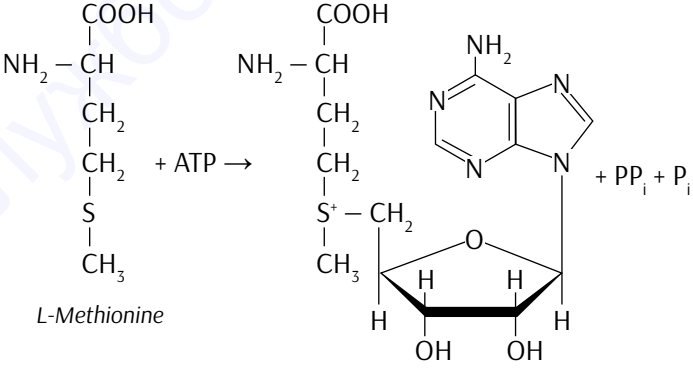
In human organism **L-methionine** is synthesized from **L-homocysteine** in a reaction which requires **N^5-methyl-H_4-folate:**



L-Homocysteine *L-Methionine*

Active-methyl cycle

(1). The preferred cofactor for methyl group biochemical transfers is an "active" form of methionine, that is **S-adenosylmethionine (adoMet).** The compound is synthesized from methionine and ATP by the action of ***methionine adenosyl transferase.***



L-Methionine

S-Adenosylmethionine

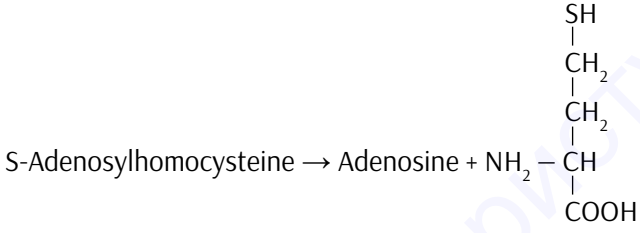
In the reaction triphosphate is cleaved to P_i and PP_i on the enzyme, and

thereafter PP_i molecule is cleaved by inorganic ***pyrophosphatase.*** Thus, three bonds, including two bonds of high-energy phosphate groups, are broken in this reaction.

(2). Transfer of the methyl group from ***S-adenosylmethionine*** to an acceptor yields ***S-adenosylhomocysteine:***

S-Adenosylmethionine + R — H → S-Adenosylhomocysteine + R — CH_3

(3). ***S-Adenosylhomocysteine*** is subsequently broken down to adenosine and ***homocysteine:***



Homocysteine

(4) ***Methionine*** is regenerated by transfer of a methyl group to ***homocysteine*** in a reaction catalyzed by *methionine synthase*, and the reconversion of methionine to 5-adenosylmethionine completes an **activated-methyl cycle** (Figure 16.3).

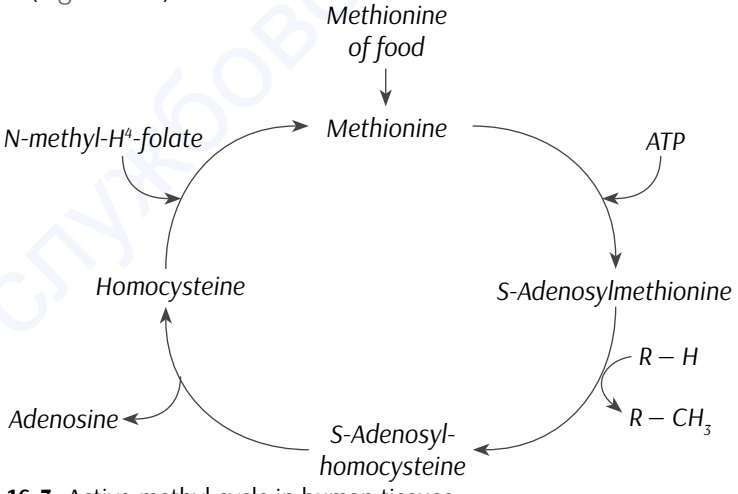
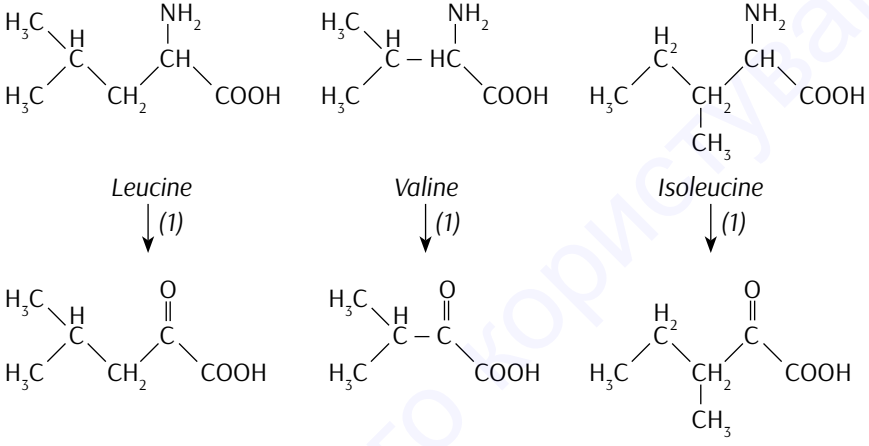


Figure 16.3. Active-methyl cycle in human tissues

16.2.3. Metabolism of the branched-chain amino acids

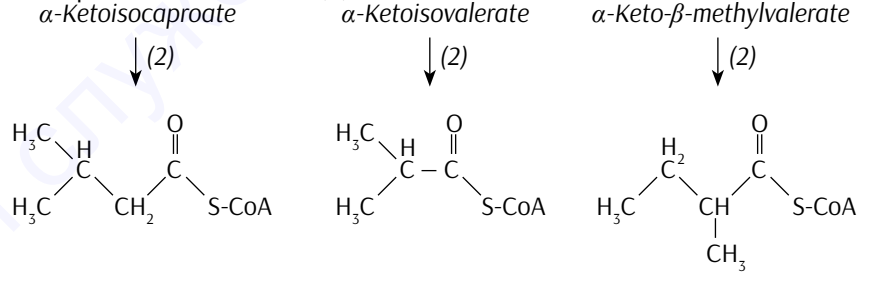
The branched-chain amino acids are **leucine, isoleucine,** and **valine.**

1. The catabolism of branched-chain amino acids begins with **transamination** that occurs in skeletal muscle, brain and adipose tissue. Liver cells lack the specific α-ketoglutarate-dependent *amino-transferase,* needed for their transamination.



α-Ketoisocapronate *α-Ketoisovalerate* *α-Keto-β-methylvalerate*

2. The **α-keto acids** that were formed in transamination reactions (1) are subjected to **oxidation and decarboxylation**. The **acyl-CoA ethers** of corresponding branched-chain amino acids derivatives are products of reaction (2).



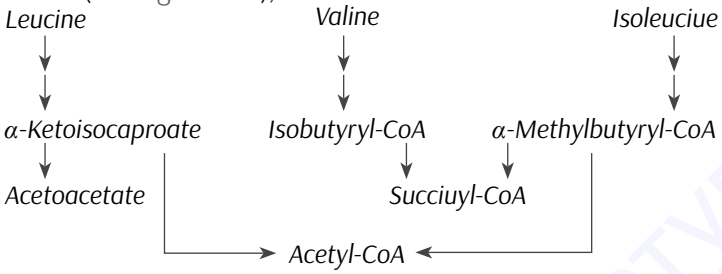
Isovaleryl-CoA *Isobutyryl-CoA* *α-Methylbutyryl-CoA*

The ***α-keto dehydrogenase complex,*** which catalyses the process, is similar to that responsible for oxidative decarboxylation of pyruvate and

α-ketoglutarate.

3. The subsequent reactions include multi-step oxidation similar to mitochondrial β-oxidation of fatty acyl CoAs.

And the following catabolic reactions yield acetyl-KoA, succinyl-CoA and acetoacetate (see Figure 10.1), that is:



Of considerable interest are concluding steps of **isoleucine** and **valine** catabolism which very largely coincide with the final catabolic reactions of **methionine,** the principal CH_3-group transporter (Figure 10.4).

As can be seen from the Figure 10.4, the carbon skeletons both of isoleucine and methionine decay to yield **propionyl-CoA.**

The latter metabolite is then carboxylated by a biotin (vitamin H)-requiring enzyme (E_1) to form **methylmalonyl-CoA.**

It is of interest that **methylmalonyl-CoA** is also a metabolite of **valine**, another branched-chain amino acid.

Methylmalonyl-CoA is isomerized by the action of vitamin B_{12}-dependent enzyme (E_2) to form **succinyl-CoA.**

Maple syrup urine disease

This is the inherited biochemical defect which consists in the lack of ***α-keto dehydrogenase complex,*** that is of enzyme catalyzing the conversion of branched-chain ketoacids into corresponding acyl-CoA esters – reaction (2) (in branched-chain amino acids transformation (see above). In the injured patients plasma and urinary levels of leucine, isoleucine, valine and α-ketoacids are greatly elevated. It is characteristic for disease that urine and sweat smell like maple syrup or burnt sugar. The children suffer from vomiting, convulsions and mental retardation.

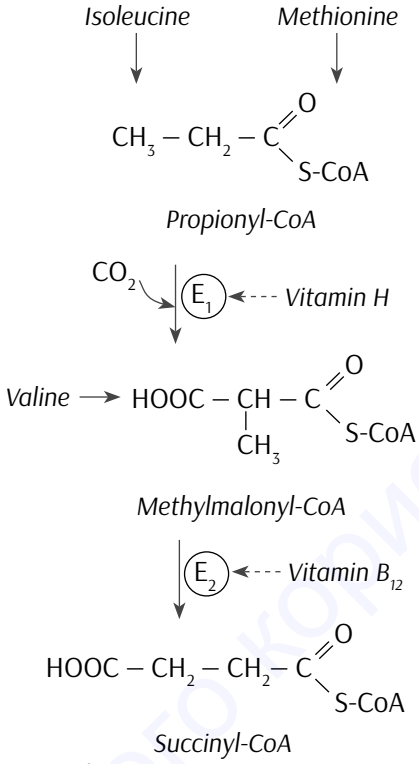


Figure 16.4. The find steps of branched chain amino acids (isoleucine, valine) and amino acid metluoitine catabolism

16.2.4. Metabolism of cyclic amino acids

The cyclic amino acids, and namely **tyrosine, thryptophan** and **histidine** serve as potent sources of vitally important biomolecules, among them hormones and neurotransmitters. The catabolism of cyclic amino acids carbon skeleton proceeds via citric acid cycle, as was shown in the Figure 10.1.

Catabolism of phenylalanine and tyrosine

1. **Tyrosine** is formed in human body by the hydroxylation of **phenylalanine,** which is nutritionally essential amino acid.

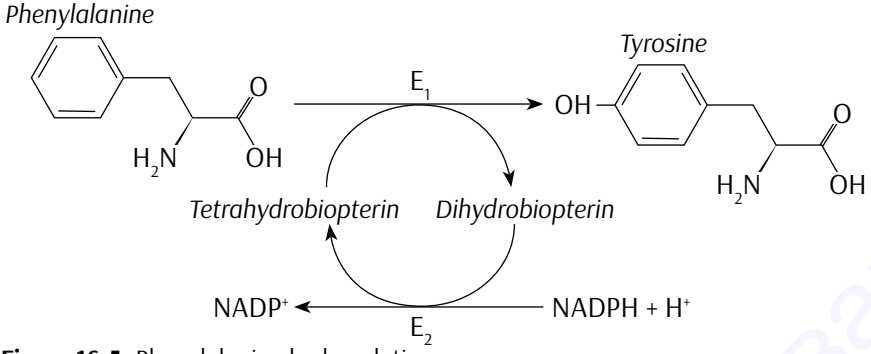


Figure 16.5. Phenylalanine hydroxylation

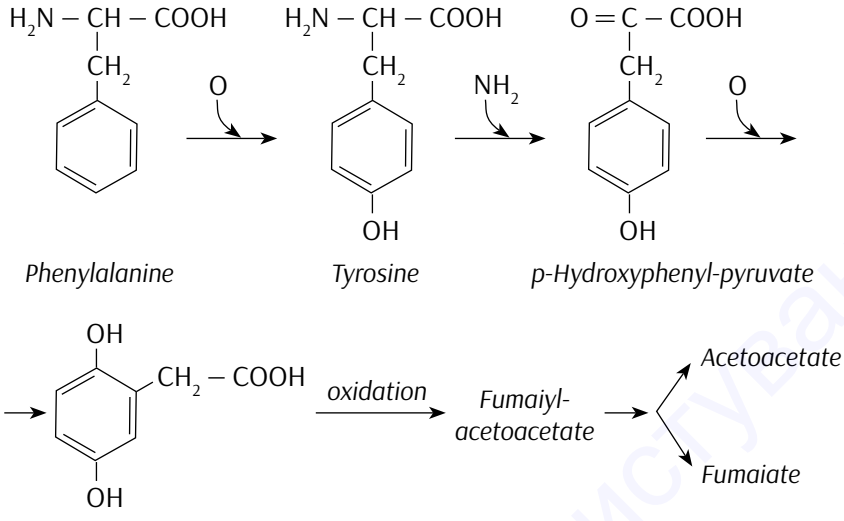
The reaction is catalyzed by *phenylalanine hydroxylase* (E_1), which requires as a cofactor **tetrahydrobiopterin**. The cofactor is oxidized in the reaction to **dihydrobiopterine** and must be regenerated by *dihydrobiopterin reductase* (E_2) which uses NADPH as the reducing coenzyme.

2. The next step is the transamination of tyrosine with α-ketoglutarate by a specific aminotransferase to form **p-hydroxyphenylpyruvate.**
3. **p-Hydroxyphenylpyruvate** is transformed into homogentisate, which is catabolized through the degradation of phenolic ring to yield **fumarylacetoacetate,** which is split to give **fumarate** and **acetoacetate** (Figure 10.6).

Hyperphenylalaninemias

There are certain types of genetic defects which are responsible for the enzymatic disturbances of **phenylalanine hydroxylation.**

These kinds of metabolic abnormalities result in the enzymatic block of ***phenylalanine*** into ***tyrosine*** transformation and the accumulation in the body of phenylalanine itself and of alternative phenylalanine catabolites, such as ***phenylpyruvate*** and ***phenylacetate,*** from which comes the classic name of disease, that is **phenylketonuria.**



Fioiue 16.6. Intermediates in nhenvlalanine and tyrosine catabolism

16.3. Amino acids as precursors of biologically important compounds

As was reported already, free amino acids serve as precursors of many physiologically essential biomolecules.

In particular, the cyclic amino acids are precursors of many hormones and neurotransmitters. The examples are:

- Amino acid **tyrosine** is a precursor of:
  - ***catecholamines (dophamine, epinephrine (adrenaline)*** and ***norepinephrine (noradrenaline),*** which carry out physiological functions of neu rotransmitters (particularly norepinephrine and dophamine) and adrenal medulla hormones (particularly epinephrine);
  - ***iodinated thyronines (thyroxine*** and ***3,3`,5-triiodthyronine)*** which are secreted by the thyroid gland and function as thyroid hormones.
- Amino acid **tryptophan** is a precursor of ***serotonin,*** that is a physiologically active compound with extremely wide spectrum of activity.

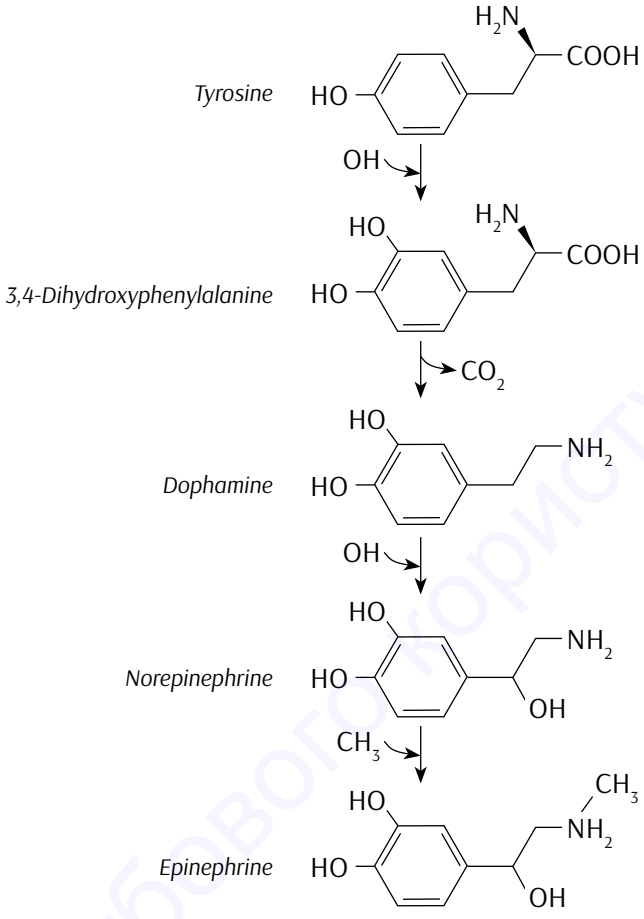


Figure 16.7. Catecholamines biosynthesis

Catecholamines biosynthesis

The general scheme of of catecholamines biosynthesis from tyrosine is presented. The synthesis begins from tyrosine and is realized through successive hydroxylations, decarboxylation and methylation of intermediary products (Figure 16.7).

16.4. Diseases associated with abnormal amino acid metabolism

Many genetic defects of amino acid metabolism have been identified in humans and they are listed in Table 16.1.

Table 16.1. Some inherited diseases of amino acid metabolism

Inherited disease	Defective enzyme	Biochemical process disturbed	Clinical symptoms
Albinism	Tyrosinase	Synthesis of melanin from tyrosine	Lack of skin and hair pigmentation
Alkaptonuria	Homogentisate oxygenase	Catabolism of tyrosine	Dark pigment in urine
Carbamoyl phosphate synthetase eficiencyd	Carbamoyl phosphate synthetase	Urea synthesis	Convulsions, lethargy, early death
Maple syrup urine disease	Branched-chain α-ketoacid dehydrogenase	Catabolism of branched-chain amino acids	Vomiting, convulsions, mental disorders, death
Phenylketonuria	Phenylalanine hydroxylase	Conversion of phenylalanine to tyrosine	Neonatal vomiting, mental retardation

Alkaptonuria was first described in the 16th century. It was clinically and biochemically characterized in 1902, which gave to **Archibald Garrod** an idea concerning heritable metabolic disorders.

Among the wide-spread metabolic disturbances, related to the lack or partial absence of a specific enzyme activity, it is noteworthy to mention inherited deficiencies of catalyst responsible for urea synthesis (see above), tyrosine transformations ***(phenylketonuria, albinism, alkaptonuria),*** branched-chain amino acids catabolism ***(maple syrup urine disease).***

16.5. Biosynthesis and catabolism of porphyrins. Gout

Porphyrins: structure, biochemical functions

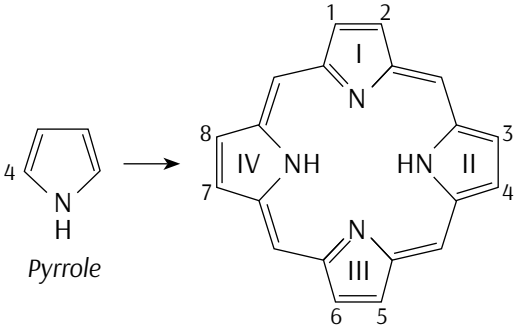
Porphyrins and their derivatives are a class of biomolecules that occupy an essential place as prosthetic groups of many vitally important proteins.

From the chemical viewpoint, ***porphyrins*** are derivatives of **porphins,** that are cyclic compounds constructed from four **pyrrole rings:** Inside porphin cycle, individual pyrrole rings are linked by methenyl (– CH =) bridges:

The **porphyrins** are compounds which are made through the substitution of the eight hydrogen atoms in the porphin nucleus by various side chains (Figure 16.8).

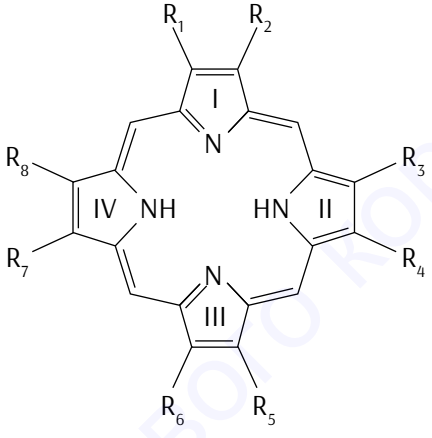
The porphyrins are named and classified by the side chains of their constituent pyrroles. The differences in the side chains are indicated by a Roman numeral (I through XV).

The distinguishing property of porphyrins is their ability to make up **complexes with metal ions,** in which metals are bound to the nitrogen atoms of the pyrrole rings. There are complexes formed with iron, cuprum and magnesium.



Pyrrole

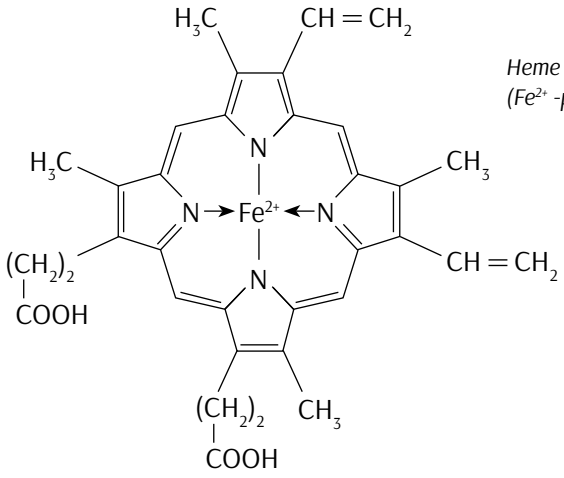
Porphin (tetrapyrrole)



Porphyrin

Figure 16.8. Structure of poiphin and porphyrins

The outstanding biomedical significance have metalloporphyrins which contain **iron.** They are **heme** of **hemoglobin,** prosthetic groups of **cytochromes** and other oxidative enzymes (Table 16.2). The conjugated proteins, that include metalloporphyrins with iron ion, are customarily designated as **hemoproteins.**



Heme of hemoglobin
(Fe^{2+} -protopoiphyrine IX)

Table 16.2. Some vitally important hemoproteins of human and animal tissues and their biochemical functions

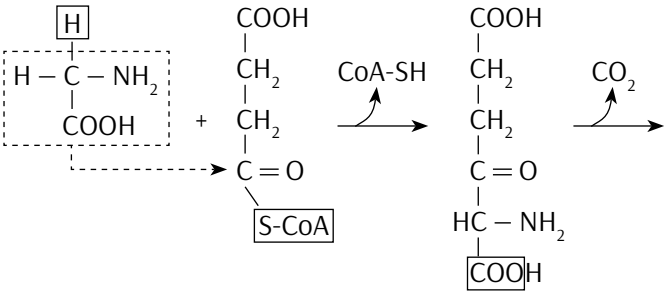
Hemoprotein	Biochemical functions
Hemoglobin	Transport of O_2 in erythrocytes
Myoglobin	Storage of O_2 in muscle
Cytochromes of mitochondria	Components of electron transport chain
Cytochrome P-450	Hydroxylation of xenobiotics and steroids
Catalase	Degradation of hydrogen peroxide
Peroxidases	Reducing of organic peroxides
Tryptophan pyrrolase	Catabolism of tryptophan

Biosynthesis of porphyrines and heme

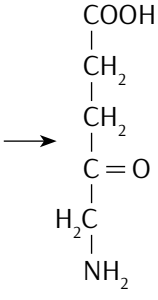
The precursors in porphyrin biosynthesis are amino acid **glycine** and citric acid cycle intermediate **succinyl-CoA.**

(1) The first enzymatic step in porphyrin biosynthesis is the condensation of glycine and succinyl-CoA which is catalyzed by ***δ-aminolevulinate synthase (ALA synthase).***

The product of the reaction is **δ-aminolevulinate.**

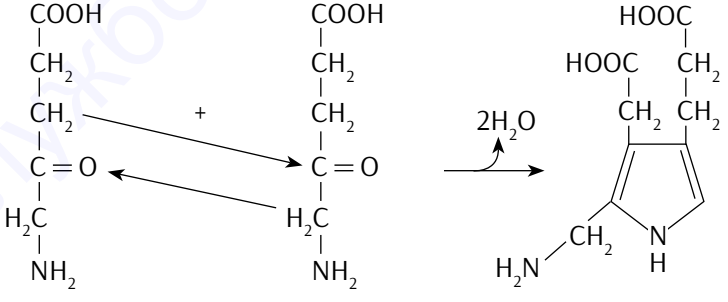


Glycine *Succinyl-CoA* *α-Ainino-β-ketoadipate*



δ-Aminolevulinate

(2) Then **porphobilinogen,** which has a pyrrole ring, is formed from two molecules of δ-aminolevulinate. The enzyme is ***δ-aminolevulinate dehydratase (ALA dehydratase)*** designated also as ***porphobilinogen synthase.***



δ-Aminolevulinate *δ*-Aminolevulinate Porphobilinogen

(3) The next step in the pathway is the condensation of four porphobilinogen molecules which brings about formation of the tetrapyrrole structure that is characteristic of the porphyrins.

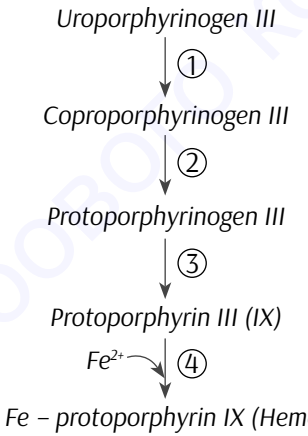
The product of the reaction is **uroporphyrinogen III** (Figure 10.9). To form the product, the enzyme ***uroporphyrinogen III synthase,*** and additional protein, **cosynthase,** are needed.

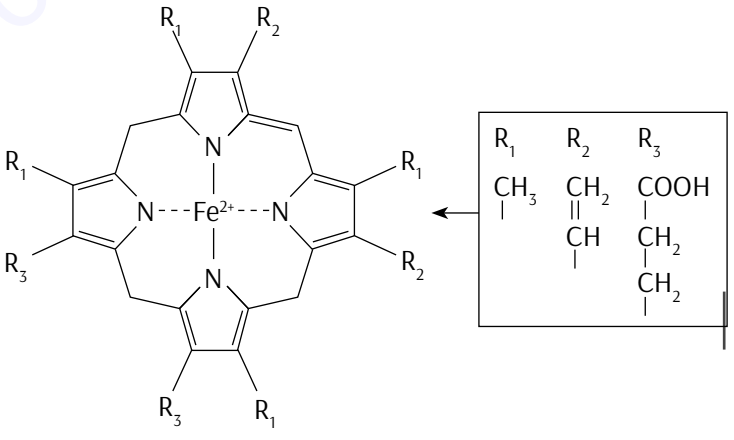
The inborn absence of **cosynthase** results in accumulation of another form of porphyrin, **uroporphyrinogen I,** that is characteristic for a certain form of **porphyria** (see below).

Porphyrin biosynthesis on the whole is regulated in animals by the concentration of the **heme,** which serves as a feedback inhibitor of early steps in the synthetic pathway (see below). Genetic defects in the biosynthesis of porphyrins can lead to the accumulation of pathway intermediates, causing a variety of human diseases known collectively as *porphyrias.*

Heme synthesis

Most of aerobic mammalian cells can synthesize **heme** (Figure 16.9), which is needed for hemoglobin, heme containing cytochromes and some other oxidative enzymes.





Heme

Figure 16.9. Biosynthesis of heme

Because the tetrapyrrole structure of **uroporphyrinogen III** is very different from that of porphyrin moiety of **heme,** additional enzymatic reactions take place, and namely:

- the biochemical modifications of porphyrin side chains. They include the conversion of ***uroporphyrinogen III*** into such tetrapyrrole structures as **coproporphyrinogen III, protoporphyrinogen III** and **protoporphyrin III (IX);**
- the incorporation of ferrous iron (Fe^{2+}) into the heterocyclic ring **protoporphyrin III (IX)** to yield **protoheme IX** which is customarily designated as **heme.** The reaction is catalyzed by the mitochondrial enzyme ***ferrochelatase.*** And the heme, thus formed, is the prosthetic group of hemoproteins.

The enzymic steps in the **heme** formation from **uroporphyrinogen III** are summarized in the outline presented in the Figure 16.10. The enzyme involved are:

- ***uroporphyrinogen decarboxylase*** **(1);**
- ***coproporphyrinogen oxidase*** **(2);**
- ***protoporphyrinogen oxidase*** **(3);**
- ***ferrohelatase*** **(4).**

Especially high concentrations of heme synthesis enzymes are found in the cells that are responsible for hemoglobin production, and particularly in nucleated erythrocytes *(reticulocytes),* bone marrow, liver cells and intestinal mucosa.

Control of heme synthesis

It is noteworthy that in human tissues ***ALA synthase*** exists in two isoensymic forms which are **hepatic (ALAS 1)** and **erythroid (ALAS 2)** enzymes.

The hepatic form, that is ALAS 1, is a regulatory enzyme, and thus, the reaction of δ-aminolevulinate production is the rate-limiting step in the overall process of heme synthesis. The rate of ALAS 1 biosynthesis increases markedly in the absence of heme and is inhibited in the presence of the latter.

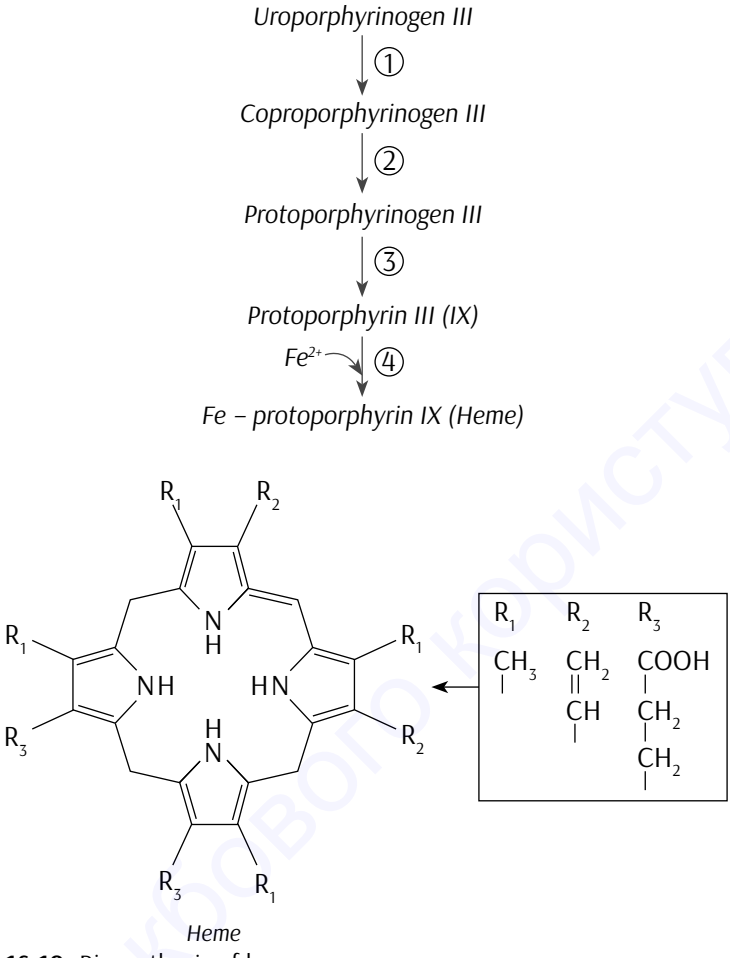


Figure 16.10. Biosynthesis of heme

Heme and hemoproteins

The **heme,** formed as reported above, can be incorporated into such hemoproteins as hemoglobin of erythrocytes, myoglobin of muscle, cytochromes and other, vital important, oxidation-reduction enzymes (Table 10.2).

Porphyrias: biochemical and clinical features

Porphyrias are a group of genetic diseases due to the disorders in physiological pathways of porphyrins biosynthesis.

Under porphyrias, the abnormal types of porphyrins and their precursors are accumulated in human tissues, especially in erythrocytes, body fluids, and the liver. This is generally due to the genetic defects in specific enzymes production or, sometimes, to the action of harmful external factors, such as certain toxic substances (*acquired porphyrias*).

Genetically defective reactions of porphyrin synthesis may occur in the liver **(hepatic porphyrias)** or in the erythropoietic tissues **(erythropoietic porphyrias).** So far, some different clinical types of inborn porphyria have been recorded. All they result from the lack of or depression in the activities of certain enzymes in the porphyrin biosynthesis pathway (Table 10.3).

The usual **clinical manifestations of porphyrias** are anemia, abdominal pains, photosensitivity of the skin and severe neurological disorders. There is a speculation that some medieval folk legends describing human vampires, or so-called werewolves, have their origin in the behaviour of porphyria sufferers, being based on their light-shyness, strange appearance and drinking of blood as the way to compensate for the heme deficiency.

The mostly widespread is **acute intermittent porphyria.** The enzyme involved is ***uroporphyrinogen III synthase.*** Because the affected individu-als are usually heterozygotes as to the abnormal gene, the disease may not show clinical symptoms for a long time and manifest only under unfavourable nutritional or environmental conditions. There is an opinion that the periodic attacks of madness, typical of the British monarch King George III, were due to the kind of porphyria under discussion.

The other common form of disease, ***congenital erythropoietic porphyria,*** results from the insufficiency of **cosynthase,** that is a special protein required for the regular production of **uroporphyrinogen III** (Figure 11.2). Under this pathology, **uroporphyrinogen I,** which is the isomeric form of above-mentioned **uroporphyrinogen III,** is formed and accumulated in tissues concurrently with uroporphyrin I, coproporphyrin I and other symmetric porphyrins. In this disease, erythrocytes are prematurely destroyed, and the urine of patients is red because of the excretion of large amounts of uroporphyrin I. It is characteristic that the teeth of the sufferers exhibit a strong red fluorescence under UV-light, and the skin is unusually photosensitive.

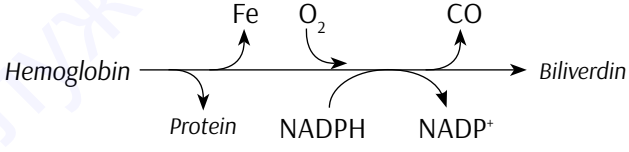
Table 16.3. The major clinical findings in the inborn porphyrias (after R. K. Murray et al., 2003; modified)

Enzyme deficiency	Clinical symptoms prevalent
1. ALA synthase	Anemia
2. ALA dehydratase	Abdominal pain, neuropsychiatric disorders
3. Uroporphyrinogen III	Uroporphyrin I accumulation
4. Uroporphyrinogen decarboxylase	Photosensitivity
5. Coproporphyrinogen oxidase	Photosensitivity, abdominal pain, neuropsychiatric disorders
6. Protoporphyrinogen oxidase	Photosensitivity, abdominal pain, neuropsychiatric disorders
7. Ferrochelatase	Photosensitivity

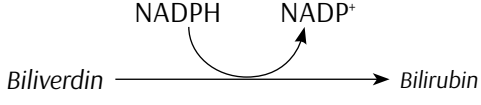
Catabolism of hemoglobin and heme

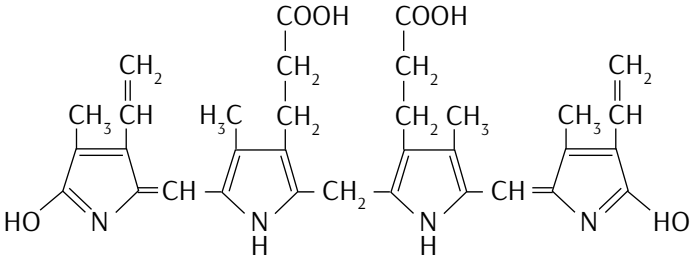
In the human body, approximately 100–200 million erythrocytes are broken down every hour. Red cells destruction occurs predominantly in the spleen, bone marrow and in the reticuloendothelial cells of the liver, which are designated as Kupffer`s cells.

1. Hemoglobin degradation begins from the protein part of the molecule, that is globin, removing, and iron ion (Fe^{2+}) release.
2. The porphyrin moiety of the molecule is subjected to the action of the NADPH-dependent ***heme oxygenase*** which cleaves carbon monooxide (CO) to give the green pigment **biliverdin.**



3. The reduction of biliverdin into the reddish-brown pigment, **bilirubin,** then follows. The reaction is catalyzed by the *biliverdin reductase*, which is also a NADPH-requiring enzyme.

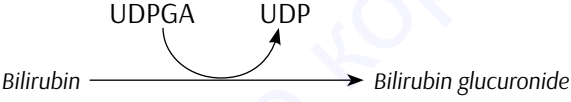




Bilirubin

Both bilirubin and biliverdin are referred to as **bile pigments.**

4. Inside liver cells, hepatocytes, bilirubin is conjugated with ***glucuronic acid*** to yield **bilirubin diglucuronide,** and this reaction is thought to be the detoxication of bilirubin, which is a toxic compound for most human cells, particularly, brain neurons. **Uridine diphosphate glucuronic acid (UDGA)** is the active form of glucuronate which takes part in the reaction.



5. Via the bile, bilirubin glucuronide is excreted into small intestine, and its further transformation occurs inside bowels lumen. The human bowel micro-organisms convert bilirubin and bilirubin diglucuronide into **urobilin** and **stercobilin**, that are excreted in the faeces (*stercobilin*) and partially in the urine (*urobilin*).

According to the modern conception, **certain intermediates of heme breakdown pathway play significant roles in protecting cells from oxidative damage.** Thus, it is shown, that **CO** produced by ***heme oxygenase,*** and which is toxic at high concentrations, appears to have some regulatory and/or signaling functions. Moreover, **bilirubin proved to be the potent antioxidant,** which is responsible for most of the antioxidant activity in human serum.

On the other hand, the disorders in bile pigments metabolism, which occur under many hepatic diseases and the massive erythrocytes breakdown (hemolysis), result in the great rise of tissues and plasma bilirubin concentration. This **hyperbilirubinemia** usually leads to **jaundice**, that is the yellowing of the skin and sclera, under which condition the severe brain cells damage is observed (Lecture 18).

The condition of **hyperbilirubinemia** and **jaundice** may be also observed in newborn infants, in the liver cells of which ***glucuronyl bilirubin transferase*** is not yet produced in quantities required for processing bilirubin into bilirubin diglucuronide that is more soluble and thence more liable for bile excretion.

Chapter 17.

METABOLISM OF PURINE AND PYRIMIDINE NUCLEOTIDES. PURINES DEGRADATION. GOUT

Contents

17.1. Nucleotides: structure, biochemical functions

Nucleotides are constituents from which nucleic acids **DNA** and **RNA** are made. Chemically, **nucleic acids** are polymers formed of the single nucleotides, thus nucleic acids are polynucleotides.

In turn, every **nucleotide** consists of a *nitrogenous base* (*nucleobase*), a *sugar* (pentose) and a *phosphate residue*. The **nucleosides** contain a nitrogenous base and a pentose residue (see **Bioorganic Chemistry**).

DNA (deoxyribonucleic acid) and **RNA (ribonucleic acid)** differ from one another in the type of the sugar (*2-deoxyribose* in DNA and *ribose* in RNA) and in one of the bases, which they contain.

And the nucleobases are heterocyclic compounds derived from either **purine** or **pyrimidine.**

There are **five major purine and pyrimidine bases** which are found in all living organisms:

- the **purine bases** of nucleotides are **adenine (Ade)** and **guanine (Gua);**
- these are present in both **RNA** and **DNA** (Figure 11.1);
- the **pyrimidine bases** of nucleotides are **cytosine (Cyt), uracil (Ura)** and **thymine (Thy).** Among these, the *cytosine* is present both in RNA

and DNA, the *uracil* is found only in RNA, and the *thymine* in DNA (Figure 11.2).

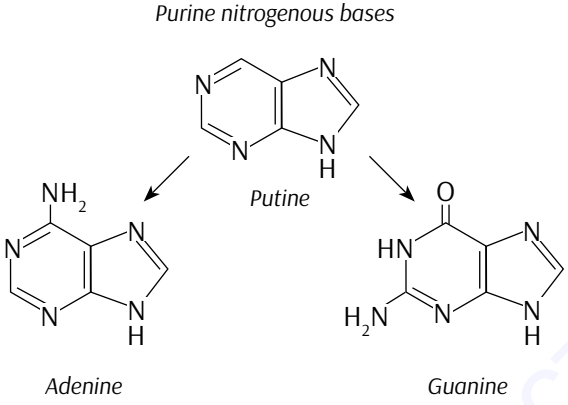


Figure 17.1. Purine nueleobases

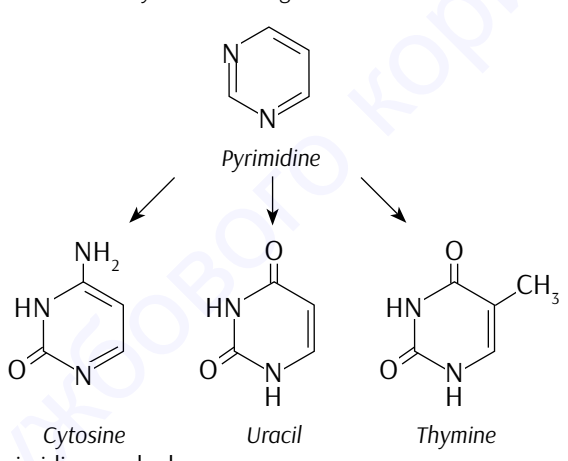
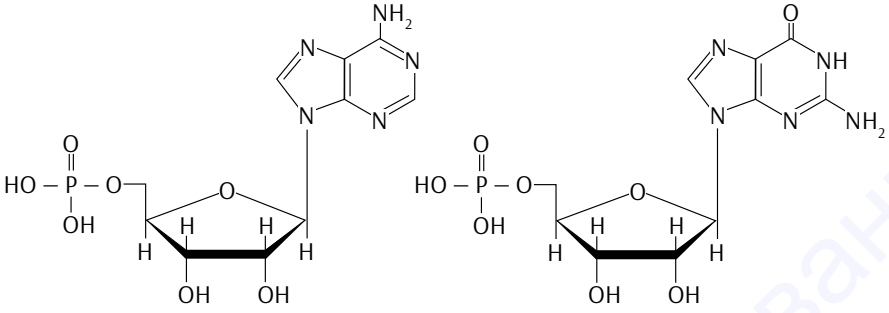


Figure 17.2. Pyrimidine nucleobases

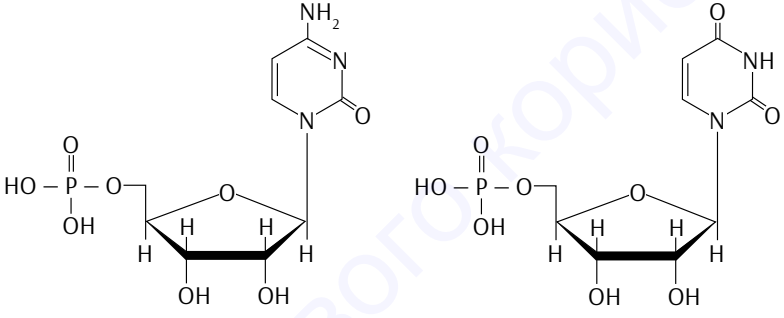
Purine and **pyrimidine nucleotides** of RNA are presented in Figure 11.3. As was reported already, in DNA the sugar is 2-deoxyribose.

Purine nucleotides of RNA



Adenine nucleotide *Gunnine nucleotide*

Pyrimidine nucleotides of RNA



Cytidine nucleotide *Uridine nucleotide*

Figure 17.3. Purine and pyrimidine nucleotides

17.2. Biosynthesis of purine nucleotides

Purines and **pyrimidines** are nutritionally nonessential biomolecules. It means that human tissues can synthesize both purines, pyrimidines and their biologically essential derivatives from simpler compounds.

It is noteworthy that purines are synthesized of building blocks predominantly in the form of completely organized nucleotides.

There are two ways of purine nucleotides biosynthesis:

1. Synthesis from other intermediates, including amino acids, NH_4^+, CO_2 and one-carbon groups in the form of tetrahydrofolate derivatives. This is called ***synthesis de novo*** and serves as the principal pathway

of purine nucleotides biosynthesis.

2. Synthesis of nucleotides by the recycling of preformed nitrogenous bases and other nucleotides components, that is by phosphoribosylation of purines and pyrimidines bases. It is designated as ***salvage synthesis.***

The origin of purine ring N- and C-atoms.

Sources of the nitrogen and carbon atoms of the purine ring are presented in the Figure 11.4. **They are:**

1. **N-1** is from **aspartate.**
2. **N-3** and **N-9** are from the amide nitrogen of **glutamine.**
3. **C-4**, **C-5** and **N-7** are from **glycine.**

C-2 and **C-8** are from **formiate** via tetrahydrofolate derivatives.

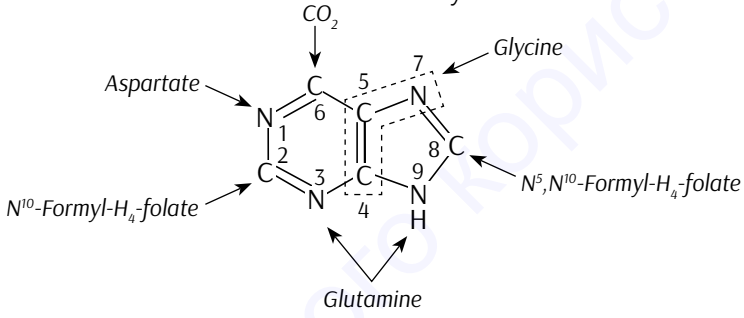


Figure 11.4. Schematic image of the sources of the individual N- and C-atoms of the purine ring

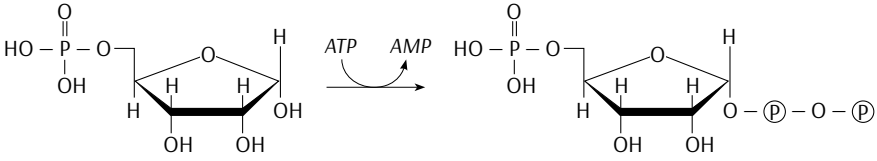
Prines biosynthesis *de novo* reactions

The first purine nucleotide formed is **inosine 5`-monophosphate (IMP).**

Its synthesis begins from the phosphorylated sugar, **α-D-ribose 5-phosphate,** on which molecule the heterocyclic structure of purine is assembled.

The reactions are as follows:

1. the formation of **5-phosphoribosyl-1-pyrophosphate** (PRPP), the activated donor of the sugar unit in the purine nucleotide:

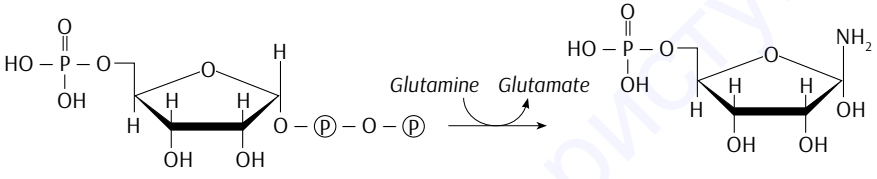


α-D-Ribose 5-phosphate *5`-Phosphoribosyl-1-pyrophosphate*

Enzyme is ***PRPP synthetase.***

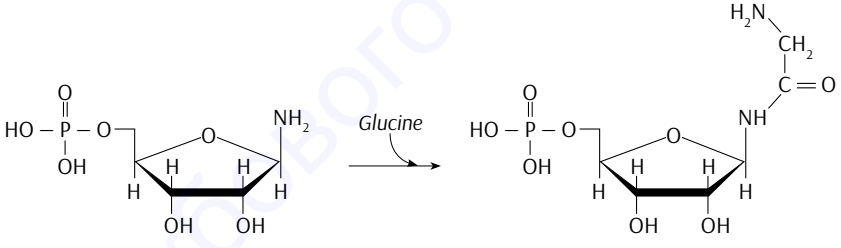
2. PRPP is the immediate precursor of **5-phosphoribosylamine,** which molecule is formed by the addition of amino group, the precursor of purine:

N-9:



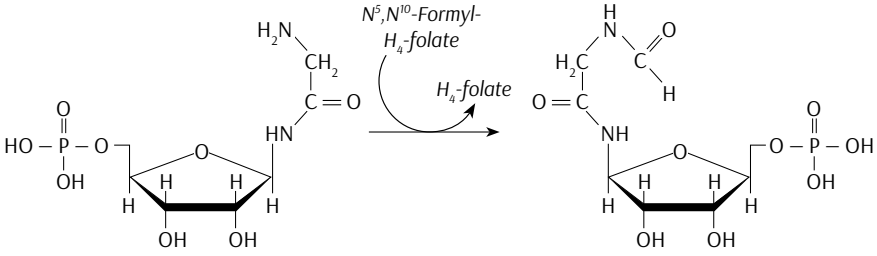
5`-Phosphoribosyl-1-pyrophosphate* *5`-Phosphoribosylamine

3. The addition of glycine molecule gives **5`-phosphoribosylglycinamide:**



5`-Phosphoribosylamine* *5`-Phosphoribosylglycinamide

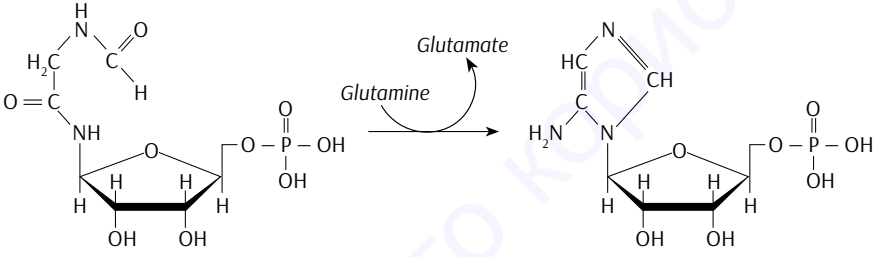
4. A formyl group is transferred from N^5,N^{10}-formyltetrahydrofolate to form **5`-phosphoribosyl-N-formylglycinamide:**



5`-Phosphoribosylglycinamide* *5`-Phosphoribosyl-N-formyl-glycinamide

5. The transfer of another N-atom from glutamine and dehydration give ring closure.

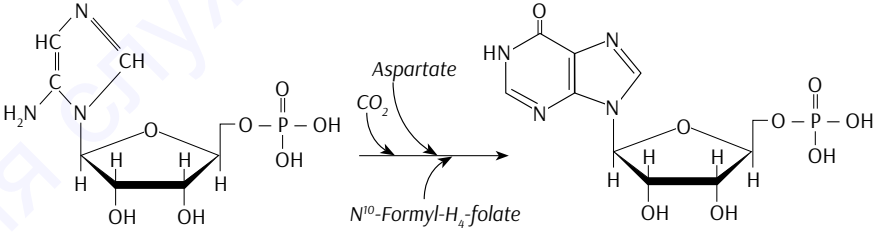
The product of the reaction is **5-aminoimidazole ribonucleotide,** which contains the complete five-membered ring of the purine skeleton:



5`-Phosphoribosyl-N-formyl-glycinamide *5-Aminoimidazole ribonucleotide*

6. Then a succession of reactions follows, in which the addition of ***CO_2***, the transfers of amino group from ***aspartate*** and of another one-carbon group from ***N^{10}-formyl-H_4-folate*** occur.

The closure of six-membered ring takes place, and the formation of purine nucleotide, **IMP,** is completed:



5-Aminoimidazole ribonucleotide *Inosine 5`-monophosphate*

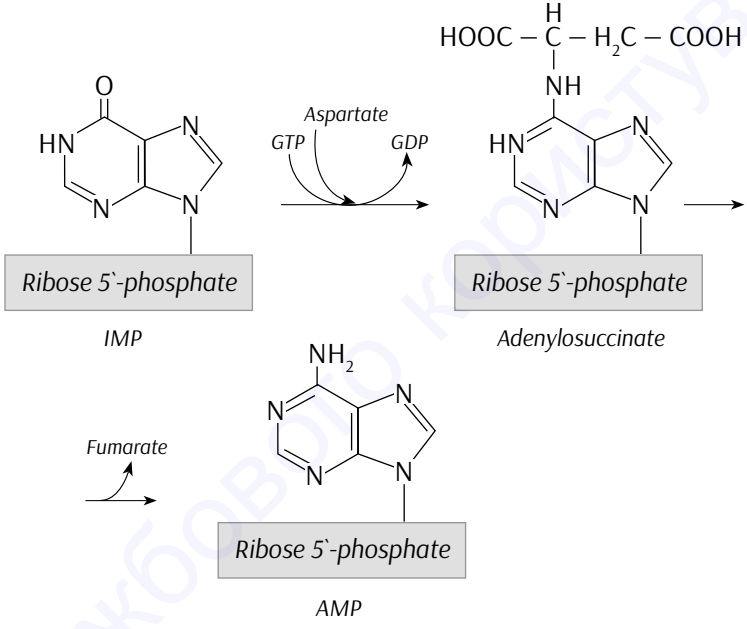
Then follows the conversion of **inosine 5`-monophosphate (IMP)** into purine nucleotides **AMP (adenosine monophosphate)** and **GMP (guano-**

sine monophosphate).

Synthesis of AMP and GMP

1. **Transformation of IMP into AMP** is achieved by the substitution of an amino group for the carbonyl oxygen atom at C-6 of the purine ring.

This is realized by the addition of ***aspartate*** to yield **adenylosuccinate** followed by the elimination of ***fumarate:***

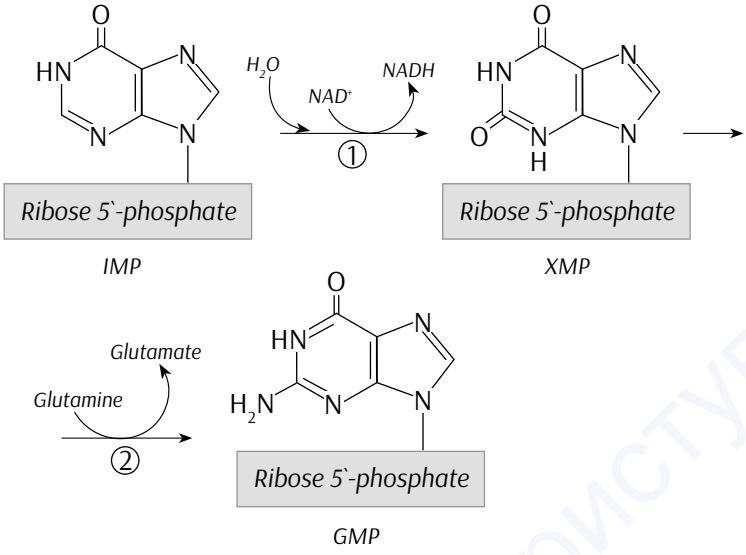


2. **Transformation of IMP into AMP** is achieved in two steps:

Step one is the formation of **xanthosine 5`-monophosphate (XMP)** from **IMP.**

Step two is the conversion **XMP** into **GMP.**

The metabolic chart of AMP and GMP synthesis from IMP is presented in Figure 11.5.



AMP and **GMP** are utilized for the biosynthesis of nucleic acids **DNA** and **RNA** and, besides, can be used as coenzymes and regulatory biomolecules.

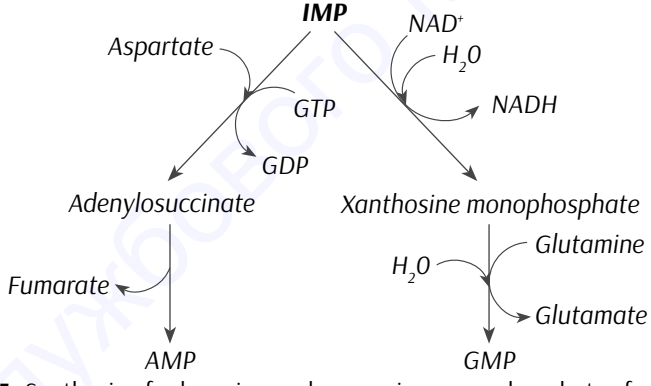


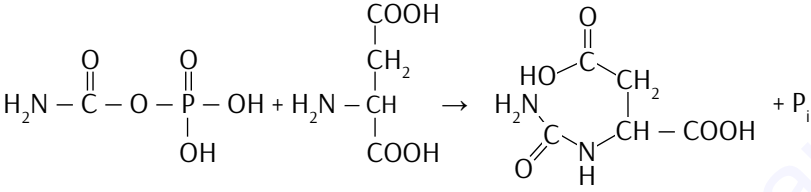
Figure 17.5. Synthesis of adenosine and guanosine monophosphates from IMP

17.3. Biosynthesis of pyrimidine nucleotides

The pyrimidine ring, unlike the purine ring, is not built up on a ribose phosphate moiety. Rather, the pyrimidine ring is first formed, and then PRPP

is used to from the nucleotide.

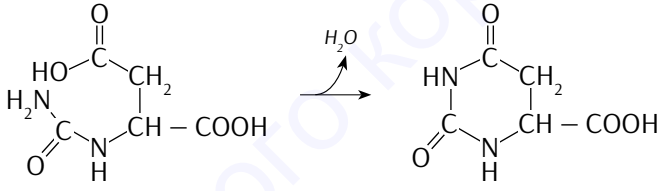
1. The first step in the biosynthesis of pyrimidines is the formation of **N-carbamoylaspartate** from ***aspartate*** and ***carbamoylphosphate.***



Carbamoylphosphate *Aspartate* *N-Carbamoyl aspartate (ureidosuccinate)*

The reaction is catalyzed by ***aspartate transcarbamoylase.*** This is an especially interesting regulatory enzyme which is feed-back inhibited by ***cytidine triphosphate (CTP),*** that is the final product in the pathway.

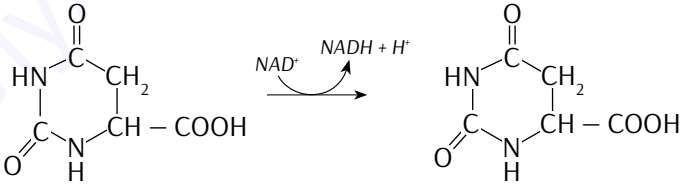
2. In the next reaction ***N-carbamoylaspartate*** cyclizes with the loss of water to yield **dihydroorotate.**



N-Carbamoyl aspartate *Dihydroorotate*

Thus, the pyrimidine ring is formed. The reaction is catalyzed by ***dihydroorotase.***

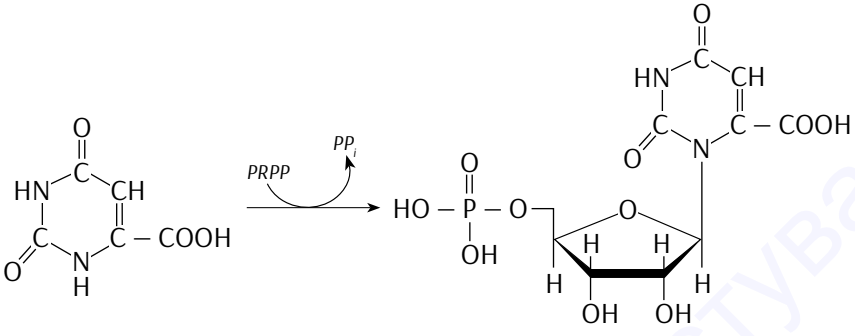
3. ***Dihydroorotate*** is subjected to dehydrogenation to give **orotate.**



Dihydroorotate *Orotate*

The reaction is catalyzed by NAD-required ***dihydroorotate dehydrogenase.***

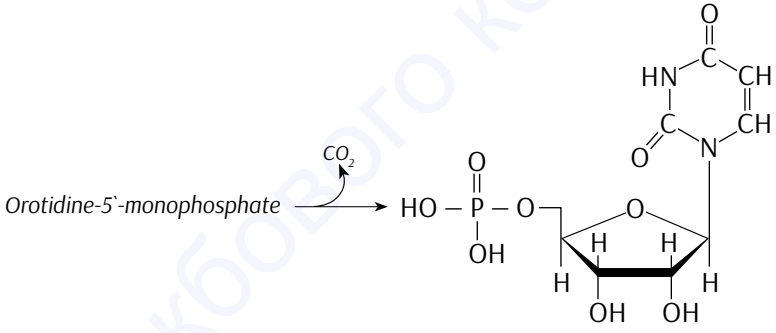
4. ***Orotate*** acquires a ribose phosphate group from ***5-phosphoribosyl-1-pyro-phosphate (PRPP),*** and the first pyrimidine nucleotide, **orotidine 5`-monophos-phate (orotidylate),** is formed.



Orotate *Orotidylate*

The enzyme is ***orotate phosphoribosyl transferase***.

5. Decarboxylation of ***orotidine 5`-monophosphate*** forms **uridine 5`-monophosphate (UMP).**



Uridine-5`-monophosphate

The enzyme is ***orotidylate decarboxylase***.

Nucleosides di- and triphosphates

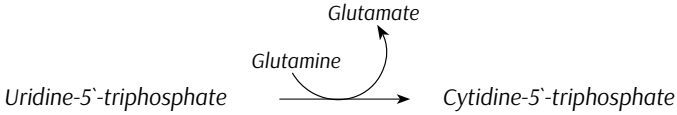
To take part in biosyntheses and energy transformations, nucleoside monophosphates need to be converted into **di- and triphosphates.**

This is accomplished in the reactions that utilize ATP as the phosphoryl groups donor.

For example:

$$UMP + ATP \rightarrow UDP + ADP;$$
$$UDP + ATP \rightarrow UTP + ADP$$

In its turn, **UTP** can be transformed into **cytidine nucleotide, CTP:**



17.4. Catabolism of purine and pyrimidine nucleotides. Gout

The **purine** and **pyrimidine nucleotides** arise in human tissues from the hydrolytic degradation of cellular nucleic acids and by means of biosynthesis *de novo* (see above).

1) The hydrolysis of nucleic acids, **RNA** and **DNA,** is carried out via the catalytic action of specific ***nucleases***, and specifically ***ribonucleases*** and ***deoxyribonucleases***, which break down the polynucleotide chains to **(mono)nucleotides.**
2) The **nucleotides** are subjected to the removal of phosphate in ***nucleotidases*** catalyzed reactions, and **nucleosides** are produced.
3) The degradation of free **purine** and **pyrimidine nucleosides** occurs by very specific pathways.

Catabolism of purine nucleotides

In human tissues, the **purines** are degraded to **uric acid,** which is excreted through the kidneys. Thus, the **purine ring** remains intact in the process of catabolism (Figure 17.6).

1) The degradative pathway for **AMP** begins from its conversion to nucleoside **adenosine,** and after that the adenosine deamination takes place to yield **inosine.** Inosine undergoes *phosphorolysis* to give **hypoxanthine**, which is oxidized to **xanthine** under the action of ***xanthine oxidase.***
2) **GMP** is converted into nucleoside **guanosine,** which is subjected to *phosphorolysis* to give **guanine.** Guanine is deaminated, and **xan-**

thine is produced.

3) After **xanthine** generation, the catabolic pathways of **AMP** and **GMP** converge.

Xanthine is subjected to oxidation to uric acid. The enzyme is *xanthine oxidase,* which is **FAD**-dependent catalyst, that requires also iron **(Fe^{3+})** and molybdenum **(Mo)** ions (Figure 17.7).

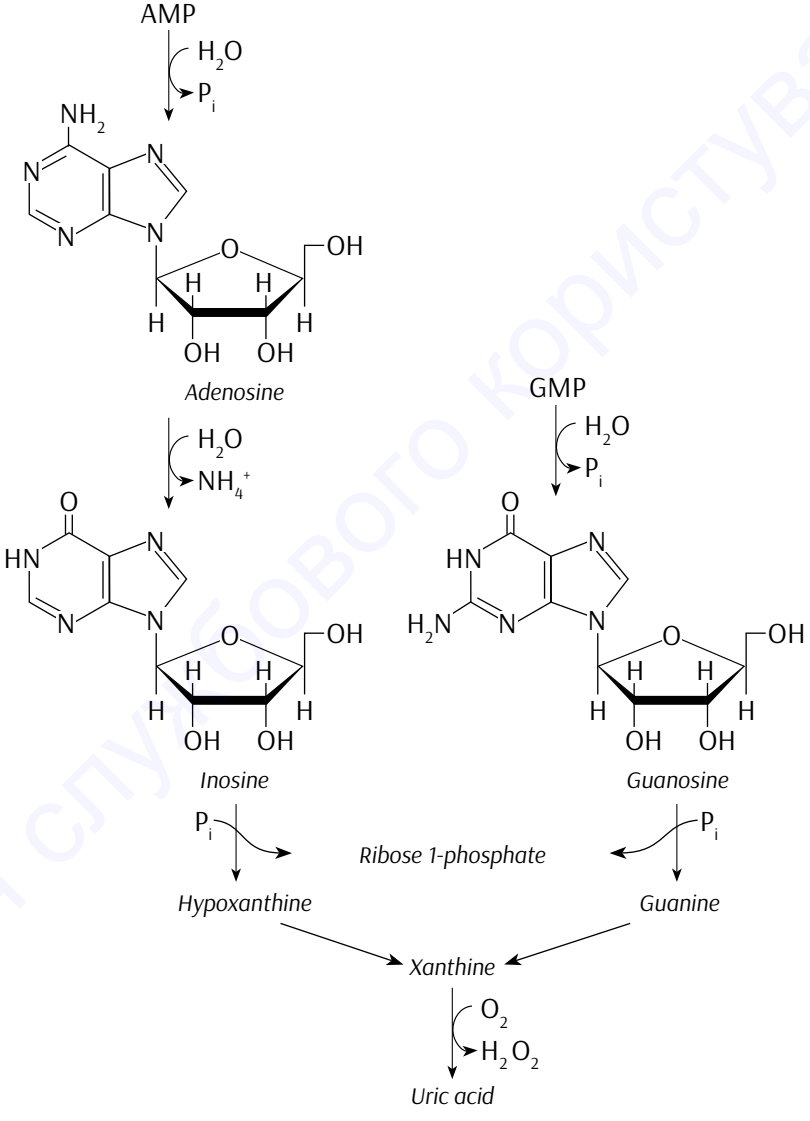


Figure 17.6. Metabolic chart of purines catabolism

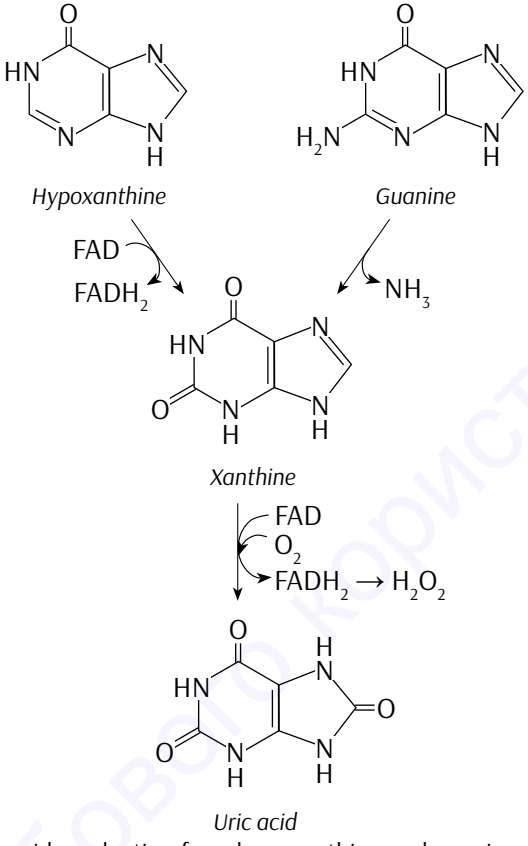


Figure 17.7. Uric acid production from hypoxanthine and guanine

Gout

Gout is a metabolic disorder of purine catabolism. Primary goat is caused by an excessive formation of **uric acid** due to an overproduction of purine nucleotides. **The genetic defect in gout is the abnormally high activity of PRPP synthetase,** the enzyme which launches purines biosynthesis reactions (see above).

Under gout, the serum level of uric acid exceeds its solubility limit (6.8 mg/dl), and crystals of sodium urate are deposited in the joints of the extremities. These deposits are known in clinical medicine as ***tophi urici*,**

which cause deformations of the joints and give severe pains.

Catabolism of pyrimidine nucleotides

In contrast to purines, the **pyrimidine rings** of ***uracil***, ***thymine*** and ***cytosine*** are broken down to small fragments, in particular **CO_2, NH_3, β-alanine** and **β-aminoisobutyrate,** which are also excreted in the urine. The principal enzymatic steps of pyrimidines catabolism are presented in the Figure 17.8.

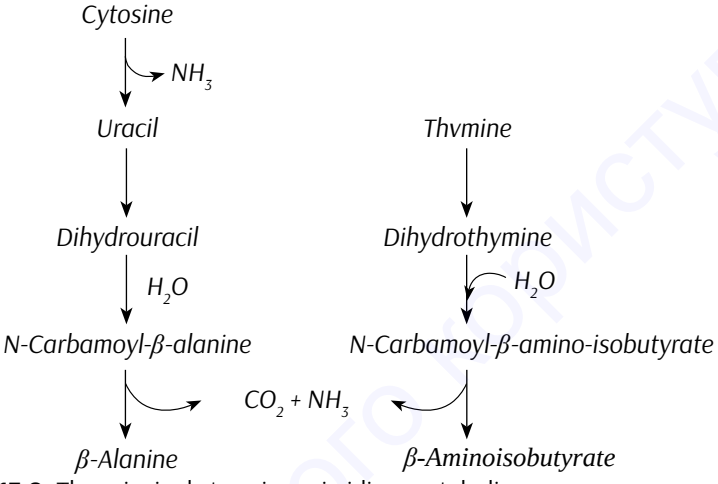


Figure 17.8. The principal steps in pyrimidines catabolism

The excretion of pyrimidine nucleotides catabolites, especially **β-amino-isobutyrate,** greatly rises in patients who suffer from leukemia and after x-ray radiation exposure due to the increased destruction of cellular DNA.

Part 4

FUNDAMENTALS OF MOLECULAR BIOLOGY AND GENETICS

Chapter 18.

GENE, GENOME. DNA REPLICATION

18.1. Gene, genome. DNA, RNA: structure, properties

Gene. Genome

The fundamental physical and functional unit of heredity is **gene.** From the modern point of view, **gene is an ordered sequence of nucleotides located in a particular position of genetic DNA.** Gene, found on a definite chromosome, encodes a specific functional product (i.e., **RNA** molecules and proteins).

All the genetic material in the chromosomes of a particular organism constitutes a **genome.** Genome`s size is generally given as its total number of base (or *nucleobase*) pairs.

Nucleic acids: DNA, RNA

Recall that **nucleic acids**, both **DNA** (deoxyribonucleic acid) and **RNA** (ribonucleic acid), are polymers consisting of **nucleotides.** In turn, nucleotides are formed from nitrogenous bases (or nucleobases), which are purine or pyrimidine derivatives, pentoses and phosphoric acid residue (see Figures 13.1–13.2, and **Bioorganic chemistry**).

As noted already in Lecture 12, the two kinds of nucleic acids are distinctive as to their nucleobases and sugar components (Figure 13.3) and biological properties, and namely:

- Purines, obtained both from DNA and RNA are ***adenine*** (6-aminopurine) and ***guanine*** (2-amino-6-oxypurine);
- Pyrimidines from DNA are ***cytosine*** (2-oxy-4-aminopyrimidine) and ***thymine*** (5-methyl-2,4-dioxypyrimidine); pyrimidines of RNA are ***cytosine*** and ***uracyl*** (2,4-dioxypyrimidine).

- The sugar in DNA is 2`-deoxyribose (deoxyribose); the sugar in RNA is ribose;
- The molecule that encodes genetic information is DNA (in certain biological species, namely viruses, RNA).
- RNA molecules serve as the template for protein synthesis (mRNA – see below), and take part in certain essential steps of ribosomal translation.

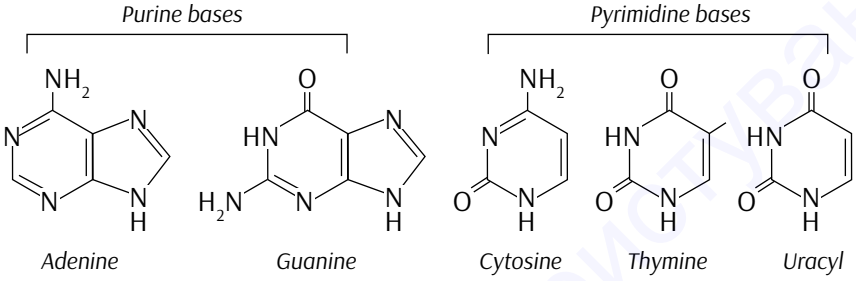


Figure 18.1. Nucleobases of DNA and RNA

Formation of nucleosides and nucleotides

Nucleosides consist of a purine or pyrimidine base, which are linked to ribose or deoxyribose. Speaking in chemical way, nucleosides are N-glycosides of sugars, and called ***ribonucleoside***s and ***deoxyribonucleosides*,** correspondingly.

Nucleotides are phosphate esters of nucleosides that are formed via esterification of sugar hydroxyl group in 5`- or 3`-position.

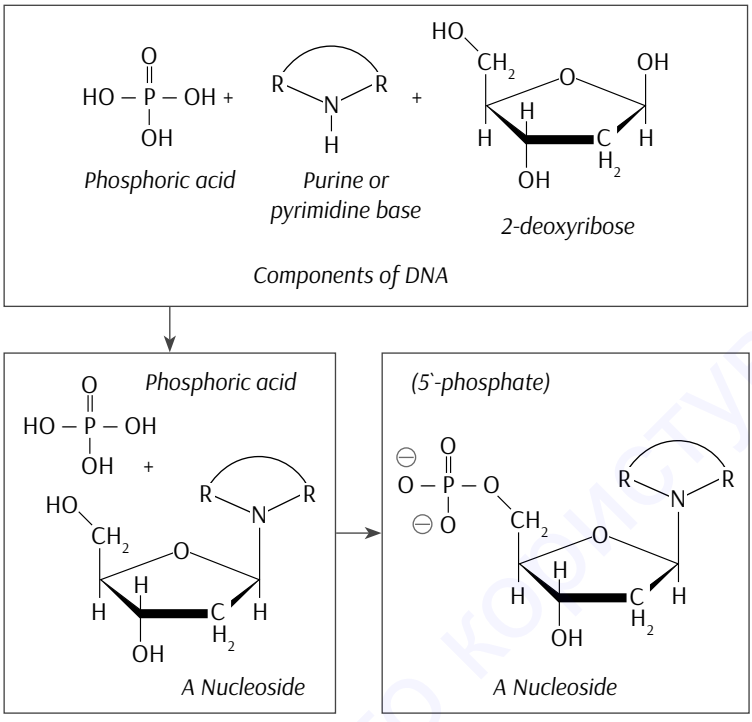
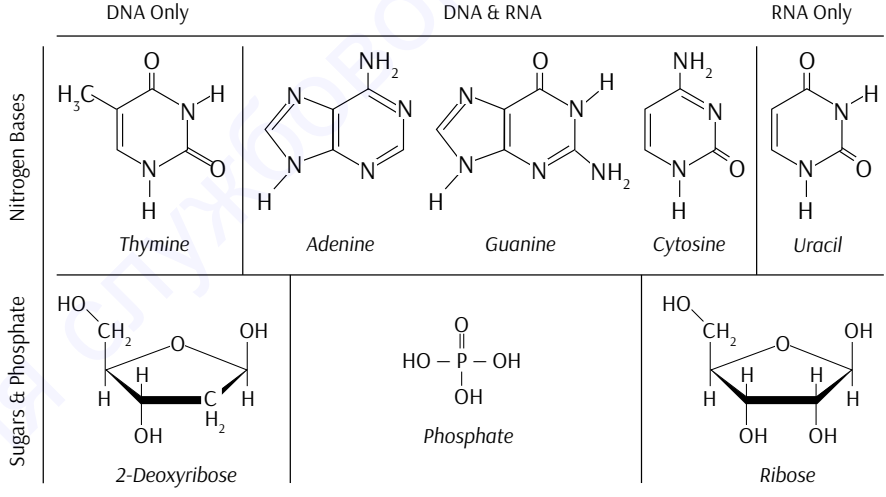


Figure 18.2. Nucleosides and nucleotides formation



DNA (deoxyribonucleic acid)

DNA is a double-stranded polymeric molecule, each strand of which is a polynucleotide composed of nucleotides **A** (adenosine), **T** (thymidine), **C** (cytidine), and **G** (guanosine) residues (Figure 13.3 *a*). To make up polynucleotide strands, polymerization takes place through the dehydration reaction, which forms linear chains with specific nucleotide sequences.

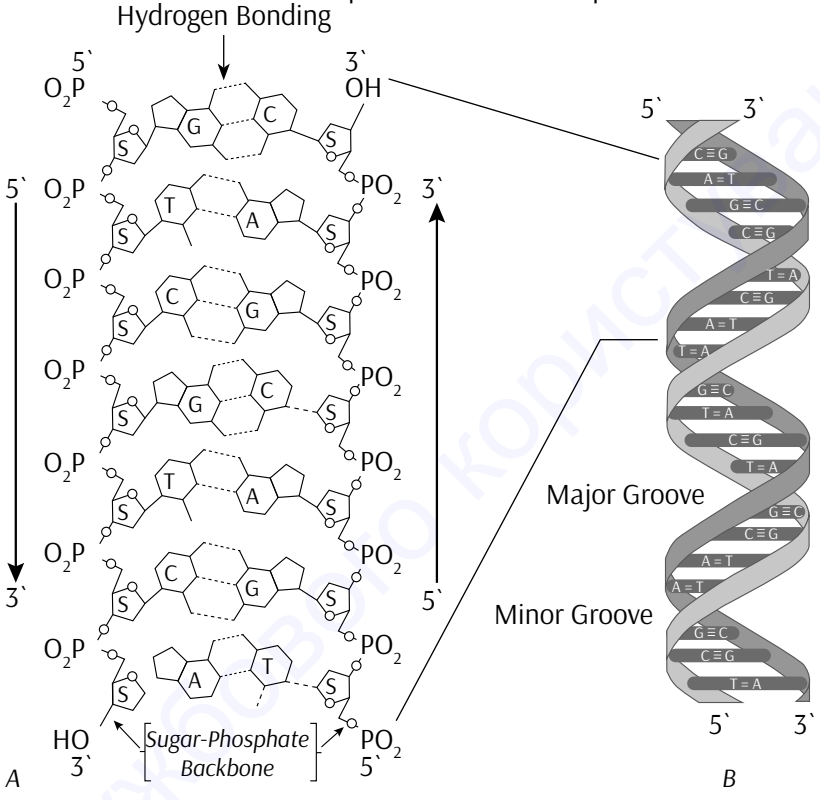


Figure 18.3. DNA image as two-strand polynucleotide molecule

Two strands in a single DNA molecule are held together by **hydrogen bonds** between base pairs of nucleotides. Owing to these weak bonds, base pairing occurs between appropriate nucleotides, that is A and T and between G and C;

The corresponding nucleotides that ale held together by hydrogen bonds are called ***complementary nucleotides;*** and the nucleobases which are included are called ***complementary nucleobases*** correspondingly.

Thus, the base sequence of each single strand of DNA can be deduced from that of its partner, and this fundamental chemical property is crucial

for the mechanism of biological replication of the whole DNA molecule.

Polarity of DNA strands.

Each strand of DNA has **polarity,** such that the 5'-hydroxyl (or 5'-phospho) group of the first nucleotide begins the strand and the 3'-hydroxyl group of the final nucleotide ends the strand; accordingly, we say that this strand runs 5' to 3' *("Five prime to three prime").* It is noteworthy to state that the two strands of DNA run *antiparallel* such that one strand runs 5'→3' while the other one runs 3'→5' *("Three prime to five prime").*

Coiling the coupled strands leads to a **double helix** structure, shown as cross-linked ribbons in part **(b)** of the Figure 13.3, and which resembles a spiral staircase. A space-filling molecular model of a short segment of DNA is displayed in the Figure 18.4.

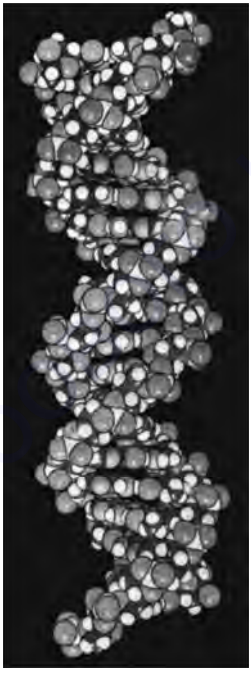


Figure 18.4. Spacefilling model of DNA molecule

DNA replication: enzymes, mechanisms

From the information presented, it is clear that genetic information is

encoded in the nucleotides sequences that constitute DNA molecule.

As far as cells do not live forever, and in light of this, they must pass their genetic information on to new cells, and this is made by cellular division. As far as genetic information is concentrated inside DNA molecules, before a cell can divide, it must duplicate all its DNA. In eukaryotes, this occurs during S phase of the cell cycle, preceding *mitosis* and *meiosis*. In other words, every living cell has to be able to replicate the DNA to be passed on to offspring.

Thus, to reproduce, a cell must copy and transmit its genetic information (DNA) to all of its progeny. To do so, DNA replicates.

DNA replication: general consideration

In humans and other eukaryotes, DNA replication occurs in the cell nucleus. And this is accomplished through the process of **semiconservative replication**. It means that each strand of the original molecule acts as a template for the synthesis of a new complementary DNA molecule (see below).

A bit of history...

Modern biochemical research on gene structure and function has brought to biology a revolution comparable to that stimulated by the publication of Darwin's theory on the origin of species nearly 150 years ago. An understanding of how information is stored and used in cells has brought penetrating new insights to some of the most fundamental questions about cellular structure and function. This was epitomized by the discovery of the double-helical structure of DNA, postulated by James Watson and Francis Crick. Their landmark paper was published in ***Nature*** magazine in 1953: *J. D. Watson and F. H. C. Crick (1953). Molecular structure of Nucleic Acids: A structure deoxyribose nucleic acid. Nature 171: 737–738.*

J. Watson and Fr. Crick were the first to propose that during the replication of DNA one of the strands of each new DNA molecules ("daughter" DNA) is newly synthesized, whereas the other strand is passed on unchanged from the "parent" DNA molecule. In 1962 they were awarded Nobel prize in Physiology or Medicine for there outstanding discovery.

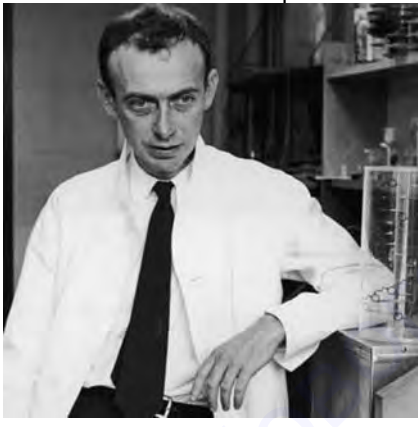
Here is the abstract from the basic article, where the fundamental idea is conveyed in the most simple, nevertheless perspicuous manner.

"If the actual order of the bases on one of the pairs of chains were given,

one could write down the exact order of the bases on the other one, because of the specific pairing. Thus one chain is, as it were, the complement of the other, and it is this feature which suggests how the deoxyribonucleic acid molecule might duplicate itself."

And further:

"Now our model for deoxyribonucleic acid is, in effect, a *pair* of templates, each of which is complementary to the other. We imagine that prior to duplication the hydrogen bonds are broken, and the two chains unwind and separate. Each chain then acts as a template for the formation onto itself of a new companion chain, so that eventually we shall have *two* pairs of chains, where we only had one before. Moreover, the sequence of the pairs of bases will have been duplicated exactly".



James Watson (born 1928) in the Cavendish Laboratory at the University of Cambridge

Francis Crick (1916–2004) is lecturing at Cambridge University

Flow of genetic information

The revolution in our understanding of the structure of DNA inevitably renewed the discussion about its biological function. The double-helical structure itself clearly suggested how DNA might be copied so that the information it contains can be transmitted from one generation to the next. And the clarification of how the information in DNA is converted into functional proteins came with the discovery of both messenger RNA (mRNA) and transfer RNA (tRNA) and with the deciphering of the genetic code.

Francis Crick proposed that information flows from DNA to RNA in a

process called **transcription,** and is then used to synthesize polypeptides by a process called **translation,** and this concept was called **The Central Dogma of Molecular Biology** (Figure 18.5).

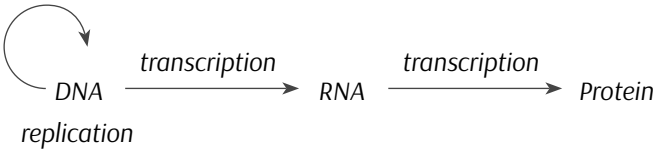


Figure 18.5. Central Dogma of Molecular Biology

Thus, according to **The Central Dogma,** the three major processes take part in the cellular deciphering and utilization of genetic information. The first is **replication,** that is the copying of parental DNA to form daughter DNA molecules with identical nucleotide sequences. The second is **transcription,** the process by which parts of the genetic message encoded in DNA are copied precisely into mRNA. The third is **translation,** whereby the genetic message encoded in messenger RNA is translated on the ribosomes into a polypeptide with a particular sequence of amino acids.

DNA replication: mechanisms, enzymes

DNA replication is the **DNA synthesis, in which process the copying of a double-stranded DNA molecule takes place.** DNA replication begins in cellular nucleus, prior to cell division. As was stated above, in eukaryotes, this proceeds during the **S-phase** of the cell cycle, preceding mitosis and meiosis. The two resulting double strands are identical (if the replication went well), and each of them consists of one original and one newly synthesized strand. This is called ***semiconservative replication.*** The process of replication consists of three steps, *initiation, replication* and *termination*

Semiconservative replication of DNA

To explain the phenomenon of heredity, biological information must be accurately copied and transmitted from each cell to all of its progeny. And this is made via DNA replication. As the result of DNA replication, two strands of DNA are obtained from one, having produced two daughter molecules which are identical to one another and to the parent molecule.

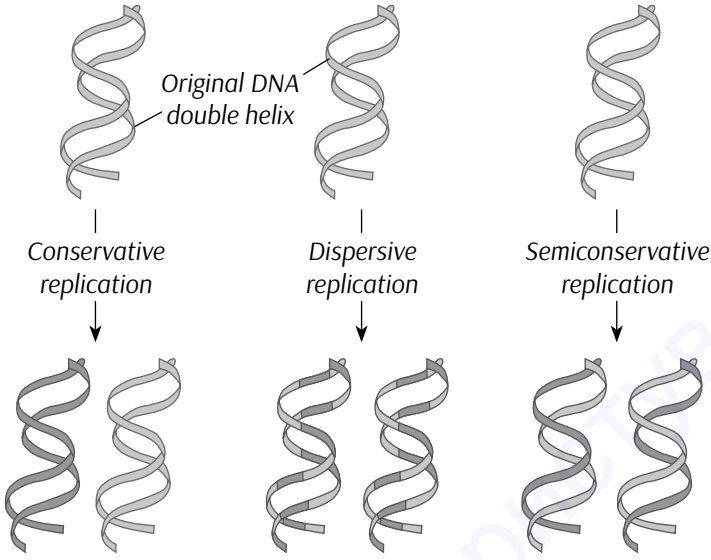
Three ways for DNA molecules to replicate may be considered, each

obeying the rules of complementary *base pairing*. In the Figure 13.6. three possible ways in which DNA can replicate are illustrated. The two original strands of DNA are shown in yellow (light); newly synthesized DNA is blue (dark).

- **Conservative replication** would leave intact the original DNA molecule and generate a completely new molecule.
- **Dispersive replication** would produce two DNA molecules with sections of both old and new DNA interspersed along each strand.
- **Semiconservative replication** would produce molecules with both old and new DNA, but each molecule would be composed of one old strand and one new one.

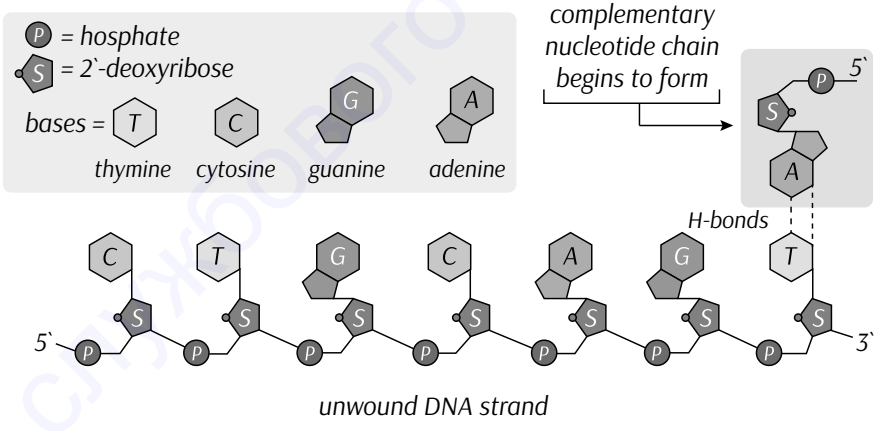
The investigations carried out by **M. S. Meselson** and **F. W. Stahl** (1957) shown that the replication of DNA is exactly semiconservative.

During the semiconservative replication, each strand acts as a template for the synthesis of a new DNA molecule by the sequential addition of complementary base pairs, thereby generating a new DNA strand that is the complementary sequence to the parental DNA. Each daughter DNA molecule ends up with one of the original strands and one newly synthesized strand (Figures 13.6–13.7).



Passible Models of DNH Replication

Figure 18.6. Three possible ways in which DNA can replicate



unwound DNA strand

Figure 18.7. General scheme of DNA strand synthesis

Enzymes in DNA replication

The two strands of the double helix are first separated by enzymes. With the assistance of other enzymes, spare parts available inside the cell are bound to the individual strands following the rules of *complementary base*

pairing: adenine (A) to thymine (T) and guanine (G) to cytosine (C).

The first enzyme discovered to catalyze the formation of polydeoxynucleotides from free dNTPs (deoxynucleoside triphosphates) was ***DNA polymerase***, studied by **Arthur Kornberg** in 1956 in his research of microorganism Escherichia coli.

DNA pollymerase can catalyze the polymerization of deoxyribonucleo-tides alongside a DNA strand, which an enzyme is able to "read" and use as a template. And the newly-polymerized molecule is complementary to the template strand and identical to the template's partner strand. And like other DNA polymerases, "Kornberg`s" enzyme, which was afterwards designated as **DNA polymerase I,** synthesizes DNA in the 5' to 3' direction.

But, before long, it was shown, that "Kornberg`s enzyme", which was afterwards designated as **DNA polymerase I,** cannot begin a new polydeoxyribonucleotide chain, that is catalyze DNA strands synthesis *de novo*. **DNA polymerase I, similar to other shortly after discovered DNA polymerases, can only add a nucleotide onto 3'-OH-group of the preexisting polynucleotide chain.**

Hence, a chemical equation that represents the process can be written in the following way:

$$(DNA)_n + dNTP \leftrightarrow (DNA)_{n+1} + PP_i$$

Thus, DNA polymerase I is not a single and sufficient enzyme to realize the whole course of DNA replication, but the essential additional enzymes are required, that catalyze the successive steps of the process. Moreover, to begin the polymerization, DNA polymerase needs a **primer,** at which it can add the first nucleotide. Primers consist of RNA and DNA bases with the first two bases always being RNA, and are synthesized by another enzyme called ***primase.*** An enzyme known as a ***helicase*** is required to unwind DNA from a double-strand structure to a single-strand structure to facilitate replication of each strand consistent with the semiconservative model of DNA replication.

The Steps of DNA replication (Figure 13.8).

1. In the first step, a portion of the double helix shown above in blue is unwound by an enzyme that is called ***helicase.***
2. Next, a molecule of **DNA polymerase** shown in green binds to one strand of the DNA. It begins moving along the strand in the 3' to 5' di-

rection, using it as a template for assembling of free nucleotides a so-called **leading strand** shown in red. In eukaryotes, enzyme molecule that catalyzes this step is designated as **DNA polymerase delta (δ).**

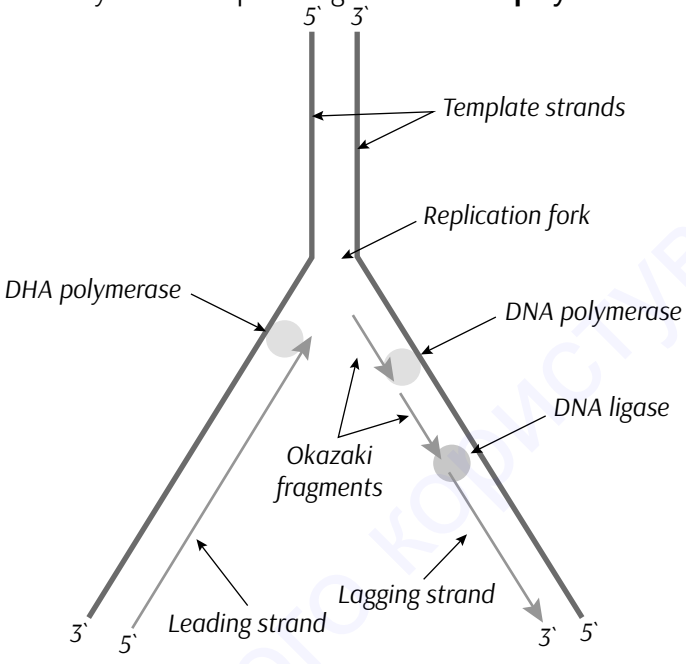


Figure 18.8. Steps and enzymes in DNA replication

3. Because DNA synthesis can only occur 5' to 3', **DNA polymerase** of a second type – **DNA polymerase epsilon, (ε),** in eukaryotes, binds to the other, the opposite, template strand – **lagging strand.** This **DNA polymerase** synthesizes discontinuous segments of polynucleotides which are called "Okazaki fragments".

The existence of such DNA fragments was originally discovered by Japanese scientist **Reiji Okazaki** and his wife **Tsuneko Okazaki** while studying replication of bacteriophage DNA inside Escherichia coli cells.

4. Yet another enzyme, **DNA ligase I** then stitches Okazaki fragments together into the **lagging strand,** thus completing a two-strand DNA formation.

18.3. Telomeres. Telomerase

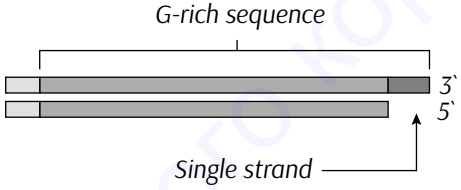
Telomeres

A ***telomere*** is a region of repetitive noncoding nucleotide sequences located at each end of chromosome, which protects the ends of the chromosomes from gradual deterioration for the space of every cellular division and DNA replication cycle. Its name is derived from the Greek nouns *telos* (*τέλος*) "end" and *meros* (*μέρος*, root) "part".

Structure of telomeres

Chemically, telomeres are repetitive nucleotide sequences located at the termini of eukaryotic organisms linear chromosomes. For vertebrates, the succession of nucleotides in telomeres makes the six-nucleotide sequence, 5¹-TTAGGG-3¹.

Telomeric DNA of mammalian cells contains double-stranded tandem repeats of TTAGGG followed by terminal 3¹G-rich single-stranded (ss) DNA overhang.



In humans this TTAGGG sequence is repeated approximately 2.500 times. Each human diploid cell encompasses 46 metaphase chromosomes which (in metaphase stage of mitosis) forms 92 chromatides and, accordingly, 92 telomeres (one at each end).

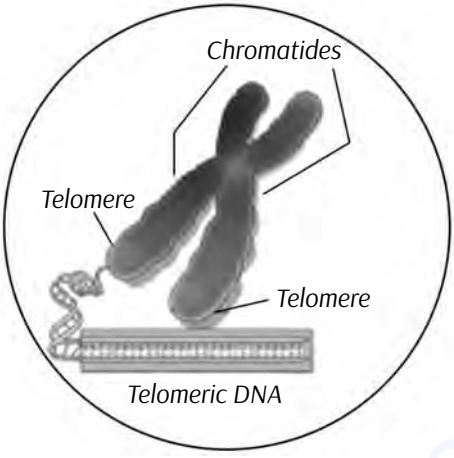


Figure 18.9. Telomeres at the ends of chromatides

The evidences exist that telomeric DNA-ends make up the three-dimensional T-loop structures, where the telomere ends fold back on themselves, and the 3[1] G- strand overhang invades into the double-stranded DNA (D-loop).

Additionally to the appointed nucleotides sequences, the telomeric ends of linear chromosomes (both the double and single-stranded DNA-sequences) contain binding sites for particular proteins, which, in complex, form on the ends of chromatides special protecting "caps" (*Elizabeth Burns*, 2008)*.

** In 1975–1977, Elizabeth Blackburn, working as a postdoctoral fellow at Yale University with Joseph Gall, discovered the unusual nature of telomeres, with their simple repeated DNA sequences composing chromosome ends. E. Blackburn, C. Greider, and J.Szostak were awarded the 2009 Nobel Prize in Physiology or Medicine for the discovery of how chromosomes are protected by telomeres and the enzyme telomerase.*

Till now, it is shown that six telomere-associated proteins – TRF1, TRF2, POT1, RAP1, TIN2 and TPP1 in mammalian cells form a complex known as the *telosome*, or *shelterin* complex. Shelterin is essential for telomere overall functions and, highly probably, impedes the fusion with neighboring chromosomes.

Biological functions of telomeres

The essence of the problem arises from the fact that, of the reason of double-strand DNA replication peculiarities (see Figure ...), DNA duplicating machinery is unable to replicate faithfully the 3'- ends of the lagging DNA strands. The phenomenon takes place because during chromosome DNA

replication, the enzymes that duplicate DNA cannot continue duplication process all the way till the end of a chromosome

Because the synthesis of Okazaki fragments requires RNA primers attaching ahead on the lagging strand and after each DNA duplication the end of the chromosome is shortened. Because of this, over time due to each cell division cycle, the telomere ends of chromosomes become shorter.

Thus, telomeres are considered to function as "disposable buffers" at the ends of chromosomes which are normally shortened, or "truncated" as the result of every cycle of cell division. Their presence protects the genes located before them on the chromosome thread.

Telomerase

The ends of chromosomes, shortened in successive rounds of DNA replication, are constantly replenished by a special enzyme, *telomerase, that* functions by adding back telomeric DNA to the ends of (shortened-UG) chromosomes, thus compensating for the loss of telomeres that normally occurs as cells divide.

Telomerase is a multisubunit RNA-containing complex, evolutionary related to viral reverse transcriptases class *(RNA-dependentDNA-polymerases).*

Telomeres and aging

Once the telomere shrinks to a certain level, the cell can no longer divide. Its metabolism slows down, it ages, and dies. Because of this, healthy animal and human cells are mortal because they can divide only a finite number of times *(Hayflick limit).*

Thus, the shortened telomeres in mitotic (dividing) cells may be responsible for some of the cellular changes associated with normal aging. Because the cells in an elderly person are much older than cells in an infant, it has been proposed that telomere shortening may be a molecular clock mechanism that counts the number of times a cell has divided and when telomeres are short, cellular and the whole body senescence occurs.

Telomeres and cancer

The majority of adult humans body cell do not produce telomerases. In humans, telomerases are active in germ cells, in some stem cells, in vitro immortalized cells and in the majority of cancer cells. That is why, the modern

conceptions are presented which bind the development of some types of human cancer.

18.4. DNA technologies

DNA technologies, or "Gene technologies" are laboratory (and nowadays – industrial, in particular pharmaceutical) procedures which involve the isolation of definite fragments from DNA of different sources to make new polynucleotide combinations with distinctive biological properties.

Recombinant DNA (rDNA) is the general name for DNA molecule that has been created by the combination of at least two strands of polynucleotides isolated from different biologic sources. Recombinant DNA molecules are sometimes called ***chimeric DNAs,*** because they can be made of the material from two different species, like mythical Greek monsters – *chimeras*. Proteins that result from the expression of recombinant DNA within living cells are termed ***recombinant proteins.***

Thus, recombinant DNAs are DNA molecules formed by methods of genetic recombination (such as *molecular cloning*) which enable bringing together genetic material from multiple sources, creating new sequences that would not otherwise be found in the natural genomes. Recombinant DNAs are possible to construct because DNA molecules from all organisms share the same pattern of chemical structure and differ only in polydesoxynucleotide sequences within identical overall structure. To make these procedures, it is necessary to use special enzymes that cut and rejoin different DNA fragments. For this purpose, special bacterial enzymes, named *restriction endonucleases*, are used.

DNA cloning

To produce rDNA in quantities which are sufficient for their further investigation and usage in biological researches or in medicine, the special procedure of molecular genetics – *DNA cloning* is applied. As mentioned above, to initiate the process, specific enzymes that cut and rejoin DNA molecules in specific sites of polynucleotide sequences are required. For this purpose, sequence-specific bacterial enzymes, *restriction endonucleases*, most frequently enzymes derived from bacterium *Escherichia Coli*, are applied.

Restriction endonucleases "cut" duplex DNA molecules in the specific sites between definite nucleotides in the middle of nucleotide chain, not at the ends. Over a hundred of restriction enzymes are known, each cleaves the link inside a very specific base sequence of the DNA molecule. For example, isolated from E.Coli nuclease EcoRI, recognizes the sequence 5"-GAATTC-3" and cleaves both DNA strands between G and A in *palindromic* sequences, that is in chains which are equally read forwards and backwards.

As the result of restriction enzyme action, two overhanging or "sticky" ends of DNA molecule are formed. This "sticky ends" from two DNA molecules of different biological sources can hybridize together; then the nicks are sealed using enzyme *ligase*. The result of the procedure is recombinant DNA (see Figure 18.10).

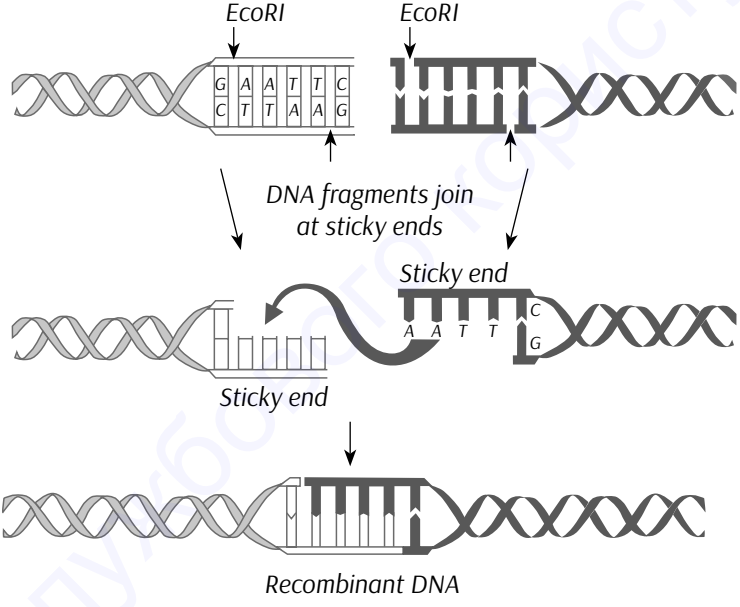


Figure 18.10. Steps of recombinant DNA construction

The subsequent operations consist in producing a large quantity of identical copies of hybrid DNA molecules (clones). And this is achieved by using special transporters (vehicles) of polynucleotide fragments, the so-called *vectors*. As cloning vectors, are generally used *plasmids* (circular DNA fragments) or viruses, into which small segments of DNA, that contain necessary genetic material, are inserted, as well as biochemical signal molecules

for replication and expressing the foreign DNA (Figure 18.11).

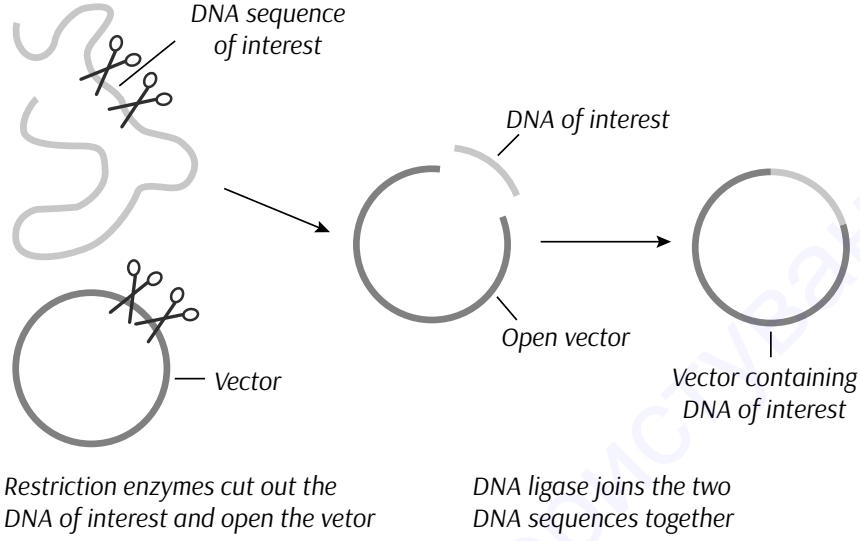


Figure 18.11. Using vector for DNA cloning

When recombinant vector is inserted into E. coli, the cell becomes able to produce great quantities of DNA molecules which make interest for investigator or for practical usage.

DNA sequences used in the construction of recombinant DNA molecules can originate from any biological objects. For example, plant DNA may be joined to bacterial DNA, or human DNA may be joined with fungal DNA. In addition, DNA sequences that do not occur anywhere in nature may be created by the chemical synthesis of DNA, and incorporated into recombinant molecules. Using recombinant DNA technology and synthetic DNA, practically any DNA sequences may be created and introduced into very wide range of living organisms.

Applications of DNA technologies, or recombinant DNA technologies

The most common application of recombinant DNA technologies is fundamental biological and biomedical researches. For example, recombinant DNAs are used in identifying, mapping and sequences genes, in analyzing

gene expression within individual cells and throughout the tissues of whole organisms (DNA probes). Recombinant proteins are widely used as reagents in laboratory practice and to generate antibody probes for examining protein synthesis within cells and organisms. In practical medicine and pharmaceutics an array of newly synthesized medicines based on DNA technologies is widely used.

Some examples of recombinant DNA technologies medical applications are presented.

Recombinant human insulin
In modern clinical practice, for the treatment of insulin-dependent diabetes, biotecnological insulin has almost completely replaced the pharmaceutical obtained from animal sources (e.g. pigs and cattle). The modern technology applies the insertion of human insulin gene into E. coli, or yeast (Saccharomyces cerevisiae) and its subsequent cloning.

Recombinant human growth hormone (somatotropin)
Preparation is used for administering to patients whose pituitary glands generate insufficient quantities of hormone to support normal body growth. Previously, somatotropin for therapeutic use was obtained from pituitary glands of cadavers, that, in some cases, led to dangerous complications, such as developing of Creutzfeldt–Jakob disease.

Recombinant blood clotting factor VIII
Some clinical forms of hemophilia are reasoned by inborn genetic deficiency of blood clotting factor VIII. A blood-clotting protein is vitally necessary for patients with forms of the hemophilia, who are unable to produce factor VIII in quantities sufficient to support normal blood coagulation. Before the introducing of recombinant factor VIII, the blood-clotting protein was obtained by processing large quantities of human blood from multiple donors, which carried a very high risk of transmission of blood borne infectious diseases, and namely HIV and hepatitis B.

Diagnoctics of HIV (Human Immunodeficiency Virus) infection
Each of modern methods for HIV infection diagnostics has been developed using recombinant DNA. They are:

- *antibody presence test (ELISA method) uses a recombinant HIV protein to test for the presence of antibodies that the body has produced in response to an HIV infection;*
- *the DNA test looks for the presence of HIV genetic material using reverse transcription polymerase chain reaction (RT-PCR). Development of the RT-PCR test was made possible by the molecular cloning and sequence analysis of HIV genomes.*

Many additional applications of recombinant DNA technologies are found in industry, food production, human and veterinary medicine, agriculture, and bioengineering.

Chapter 19.

mRNA TRANSCRIPTION. RIBOSOMAL TRANSLATION

19.1. mRNA. Transcription: enzymes, mechanisms

The genetic information is found in the nucleus, though protein synthesis actually occurs in **ribosomes** found as the free particles in the cytoplasm and on the surface of rough endoplasmic reticulum. If protein is to be synthesized, then the genetic information held in the nucleus must be transferred to these ribosomes. This is done by **mRNA (messenger ribonucleic acid)** via the process of ***transcription*.**

Thus, **transcription** is the synthesis of an RNA which copyes a sequence of DNA (a gene), and that is the first step in gene expression.

mRNA, which is the product of transcription, is very similar to DNA, but fundamentally differs in two ways:

- A base called ***uracyl*** replaces all ***thymine*** bases in mRNA.
- The deoxyribose sugar in DNA in is replaced by ribose sugar in mRNA.

Transcription proceeds in much the same fashion as the replication of DNA and also follows the base pairing principle. Again, at the beginning of transcription, a section of DNA double helix is uncoiled and only one of the DNA strands serves as a template for RNA polymerase enzyme to guide the synthesis of RNA. For example, if the code of DNA looks like this: **G–G–C–A–T–T,** then the mRNA would look like this **C–C–G–U–A–A** (remembering that uracil replaces thymine) – Figure 19.1.

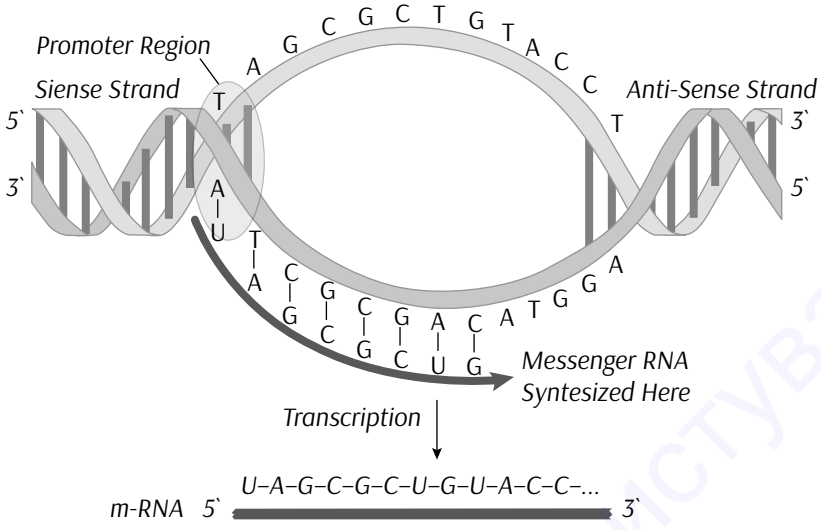


Figure 19.1. General view of transcription and RNA polymerase action

After the transcription is complete, the RNA separates from the DNA and the DNA recoils into its helix. With the genetic information responsible for creating proteins now available on the mRNA strand, the mRNA moves away from the DNA and out of the nucleus towards the ribosomes.

RNA polymerase

A process termed **transcription** is realized by the enzyme called **RNA polymerase (RNAP), which constructs mRNA chains. RNAP** is present in all procaryotic cells, eucaryotic cells and many viruses and is responsible for the reading the genetic code stored in DNA. RNAP was discovered independently by **Sam Weiss** and **Jerard Hurwitz** in 1960. By the chemical mechanism action, RNAP is a ***nucleotidyltransferase*** that polymerises ribo-nucleotides in accordance with the information present in DNA.

As can be seen from the image prese1nted (Figure 13.9), RNAP can initiate transcription at specific DNA sequences known as **promoters.** It then produces an RNA chain which is complementary to the DNA strand used as a template. The process of adding nucleotides to the RNA strand is known as **elongation,** and in eukaryotes RNAP can build chains as long as 2.4 million nucleosides. RNAP will release its RNA transcript at specific DNA se-

quences encoded at the end of genes that are known as **terminators.**

RNA polymerases in prokaryotes

In prokaryotes, the same enzyme catalyzes the synthesis of three types of RNA: mRNA, rRNA and tRNA.

RNAP is a relatively large molecule. The core enzyme has 5 subunits (~400 kDa):

- α_2: the two α subunits assemble the enzyme and recognize regulatory factors.
- β: this has the polymerase activity (catalyzes the synthesis of RNA).
- β′: binds to DNA (nonspecifically).
- ω: function not known clearly. However it has been observed to offer a protective function to the β› subunit in M. megmatis.

In order to bind promoter-specific regions, the core enzyme requires another subunit, **sigma (σ).** The ***sigma factor*** greatly reduces the affinity of RNAP for nonspecific DNA sequences while increasing specificity for certain promoter regions. The complete holoenzyme therefore has 6 subunits: $\alpha_2\beta\beta'\sigma\omega$ (~480 kDa).

RNA polymerase in eukaryotes

Eukaryotes have several types of RNAP:

- **RNA polymerase I** synthesizes a **pre-rRNA** 45S, which matures into 28 S, 18S and 5,8S **rRNAs** that constitute the RNA molecules of the whole ribosome.
- **RNA polymeraze II** synthesizes precursors of **mRNAs** and most snRNA. This is the most studied type, and due to the high level of control required over transcription a range of *transcription factors* are required for its binding to promoters.
- **RNA polymeraseIII** synthesizes **tRNAs, rRNA 5S** and other small RNAs found in the nucleus and cytosol.
- Other RNA polymerase types of mitochondria and chloroplasts.

Control of the process of transcription affects patterns of ***gene expression*** and thereby allows a cell to adapt to a changing environment, perform specialized roles within an organism, and maintain basic metabolic processes necessary for survival. Therefore, it is hardly surprising that the activity of RNAP is both complex and highly regulated. In *Escherichia Coli*

bacteria, more than 100 factors have been identified which modify the activity of RNAP.

RNA maturation. Splicing. Introns and exons

The immediate products of transcription are primary RNA-transcripts (so-called hnRNAs, or "high molecule weight nuclear RNA"), which include the RNA-sequences that do not contain coding information.

To settle the task, process of *maturation* is going on, which consists in hnRNA splicing, that is removing of non-coding sequences (*introns*) from primary trancripts to produce mature mRNA molecules, containing *exons*, which encode information for cellular proteins (Figure 19.2).

In the process of splicing special low molecular weight RNAs (snRNAs – "small nuclear RNAs") and an array of protein molecules take place.

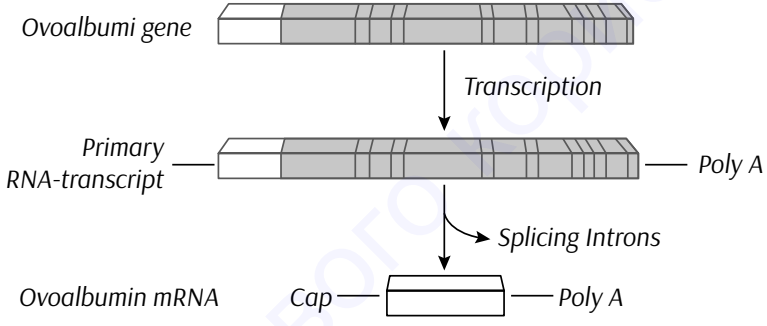


Figure 19.2. Maturation of primary transcript – hnRNA

19.2. Genetic code. Translation in ribosomes

As was already discussed, the genetic information carried by an organism – its genome – is included in one or more DNA molecules. Each functional portion of a DNA molecule is referred to as a **gene.** Each gene is transcribed into a short template molecule of the related polymer RNA, which is better suited for protein synthesis. This in turn is translated by mediation of a machinery consisting of ribosomes and a set of transfer RNAs and associated enzymes into an amino acid chain (polypeptide), which will then be folded into a protein.

To translate nucleotides (or nucleobase) sequence of DNA and then mRNA molecule into the amino acid (protein) polypeptide sequence, the specific **biological (genetic) code** has to be deciphered – Figure 13.10.

The **genetic code** is a set of rules that maps DNA sequences to proteins in the living cell, and is employed in the process of protein synthesis. Nearly all living things use the same genetic code, called the **standard genetic code,** although a few organisms use minor variations of the standard code.

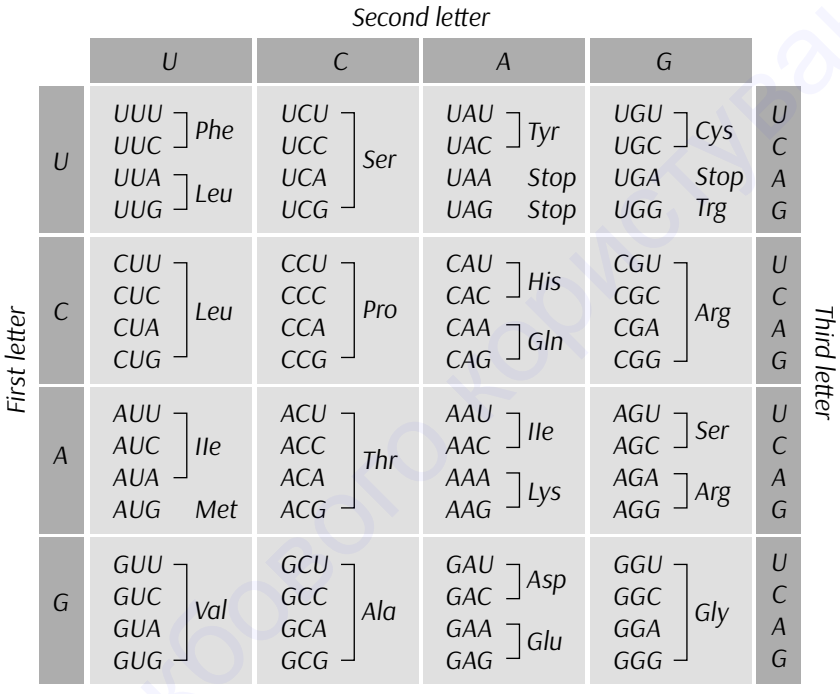


Figure 19.3. The Table of genetic code

The gene sequence inscribed in DNA, and in mRNA, is composed of tri-nucleotide units called **codons,** each coding for a single amino acid. Overall, there are $4^3 = 64$ different codon combinations. For example, the RNA sequence UUUAAACCC contains the codons UUU, AAA and CCC, each of which specifies one amino acid. So, this RNA sequence represents a peptide sequence, three amino acids residues long.

Historical hints...

Marshall W. Nirenberg and **Heinrich J. Matthaei** at the National Insti-

tute of Health (USA) were the first to perform the experiments that elucidated the correspondence between the codons and the amino acids that they code. **Har Gobind Khorana** expanded on Nirenberg's work and found the codes for the amino acids that Nirenberg's methods could not find. Khorana and Nirenberg won a share of the 1968 Nobel Prize in Physiology or Medicine for this work.

Marshall W.Nirenberg (born 1927)

Har Gobind Khorana (born 1922)

Nobel Prize winners, 1968 "for describing the genetic code and how it operates in protein synthesis"

19.3. Ribosomal molecular machinery

The components of translation

Ribosomes

Ribosomes are the small but complex intracellular organelles, the main site of protein synthesis. The individual ribosomes are made up of two distinct and separable subunits (one about twice the size of the other) – Figure 13.11. Each subunit is composed of one or two RNA molecules (60–70 %) associated with 20 to 40 small proteins (30–40 %).

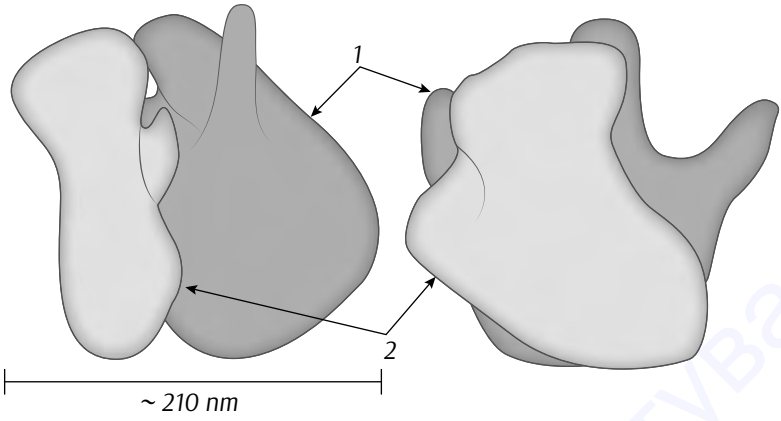


Figure 19.4. General image of ribosome including large (1) and small (2) subunits

Transfer RNAs (tRNAs)

Transfer RNAs are a class of relatively small RNA molecules which contain about 70 nucleotides.

The biochemical role of tRNAs in protein synthesis is to transfer the amino acids to the ribosomes, where proteins are assembled according to the genetic code carried by mRNA. There are more than 20 different tRNA molecules.

These RNA's have distinctive three-dimensional structures consisting of loops of single-stranded RNA connected by double stranded segments. The **clover-leaf** resembling secondary structure of tRNAs is further wrapped into an "L-shaped" assembly, having the amino acid linking end **(acceptor arm),** and a characteristic **anticodon arm** at the other end. The individual amino acids are being attached by an ester bond at the free 3'-end of tRNAs. And the anticodon arm includes the triplet nucleotide sequencies that are complementary to the triplet nucleotide coding sequences of mRNA.

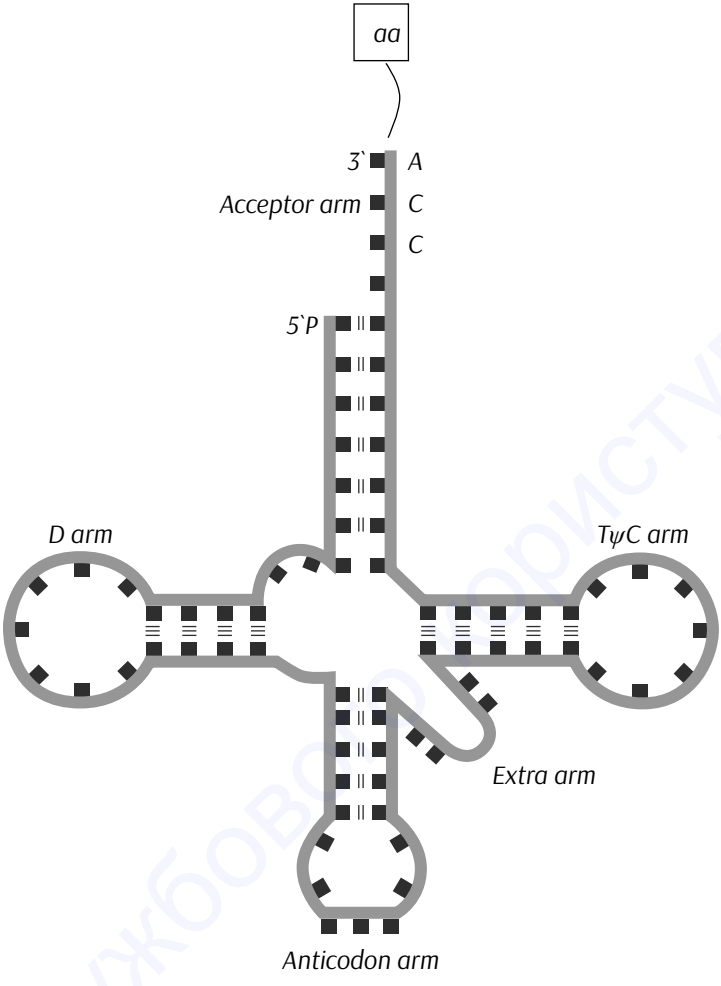


Figure 19.5. Presentation of two-dimensinal structure of a typical tRNA molecule

The clover-leaf resembling presentation of a typical tRNA in which amino acid (aa) is attached to the acceptor arm (ACC) is presented in Figure 13.12 cleotide triplet that is the complement of the amino acid's codon(s). Space-filling models of two tRNA molecules are shown in Figure 13.13.

Transfer RNA Molecules

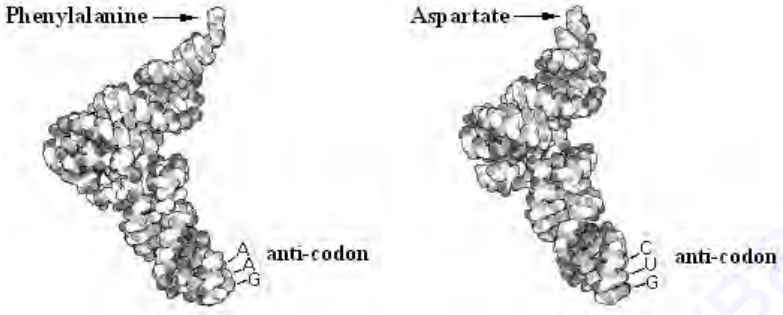


Figure 19.6. tRNA molecules for phenylalanine and aspartate

Ribosomal RNAs (rRNAs)

These are a class of RNA found in the ribosomes of cells that take part in the ribosomal molecular architecture and biochemical functions.

The translation process

The translation process requires the participation of ribosome itself, mRNA and tRNA molecules, amino acids, ATP, GTP and at least ten specific proteins that in eukaryotic cells are designated as **eukaryotic initiation factors (eIFs).**

Initiation of translation

The first phase in the process of translation is **initiation**. During the initiation, the association of individual (large and small) ribosome subunits with mRNA takes place. In case of eukaryotes, these are 30S and 50S subunits (which make up 70S ribosomes), in prokaryotic cells – 40S and 60S subunits, making up 80S ribosome, correspondingly.

The 5` terminal of mRNA is "capped" which means that a special chemical group, and specifically methyl-guanosyl triphosphate **(G^mTP)** is bound to a polynucleotide chain. It is thought that the "capping" facilitates the binding of mRNA to the whole initiation complex.

mRNA which binds with the ribosome, bears the codons transcribed of DNA and required for orderly and successive translation. To provide this, mRNA binds to the ribosome in a pattern ensuring the attachment with

mRNA a definite tRNA molecule with the appropriate anticodon.

The anticodon bearing tRNA links with the mRNA at a characteristic nucleotide sequence at the 5'-end, that is called **initiation codon.** This codon designates **a starting point of translation,** and makes polypeptide synthesis being carried out in a way providing the construction of a new polypeptide according to the genetic code of a cell. In eukaryotes, the **initiation codon** constitutes the mRNA sequence **AUG,** and this codon specifies **L-methionine**, which is, consequently, the first amino acid used in the translation process.

Methionine specific tRNA, which binds methionine, **$tRNA^{Met}$,** attaches to a ribosome at a special tRNA-binding site that is referred to as the **peptidyl site (P-site).** A second binding site of ribosome, the **acceptor site (A-site),** is not yet occupied during this phase of translation.

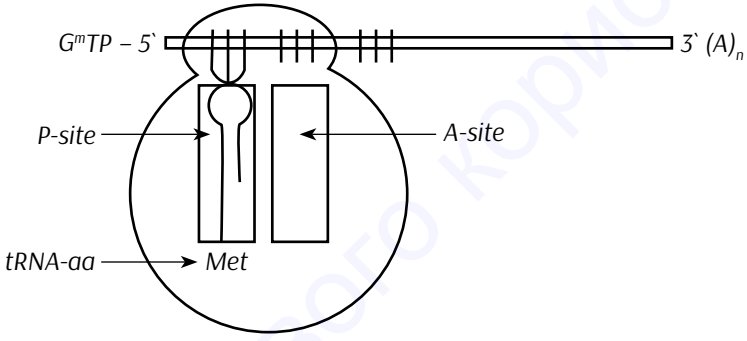


Figure 19.7. Formation of initiation complex

The formation of initiation complex is presented in Figure 19.7.

Elongation, termination of translation

Elongation is a cyclic process realized on the ribosome in which one amino acid at a time is added to the nascent peptide chain.

The peptide sequence of any protein is determined by the order of the codons in the mRNA chain. Hence, elongation involves several steps including series of appropriate tRNA molecules attachments, peptide bond formations and shifts of the ribosome along the mRNA chain to expose new codons to the ribosomal machinery – Figure 19.8 *a, b.*

Thus, the sequential steps of elongation include:

- Binding of specific aminoacyl-tRNA to the A-site (position A);

- Peptide bond formation (position B). The reaction is catalyzed by a ***peptidyltransferase*** which is the component of the 60S ribosomal subunit;
- Translocation, that is displacement of the peptidyl-tRNA from A-site to the P-cite. The step requires the participation of the **elongation factor 2** (eIF2).

The execution of multiple cycles of elongation accomplishes the poly-merization of the specific amino acids into a protein molecule, and after that the **termination** of translation takes place.

The termination occurs when the "stop codon" or **terminating codon** appears in the A-site of a ribosome. In another words, in a process of shifting along mRNA chain from 5` end to 3` end, the ribosome encounters one of the following triplets – **UAA, UAG, UGA** which are recognized as termination signals. After that, the protein **releasing factors** (RF1 to RF3) operate, which promotes the hydrolysis of the bond between the peptide formed during translation and the tRNA occupying the P-site.

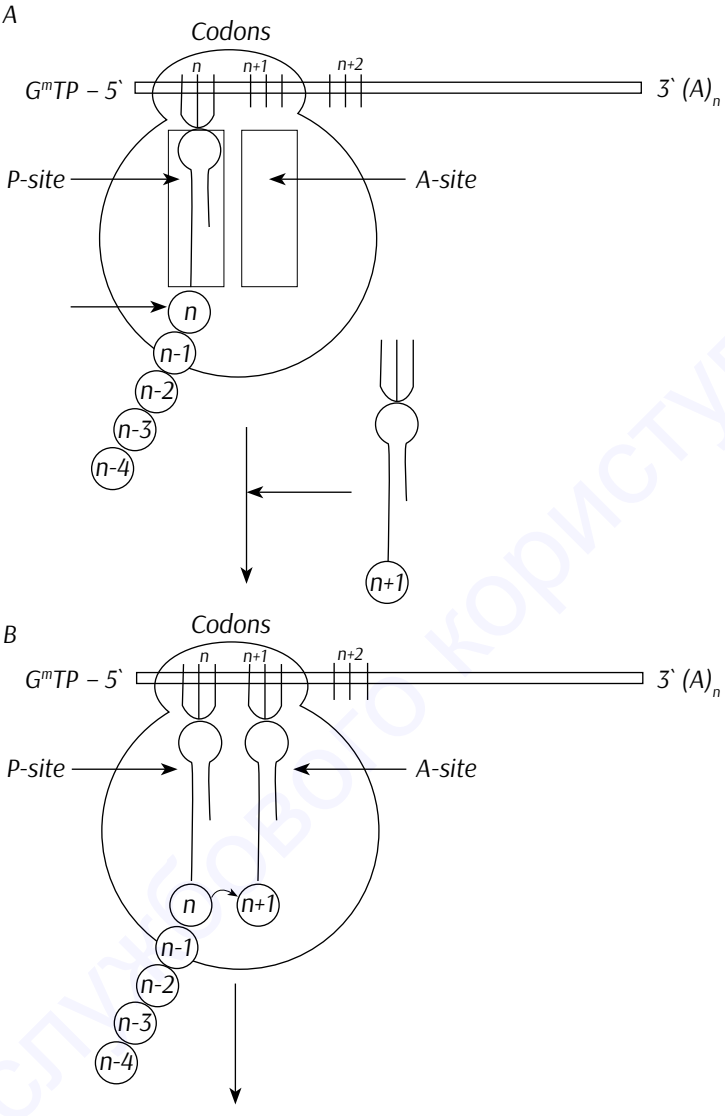


Figure 19.8 a. Steps of translation (positions A, B)

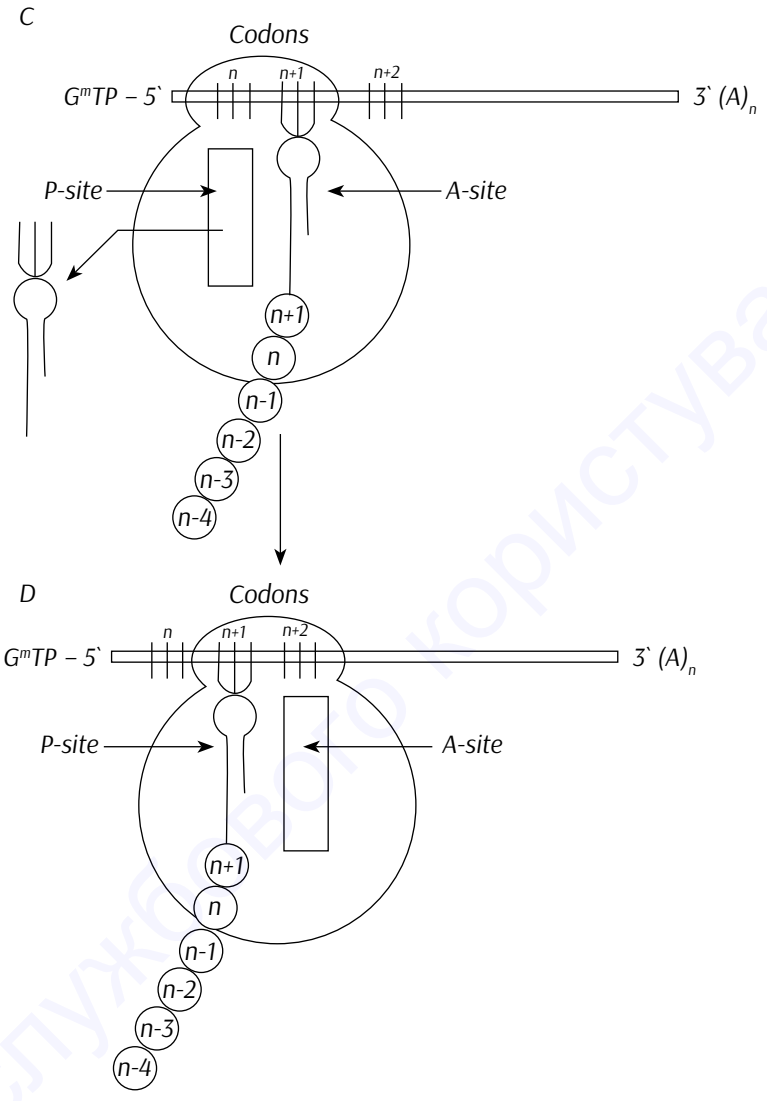


Figure 19.8 b. Steps of translation (positions C, D)

And the outcome of the whole process is the release of a polypeptide chain corresponding to the mRNA blueprint and to the genomic locus which is expressed in a cell.

Part 5

METABOLIC CONTROL. HORMONES AND VITAMINS

Chapter 20.

HORMONES-1. BIOCHEMICAL AND CELLULAR MECHANISMS OF HORMONAL REGULATION

20.1. Hormones: general definitions

The **coordination of metabolism** in mammalian organism is realized by the function of *neuroendocrine system,* which produces **neuronal signaling,** carried out by neurotransmitters (acetylcholine, norepinephrine etc.) and **hormonal signaling.**

Hormones are chemical signaling substances that are synthesized by specialized cells of endocrine glands. After releasing into bloodstream, hormones are transported to their target organs, where they exert specific physiological and biochemical effects inherent to a certain hormonal molecule. Virtually every physiological and biochemical process in mammalian organism, including maintenance of blood pressure, blood volume, and electrolyte balance, embryogenesis, sexual differentiation and behavior, metabolic fuel allocation etc., is regulated by one or more hormones.

To define on the whole, the hormone-producing system of the human body includes the chain of endocrine glands which transmit information, primary received by the central nervous system, in the form of chemical signals to the specific high sensitive (target) cells.

In hormonal signaling, the intercellular messengers, that is hormones, are carried in the bloodstream to neighboring cells or to distant organs and tissues; they may travel a meter or more before encountering their target cell. When transported in blood to the distant organs or tissues, hormones are associated with special plasma proteins that serve as hormone carriers.

A bit of history...

Ernest Henry Starling

- Starling's best-known work (1902) was his collaboration with W. Bayliss in the discovery of the pancreatic substance secretin which is released into duodenum to aid in the process of digestion.
- Starling and Bayliss were the first to show that the release of the juices from the pancreas was not under the nervous but rather under the chemical control.
- For the general class of such chemicals Starling proposed, in 1905, the term *"hormone"*, from the Greek root meaning "to excite".
- In this way *"endocrinology"* – a major branch of medicine, physiology and biochemistry – had been created.

Hormones are extremely potent physiologically active substances, and they are produced in very small amounts. The blood concentrations of hormones that act as signal molecules are very low, commonly between 10^{-7} and 10^{-12} M. For example, to detect peptide hormones concentration in human blood, urine or tissue extracts, nowadays, the extraordinarily sensitive **radioimmunoassay (RIA)** is used. The method was developed by **Rosalyn Yalow** and **Solomon A. Berson,** and the former was awarded by the Nobel Prize (1977) for this brilliant discovery.

Biochemical nature of hormones

Hormones synthesized in mammalian cells are chemically diverse molecules. The number of different hormones that are produced inside human body equals to approximately 50 specimens. According to their chemical structure, properties and essential peculiarities of biochemical effects, hormones are divided into two major classes, and they are:

- **Hydrophilic hormones,** which involve peptide hormones, proteohor-mones and catecholamines.
- **Lipophilic hormones,** which include the steroid hormones, thyroid hormones, eicosanoids and retinoids.

20.2. Basic principles of hormone effects

Transport of Hormones

Being synthetized inside endocrine glandule cells, molecules of hormones are secreted into circulation and are transported throughout the body by the bloodstream, where the majority of hormonal molecules bind to special transport proteins.

Target cells. Receptors

In the organs and tissues that are sensitive to hormonal action, the specific hormonal signals are received by the target cells.

Inside target cells, hormones realize their biochemical effects through specific cellular **receptors**, to which the hormones bind with high affinity. This is the high affinity of the interaction that allows cells to respond to very low concentrations of hormone.

At that, the specificity of hormone action results from ***structural complementarity*** between the hormone and its receptor; this interaction is extremely selective to the three-dimensional conformation of hormonal molecule. Moreover, each cell type has its own combination of hormone receptors, which define the range of its hormone responsiveness.

The localization of the hormone receptor may be extracellular, cyto-solic, or nuclear, depending on the hormone type.

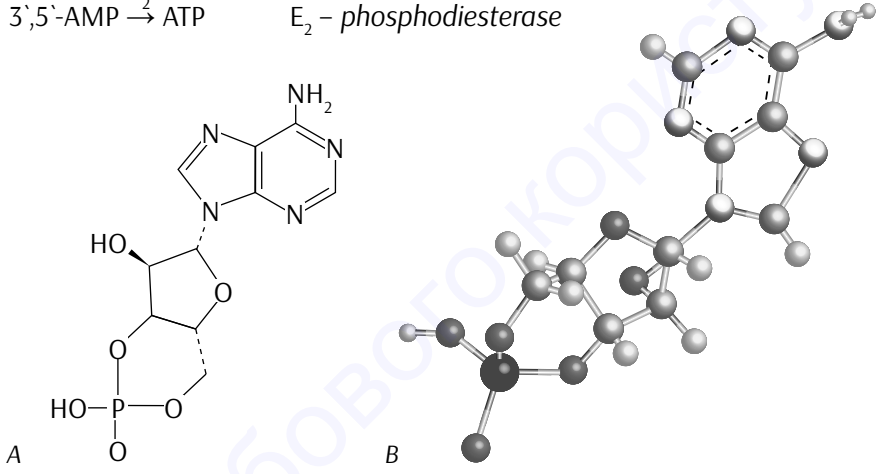
Principles of hormonal signal transduction

The intracellular consequences of hormone-receptor interaction are of the following general types:

1) receptor protein is activated by the extracellular hormone, and this triggers the generation of so-called **second messengers** (such as cAMP (Figure 20.1), cGMP, intracellular release of Ca^{2+}-ions and/or activation of phosphatidylinositol cycle), which bring about the specific responses of the hormone-sensitive cells.

$$ATP \xrightarrow{E_1} 3`,5`\text{-}AMP + PP_i \qquad E_1 - \textit{adenylyl cyclase}$$

$$3`,5`\text{-}AMP \xrightarrow{E_2} ATP \qquad E_2 - \textit{phosphodiesterase}$$



A *B*

Figure 20.1. Adenosine 3\5"-monophosphate (cyclic AMP; cAMP)
A – common structural formula; B – ball-and-stick model

2) change in membrane potential results from the opening or closing of a hormone-gated ion channel; the effect is more characteristic for substances that share physiological properties of hormones and neurotransmitters, such as catecholamines, histamine etc.
3) hormonal molecule causes a change in the level of expression of one or more genes (transcription of DNA into mRNA), mediated by a cytosolic or nuclear hormone receptor proteins.

To transduce chemical signal transmitted by hormone molecule into specific metabolic response that is characteristic for certain type of target cell,

the special biochemical transducing device exists. For a class of peptides and amino acid derived hormones, the central constituent of this device is a special **G protein** which accomplishes the hormone signal transmission from the cell membrane bound receptor to the inside of the cell (see below).

Signal amplification

It is noteworthy that a **single** hormone molecule, in forming a hormone-receptor complex, activates a receptor and a catalytic protein so that it produces a **great many** molecules of the second messenger. According to this, the hormonal receptors serve not only as **signal transducers** but also as **signal amplifiers.** Moreover, inside the target cell, the chemical signal may be further amplified by a **signaling cascade,** which is formed through a series of steps in which one catalyst activates a next catalyst, resulting in very large amplifications of the original humoral stimulus, generated by endocrine cell.

The generalized scheme of hormonal signal transducing and amplification is presented in Figure 20.2.

Hydrophilic (water-soluble) hormones, that is peptides, proteins and some amine acid derived hormones (glucagon, adrenocorticotropic hormone and epinephrine, for example) act extracellularly by binding to cell surface receptors that span the plasma membrane **(mechanisms 1 and 2).**

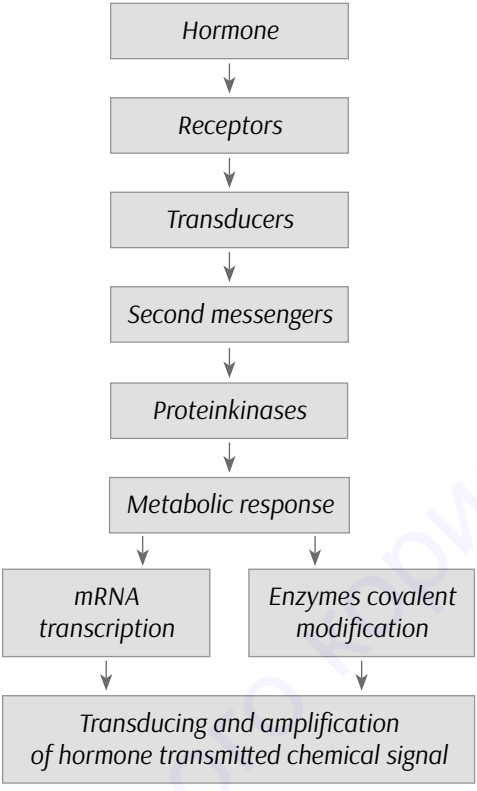


Figure 20.2. The consequence of biochemical events underlying the transformation of hormone induced regulatory signal to the target cells response

When hormone binds to the receptor extracellular domain, the receptor protein complex undergoes a conformational change that triggers the biochemical reactions constituting the downstream effects of the hormone.

The key event in the stimulation of the biochemical reactions cascade is the interaction of the hormone-bound receptor with a special transducing device which role is played by **G proteins.**

G proteins are heterotrimers consisting of three different types of subunits, α, β and γ. The α subunit can bind the nucleotide GTP, and this binding is the key molecular event which activates the G protein and makes it capable of the second messenger formation.

In case of cAMP as the second messenger, the G protein activation leads to the ***adenylyl cyclase*** catalytic activity modification, and that is accompanied by the hydrolysis of GTP to GDP and P_i. Depending on different hor-

mone-target cell systems, there are two distinctive families of G proteins which activation results in the stimulation **(G_s)** or inhibition **(G_i)** of ***adenylyl cyclase*** activity (Figure 20.3).

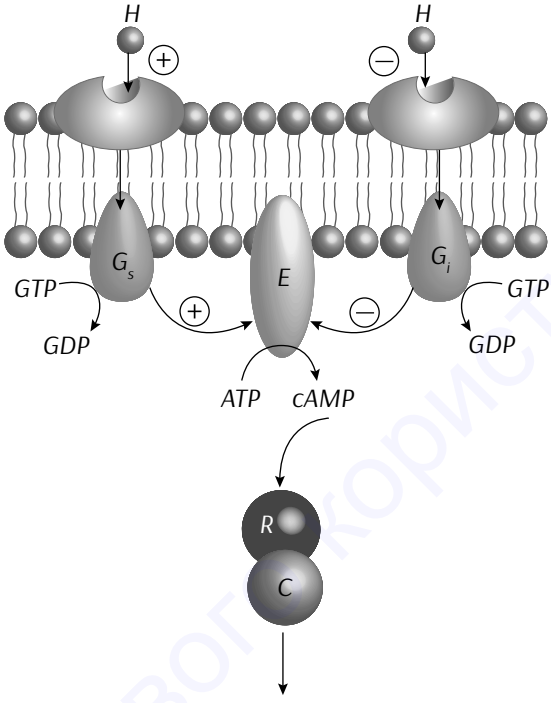


Figure 20.3. G proteins as transducers of hormone signal transmission

The examples of hormones, which interaction with membrane receptors activates *adenylyl cyclase* and, consequently, leads to the essential rise of cellular cAMP production, present the **majority of hypothalamus and hypophysis peptide and proteohormones, glucagon, vasopressin**, **calcitonin, epinephrine, dophamine** (when affecting D_1-receptors) etc. The opposite (inhibiting) effects give **angiotensin II**, **somatostatin**, **opioid peptides**, **norepinephrine** (when affecting α_2-receptors), **dopamine** (affecting D_2-receptors), **acetylcholine** (affecting m-cholinergic receptors).

An enzyme cascade of this type was discussed in the previous lectures, when studying the mechanisms of the regulation of glycogen and/or triacylglycerols synthesis and degradation by **adrenaline *(epinephrine)*** and **glucagon.** Epinephrine activates (through its specific receptor) ***adenylyl cyclase,*** which produces many molecules of cAMP for each molecule of re-

ceptor-bound hormone, and this brings about the activation of ***cAMP-dependent protein kinases.*** The latter enzymes, in their turn, stimulate ***glycogen phosphorylase*** or ***triacylglycerol lipase,*** in muscle, hepatocytes or adipocytes, correspondingly, that causes the production of many thousands of molecules of the metabolite desired (glucose, fatty acid etc).

Lipophilic (water-insoluble) hormones, that is steroids, and thyroid hormones and retinoids readily pass through the plasma membrane of their target cells to reach their receptor proteins in the nucleus. In its turn, the hormone-receptor complex interacts with certain DNA loci to alter the expression of specific genes that virtually changes the enzyme set of the cell and, thereby, the cellular metabolism.

The physiological and biochemical responses to the hormones which act through the plasma membrane receptors develop rather rapidly, amounting commonly some seconds. This is the case, for example, of muscle glycogen breakdown following additional adrenalin secretion. On the contrary, the steroid hormones induce metabolic and cellular changes inside their target tissues only within few hours time, which is conditioned by the period of time needed for mRNA transcription and the synthesis *de novo* of new enzyme molecules.

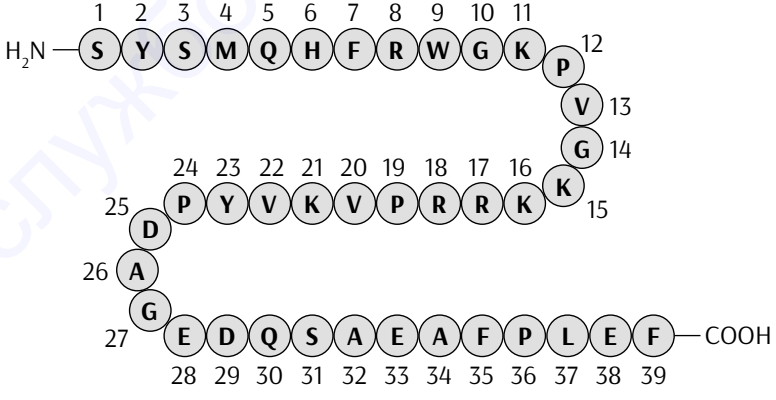
Chapter 21.

HORMONES-2. HORMONES OF PEPTIDE AND PROTEIN NATURE

21.1. Peptide and protein hormones of hypothalamus and hypophysis

Peptide and protein hormones (proteohormones) are presented, chemically, by a group of peptides that may be composed of 3 to 200 or more amino acid residues. They include all the hormones of the hypothalamus and pituitary gland, the pancreatic hormones insulin, glucagon, and somatostatin, the gastro-intestinal hormones, the parathyroid hormone, calcitonin and certain hormone-like substances – Figure 21.1.

PyrGlu — His — Pro
Thyrotropin-releasing hormone (TRH)



Adrenocorticotropic hormone (ACTH)

Figure 21.1. Examples of peptide hormones primary structure: TRH and ACTH

Hormones production

Peptide hormones and proteohormones are synthesized on ribosomes of endocrine gland cells in the form of longer precursor proteins ***(prohormones).*** The following steps include packaging of peptides inside secretory vesicles where proteolytic cleavage to form the active substances and glycosylation (if necessary) occurs, and secretion into bloodstream. The release of peptide and protein hormones out of the secretory granules is released by exocytosis.

Commonly, prohormone proteins yield a single active peptide hormone, but in some cases several active hormones are carved out of the same prohormone. The most distinguished example of the latter is the production of nine biologically active peptides out of **proopiomelanocortin (POMC).** In that case the single **POMC gene** encodes a large polypeptide that is progressively broken into active hormone molecules – see below.

After entering the bloodstream, the hormone molecules are transported through the blood plasma until they encounter the target cells. As was already noted, all peptide hormones and proteohormones act by binding to the receptors located in the plasma membrane. They cause the generation of a second messenger in the cytosol (presumably cAMP and cGMP), which changes the activity of an intracellular enzymes, thereby altering the cell's metabolism.

Hormones of hypothalamus

The coordination center of the endocrine system is the hypothalamus, which is a small region inside the brain, that receives and integrates messages from the central nervous system and transmits the chemical messages to the "subordinate" glands of which the hypophysis is the principal one.

To realize its physiological functions, hypothalamus produces a set of releasing and inhibiting hormones which are carried to the anterior pituitary gland via hypothalamic-hypophyseal portal veins. Hypothalamic hormones are peptides that bind to receptors on specific anterior pituitary cells, modulating the release of the hormone they produce.

The major representatives of hypothalamus hormones are as follows.

Somatoliberin *(Growth hormone-releasing hormone; GHRH)* is a

hypothalamic peptide that stimulates both the synthesis and secretion of growth hormone by the cells of the anterior pituitary gland.

Somatostatin (SS) is a peptide produced by hypothalamus and several tissues. Somatostatin counteracts to the release of growth hormone in response to GHRH and to other stimulatory factors such as low blood glucose concentration.

Thyroliberin *(Thyrotropin-releasing hormone; TRH)* is the smallest peptide hormone ($M_r \approx 362$ D). It is the tripeptide, consisting of three amino acid residues, including glutamate derivative (pyroglutamate), histidine and proline which is modified to amide (Figure 21.2).

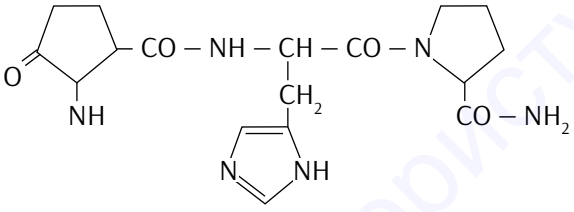


Figure 21.2. Thyrotropin-releasing hormone

It is interesting to note, that when Roger Guillemin and Andrew Schally independently purified and characterized thyrotropin-releasing hormone (TRH) from the hypothalamus, Schally's group processed about 20 tons of hypothalamus from nearly two million sheep, and R.Guillemin's group extracted the hypothalamus from about a million pigs! TRH proved to be a simple derivative of the tripeptide Glu-His-Pro. For their work on hypothalamic hormones, A.Schally and R.Guillemin shared the Nobel Prize in Physiology or Medicine in 1977.

Being produced in hypothalamus, TRH is responsible for the **thyrotropin** (TSH) secretion by the cells of the anterior pituitary, *adenohypophysis*.

Gonadoliberin *(Gonadotropin-releasing hormone*; *GnRH*, also known as *LH-releasing hormone)* is the principle regulator of **luteinizing hormone** (LH) and **follicle-stimulating hormone** (FSH) secretion. GnRH is a ten amino acid peptide that is synthesized and secreted from hypothalamic neurons and binds to receptors on specialized cells of anterior pituitary gland, *gonadotrophs*.

Corticoliberin *(Corticotropin-releasing hormone; CRH)* is hypothalamus hormone which controls the synthesis and secretion of **adrenocorticotropic hormone *(corticotropin)*** by anterior pituitary gland. Corticotro-

pin-releasing hormone production is increased in response to many types of stress.

Hormones of adenohypophysis

The anterior part of the pituitary gland (adenohypophysis) produces and secretes into blood a great amount of hormones which can be divided into three distinctive subclasses clearly differing in the biochemical and physiological properties.

Subclass I is the protein family, which includes **growth hormone, prolactin** and **chorionic somatomammotropin.** The members of the family have essential similarities, or *homologies*, in their amino acid sequences, and, thence, are rather similar as to their biological effects.

Subclass II includes ***tropic hormones,*** or ***tropins*** (from the Greek tropos, "turn"). These relatively long polypeptides are secreted mainly in response to hypothalamic hormones, and activate the next rank of endocrine glands, which includes the adrenal cortex, thyroid gland, ovaries, and testes. These glands in turn secrete their specific hormones, which are carried in the bloodstream to the cellular receptors in the target tissues.

Subclass III consists of a family of ***proopiomelanocortin*** derivatives.

A. Subclass I

Growth hormone, also known as ***somatotropin,*** is a protein hormone that consists of 190 amino acids residues. Somatotropin is synthesized and secreted by cells called ***somatotrophs*** in the anterior pituitary. It is a major participant in control of several complex physiologic processes, including growth and metabolism. The hormone has two distinct types of biological effects:

- *Direct effects* which are the result of growth hormone binding to its receptors on target cells. For example, the interaction of somato-tropin with hormone receptors located in plasma membranes of adipocytes stimulates the latter to break down triglycerides and supresses their ability to take up and accumulate circulating lipids.
- *Indirect effects* that are mediated primarily by an *insulin-like growth factor I (IGF-I)*, a tissue hormone which is secreted from the liver and other tissues in response to somatotropin. It is established that a majority of the growth promoting effects of growth hormone is actually

due to IGF-I acting on its target cells.

Effects of somatotropin on growth. Growth is a very complex process, and requires the coordinated action of several hormones. **The major role of growth hormone in promoting body growth is to stimulate the liver and other tissues to secrete IGF-I.** IGF-I stimulates proliferation of *chondrocytes* (cartilage cells), resulting in bone growth. Besides, growth hormone has a direct effect on bone growth in stimulating differentiation of chondrocytes.

Moreover, IGF-I also affects the muscle growth**.** It stimulates both the differentiation and proliferation of *myoblasts*, as well as the amino acid uptake and protein synthesis in muscle and other tissues.

Metabolic effects of somatotropin:

- **Protein metabolism:** In general, growth hormone stimulates protein anabolism in many tissues. This effect reflects increased amino acid uptake, increased protein synthesis and decreased catabolism of proteinogenic amino acids.
- **Fat metabolism:** Growth hormone enhances the utilization of fats by stimulating triacylglycerols breakdown in adipocytes and fatty acids oxidation.
- **Carbohydrate metabolism:** Growth hormone is one of a set of hormones that control blood glucose level within a normal range. Growth hormone is often said to have "anti-insulin activity", because it supresses the ability of insulin to stimulate uptake of glucose in peripheral tissues and enhance glucose synthesis in the liver. On the other hand, growth hormone administration stimulates insulin secretion, leading to *hyperinsulinemia.*

Control of growth hormone secretion

The primary controllers of growth hormone production are two hypotha-lamic hormones and one hormone from the gastrointestinal group.

- **Growth hormone-releasing hormone** (GHRH; see above) stimulates both the synthesis and secretion of growth hormone.
- **Somatostatin (see above)** inhibits growth hormone release in response to GHRH and to other stimulatory factors such as low blood glucose concentration.
- **Ghrelin** is a peptide hormone secreted from the stomach which stim-

ulates secretion of growth hormone.

Besides, growth hormone biosynthesis and secretion are modulated by many factors of physiological importance, including stress, exercise, nutrition, and sleep. It is extremely interesting that somatotropin production is largely activated during sleeping which proves the folk proverb "The children are growing when sleeping"!

Disease states. States of both growth hormone deficiency and excess provide very visible clinical manifestations.

- The deficiency in growth hormone production is commonly clear visible as growth retardation or ***dwarfism.*** The degree of the manifestations depends upon the age of the disorder onset and arises from either heritable or acquired disease. It is noteworthy that a deficiency state can result not only from a deficiency in production of the hormone, but in the target cell's response to the hormone.
- The effect of excessive growth hormone secretion also greatly depends on the age of disease onset and is seen as two distinctive disorders:

Gigantism is the result of excessive growth hormone secretion that begins in young children or adolescents. The disorder usually results from a tumor of somatotrophs and has rather rare occurrence.

One of the most famous giants was a man named ***Robert Pershing Wadlow****, who was born, educated and buried in Alton, Illinois (USA). His height of 8 feet 11 inches qualifies him as the tallest person in history, as recorded in the Guinness Book of Records.*

Robert was born on February 22, 1918, and weighed a normal eight pounds, six ounces, but by 5 years of age was 105 pounds and 5 feet 4 inc hes tall. Robert reached an adult weight of 490 pounds and 8 feet 11 inches in height. Trying to maintain a normal life, Robert enjoyed collecting stamps, photography, and become the world's tallest Boy Scout at seven feet, four inches, when he was 13 years of age.

In 1984 a citizens committee organized efforts to immortalize Robert, and in 1985 a bronze statue (photo), was erected on the campus of the Southern Illinois University School of Dental Medicine. At the time of his death Robert Wadlow weighed 490 pounds.

It is a human`s tragedy...

Acromegaly results from excessive secretion of growth hormone in adults. The onset of the disorder is typically insidious. But, little by little, an overgrowth of bone and connective tissue leads to a change in human appearance that might be described as having "coarse features".

Prolactin

Prolactin is a single-chain protein hormone closely related to growth hormone. It is secreted by so-called *lactotrophs* in the anterior pituitary.

Prolactin is synthesized as a prohormone, and, following cleavage of the

signal peptide, the length of the mature hormone is between 194 and 199 amino acids, depending on species. Hormone structure is stabilized by three intramolecular disulfide bonds.

The major target organ of prolactin is the mammary gland, and the principal physiological function of the hormone is the stimulating of mammary gland development and lactogenesis. Thus, prolactin has two major roles in milk production:

- **Prolactin induces lobuloalveolar growth of the mammary gland**. The mammary gland alveoli are the clusters of cells that actually secrete milk, and their development is greatly stimulated after prolactin treatment.
- **Prolactin stimulates milk production** after giving birth. The hormone, along with cortisol and insulin, act together to stimulate transcription of the genes that encode milk proteins.

Control of prolactin secretion. The major prolactin-inhibiting factor or brake on prolactin secretion is catecholamine ***dopamine***. Dopamine is secreted into portal blood by hypothalamic neurons, binds to receptors on lactotrophs, and inhibits both the synthesis and secretion of prolactin. Agents and drugs that interfere with dopamine secretion or receptor binding lead to enhanced secretion of prolactin.

Hyperprolactinemia. Excessive secretion of prolactin – *hyperprolactine-mia* – is a relatively common disorder in humans. This condition has various causes, including prolactin-secreting tumors.

Clinical manifestations of hyperprolactinemia in women include ***amenorrhea*** (lack of menstrual cycles) and ***galactorrhea*** (excessive or spontaneous secretion of milk). Men with hyperprolactinemia typically show ***hypogonadism,*** with decreased sex drive, decreased sperm production and impotence. Such men also often show breast enlargement ***(gynecomastia),*** but very rarely produce milk.

It needs also to emphasize that prolactin is a multifunctional hormone. Moreover, it is difficult to point to a tissue that does not express prolactin receptors, and, besides anterior pituitary, the hormone is synthesized and secreted in many other tissues. The prolactin receptors are extremely widely expressed by immune cells, and some types of lymphocytes synthesize and secrete prolactin. These observations suggest that prolactin may act as an autocrine or paracrine modulator of immune activity. It appears that

prolactin has a modulatory role in several aspects of normal and pathologic immune responses.

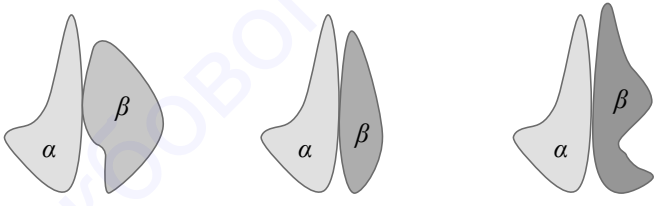
Chorionic somatomammotropin, also called ***placental lactogen,*** is a polypeptide hormone that promotes maternal breast development during pregnancy.

After being secreted by the placenta, the hormone enters the maternal circulation and disappears from the circulation immediately after delivery. It is immunologically similar to human *growth hormone*, and has growth-promoting activity. Placental *lactogen* also hampers maternal insulin activity during pregnancy and, by inhibiting glucose oxidation in maternal body, it increases the glucose supply to a foetus.

B. Subclass II

Thyrotropin, **lutropin** and **follitropin** are a group of proteohormones which are produced in adenohypophysis. All they are essential for the synthesis and secretion of the appropriate hormones from the "subordinate" endocrine glands.

Chemically, these hormones are glycoproteins. They are composed of two polypeptide chains (α- and β-) with the carbohydrate (oligosaccha-rides) units attached.



Thyroid-stimulating hormone | *Luteinizing hormone* | *Follicle-stimulating hormone*

As can be seen from the image presented, all the three hormones have the identical alpha subunit, but **each of them has a unique beta subunit**, which provides receptor specificity. Free alpha and beta subunits have essentially no biological activity.

Thyrotropin *(thyroid-stimulating hormone, TSH),* is secreted from cells in the anterior pituitary called *thyrotrophs* and finds its receptors on epithelial cells in the thyroid gland. The hormone stimulates the synthesis

and secretion of thyroid hormones by the thyroid gland.

Lutropin *(luteinizing hormone, LH)* and **Follitropin *(follicle-stimulating hormone, FSH)*** are called ***gonadotropins*** because they stimulate the gonads – in males, the testes, and in females, the ovaries. As was stated above, the principle regulator of **LH** and **FSH secretion is gonadotropin-releasing hormone or GnRH** (also known as LH-releasing hormone). GnRH stimultes secretion of **LH**, which in turn stimulates gonadal secretion of the sex steroids **testosterone**, **estrogen** and **progesterone**. In a classical negative feedback loop, sex steroids inhibit secretion of **GnRH** and also appear to have direct negative effects on gonadotrophs.

C. Subclass II.

This a subclass of proopiomelanocortin derivatives. Chemically, this is the peptide hormones family, constituted by the hormones which are the products of proteolytic processing of prohormone-precursor – **proopio-melanocortin** (**POMC**, "Big Mama") – Figure 21.3.

The major hormones representative that are produced in this process are summarized as follows:

- **Adrenocorticoptropic hormone *(ACTH; corticotropin),*** which, as its name implies, stimulates the adrenal cortex. More specifically, it stimulates secretion of ***glucocorticoids*** such as **cortisol,** and has little control over secretion of **aldosterone,** the other major steroid hor-mone from the adrenal cortex. ACTH is secreted from the anterior pituitary in response to corticotropin-releasing hormone from the hypothalamus.
- **Lipotropin:** originally described as having weak lipolytic effects, its major importance is as the precursor to beta-endorphin.
- **Beta-endorphin** and **Met-enkephalin:** opioid peptides with pain-alleviation and euphoric effects.
- **Melanocyte-stimulating hormone (MSH):** known to control melanin pigmentation in the skin of most vertebrates.

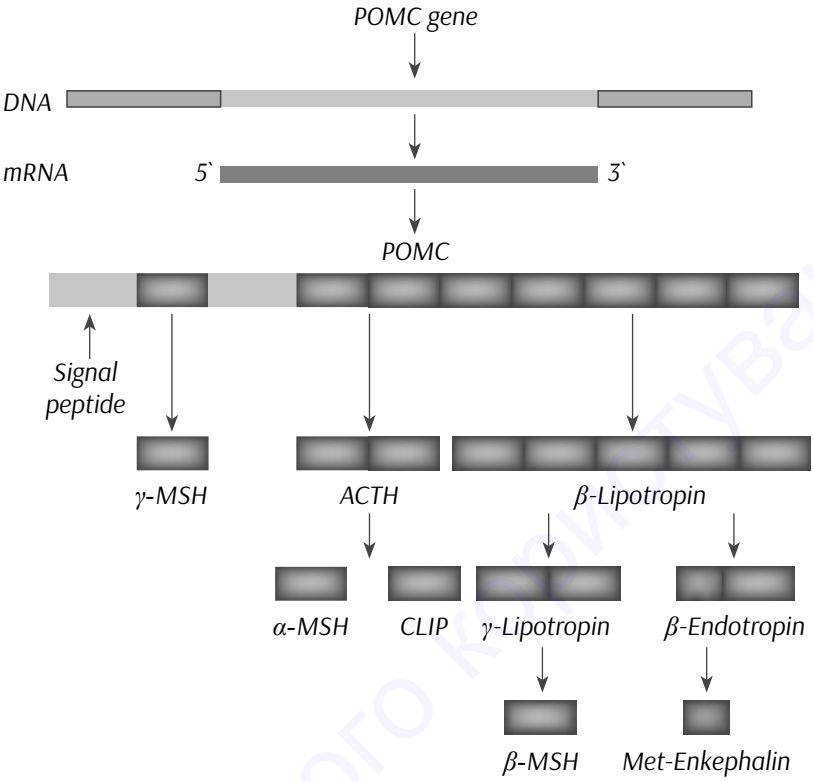


Figure 21.3. Production of active hormone molecules via proteolytic processing of proopiomelanocortin (POMC). ACTH – adrenocorticotropic hormone; MSH – melanocyte-stimulating hormone; CLIP – corticotropin-likeintermediatelobepeptide

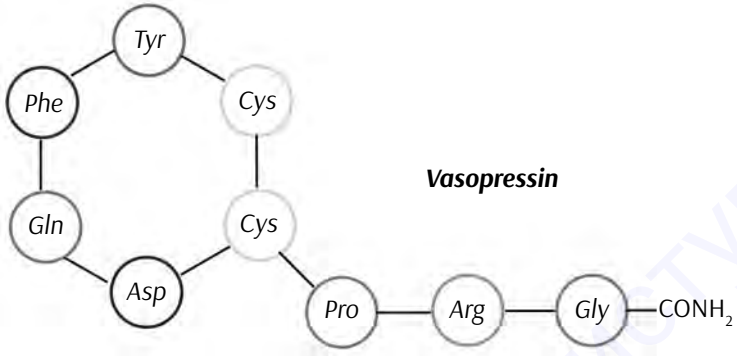
Hormones of neurohypophysis

The posterior part of the hypophysis is called ***neurohypophysis.*** This part of the pituitary contains the axonal endings of many neurons that originate in the hypothalamus. Virtually, it is an individual gland which produces two short peptide hormones **vasopressin** and **oxytocin.**

Both hormones are synthesized in hypothalamic neurons and transported down axons of the posterior pituitary for secretion into blood. Vasopressin and oxytocin equally are packaged into secretory vesicles and secreted along with carrier proteins called ***neurophysins.***

Vasopressin (antidiuretic hormone; ADH)

Vasopressin, also known as ***antidiuretic hormone***, is a nine amino acid cyclic peptide secreted from the posterior part of pituitary gland.



Vasopressin

Physiological effects of ADH

The principal physiological effect of antidiuretic hormone is to conserve body water by reducing the output of urine.

Antidiuretic hormone binds to receptors in the distal or collecting tubules of the kidney and promotes reabsorbtion of water back into the circulation. This is realized via the stimulating of the formation inside the kidney tubules membranes of the special "water channels" or ***aquaporins.*** These channels transport solute-free water through tubular cells and back into blood, leading to a decrease in plasma osmolarity and an increase osmolarity of urine.

Control of ADH secretion. Diseases state

The most important variable regulating antidiuretic hormone secretion is plasma **osmolarity,** or the concentration of solutes in blood. The rise of osmolarity is sensed by the hypothalamus neurons known as an **osmoreceptors,** and this, in turn, simulates the production of the ADH in the hypothalamus and its secretion from the cells of the posterior pituatory gland.

In the absense of antidiuretic hormone, the kidney tubules are virtually impermiable to water, and it flows out as urine. The most common disease of man and animals related to antidiuretic hormone deficiency is **diabetes insipidus**. This condition can arise from either of two situations:

- **Hypothalamic ("central") diabetes insipidus** results from a deficiency in secretion of ADH hormone from the posterior pituitary. Causes

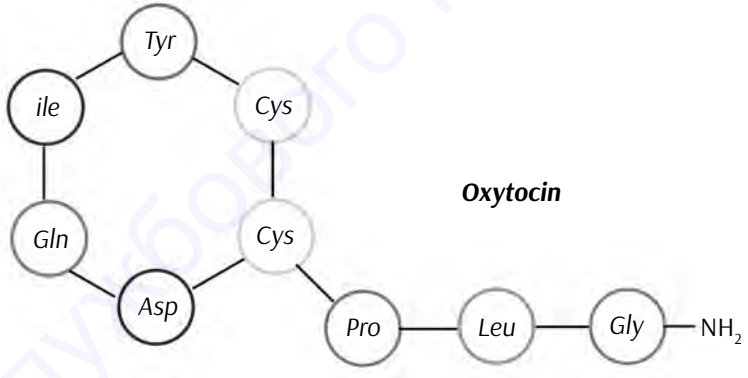
of this disease include head trauma, and infections or tumors involving the hypothalamus.

- **Nephrogenic diabetes insipidus** occurs when the kidney is unable to respond to ADH. Most commonly, this results from some type of renal disease; at the same time, in affected humans, mutations in the ADH receptor gene or in the gene encoding aquaporin-2 have been demonstrated

The major sign of either type of diabetes insipidus is excessive urine production. Some human patients produce as much as 16 liters of urine per day! If adequate water is available for consumption, the disease is rarely life-threatening, but withholding water can be very dangerous. Hypothalamic ***diabetes insipidus*** can be treated with exogenous antidiuretic hormone.

Oxytocin

Oxytocin in a nine amino acid cyclic peptide that differs from vasopressin in two of the nine amino acid residues.



Similar to vasopressin, oxytocin is synthesized in hypothalamic neurons and transported down axons of the posterior pituitary for secretion into blood. The hormone is also secreted within the brain and from a few other tissues, including the ovaries and testes.

Physiologic effects of oxytocin

In females oxytocin mediates three major physiological functions, and

namely:

- **Stimulation of milk ejection (milk letdown).** Milk is initially secreted into small sacs within the mammary gland called alveoli, from which it must be ejected for consumption or harvesting. Mammary alveoli are surrounded by smooth muscle (myoepithelial) cells which are target cell for oxytocin. Oxytocin stimulates contraction of myoepithelial cells, causing milk to be ejected into the ducts and cisterns.
- **Stimulation of uterine smooth muscle contraction at birth.** At the end of gestation, the uterus must contract vigorously and for a prolonged period of time in order to deliver the foetus. During the later stages of gestation, there is an increase in abundance of oxytocin receptors on uterine smooth muscle cells, which leads to the increased sensitivity of the uterus to the hormonal stimuli. Oxytocin is released during labor, and this enhances contraction of uterine smooth muscle to facilitate parturition or birth. Thus, in cases where uterine contractions are not sufficient to complete delivery, oxytocin is sometimes administered by physicians to facilitate the birth of the foetus.
- **Establishment of maternal behavior.** Everybody knows that the successful reproductive function in mammals demands that mothers nourish their infants immediately after birth. And it is noteworthy that oxytocin affects not only the uterus and mammary gland at the time of birth but also influences the brain. During parturition, there is an increase in concentration of oxytocin in cerebrospinal fluid, and there is commonly accepted scientific concept that **oxytocin acting within the brain plays a major role in establishing maternal behavior.**

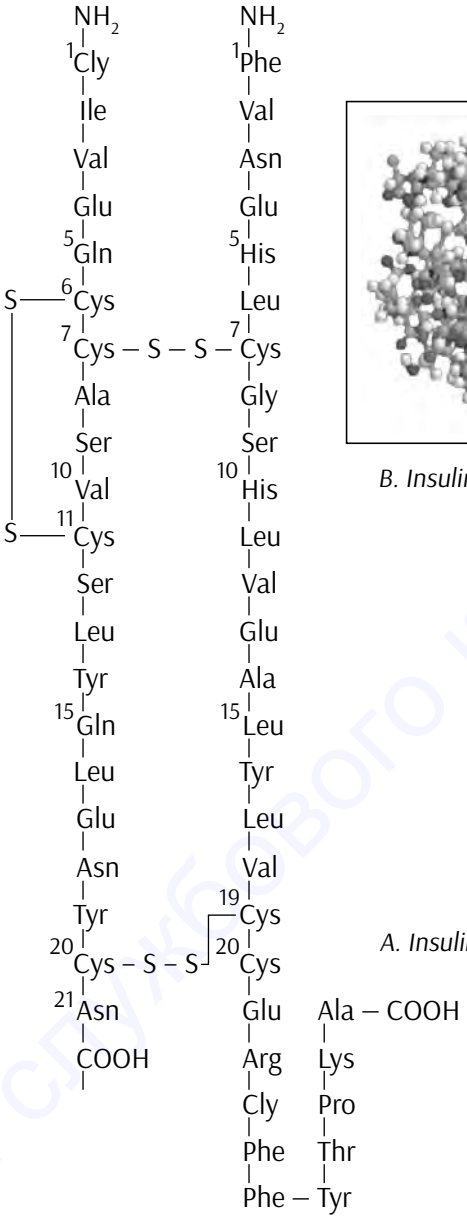
21.2. Protein hormones of pancreatic gland

Hormones of pancreas

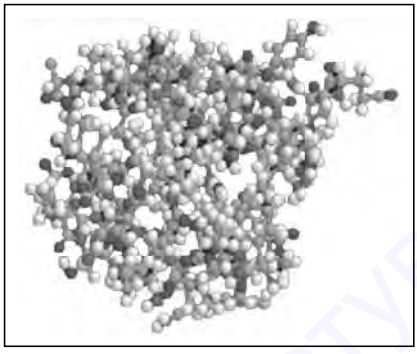
Insulin

Insulin is a low molecular mass protein ($M_r \approx 5{,}8$ kD) which includes two polypeptide chains, **A** and **B,** joined by two disulfide bonds (Figure 21.4).

Insulin is synthesized in the special β-(beta) cells of the pancreatic **islets of Langerhans.** As is usual with secretory proteins, the hormone is originally produced in the form of inactive single-chain precursor, **preproinsulin,** that includes an amino-terminal "signal sequence" directing its passage into cisterns of endoplasmic reticulum. There, after the proteolytic removal of the signal sequence and formation of three disulfide bonds, **proinsulin** is formed. Proinsulin is transported to the secretory granules which are components of Golgi apparatus. When elevated blood glucose level triggers insulin secretion, proinsulin is converted to active hormone by specific proteases, which cleave two peptide bonds to form the mature insulin molecule.



A. Insulin: primary structure



B. Insulin: tertiary structure

Figure 21.4. The primary and tertiary structure of insulin molecule. A chain – 21 a a; B chmin – 30 an

Insulin receptor and mechanism of hormone action

Similar to receptors for other protein hormones, the receptor for insulin is embedded in the plasma membrane. Nevertheless, this receptor has some unusual peculiarities in its molecular architecture and biochemical proper-ties.

Contrariwise to the majority of peptides and proteohormones, insulin receptor has is own, intrinsic enzyme activity. It is, essentially, the representative of the distinctive class of enzymatic proteins, which are called ***receptor tyrosine kinases.*** In other words, it functions not only as a binding site for hormone molecule, but as an enzyme that transfers phosphate groups from ATP to tyrosine residues on intracellular target proteins.

The insulin receptor is composed of two **α subunits** and two **β subunits** linked by disulfide bonds. The alpha chains are entirely extracellular and contain **insulin binding domains,** while the beta chains penetrate through the plasma membrane (Figure 14.8).

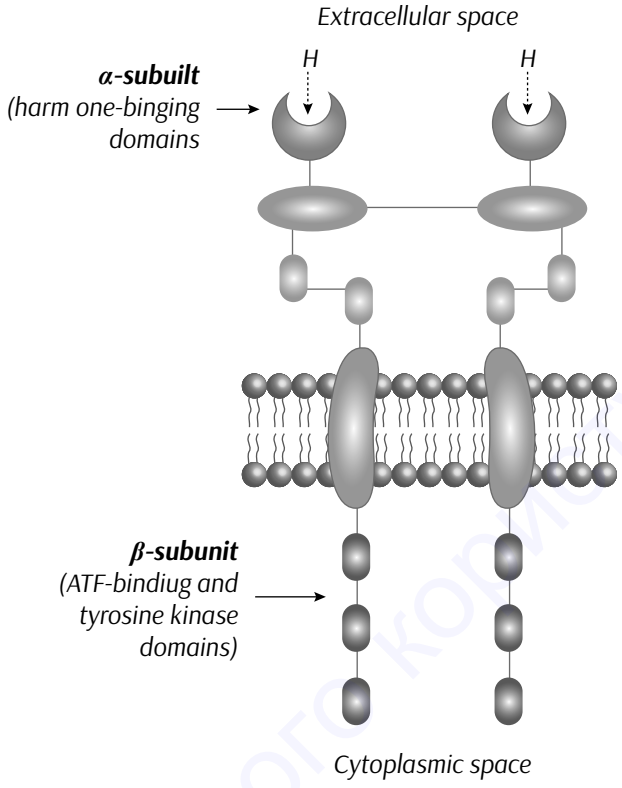
Binding of insulin to the alpha subunits causes the beta subunits to phosphorylate themselves (autophosphorylation), thus activating the catalytic activity of the receptor. The activated receptor then phosphorylates a number of intracellular proteins, which in turn alters their activity, thereby generating a biological response of the cell to the insulin action.

Biochemical effects of insulin

The most prominent and clinically detectable biochemical effects of insulin are apparent in his influence on carbohydrate metabolism, though under hormone deficiency the severe disturbances of lipid and amino acid metabolism occur. In a simplified fashion, the **effects of insulin as to the carbohydrates turnover** can be described as **stimulation of glucose utilization and the inhibition of glycogen breakdown and glucose *de novo* synthesis.** This is accomplished via the following biochemical mechanisms.

1) Insulin greatly promotes the uptake of glucose by muscle and fat tissue cells.

It is known that the downhill (concentration gradient directed) movement of glucose molecules across the plasma membranes of animal cells is mediated by special *glucose transporters.* These are protein family, designated as **GLUT1** to **5,** either of which consists of a single polypeptide chain (500 aa residues) that spans the plasma membrane.



Insulin stimulates the biosynthesis of a transporter peptide, **GLUT4,** that leads to a rapid entry of additional glucose molecules into cells.

2) Insulin stimulates, by the way of genetic induction, the expression of ***glycogen synthase,*** as well as of several key enzymes of glycolysis.

This leads to the considerable increase of intracellular glucose utilization via the routes of glycogen synthesis and that of glucose oxidation.

3) At the same time, insulin inhibits the biosynthesis of key gluconeogenesis enzymes. This results in the decrease of pyruvate and amino acids into gluconeogenetic pathway entry, that is the substantial delay in glucose *de novo* synthesis.

The principal consequence of the biochemical events listed in (1), (2), (3) is the considerable fall in intracellular free glucose concentration which can become an unfavorable consequence of a momentary insulin high dose injection.

4) Concurrently with the stimulation of glucose utilization, insulin substantially reduces the catabolic use of lipids. It is achieved by the inhibiting of adipocytes ***lipase,*** which is the additional metabolic effect of insulin. Moreover, insulin activates the synthesis of fatty acids from glucose that is achieved by the activation of ***acetyl-CoA carboxylase.*** On the whole, these two processes summarily lead to the stimulation of the fat anabolism and the additional deposition of triacylglycerols inside fat tissue.

Diabetes mellitus

The impair of insulin control over carbohydrate and lipid metabolism results in ***diabetes mellitus*** development.

Under the disease, which is the consequence of an absolute or a relative insulin deficiency, the entry of glucose molecules into cells is substantially impaired. This, concurrently with the diminishing of glucose utilization via glycogen synthesis and glycolytic oxidation, leads to the large rise of glucose plasma content, usually from 5 mM to 9 mM and above. This is called ***hyperglycemia*** ("elevated blood glucose level") and is in itself the characteristic clinical manifestation of ***diabetes mellitus*** (Lecture 6).

The insulin deficiency results also in the enhanced degradation of lipids. The excess accumulation of free tatty acids that results from the lipolysis activation, together with the inhibition of acetyl-CoA oxidation in the citric acid cycle, entails the considerable rise of **ketogenesis** (Lecture 7). The increased breakdown of tissue, predominantly muscle, proteins, which occurs under the disease, and the resulting accumulation of ketogenic amino acids, adds to the increase of ketone bodies production.

Ketone bodies (acetoacetate and 3-hydroxybutyrate), which are actively produced in hepatocytes and extensively released into blood, dissociate in plasma to give respective anions and free protons. Thus, high ketone body production, which is the case in diabetes mellitus, may result in overloading of the plasma buffer systems, that leads to the consequent decrease in plasma pH, characteristic for severe **diabetic ketoacidosis.**

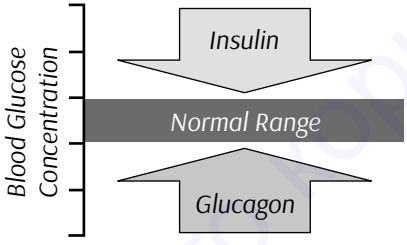
The long duration of major metabolic disturbances which take place in diabetes mellitus, produce a variety of secondary clinical complications. They are ***diabetic angiopathy,*** which includes structural changes in blood vessels walls, ***diabetic nephropathy,*** resulting from kidneys damage and

diabetic cataract that comes from the biochemical disturbances in eyes tissue metabolism.

Glucagon

Glucagon is a single chain peptide consisting of 29 amino acid residues. The hormone is primarily synthesized within α-(alpha) cells of the pancreatic islets as ***proglucagon*** and then proteolytically processed to yield the mature molecule of **glucagon**.

Glucagon secretion rises when there is a decline of blood glucose concentration, commonly as a result of fasting. The principal metabolic effect of glucagon is to stimulate the liver glycogen breakdown through the ***glycogen phosphorylase*** activation.



The release of glucose from the liver leads to the sugar blood concentration increase, which permits to regard glucagon as the essential antagonist of insulin.

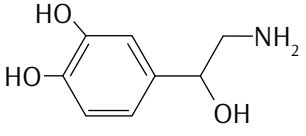
Chapter 22.

HORMONES-3. AMINO ACID DERIVED HORMONES: CATECHOLAMINES; THYREOIDS. LIPOPHILIC HORMONES

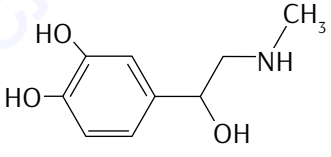
22. 1. Catecholamines and other biogenic amines

Catecholamine Hormones

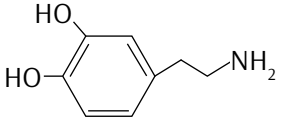
Catecholamines are a group of water-soluble compounds that are named for their structural relation to cyclic alcohol ***catechol.*** Catecholamines include **epinephrine (adrenaline), norepinephrine (noradrenaline)** and **dopamine.**



Norepinephrine



Epinephrine



Dopamine

In the human body catecholamines serve as hormones or as neutrotransmitters that mediate signaling in the *sympathetic nervous system.*

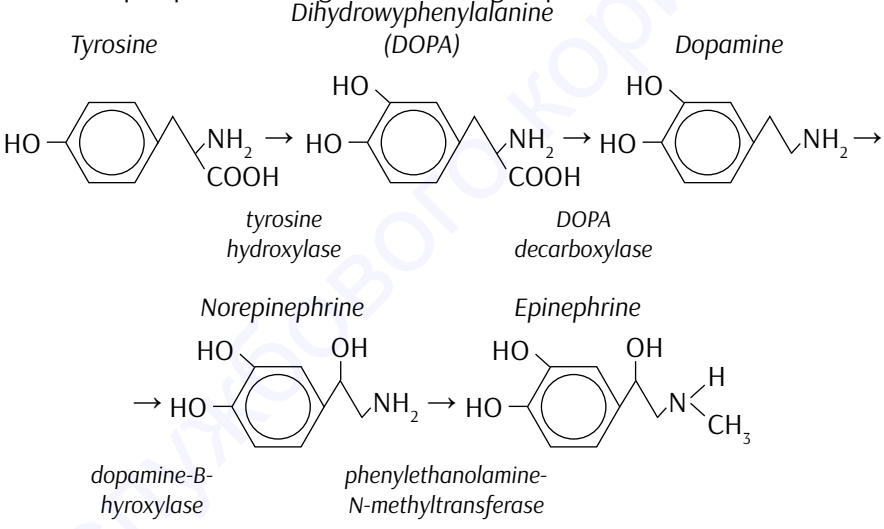
Epinephrine and **norepinephrine** are hormones that are synthesized and secreted by the *chromaffin cells* in the **adrenal medulla.** These substances prepare the body to meet emergencies such as cold, fatigue, and shock. The most prominent hormone activity is inherent to **epinephrine.**

Norepinephrine is also produced by the brain and some other neural tissues, and is also a chemical transmitter at noradrenergic nerve synapses.

Synthesis and secretion of catecholamines in adrenal medulla

As was noted above, cells in the adrenal medulla synthesize and secrete both epinephrine and norepinephrine. The ratio of these two catecholamines differs considerably among various animal species, but in humans epinephrine amounts about 80 % of the total catecholamine output.

Synthesis of catecholamines begins with the amino acid tyrosine, which is taken up by chromaffin cells in the medulla and converted to norepinephrine and epinephrine through the following steps:



Norepinephine and epinephrine are stored in electron-dense granules of chromaffin cells which also contain ATP and several neuropeptides. They are released from secretory granules by exocytosis. The secretion of catecholamines from adrenal medulla is stimulated by acetylcholine and many kinds of emotional and traumatic stress, physical exercise, hypoglycemia etc. Following secretion into blood, the catecholamines bind loosely to serum albumin and are carried in the circulation before interacting with the target cells.

Physiological and biochemical effects of catecholamines

The physiologic and biochemical effects of epinephrine and norepinephrine are initiated by their binding to ***adrenergic receptors*** on the surface of target cells. Similar to peptides and proteohormones, catecholamines affect the metabolism of sensitive cells through **G proteins-trancducers** and generation the second messengers, predominantly **cAMP.**

In general, circulating **epinephrine** and **norepinephrine** released from the adrenal medulla have the same effects on target organs as direct stimulation by sympathetic nerves, although their effect is longer lasting. **The physiologic consequences of medullary catecholamine release are directed to manage with stress.** Thus, in accordance with their principal effects on the blood vessels, the heart and intermediary catabolism, epinephrine and norepinephrine are called the hormones of "fight or flight".

A bit of history...

Earl W. Sutherland
(1915–1974)
1971 Nobel Laureate in Medicine for his discoveries concerning the mechanisms of the actions of hormones

In collaboration with the Nobel Laureate Carl Cori, E. Sutherland studied the mechanism by which epinephrine regulates the degradation of glycogen to glucose in the liver. Before that, the mechanisms by which various hormones exert their extremely important functions have been a complete

enigma. Because of the work of Sutherland we can today understand the general mode of action of many of them.

Sutherland was the first to discover that epinephrine acts by activating the enzyme (phosphorylase) which leads to the formation of glucose from glycogen. Later he found that this activation took place by means of a hitherto unknown substance which occurs as an intermediate during the process. The discovery and chemical characterization of the intermediate, which has been termed "the second messenger" by Sutherland (the hormone itself is the first messenger) was of crucial importance for an understanding of the mechanism of action of epinephrine and of many other hormones. The newly identified substance proved to be a nucleotide, and was named cyclic adenosine phosphate or cyclic AMP.

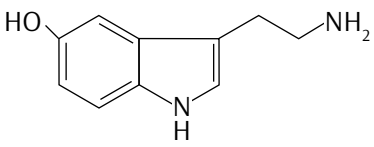
A brief listing of major effects mediated by epinephrine and norepinephrine includes:

- **Increased rate and force of contraction of the heart muscle:** this is pre- dominantly an effect of epinephrine.
- **Constriction of blood vessels:** norepinephrine, in particular, causes widespread vasoconstriction, resulting in increased resistance and hence arterial blood pressure.
- **Dilation of bronchioles,** which assists in pulmonary ventilation.
- **Stimulation of glycogenolysis in muscle and liver cells** which, promotes the release of additional glucose into blood. Simultaneously with that, the inhibition of glycogen synthesis occurs.
- **Stimulation of lipolysis in adipocytes:** this provides fatty acids for energy production in many tissues and aids in conservation of sugars reserves.

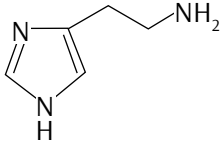
Other biogenic amines

There are two major representatives of biogenic amines, distinctive from catecholamines, which have the functional properties of signaling molecules, and they are **serotonin** and **histamine.**

Serotonin is a derivative of amino acid L-tryptophan (5-hydroxy-tryptophan), and **histamine** is a derivative of amino acid **L-hystidine.**



Serotonin



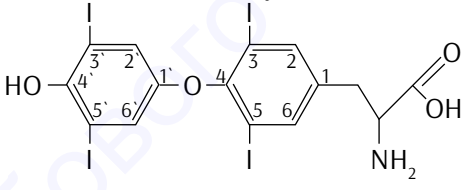
Hystamine

Similarly to catecholamines, serotonin and histamine share the properties of tissue hormones and neurotransmitters. The roles of serotonin in nerve system signals transmission, as well as the determination of some severe mental disorders in human brain, will be discussed in Lecture 20.

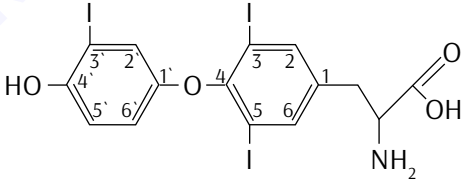
22.2. Thyroid hormones: representatives, pathology

Thyroid hormones are derivatives of the amino acid tyrosine bound covalently to iodine. The two principal thyroid hormones are:

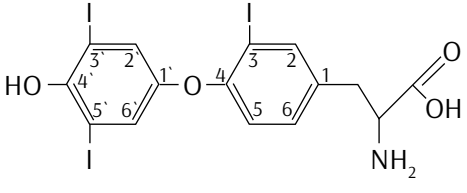
- **thyroxine** (L-3,5,3',5'-tetraiodothyronine, known as T4);
- **triiodotyronine** (L-3,5,3'-triiodothyronine, known as T3).



3,5,3`,5`-Tetraiodothyronine (Thyroxine; T4)



3,5,3`-Triiodothyronine (T3)



3,3`,5`-Triiodothyronine (reverse T3; inactive)

As can be see from the formulae shown, the **thyroid hormones are basically two tyrosines linked together with the critical addition of iodine at three or four positions on the aromatic rings.** The number and position of the iodines is essential for the availability of hormonal activity. Several other iodinated molecules which can be generated from two tyrosines have little or no biological activity; the so called "reverse T3" (3,3',5'-T3) is such an example.

Synthesis and secretion of thyroid hormones

The microscopic structure of the thyroid glands reveales the occurrence of thyroid epithelial cells which are responsible for synthesis of thyroid hormones. As can be seen from the microscopic images of the gland, the thyroid cells are arranged in spheres called ***thyroid follicles*** that are filled with a so-called ***colloid*** (Figure 22.1).

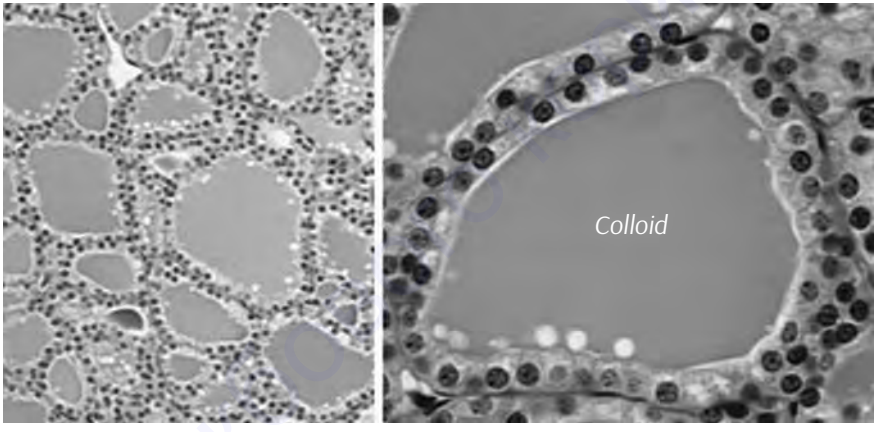


Figure 22.1. Microscopic images of the thyroid gland

The colloid is a depot of thyroid hormone protein precursor, **thyroglobulin.** A molecule of thyroglobulin contains 134 tyrosine residues, and a portion of them are used to synthesize T4 and T3.

To make up thyroid hormones, **iodine,** or more accurately **iodide (I^-),** is required. Iodide is taken up from blood by thyroid epithelial cells, which have a special *sodium-iodide symporter* or "*iodine trap*" on their outer plasma membrane. The formation of thyroid hormones properly is conducted by the enzyme ***thyroid peroxidase,*** which is located in the apical (colloid-facing) plasma membrane of thyroid epithelial cells. And the enzyme catalyzes

two sequential reactions:

1) iodination of tyrosines linked to thyroglobulin molecule; the products of reactions are ***iodotyrosines;***
2) synthesis of **thyroxine** or **triiodothyronine** from two iodotyrosine molecules.

But it needs to state, that at this stage of hormone production, the iodated thyronins remain linked to the thyroglobulin molecule. The liberation of hormone molecules and their entry into blood circulation take place as the response of the thyroid gland to the physiological stimuli promoted by *thyroid-stimulating hormone* from the anterior pituitary gland. Binding of TSH to its receptors on thyroid epithelial cells stimulates synthesis of the iodine transporter, thyroid peroxidase, iodated thyroglobulin formation and the release of thyroid hormones into the circulation.

Physiological and biochemical effects of thyroid hormones

It is established that practically all cells in the human body are targets for thyroid hormones. According to this, thyroid hormones have profound effects on such vital physiologic and biochemical processes as development, growth and metabolism.

Thyroid hormone receptors

Thyroid hormones enter target cells through membrane transporter proteins, and thereafter penetrate into cell nucleus. **Recep-tors for thyroid hormones are intracellular DNA-binding proteins that function as hormone-responsive transcription factors,** very similar conceptually to the receptors for steroid hormones.

Once inside the nucleus, the thyroid hormone binds its receptor, and **the hormone-receptor complex interacts with specific sequences of DNA in the promoter regions of responsive genes.** The effect of receptor binding to DNA is to modulate certain genes expression, either by stimulating or inhibiting transcription of specific genes. For example, it is well known that **thyroid hormones increase the strength of contraction of the heart.**

Cardiac contractility depends, in part, on the relative ratio of different types of myosin proteins inside cardiac muscle. So, transcription of some myosin genes is stimulated by thyroid hormones, while transcription of others is inhibited. The net effect is to alter the ratio toward increased contractility.

Effects on metabolism

Thyroid hormones treatment leads to the considerable **increase in basal metabolic rate.** The consequence of this activity is the essential rise of body heat production, which results, at least in part, from increased oxygen consumption and uncoupling of oxidative phosphorylation.

- **Carbohydrate metabolism:** Thyroid hormones stimulate almost all pathways in carbohydrate metabolism, including increased gluconeogenesis and glycogenolysis to generate free glucose and the enhancement of insulin-dependent entry of glucose into cells.
- **Lipid metabolism:** Increased thyroid hormone levels stimulate fat mobilization, leading to increased concentrations of fatty acids in plasma, along with the enhance of fatty acids oxidation. Finally, plasma concentrations of cholesterol and triglycerides are inversely correlated with thyroid hormone levels. And hence, the increased blood cholesterol concentration serves the diagnostic indication of hypothyroidism.

***Growth*:** Thyroid hormones are necessary for normal growth in children and young animals. And this is clearly evidenced by the growth-retardation observed in thyroid deficiency.

***Development*:** A classical experiment in endocrinology was the demonstration that tadpoles deprived of thyroid hormone failed to undergo metamorphosis into frogs. Of critical importance in mammals is the fact that normal levels of thyroid hormone are essential to the development of the foetal and neonatal brain.

***Other effects*:** A few additional, well-documented physiological effects of thyroid hormones include:

- **Cardiovascular system:** Thyroid hormones increases heart rate, cardiac contractility and cardiac output. They also enhance blood flow to many organs.
- **Central nervous system:** Both decreased and increased concentra-

tions of thyroid hormones lead to alterations in mental state. Too little thyroid hormone, and the individual tends to feel mentally sluggish, while too much induces anxiety and nervousness.

- **Reproductive system:** Normal reproductive behavior and physiology is dependent on having essentially normal levels of thyroid hormone. Hypothyroidism in particular is commonly associated with infertility.

Thyroid disease states. Both insufficient production and overproduction of thyroid hormones are associated with severe disease states.

Hypothyroidism is the result from any thyroid hormone deficiency. Two well-known examples include:

- **Iodine deficiency:** iodide is absolutely necessary for production of thyroid hormones; without adequate iodine intake, thyroid hormones cannot be synthesized.
- **Primary thyroid disease:** an important cause of hypothyroidism constitute inflammatory diseases of the thyroid that destroy epithelial cells of the gland.

Common clinical symptoms of hypothyroidism include lethargy, fatigue, cold-intolerance, weakness, hair loss and reproductive failure. The specific disease states of hypothyroidism include **myxedema** and **goiter.** The latter develops in the case of prolonged iodide deficiency, when the thyroid gland becomes inadequately large in size. **The most severe form of hypothyroidism develops in young children.** If that condition is not corrected by supplemental therapy soon after birth, the child will suffer from **cretinism**, a disease that includes considerable mental retardation.

Hyperthyroidism results from hypersecretion of thyroid hormones. In humans the most common form of hyperthyroidism is **Graves disease**, an immune disease in which the abnormal and prolonged activation of the thyroid-stimulating hormone receptor occurs, that leads to continual stimulation of thyroid hormone synthesis.

Common manifestations of hyperthyroidism are basically the opposite of those seen in hypothyroidism, and include nervousness, insomnia, high heart rate, eye disease and anxiety.

22.3. Steroid hormones: representatives, effects, pathology

The steroid hormones are of two general types, namely:

- **corticosteroids** which are synthesized in the adrenal cortex (adrenocortical hormones);
- **sex hormones** that are produced in the testes, ovaries and placenta.

All **steroids, including adrenal "corticosteroids" and sex hormones, are synthesized from cholesterol** through a series of enzyme-mediated transformations.

To generate steroid hormones, the reactions that remove the side chain from the D ring of cholesterol and introduce oxygen to form keto and hydroxyl groups are needed. Many of the reactions involve cytochrome P-450-dependent enzyme systems.

The oversimplified chart of steroid hormones synthesis is presented in Figure 22.2.

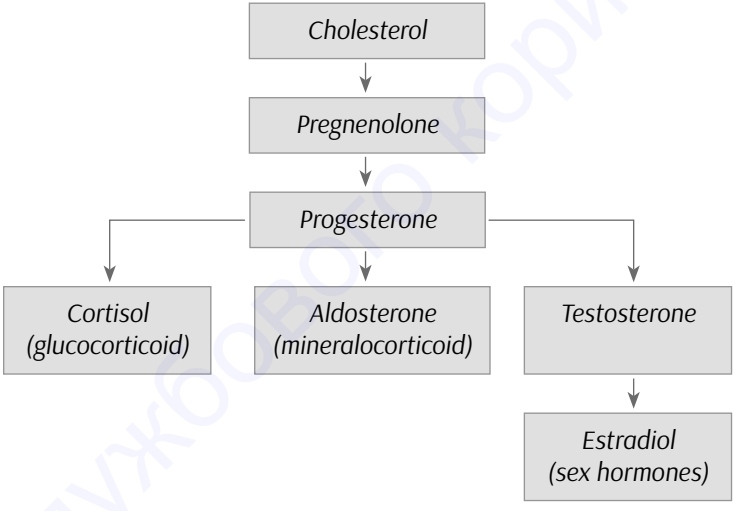
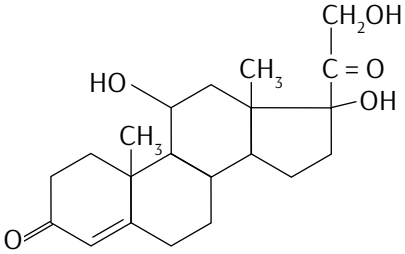


Figure 22.2. The simplified scheme of steroidogenesis

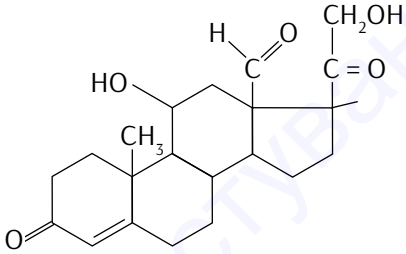
All steroid hormones act through nuclear receptors to change the level of expression of specific genes which are responsible for the synthesis of enzymes that determine specific biochemical and physiological responses of target cells to steroid hormones. There are recent investigations indicating that certain steroid hormones also have more rapid effects, mediated by receptors localized in the plasma membrane.

Corticosteroids

There are two classes of corticosteroids, namely **glucocorticoids** (the major representative is **cortisol**) primarily affecting the metabolism of carbohydrates, and **mineralocorticoids** (such as **aldosterone**) which regulate the concentrations of electrolytes in the blood and other tissue fluids.



Cortisol



Aldosterone

Cortisol, the most significant glucocorticoid, is synthesized mainly inside cells of the inner zones of the adrenal cortex *(zonae fasiculata* and *reticularis)*. It is involved in the regulation of carbohydrate and protein metabolism via promoting the conversion of amino acid to glucose (*gluconeogenesis*) and stimulation the protein catabolism.

The prolonged administration of cortisol and other glucocorticoids leads to the essential rise of blood glucose level. Because of their antiinflammatory and immunosuppressive effects, the natural and synthetic glucocorticoids are widely used medical preparations.

Aldosterone. The principal steroid with mineralocorticoid activity is aldosterone. The hormone is synthesized primarily in the cells of adrenal cortex *zona glomerulosa.*

The main physiological and biochemical effects of aldosterone are concentrated in kidneys, where **hormone displays its key role in the control of minerals – particularly sodium and potassium homeostasis.** The major target cells for aldosterone action are located in the distal tubules of the kidney, where it stimulates exchange of sodium and potassium. Here, the hormone produces three principal physiologic effects:

- **Increased resorption of sodium:** sodium loss in urine is decreased under aldosterone stimulation.
- **Increased resorption of water,** with consequent expansion of extracellular fluid volume. This is an osmotic effect directly related to

increased resorption of sodium.
- **Increased renal excretion of potassium.**

That is why, removal of the adrenal glands or any kind results in aldosterone deficiency and leads to death within just a few days, which is due to the following metabolic derangements:
- the concentration of potassium in extracellular fluid becomes dramatically elevated;
- urinary excretion of sodium is high and the concentration of sodium in extracellular fluid decreases significantly;
- volume of extracellular fluid and blood decrease;
- the function of the heart becomes collapsed, cardiac output declines and the cardiogenic shock ensues.

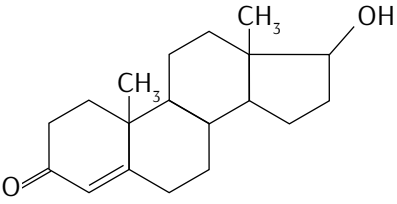
Disease States. A deficiency in aldosterone can occur by itself, or, more commonly, in conjunction with a glucocorticoid deficiency, and is known as *hypoadrenocorticism* or **Addison's disease.** Without treatment by mineralo-corticoid replacement therapy, aldosterone deficiency is lethal, due to electrolyte imbalances and resulting hypotension and cardiac failure.

Sex hormones

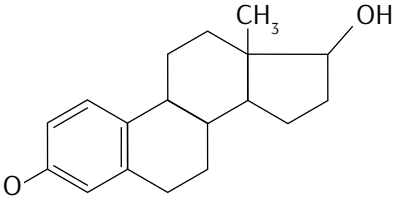
Sex hormones, also known as **sex steroids,** or **gonadal steroids,** play key roles in determining the primary and secondary sex characteristics in male and female.

Natural sex steroids are made mainly in the organs and tissues of reproductive system, that is in gonads (ovaries or testes) and placenta during pregnancy, and partially in adrenal glands, or by conversion from other sex steroids in other tissues such as liver or fat. The principal classes of sex steroids are **androgens, estrogens** and **progestagens,** of which the most important human examples are **testosterone, estradiol** and **progesterone** respectively.

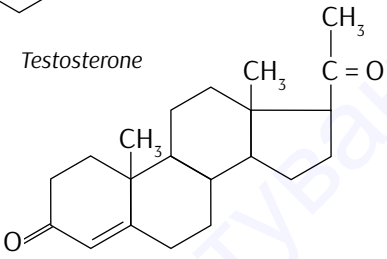
Sex hormones control sexual development of the body, patterns of sexual behavior, and a variety of other reproductive and nonreproductive functions of male and female organisms.



Testosterone



Estradiol

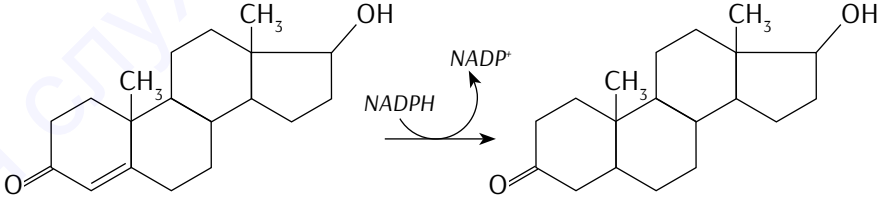


Progesterone

Testosterone

Testosterone, a C-19 steroid, is the major representative from the androgens group. It is synthesized and secreted in the Leidig cells of testes and, in some quantity, in the ovaries of women. Testosterone controls such secondary sexual characteristics of men, as the characteristics of bones, muscle and hair develop-ment and the pattern of behavior, mental and physical energy, including men`s aggressiveness etc.

Dihydrotestosterone *(5-α-dihydrotestosterone; DHT)* is the metabolite of the **testosterone**, which markedly exceeds the latter in biological activity. DHT is formed in the prostate gland, testes and adrenal glands under the action of enzyme, ***5α-reductase,*** which reduces the Δ4,5 double-bond in the A-ring of testosterone.



Testosterone *5-Dihvdrotestosterone*

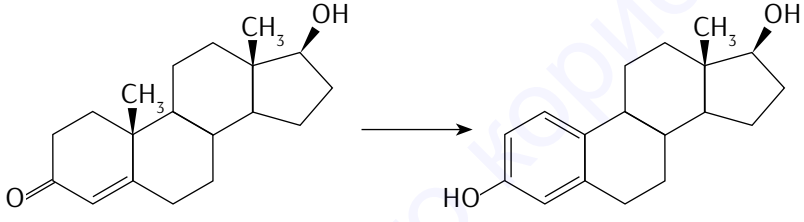
Because of its increased affinity to the androgen receptor DHT is about 30 times more potent than testosterone. It is reputed that **dihydrotestos-**

terone exactly is the genuine androgen that defines sexual characteristics generally attributed to males.

Estradiol

Estradiol, the C-18 steroid, is the most important representative of the estrogens. Despite the fact that estrogens can be found both in men and women, they are present at significantly higher levels in women of reproductive age. Like other estrogens, estradiol is synthesized by developing follicles in the *ovaries*, the *corpus luteum* and the *placenta*.

Estradiol, like other sex steroids, is derived from cholesterol, but the immediate estradiol precursor is testosterone, which molecule undergoes aromatization in the A-ring of steroid. The enzymatic conversion of testosterone to estradiol is presented:



Testosterone *Estradiol*

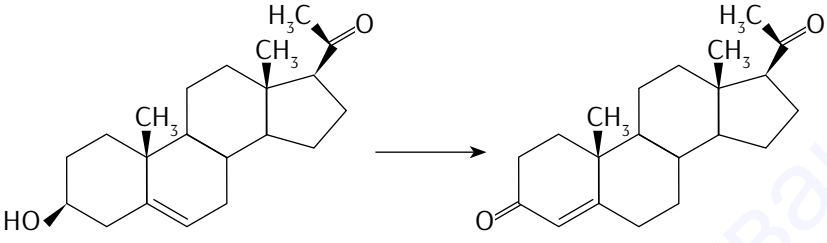
Estrogens, and estradiol primarily, together with progesterone, are the principal regulators of the menstrual cycle, involved in the proliferation of the endometrium. Besides, they control the development of distinctive female *secondary sex characteristics*, such as breasts, fat distribution, mental characteristics etc. The production of estrogens in ovulating women is regulated by the *follicle stimulating hormone* (FSH) and *luteinizing hormone* (LH) of the anterior pituitary.

Progesterone

Progesterone is a C-21 steroid hormone involved in the control of female menstrual cycle and the development of pregnancy. Progesterone belongs to a class of steroid hormones called *progestagens*, but it is the only naturally occurring human progestagen.

Progesterone is synthesized in the ovaria, specifically after ovulation in the *corpus luteum* and in the adrenal glands. The immediate precursor of

progesterone is **pregnenolone,** a derivative of cholesterol. There are two steps in the conversion: firstly, the 3-hydroxyl group is converted to a keto group and then the double bond is moved to C-4, from C-5.



Pregnenolone *Progesterone*

The function of progesterone in the menstrual cycle is to convert the *endometrium* to its secretory stage, that prepares the uterus for implantation.

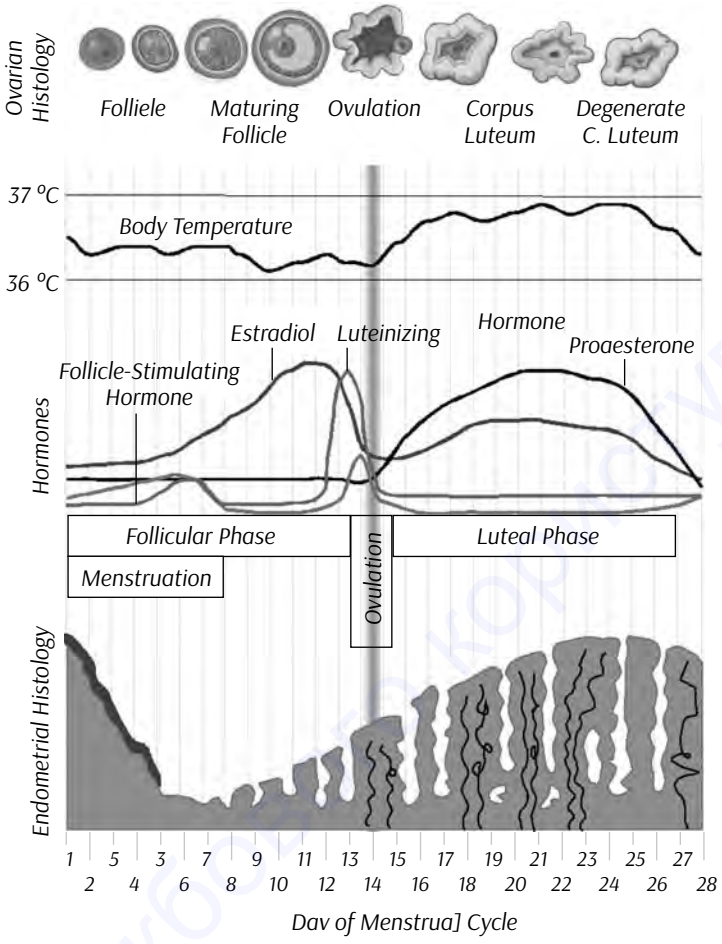


Figure 22.3. Presentation of normal menstrual cycle

If the pregnancy does not set in, progesterone levels sharply decreases and that leads to the normal menstrual bleeding (Figure 22.3). In case, if the pregnancy sets in, increasing amounts of progesterone are produced in the *placenta*.

Synthetic sex hormones. Widespread in medical practice and regulation of human reproductive function are synthetic sex steroids. For example, synthetic androgens (*Methandrostenolone, Nandrolone* etc.) are used as *anaboloc steroids*. They markedly stimulate protein synthesis from amino acids, and by this promote growth of muscle tissue, bone size and in-

crease of bodily strength. Synthetic estrogens and progestins are used as oral contraceptive pills.

22.4. Hormonal regulation of calcium homeostasis

Calcium ions (Ca^{2+}) play a crucial role in the regulation of a very great amount of physiological functions and biochemical reactions. It is said, that **"It would be very difficult to name a physiologic process that does not depend, in one way or another, on calcium".**

That is why, the control of calcium homeostasis, and specifically the maintenance of the normal blood calcium concentration, that is preventing both hypercalciemia and hypocalciemia, is vitally important cause which is realized by the concerted action of endocrine control systems.

There are three major pools of calcium in the body:

- ***Intracellular calcium*,** which is sequestered in mitochondria and endo-plasmic reticulum. The cytosolic calcium level in the unexcited cell is typically near 100 nM, much lesser than extracellular concentration of the ion. The fluctuations of calcium intracellular concentration are critical for its role in intracellular signaling, enzyme activation and muscle contraction.
- ***Calcium in blood and extracellular fluids*.** The concentration of Ca^{2+} inside these compartments is normally approximately 1 mM, or 10.000 times over the basal concentration of free calcium within cells. It is critical to maintain blood calcium concentrations within a tight normal range, because the deviations above or below the normal range lead to serious diseases.
- ***Bone calcium*.** A vast majority of body calcium is in bone, where it is closely associated with phosphates. Within bone, 99 % of the calcium is tied up in the mineral phase, but the remaining 1 % is in a pool that can rapidly exchange with extracellular calcium.

Fluxes of calcium. The maintenance of constant concentrations of blood and extracellular Ca^{2+} requires the controlled fluxes of calcium between blood and other body compartments. Three organs participate in supplying calcium to blood and removing it from blood when necessary, and

these are (Figure 22.4):

- small intestine, where dietary calcium is absorbed;
- bones of skeleton, which serve the main reservoir of calcium and phosphates;
- kidneys, where reabsorption of calcium, that enters glomerular filtrate, occurs.

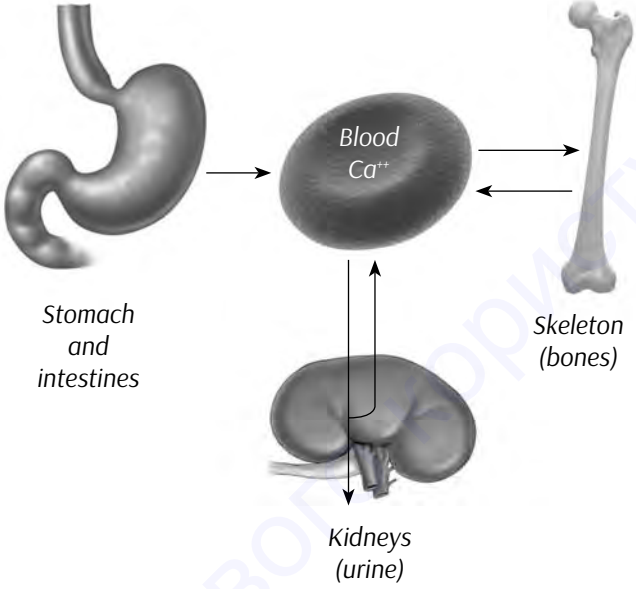
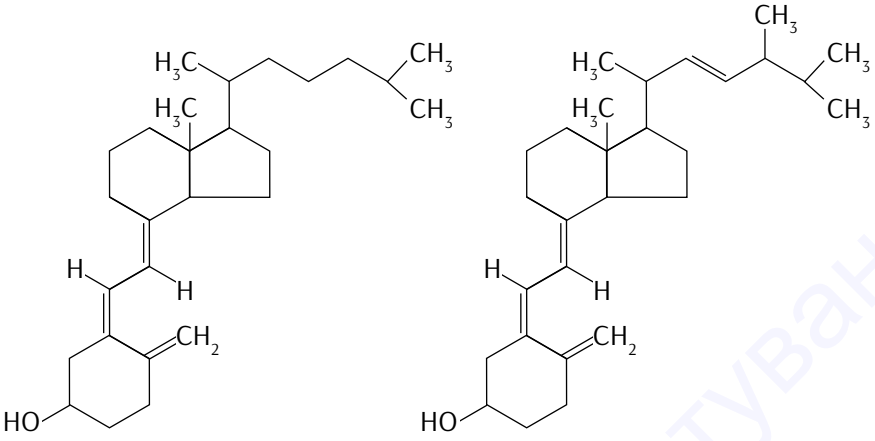


Figure 22.4. Fluxes of calcium between blood, bones, intestines and kidneys

Three hormones control calcium homeostasis via affecting ion fluxes between major compartments of calcium deposition. These are: **calcitriol** (vitamin D derivative), **parathyroid hormone,** and **calcitonin.**

Vitamin D and Calcitriol

The term "**vitamin D"** refers to a group of steroid molecules, mainly **Vitamin D_3** and **Vitamin D_2,** that have long been known for their important role in the mineralization of bones and regulation of calcium and phosphorus body levels.



Vitamin D_3 *Vitamin D_2*

Vitamin D_3, also known as ***cholecalciferol (***or ***calciol),*** is not a genuine vitamin**. It is generated in the skin of animals from a precursor molecule 7-dehydrocholesterol under the conditions of light (UV-rays) absorption.** The next compound of the D-family, that is **Vitamin D_2** or ***ergocalciferol,*** is the substance of plant origin, and is provided to human body as a component of plant food. Summarily, adequate exposure to sunlight and consumption of foodstuffs supplemented with vitamin D_2 and D_3 (egg yolk, fish and plant oils) suffice to prevent vitamin D deficiencies.

Vitamin D by itself does not have significant biological activity**.** To yield active hormone **calcitriol (1,25-dihydroxycholecalciferol; 1,25[OH]$_2$D$_3$)** the metabolic activation of Vitamin D_3 is required. This is realized via two-steps successive hydroxylation of calciol in the liver and kidneys – Figure 22.5:

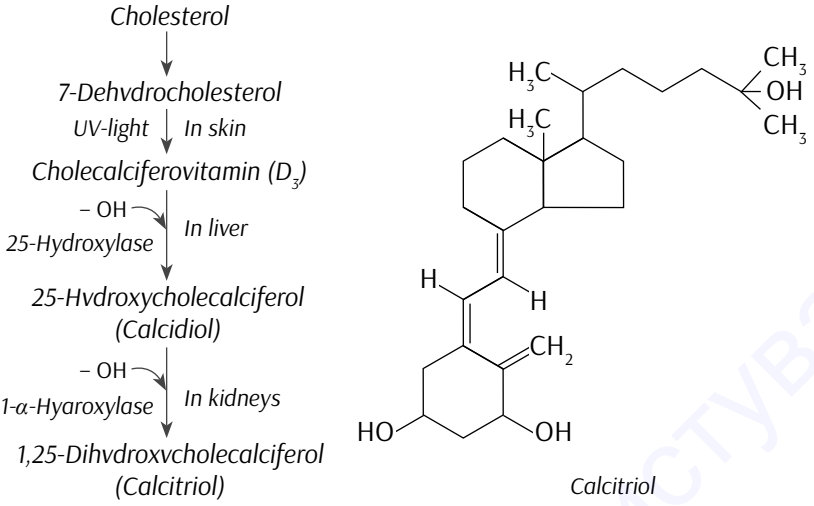


Figure 22.5. Scheme of calcitriol synthesis from vitamin D_3

The main target cells for calcitriol are enterocytes. Acting through nuclear receptors, calcitriol stimulates the biosynthesis inside enterocytes of an intestinal Ca^{2+}-binding protein which is responsible for the uptake of dietary Ca^{2+}.

Inadequate dietary vitamin D provision or defects in the biosynthesis of calcitriol by the photochemical reaction in the skin, leads to the vitamin D deficiency. This is manifested by **rickets** in children and **osteomalacia** in adults. Under these pathologies, the serious disturbances in bones mineralization take place, and the skeletal bones become weak and malformed.

In the control of Ca^{2+} homeostasis, calcitriol functions in concert with parathyroid hormone. These two hormones regulate calcium concentration in blood and the balance between Ca^{2+} deposition in bones and Ca^{2+} mobilization.

Parathyroid hormone

This is a peptide hormone containing 84 amino acid residues. Parathyroid hormone is secreted from cells of the parathyroid glands and encounters its target cells in bone and kidneys. It is the principal endocrine regulator of calcium and phosphates concentration in blood plasma and extracellular fluids.

To say it briefly, **parathyroid hormone counteracts the decrease of calcium ion concentration below normal range.** The physiological effect of hormone is accomplished through the following biochemical cellular mechanisms:

- parathyroid hormone stimulates *osteoclasts* to reabsorb bone mineral, which liberates calcium ions into blood;
- parathyroid hormone enhances calcium absorption from the small intestine, and that clearly serves to elevate blood level of calcium. Parathyroid hormone activates this process indirectly by stimulating production of the active form of **vitamin D** in the kidney. As was discussed above, vitamin D induces synthesis of a calcium-binding protein in intestinal epithelial cells that facilitates efficient absorption of calcium into blood.
- **parathyreoid hormone suppresses calcium loss in urine.** This effect is me-diated by the hormone stimulating of kidney tubular reabsorption of calcium.

Disease States. Both increased and decreased secretion of parathyroid hormone result in abnormalities of calcium homeostasis manifesting themselves as serious diseases of humans. For example, different clinical forms of **hyperpara-thyreoidism** are displayed by elevations of blood calcium concentration ***(hyper-calciemia),*** development of kidney stones and decalcification of skeleton leading to pathologic fractures of bones.

Calcitonin is a 32 amino acid peptide, containing a single disulfide bond. The major source of calcitonin is from the *parafollicular* or *C cells* in the thyroid gland, but it is also synthesized in other tissues, including the lung and intestinal tract.

The major effect of calcitonin as to the calcium homeostasis is its ability to decrease blood calcium levels. This results from the action of hormone concerning the following target organs:

- **bones:** calcitonin suppresses resorption of bone by inhibiting the activity of *osteoclasts*, a cell type that break down bone matrix, releasing calcium and phosphorus into blood.
- **kidneys:** Calcium and phosphorus are prevented from being lost in urine by reabsorption in the kidney tubules. Calcitonin inhibits tubular reabsorption of these two ions, leading to increased rates of their loss in urine.

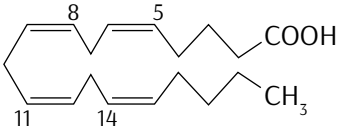
22.5. Eicosanoids. Biomedical and pharmacological aspects

Eicosanoids are a large group of biomolecules derived from polyunsaturated fatty acids, predominantly ***arachidonic*** and other ***eicosanoic acids*** **($C_{20:4}$).** The principal groups of substances of this class are **prostaglandins (PGs), prostacyclins (PCs), thromboxanes (TXs), leukotrienes (LTs)** and **lipoxins (LXs).** The prostaglandins and prostacyclins are collectively identified as **prostanoids,** which are derivatives of ***prostanoic acid.***

According to their physiological and biochemical properties, eicosanoids constitute a distinct class of signaling molecules, which have hormone-like effects as to the nearby cells of their immediate surroundings. This is called **paracrine effect.** Moreover, in some cases, the target cell and the cell synthesizing the eicosanoids are one and the same **(autocrine effect).** As distinct from the genuine hormones, eicosanoids are produced by many different types of cells, rather than being formed in specialized endocrine glands.

Practically all mammalian cells except erythrocytes synthesize eicosanoids. These molecules are extremely potent, they are able to cause profound physiological effects at very dilute concentrations. These hormones are rapidly inactivated by being metabolized, and are typically active for only a few seconds. All eicosanoids function locally at the site of synthesis, through receptor-mediated G-protein linked signaling pathways leading to an increase in cAMP levels.

Biosynthesis and biochemical characteristics. The 20-carbon polyunsaturated fatty acid **arachidonate (arachidonic acid; $C_{20:4}$)** is the abundant precursor for the most of eicosanoids. **Arachidonic acid (5,8,11,14-eicosatetraenoic acid)** is a polyunsaturated fatty acids with twenty carbons and four ***cis***- double bonds, the first at the omega-6 position (20 : 4n-6). The double bonds are the source of molecule flexibility and give it the capacity to react with an oxygen molecule.



Arachidomc acid

In animals, arachidonic acid is synthesized from essential polyunsaturated fatty acids and becomes incorporated into phospholipids of the plasma membranes. Thus, stores of arachadonic acid are present in membrane lipids and released through the action of various ***phospholipases.***

Unlike the hormones described above, eicosanoids are not synthesized in advance and stored. These substances are produced, when needed, from arachidonate that is constituent of the membrane phospholipids. To initiate the biosynthesis of every class eicosanoid, the membrane-bound ***phospholipase A_2*** is required, which releases free arachidonate from phospholipid molecules. The general scheme of different classes eicosanoids biosynthesis is presented in the Figure 22.6.

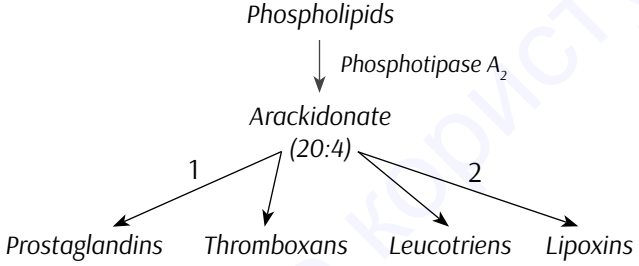
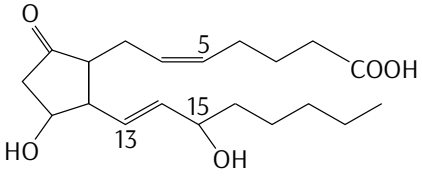


Figure 22.6. The simplified metabolic chart of eicosanoids biosynthesis

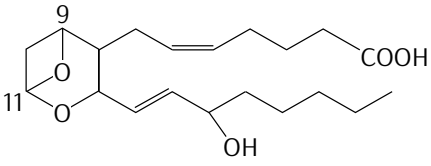
The specific array of eicosanoids synthesized in a cell are dictated by the set of processing enzymes expressed by the certain cell.

The arachidonate itself is a signal substance. However, the products of its metabolism, that is the **prostaglandins, prostacyclins, leukotrienes** and **thromboxanes** are of much greater physiological importance. There are two different pathways for the eicosanoids production. One is the ***cyclic pathway*** initiated by ***prostaglandin synthase***, and the other – the ***linear pathway*** launched by ***lipoxygenase.***

1) **Prostaglandin synthase pathway.** ***Prostaglandin synthase*** a heme protein that catalyzes both a cyclooxygenase and an endoperoxidase reactions. As the results of these reactions, endoperoxide intermediates appear, and the ring closure occurs. Subsequent steps lead to the generation of various prostanoids, that is PGs, PCs and TXs. The structural formulas of a few selected compounds are shown:



PGE_2

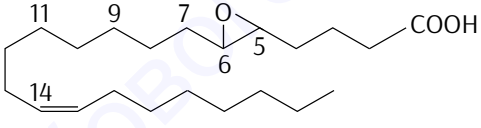


TXA_2

Prostaglandins promote the contraction of smooth muscle, including that of the intestine and uterus (and can therefore be used medically to induce labor). They also mediate pain and inflammation in all tissues. **Thromboxanes** are known for regulating platelet function and therefore blood clotting.

Many anti-inflammatory, drugs act by inhibiting steps in the prostaglandin synthetic pathway. The remarkable example is the ***acetylsalicylate***, which in the form of pharmaceutical preparation ***Aspirin*** is one of the most widespread antipyretic and antirheumatic medicines.

2) ***Lipoxygenase pathway.*** The reactions catalyzed by ***lipoxygenases*** produce **hydroperoxy** and **hydroxyl- fatty acids**. These intermediates undergo numerous dehydration and transfer reactions to produce physiologically active **leukotriens** and **lipoxins.**



LTA_4

Leucotriens and less studied lipoxins are a family of conjugated trienes, which are derivatives of eicosanoic acids produced in leucocytes, macrophages, platelets etc. in response to different damaging stimuli, including immunologically significant factors.

Chapter 23.

BIOCHEMISTRY OF NUTRITION-I. VITAMINS AS ESSENTIAL COMPONENTS OF HUMAN DIET. WATER-SOLUBLE VITAMINS

23.1. Biochemistry of nutrition. Components of human diet

Nutrients: definitions

To function, the human body has to be supplied by a balanced diet. The components of human diet are called **nutrients.**

The nutrients known to be essential for human beings are **proteins, carbohydrates, fats** and **oils, vitamins, minerals** and **water.** The absolute and relative amounts of these substances may vary greatly depending on the diet. The majority of nutrients listed are necessary for healthy life maintenance and must therefore be provided in the diet on a regular basis.

The principal essential nutrients daily requirements, recommended by WHO (World Health Organization), are presented in Table 23.1. The special daily requirements for vitamins would be studied below.

Table 23.1. Daily requirements of essential nutrients (g) for adult healthy men (after J. Koolman, K. -H. Rom, 1996)

Nutrient	Quantity in a body, average (kg)	a	b
Proteins (g)	10	55	92

Carbohydrates (g)	1	390	240–310
Fats (g)	10–15	80	130
Water (ml)	35–40	1500–2500	

a: Recommended daily intake

b: Actual daily intake in industrialized nations

Proteins as nutrients. Proteins are the molecular form in which the animal body obtains amino acids needed for the synthesis of its own proteins and peptides. Excess amino acids are degraded to provide energy. Glucogenic amino acids can be used for the synthesis of carbohydrates, and the ketogenic amino acids for the production of ketone bodies.

The minimum daily protein requirement for a man is 37 g, and for a woman 29 g. However, the recommended amount is twice these values. The minimum requirement for pregnant and lactating women are still higher.

Not only the quantity, but also the quality, of the protein is vitally important. Proteins lacking or containing only small quantities of some of the essential amino acids are said to be of *low biological value*. For human beings, these are plant derived proteins. On contrary, animal proteins are considered to be high value nutrients.

Carbohydrates as nutrients. Carbohydtares serve as a general energy source for humans. In the diet, they are present mainly as ***polysaccharides*** found in plant food (*starch*), ***disaccharides*** in milk (*lactose*) and in all foods sweetened with table sugar (*sucrose*) and as ***monosaccrarides*** (*glucose*, *fructose*) in fruits and honey.

Fats as nutrients. Animal fats and plant oils are the most energy-yielding chemical fuels in the human diet. Their energy content (9,3 kcal/g) is about twice as high as that of proteins and carbohydrates (about 4,1 kcal/g).

In addition, fats and oils are essential as carriers for lipid-soluble vitamins, and as sources of the polyunsaturated fatty acids required for the synthesis of eicosanoids (plant oils, especially).

Minerals constitute a very heterogenous group of essential elements. They can be divided into ***macroelements*** and ***microelements***, which are found in the body in the trace quantities (*trace elements*).

The daily requirement of **macroelements** is > 100 mg. They include sodium (Na), potassium (K), calcium (Ca), magnesium (Mg), chlorine (Cl), phosphorus (P) and sulfur (S). The essential microelements, which daily require-

ment is < 100 mg, include iron (Fe), zinc (Zn), manganese (Mn), copper (Cu), cobalt (Co), selenium (Se), iodine (I) and fluoride (F).

23.2. Vitamins: basic definitions, classes of vitamins

Vitamins are organic molecules that are necessary for normal metabolism in animals, but either are not synthesized in the body or are synthesized in inadequate quantities.

Consequently, vitamins need to be constantly obtained from the diet. Most vitamins function as coenzymes or cofactors of diverse biochemical reactions. Deficiency states are recognized for all vitamins, and in many cases, excessive intake of vitamins also leads to disease.

The vitamins are of two distinct types:

1) **Water-soluble vitamins**

The class includes:

Vitamin B_1 (Thiamine);
Vitamin B_2 (Riboflavin);
Vitamin PP *(Niacin);*
Vitamin B_6 *(Pyridoxine);*
Pantothenic Acid *(Vitamin B_5);*
Biotin *(vitamin H);*
Cobalamin *(Vitamin B_{12});*
Folic Acid *(Vitamin B_c);*
Vitamin C *(L-Ascorbic acid);*
Bioflavonoids *(vitamin P).*

Water-soluble vitamins and their derivatives mainly serve mainly as coenzymes of manifold enzymatic systems, that catalyze key reactions of metabolism. Specific examples of these will be considered below.

2) **Lipid-soluble vitamins**

The class includes:

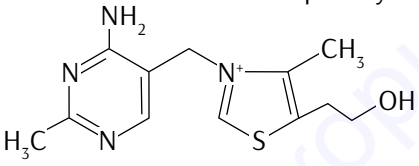
Vitamin A (Retinol);
Vitamin D (Calciferol);
Vitamin E *(α-Tocoferol);*
Vitamin K;

Lipid-soluble vitamins play diverse roles as cofactors of certain biochemical processes and signaling molecules. Some of them, and vitamin E firstly, function as essential components of biomembranes and antioxydants.

23.3. Water-soluble vitamins. Vitamins as coenzymes: structure, biochemical properties

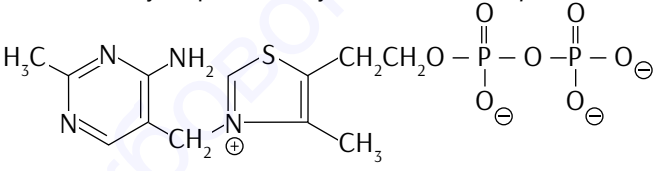
Vitamin B_1 (Thiamine)

Thiamine is also known as **vitamin B_1.** Thiamine is derived from a substituted pyrimidine and a thiazole which are coupled by a methylene bridge.



Thiamine

Thiamine is rapidly converted to its active form, *thiamine diphosphate,* in the brain and liver by a specific enzymes, *thiamine diphosphotransferase.*



Thiamine diphosphate

***Major sources*:** Present in meats, leafy green vegetables, grains and legumes.

Biochemical properties

TPP is necessary as a coenzyme for several enzymes involved in oxidadive decarboxylation reactions, namely the ***pyruvate*** and ***α-ketoglutarate dehyd-rogenase*** catalyzed reactions as well as the ***transketolase*** catalyzed reactions of the pentose phosphate pathway. Thus, thiamine is an important factor in the glucose oxidation metabolic pathways.

A deficiency in thiamin intake leads to a severely reduced capacity of

cells to generate energy as a result of its role in these reactions.

Dietary requirement

The dietary requirement for thiamine is proportional to the caloric intake of the diet and ranges from 1.0–1.5 mg/day for normal adults. If the carbohydrate content of the diet is excessive then an in thiamin intake will be required.

Clinical Significances of Thiamine Deficiency

The earliest symptoms of thiamin deficiency include constipation, appetite suppression, nausea as well as mental depression, peripheral neuropathy and fatigue. Chronic thiamin deficiency leads to more severe neurological symptoms including ataxia, mental confusion and loss of eye coordination. Other clinical symptoms of prolonged thiamin deficiency are related to cardiovascular and musculature defects.

The severe thiamin deficiency disease known as **Beriberi,** is the result of a diet that is carbohydrate rich and thiamin deficient. Beriberi is a vitamin deficiency disease caused by inadequate bodily stores of thiamine (vitamin B_1). It can damage the heart and nervous system.

Thus, there are two major manifestations of thiamine deficiency: cardiovascular disease (wet beriberi) and nervous system disease ("dry beriberi" and ***Wernicke-Korsakoff syndrome***). Both types are most often caused by excessive alcohol consumption.

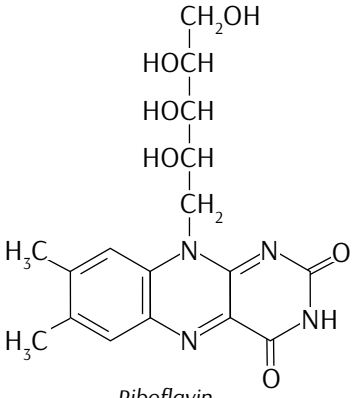
Symptoms of ***dry beriberi*** include pain, tingling, or loss of sensation in hands and feet (peripheral neuropathy), muscle wasting with loss of function or paralysis of the lower extremities, and potential brain damage and death.

Wet beriberi is characterized by swelling, increased heart rate, lung congestion, and enlarged heart related to congestive heart failure.

An additional thiamin deficiency related disease is known as **Wernicke-Korsakoff syndrome.** This disease is most commonly found in chronic alcoholics due to their poor dietetic lifestyles.

Vitamin B_2 (Riboflavin)

Riboflavin is also known as **vitamin B_2.** Chemically, it is the derivative of heterocyclic compound isoaloxazine.



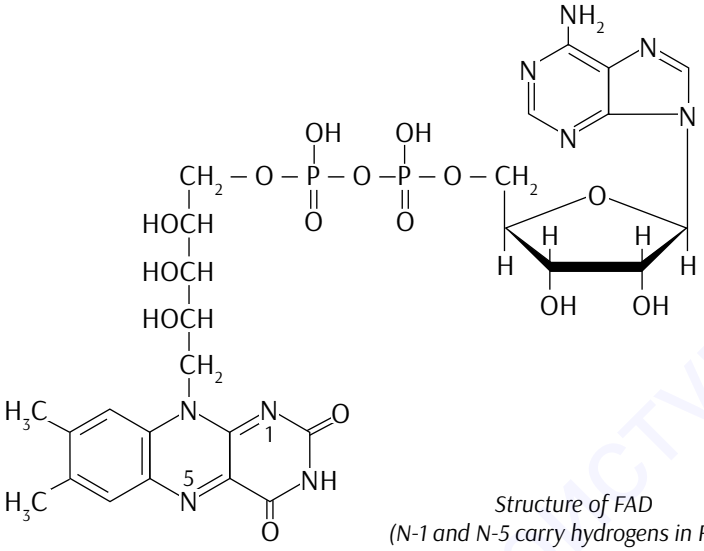
Riboflavin

***Major sources*:** Riboflavin is present in wide variety of foods, including milk, meats and grains.

Biochemical properties

Riboflavin is the precursor for the coenzymes, flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD). The enzymes that require FMN or FAD as cofactors are termed flavoproteins, which serve as hydrogen carriers in a number of important oxidation-reduction (respiration) reactions within mitochondria.

Several flavoproteins also contain metal ions and are termed metalloflavoproteins. Both classes of enzymes are involved in a wide range of redox reactions, e.g. succinate dehydrogenase and xanthine oxidase. During the course of the enzymatic reactions involving the flavoproteins the reduced forms of FMN and FAD are formed, $FMNH_2$ and $FADH_2$, respectively.



Structure of FAD
(N-1 and N-5 carry hydrogens in $FADH_2$

Dietary requirement

The normal daily requirement for riboflavin is 1.2–1.7 mg/day for normal adults.

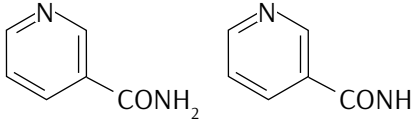
Clinical Significances of Flavin Deficiency

Riboflavin deficiencies are rare in the United States due to the presence of adequate amounts of the vitamin in eggs, milk, meat and cereals. Riboflavin deficiency is often seen in chronic alcoholics due to their poor dietetic habits.

Symptoms associated with riboflavin deficiency include, glossitis, seborrhea, angular stomatitis, cheilosis and photophobia. Riboflavin decomposes when exposed to visible light. This characteristic can lead to riboflavin deficiencies in newborns treated for hyperbilirubinemia by phototherapy.

Vitamin PP (Niacin)

Chemically, vitamin PP is nicotinamide and/or nicotinic acid.



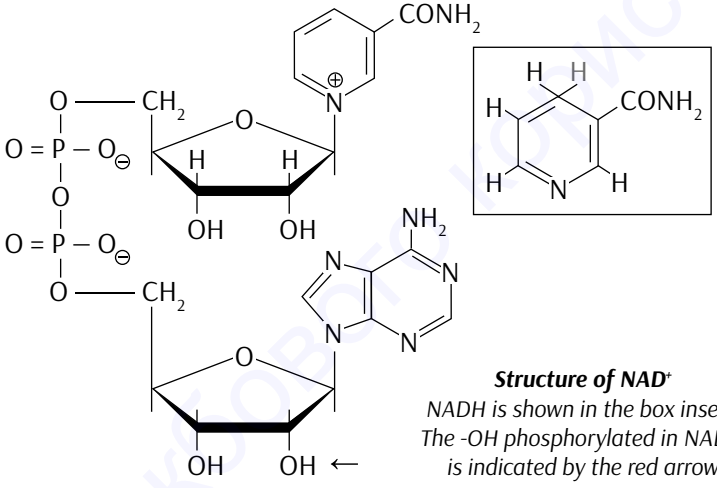
Nicotinamide Nicotinic Acid

Niacin (nicotinic acid and nicotinamide) is also known as vitamin B_3. Both nicotinic acid and nicotinamide can serve as the dietary source of vitamin B_3.

Major sources: Present in meats, leafy green vegatables, potatoes and peanuts. Can be synthesized in small amounts within the body from tryptophan.

Biochemical properties

Precursor to the coenzymes *nicotinamide adenine dinucleotide (NAD) and nicotinamide adenine dinucleotide phosphate (NADPH),* which serve as coenzymes for numerous dehydrogenase, that is hydrogen carriers in such important processes as glycolysis, Kreb's cycle and oxidative phosphorylation.



Structure of NAD⁺
NADH is shown in the box insert. The -OH phosphorylated in NADP⁺ is indicated by the red arrow

Daily requirement.

Niacin is not a true vitamin in the strictest definition since it can be derived from the amino acid tryptophan. However, the ability to utilize tryptophan for niacin synthesis is inefficient (60 mg of tryptophan are required to synthesize 1 mg of niacin). Also, synthesis of niacin from tryptophan requires vitamins B_1, B_2 and B_6 which would be limiting in themselves on a marginal diet.

The recommended daily requirement for niacin is 13–19 niacin equivalents (NE) per day for a normal adult. One NE is equivalent to 1 mg of free niacin).

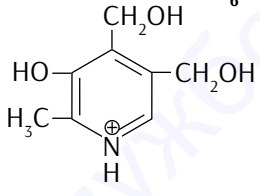
Deficiency. Clinical Significances of Niacin and Nicotinic Acid

A diet deficient in niacin (as well as tryptophan) leads to glossitis of the tongue, dermatitis, weight loss, diarrhea, depression and dementia. The severe symptoms, depression, dermatitis and diarrhea, are associated with the condition known as ***pellagra.*** Several physiological conditions (e.g. *Hartnup disease*) as well as certain drug therapies (e.g. isoniazid) can lead to niacin deficiency. In Hartnup disease tryptophan absorption is impaired and in malignant carcinoid syndrome tryptophan metabolism is altered resulting in excess serotonin synthesis. Isoniazid (the hydrazide derivative of isonicotinic acid) is the primary drug for chemotherapy of tuberculosis.

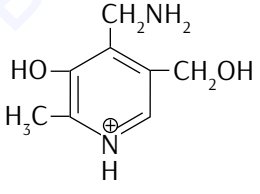
Nicotinic acid (but not nicotinamide) when administered in pharmacological doses of 2–4 g/day lowers plasma cholesterol levels and has been shown to be a useful therapeutic for hypercholesterolemia. The major action of nicotinic acid in this capacity is a reduction in fatty acid mobilization from adipose tissue. Although nicotinic acid therapy lowers blood cholesterol it also causes a depletion of glycogen stores and fat reserves in skeletal and cardiac muscle. Additionally, there is an elevation in blood glucose and uric acid production. For these reasons nicotinic acid therapy is not recommended for diabetics or persons who suffer from gout.

Vitamin B_6 (Pyridoxine)

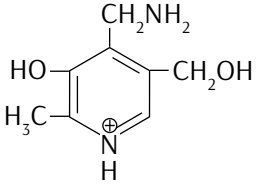
Pyridoxal, pyridoxamine and **pyridoxine (pyridoxol)** are collectively known as **vitamin B_6.**



Pyridoxine



Pyridoxal



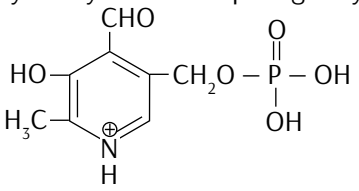
Pyridoxamine

Major sources: *P*yridoxine is present in meat from mammals, fish and poultry. Also present in a number of vegetables, including potatoes and tomatoes.

Biochemical properties

Pyridoxin, as well as **pyridoxal** and **pyridoxamine** are efficiently converted to the biologically active form of vitamin B_6, ***pyridoxal phosphate***.

This conversion is catalyzed by the ATP requiring enzyme, pyridoxal kinase.



Pyridoxal phosphate

In its turn, pyridoxal phosphate is a coenzyme for several important reactions involving protein metabolism, specifically the transamination and decarboxylation reactions. Thus, pyridoxal phosphate is necessary for synthesis and catabolism of amino acids.

Besides, pyridoxal phosphate serves as a cofactor for glycogen phosphor-rylase, thus taking part in glycogenolysis.

Dietary requirement

The requirement for vitamin B_6 in the diet is proportional to the level of protein consumption ranging from 1.4–2.0 mg/day for a normal adult. During pregnancy and lactation the requirement for vitamin B_6 increases approximately 0.6 mg/day.

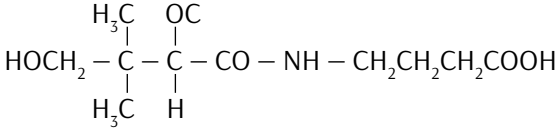
Deficiency

Deficiencies of vitamin B_6 are rare and usually are related to an overall deficiency of all the B-complex vitamins. Isoniazid (see niacin deficiencies above) and penicillamine (used to treat rheumatoid arthritis and cystinurias) are two drugs that complex with pyridoxal and pyridoxal phosphate resulting in a deficiency in this vitamin.

Pantothenic Acid

Pantothenic acid is also known as vitamin B_5.

Pantothenic acid is formed from β-alanine and pantoic acid (see chemical structure).



Pantothenic *Acid*

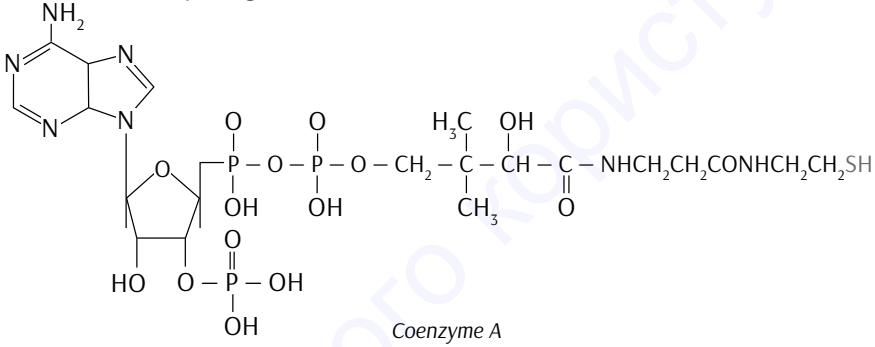
Major sources: Present in a broad variety of foods, including grain, legumes, egg yolk, and meat. Also synthesized by intestinal bacteria.

Biochemical properties

Pantothenate is a precursor required for synthesis of coenzyme A (CoA), which is the cofactor critical to many oxidations and/or syntheses of carbohydrates and fatty acids.

Besides, CoA is a component of the acyl carrier protein (ACP) domain of fatty acid synthase.

Pantothenate is, therefore, required for the metabolism of carbohydrate via the TCA cycle and all fats and proteins. At least 70 enzymes have been identified as requiring CoA or ACP derivatives for their function.



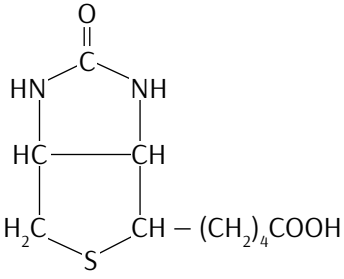
Coenzyme A

Deficiency

Deficiency of pantothenic acid is extremely rare due to its widespread distribution in whole grain cereals, legumes and meat. Symptoms of pantothenate deficiency are difficult to assess since they are subtle and resemble those of other B vitamin deficiencies.

Biotin (vitamin H)

Biotin is also known as vitamin H.



Biotin

Major sources: Biotin is found in egg yolk, legumes, nuts and liver. Also synthesized by intestinal bacteria.

Biochemical properties

Biotin functions as a coenzyme for several enzymes that catalyze carboxylation, decarboxylation and deamination reactions.

The examples are:

- pyruvate carboxylase, an essential enzyme of Kreb's cycle;
- acetyl-CoA carboxylase.

Deficiencies

Biotin is found in numerous foods, and also is synthesized by intestinal bacteria and as such deficiencies of the vitamin are rare.

Deficiencies are generally seen only after long antibiotic therapies which deplete the intestinal fauna or following excessive consumption of raw eggs. The latter is due to the affinity of the egg white protein, avidin, for biotin preventing intestinal absorption of the biotin.

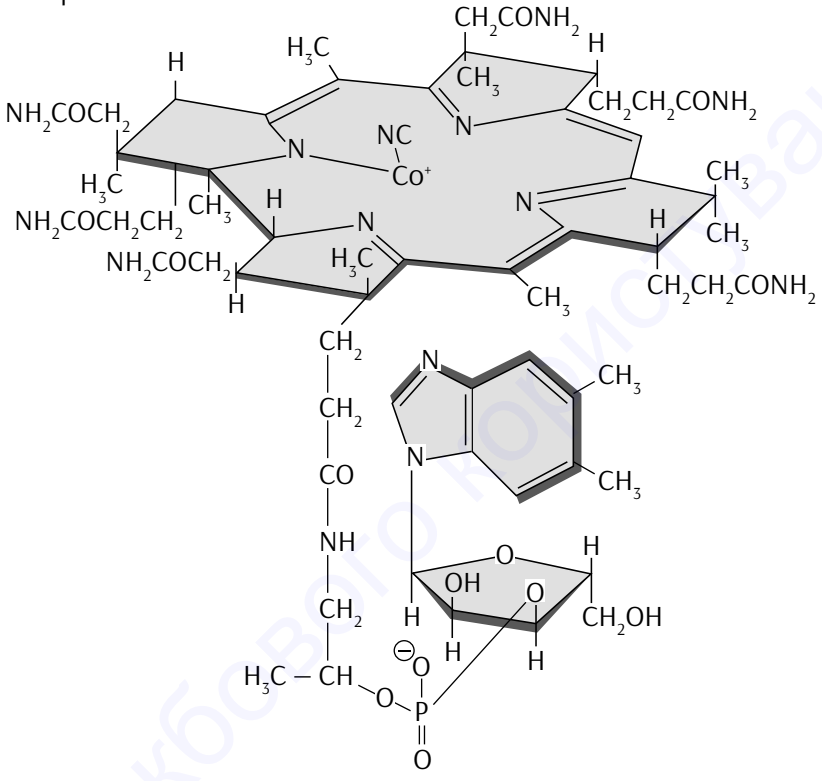
Cobalamin (Vitamin B_{12})

Cobalamin is more commonly known as vitamin B_{12}. Vitamin B_{12} is composed of a complex tetrapyrrol ring structure (corrin ring) and a cobalt ion in the center.

Major sources: Microbial synthesis is the sole source of this vitamin in nature. It is obtained almost exclusively from ingestion of animal products, and is essentially absent from plant products. In animal bodies, it is found found in the liver, bound to protein as **methycobalamin** or **5'-deoxyadenosylcobalamin.**

The vitamin must be hydrolyzed from protein in order to be active. Hy-

drolysis occurs in the stomach by gastric acids or the intestines by trypsin digestion following consumption of animal meat. The vitamin is then bound by intrinsic factor, a protein secreted by parietal cells of the stomach, and carried to the ileum where it is absorbed. Following absorption the vitamin is transported to the liver in the blood bound to **transcobalamin II.**



Cyanocobalamin

Biochemical properties

A cobalt-containing coenzyme is involved in numerous metabolic pathways.

There are two significant reactions in the body that require vitamin B_{12} as a cofactor. During the catabolism of fatty acids with an odd number of carbon atoms and the amino acids valine, isoleucine and threonine the resultant propionyl-CoA is converted to succinyl-CoA for oxidation in the TCA cycle. One of the enzymes in this pathway, methylmalonyl-CoA mutase, re-

quires vitamin B_{12} as a cofactor in the conversion of methylmalonyl-CoA to succinyl-CoA. The 5'-deoxyadenosine derivative of cobalamin is required for this reaction.

The second reaction requiring vitamin B_{12} catalyzes the conversion of homocysteine to methionine and is catalyzed by methionine synthase. This reaction results in the transfer of the methyl group from N^5-methyltetrahydrofolate to hydroxycobalamin generating tetrahydrofolate (THF) and methylcobalamin during the process of the conversion.

Deficiency. Clinical Significances of B_{12} Deficiency

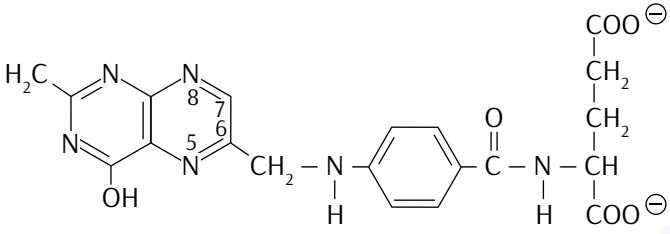
The liver can store up to six years worth of vitamin B_{12}, hence deficiencies in this vitamin are rare. Deficiency usually results from failure to absorb the molecule due to inadequate quantities of intrinsic factor, and is typically manifest as a defect in red blood cell formation (pernicious anemia).

Pernicious anemia is a megaloblastic anemia resulting from vitamin B_{12} deficiency that develops as a result a lack of intrinsic factor in the stomach leading to malabsorption of the vitamin. The anemia results from impaired DNA synthesis due to a block in purine and thymidine biosynthesis. The block in nucleotide biosynthesis is a consequence of the effect of vitamin B_{12} on folate metabolism. When vitamin B_{12} is deficient essentially all of the folate becomes trapped as the N^5-methyl-THF derivative as a result of the loss of functional methionine synthase. This trapping prevents the synthesis of other THF derivatives required for the purine and thymidine nucleotide biosynthesis pathways.

Neurological complications also are associated with vitamin B_{12} deficiency and result from a progressive demyelination of nerve cells. The demyelination is thought to result from the increase in methylmalonyl-CoA that result from vitamin B_{12} deficiency. Methylmalonyl-CoA is a competitive inhibitor of malonyl-CoA in fatty acid biosynthesis as well as being able to substitute for malonyl-CoA in any fatty acid biosynthesis that may occur. Since the myelin sheath is in continual flux the methylmalonyl-CoA-induced inhibition of fatty acid synthesis results in the eventual destruction of the sheath. The incorporation methylmalonyl-CoA into fatty acid biosynthesis results in branched-chain fatty acids being produced that may severely alter the architecture of the normal membrane structure of nerve cells.

Folic acid is a conjugated molecule consisting of a pteridine ring struc-

ture linked to para-aminobenzoic acid **(PABA)** that forms pteroic acid. Folic acid itself is then generated through the conjugation of glutamic acid residues to pteroic acid.



Folic Acid

Positions 7 & 8 carry hydrogens in dihydrofolate (DHF); positions 5–8 carry hydrogens in tetrahydrofolate (THF)

Major sources: Present in many natural foods, including dark-green vegetables (spinich!), beef, eggs, whole grains. Also synthesized by intestinal bacteria.

Folic acid is obtained primarily from yeasts and leafy vegetables as well as animal liver. Animal cannot synthesize PABA nor attach glutamate residues to pteroic acid, thus, requiring folate intake in the diet.

When stored in the liver or ingested folic acid exists in a polyglutamate form. Intestinal mucosal cells remove some of the glutamate residues through the action of the lysosomal enzyme, conjugase. The removal of glutamate residues makes folate less negatively charged (from the polyglutamic acids) and therefore more capable of passing through the basal lamenal membrane of the epithelial cells of the intestine and into the bloodstream.

Biochemical properties. Folic acid serves as a coenzyme in the synthesis of several amino acids, as well as purines and thymine, and therefore DNA. Deficiency is typically manifest as growth failure and anemia.

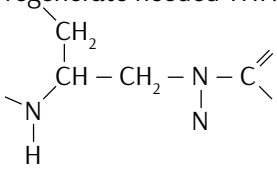
Within cells (principally the liver where it is stored) folic acid is reduced to tetrahydrofolate (THF also H_4folate) through the action of dihydrofolate reductase (DHFR), an NADPH-requiring enzyme.

The function of THF derivatives is to carry and transfer various forms of one carbon units during biosynthetic reactions. The one carbon units are either methyl, methylene, methenyl, formyl or formimino groups.

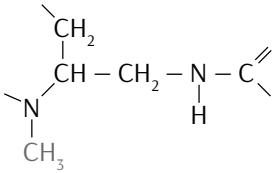
These one carbon transfer reactions are required in the biosynthesis of serine, methionine, glycine, choline and the purine nucleotides and dTMP.

The ability to acquire choline and amino acids from the diet and to

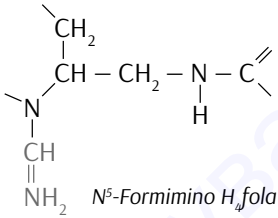
salvage the purine nucleotides makes the role of N^5,N^{10}-methylene-THF in dTMP synthesis the most metabolically significant function for this vitamin. The role of vitamin B_{12} and N^5-methyl-THF in the conversion of homocysteine to methionine also can have a significant impact on the ability of cells to regenerate needed THF.



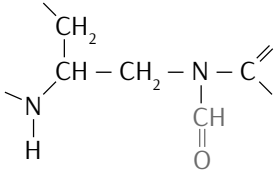
Tetrahydrofolate (H_4folate)



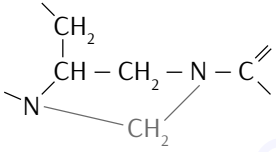
N^5-Methyl H_4folate



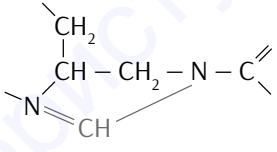
N^5-Formimino H_4folate



N^{10}-Formyl H_4folate



N^5,N^{10}-Methylene H_4folate



N^5,N^{10}-Methenyl H_4folate

Active center of tetrahydrofolate (THF). Note that the N^5 position is the site of attachment of methyl groups, the N^{10} the site for attachment of formyl and formimino groups and that both N^5 and N^{10} bridge the methylene and methenyl groups

Clinical Significance of Folate Deficiency

Folate deficiency results in complications nearly identical to those described for vitamin B_{12} deficiency. The most pronounced effect of folate deficiency on cellular processes is upon DNA synthesis. This is due to an impairment in dTMP synthesis which leads to cell cycle arrest in S-phase of rapidly proliferating cells, in particular hematopoietic cells. The result is ***megaloblastic anemia*** as for vitamin B_{12} deficiency. The inability to synthesize DNA during erythrocyte maturation leads to abnormally large erythrocytes termed ***macrocytic anemia.***

Folate deficiencies are rare due to the adequate presence of folate in food. Poor dietary habits as those of chronic alcoholics can lead to folate deficiency. The predominant causes of folate deficiency in non-alcoholics are impaired absorption or metabolism or an increased demand for the vitamin. The predominant condition requiring an increase in the daily intake of folate is pregnancy. This is due to an increased number of rapidly proliferat-

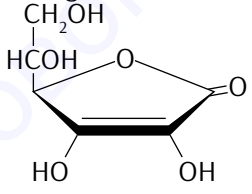
ing cells present in the blood. The need for folate will nearly double by the third trimester of pregnancy. Certain drugs such as anticonvulsants and oral contraceptives can impair the absorption of folate. Anticonvulsants also increase the rate of folate metabolism.

Chapter 24.

BIOCHEMISTRY OF NUTRITION-2. VITAMIN C. LIPID-SOLUBLE VITAMINS AS BIOREGULATORS AND ANTIOXYDANTS

24.1. Vitamin C (Ascorbic acid)

Ascorbic acid is more commonly known as **vitamin C.** Ascorbic acid is derived from glucose via the uronic acid pathway. The enzyme L-gulonolactone oxidase responsible for the conversion of gulonolactone to L-ascorbic acid is absent in primates making ascorbic acid required in the diet.



L-Ascorbic Acid

Major sources: Present in fruits and vegetables. Rich sources include citrus fruits, strawberries, tomatoes and leafy green vegetables. Most animals can synthesize ascorbic acid; those that cannot include primates (including humans), guinea pigs and Mongolian fruit bats.

Biochemical properties

The active form of vitamin C is ascorbate acid itself. The main function of ascorbate is as a reducing agent in a number of different reactions. Vitamin C has the potential to reduce ***cytochromes a*** and ***c*** of the respiratory chain

as well as molecular oxygen.

The most important reaction requiring ascorbate as a cofactor is the hydroxylation of proline residues, which are essential components in collagen structure. Vitamin C is, therefore, required for the maintenance of normal connective tissue and is important for growth of cartilage, bone and teeth,

Thus, vitamin C is required for wound healing since synthesis of connective tissue is the first event in wound tissue remodeling. Vitamin C also is necessary for bone remodeling due to the presence of collagen in the organic matrix of bones.

Several other metabolic reactions require vitamin C as a cofactor. These include the catabolism of tyrosine and the synthesis of epinephrine from tyrosine and the synthesis of the bile acids. It is also believed that vitamin C is involved in the process of steroidogenesis since the adrenal cortex contains high levels of vitamin C which are depleted upon adrenocorticotropic hormone (ACTH) stimulation of the gland.

Dietary requirement

The dietary requirement for vitamin C ranges from 50 to 70 mg/day for normal adults.

Deficiency

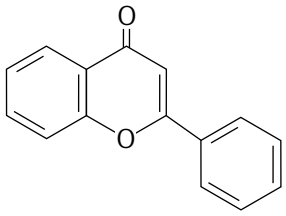
Deficiency in vitamin C leads to the disease **scurvy** due to the role of the vitamin in the post-translational modification of collagens. Scurvy is characterized by easily bruised skin, muscle fatigue, soft swollen gums, decreased wound healing and hemorrhaging, osteoporosis, and anemia. Vitamin C is readily absorbed and so the primary cause of vitamin C deficiency is poor diet and/or an increased requirement. The primary physiological state leading to an increased requirement for vitamin C is severe stress (or trauma). This is due to a rapid depletion in the adrenal stores of the vitamin. The reason for the decrease in adrenal vitamin C levels is unclear but may be due either to redistribution of the vitamin to areas that need it or an overall increased utilization.

Bioflavonoids (Vitamin P)

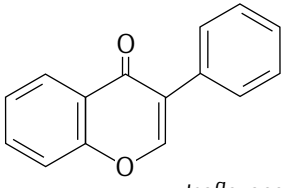
These are a group of essential nutrients that include a number of flavone derivatives and are usually referred to as vitamin P.

Chemically vitamin P comprises a class of plant pigments and metabo-

lites based around a *phenylbenzopyrone*, or *flavone* and *isoflavone* structures.

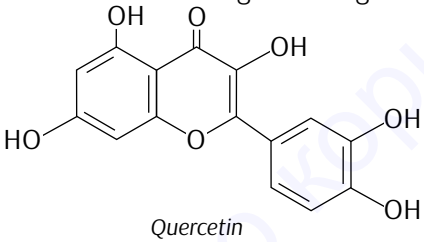


Flavone



Isoflavone

Vitamin P includes a number of components that work together – **quercetin, rutin, citrin, hesperidin, catechines** etc. Flavonoids are also commonly referred to as **bioflavonoids** – these terms are equivalent and interchangable, since all flavonoids are biological in origin.



Quercetin

Biological properties

Bioflavonoids are the water-soluble companions of vitamin C *(L-ascorbic acid)*, usually found in the same foods. Their association with *vitamin C* is the reason that natural forms of *vitamin C* are more effective than are synthetic *L-ascorbic acid* without the bioflavonoids in the equivalent amounts.

Flavonoids are characterized by their potent ***antioxidant activity***. They also protect blood vessels, reduce platelet aggregation (acting as natural blood thinners) and promote circulation. As antioxidants, some bioflavonoids, such as quercetin, protect *LDL-cholesterol* from oxidative damage. Others, such as the anthocyanidins from bilberry, may help protect the lens of the eye from cataracts. Preliminary evidence suggests that some bioflavonoids, such as naringenin, may have anticancer activity.

The main known function of the bioflavonoids, that proves their clinical usage, is to increase the strength of the capillaries and to regulate their permeability. The capillaries link the arteries to the veins. They deliver oxygen and nutrients to the organs, tissues, and cells and then pick up carbon dioxide and waste and carry them through the veins and back to the heart.

By its support of the capillaries, vitamin P helps to prevent hemorrhage and rupture of these tiny vessels, which could lead to easy bruising.

Food sources of bioflavonoids

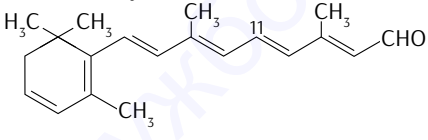
Bioflavonoids are found in the white material just beneath citrus peel, as well as in peppers, grapes, pine bark, onions, garlic, blue and red berries, green tea as well as buckwheat (source of *quercetin*). If a diet contains enough fruit and vegetables, bioflavonoids should not be deficient, but deficiency would show up as bruising.

The main source of bioflavonoids in human diet is the citrus fruits—lemons, grapefruits, oranges, and, to a lesser extent, limes. Rose hips, apricots, cherries, grapes, black currants, plums, blackberries, and papayas are other fruit sources of vitamin P. Green pepper, broccoli, and tomatoes are some good vegetable sources of bioflavonoids. The buckwheat plant, leaf and grain, is a particularly good source of bioflavonoids, especially the rutin component.

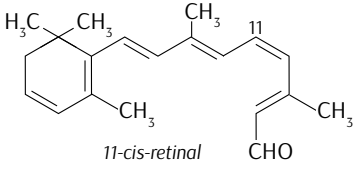
24.2. Vitamin A (retinol): structure, biochemical functions

Vitamin A (retinol)

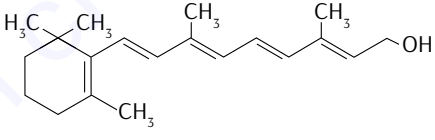
Vitamin A consists of three biologically active molecules, retinol, retinal (retinaldehyde) and retinoic acid.



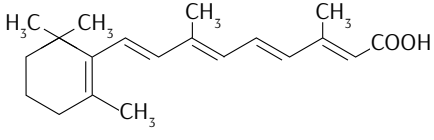
All-trans-retinal



11-cis-retinal



Retinol



Retinoic Acid

Each of these compounds are derived from the plant precursor molecule, **β-carotene** (a member of a family of molecules known as *carotenoids*). Beta-carotene, which consists of two molecules of retinal linked at their alde-

hyde ends, is also referred to as the provitamin form of vitamin A.

Major sources: Present in many animal tissues, especially fish and liver. Carotinoids in green plants serve can be converted to vitamin A following ingestion.

Ingested **β-carotene** is cleaved in the lumen of the intestine by -carotene dioxygenase to yield retinal. Retinal is reduced to retinol by retinaldehyde reductase, an NADPH requiring enzyme within the intestines. Retinol is esterified to palmitic acid and delivered to the blood via chylomicrons. The uptake of chylomicron remnants by the liver results in delivery of retinol to this organ for storage as a lipid ester within lipocytes. Transport of retinol from the liver to extrahepatic tissues occurs by binding of hydrolyzed retinol *to aporetinol binding protein (*RBP). the retinol-RBP complex is then transported to the cell surface within the Golgi and secreted. Within extrahepatic tissues retinol is bound to cellular retinol binding protein (CRBP). Plasma transport of retinoic acid is accomplished by binding to albumin.

Biochemical properties

Vitamin A is necessary for a broad range of bodily function, including production of vision pigments, resistance to infectious agents and maintenance of health in many epithelial cells. Disease results from both deficiency and excess.

Vision and the Role of Vitamin A

Photoreception in the eye is the function of two specialized cell types located in the retina; the rod and cone cells. Both rod and cone cells contain a photoreceptor pigment in their membranes. The photosensitive compound of most mammalian eyes is a protein called ***opsin*** *t*o which is covalently coupled an aldehyde of vitamin A. The opsin of rod cells is called ***scotopsin.***

The photoreceptor of rod cells is specifically called ***rhodopsin.*** This compound is a complex between scotopsin and the 11-*cis*-retinal (also called 11-*cis*-retinene) form of vitamin A. Rhodopsin is a serpentine receptor imbedded in the membrane of the rod cell. Coupling of 11-*cis*-retinal occurs at three of the transmembrane domains of rhodopsin. Intracellularly, rhodopsin is coupled to a specific G-protein called ***transducin.***

When the rhodopsin is exposed to light it is bleached releasing the 11-*cis*-retinal from opsin. Absorption of photons by 11-*cis*-retinal triggers a series of conformational changes on the way to conversion *all-trans-reti-*

nal. One important conformational intermediate is *metarhodopsin II*. The release of opsin results in a conformational change in the photoreceptor. This conformational change activates transducin, leading to an increased GTP-binding by the a-subunit of transducin. Binding of GTP releases the α-subunit from the inhibitory β- and γ-subunits. The GTP-activated α-subunit in turn activates an associated phosphodiesterase; an enzyme that hydrolyzes cyclic-GMP (cGMP) to GMP. Cyclic GMP is required to maintain the Na^+ channels of the rod cell in the open conformation. The drop in cGMP concentration results in complete closure of the Na^+ channels. Metarhodopsin II appears to be responsible for initiating the closure of the channels. The closing of the channels leads to hyperpolarization of the rod cell with concomitant propagation of nerve impulses to the brain.

Gene Control Exerted by Retinol and Retinoic Acid

Within cells both retinol and retinoic acid bind to specific receptor proteins. Following binding, the receptor-vitamin complex interacts with specific sequences in several genes involved in growth and differentiation and affects expression of these genes. In this capacity retinol and retinoic acid are considered hormones of the steroid/thyroid hormone superfamily of proteins. Vitamin D also acts in a similar capacity. Several genes whose patterns of expression are altered by retinoic acid are involved in the earliest processes of embryogenesis including the differentiation of the three germ layers, organogenesis and limb development.

Additional Role of Retinol

Retinol also functions in the synthesis of certain glycoproteins and mucopolysaccharides necessary for mucous production and normal growth regulation. This is accomplished by phosphorylation of retinol to *retinyl phosphate* which then functions similarly to dolichol phosphate.

Clinical Significances of Vitamin A Deficiency

Vitamin A is stored in the liver and deficiency of the vitamin occurs only after prolonged lack of dietary intake. The earliest symptoms of vitamin A deficiency are *night blindness.* Additional early symptoms include follicular hyperkeratinosis, increased susceptibility to infection and cancer and anemia equivalent to iron deficient anemia. Prolonged lack of vitamin A leads to deterioration of the eye tissue through progressive keratinization of the

cornea, a condition known as xerophthalmia.

The increased risk of cancer in vitamin deficiency is thought to be the result of a depletion in ***β-carotene***. Beta-carotene is a very effective antioxidant and is suspected to reduce the risk of cancers known to be initiated by the production of free radicals. Of particular interest is the potential benefit of increased -carotene intake to reduce the risk of lung cancer in smokers. However, caution needs to be taken when increasing the intake of any of the lipid soluble vitamins. Excess accumulation of vitamin A in the liver can lead to toxicity which manifests as bone pain, hepatosplenomegaly, nausea and diarrhea.

24.3. Vitamin D (calciferol) as calcium and phosphorous homeostasis regulator

Vitamin D (calciferol)

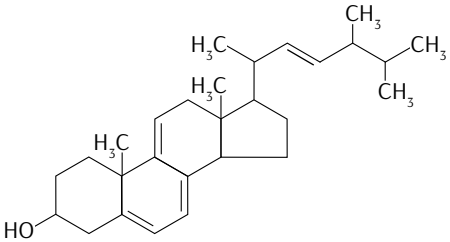
Vitamin D includes **vitamin D_2** ***(ergocalciferol)*** and **vitamin D_3** ***(chole-calciferol)*** which functions to regulate calcium and phosphorous homeostasis.

The biologically active form of vitamin D_3 which is the prevalent form for human body is *1,25-dihydroxy vitamin D_3* ($1,25\text{-}(OH)_2D_3$, also termed *calcitriol)*. Active calcitriol is derived from **ergosterol** (produced in plants) and from **7***-dehydrocholesterol* (produced in the skin). **Ergocalciferol** (vitamin D_2) is formed by uv irradiation of ergosterol. In the skin 7-dehydrocholesterol is converted to *cholecalciferol* (vitamin D_3) following UV irradiation.

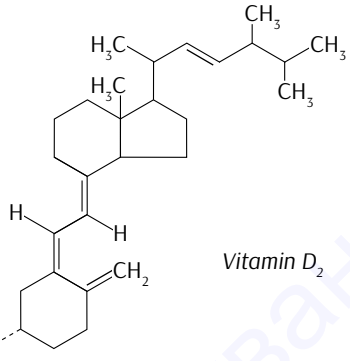
Major sources: Synthesized in the skin when exposed to sunlight (and thus not a true vitamin). Also present at low concentration in some natural foods, and in many artificially-fortified food products.

Daily requirement

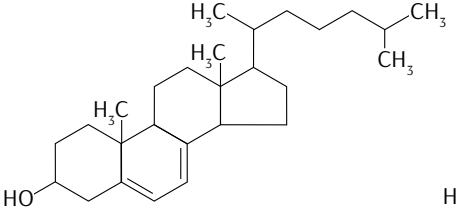
The dietary requirement of vitamin D for adults is about 0,01 mg per day, for children – 0,012–0,025 mg per day.



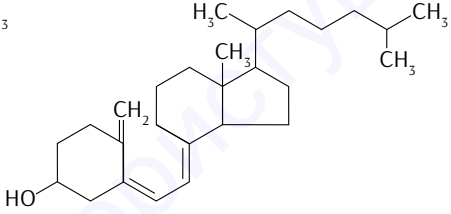
Ergosterol



Vitamin D_2



7-Dehydrocholesterol

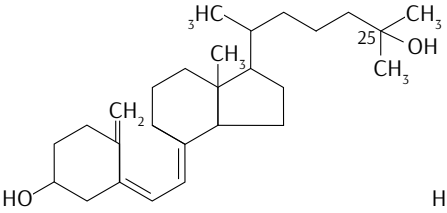


Vitamin D_3

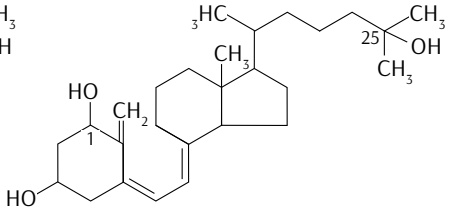
Biochemical properties

The principal biochemical effect of vitamin D is to facilitate absorption of calcium from the intestine, and thereby assist in maintaining calcium homeostasis. Receptors are present in most cells and it likely has many additional effects.

Vitamin D_2 and D_3 are processed to D_2-calcitriol and D_3-calcitriol, respectively, by the same enzymatic pathways in the body. Cholecalciferol (or egrocalciferol) are absorbed from the intestine and transported to the liver bound to a specific *vitamin D-binding protein*. In the liver cholecalciferol is hydroxylated at the 25 position by a specific D_3–25-hydroxylase generating 25-hydroxy-D_3 [25-(OH)D_3] which is the major circulating form of vitamin D. Conversion of 25-(OH)D_3 to its biologically active form, calcitriol, occurs through the activity of a specific D_3–1-hydroxylase present in the proximal convoluted tubules of the kidneys, and in bone and placenta. 25-(OH)D_3 can also be hydroxylated at the 24 position by a specific D_3–24-hydroxylase in the kidneys, intestine, placenta and cartilage.



25-hydroxyvitamin D_3



1,25-dihydroxyvitamin D_3

Calcitriol functions in concert with parathyroid hormone (PTH) and calcitonin to regulate serum calcium and phosphorous levels. PTH is released in response to low serum calcium and induces the production of calcitriol. In contrast, reduced levels of PTH stimulate synthesis of the inactive 24,25-$(OH)_2D_3$. In the intestinal epithelium, calcitriol functions as a steroid hormone in inducing the expression of ***calbindin D_{28K}***, a protein involved in intestinal calcium absorption. The increased absorption of calcium ions requires concomitant absorption of a negatively charged counter ion to maintain electrical neutrality. The predominant counter ion is Pi. When plasma calcium levels fall the major sites of action of calcitriol and PTH are bone where they stimulate bone resorption and the kidneys where they inhibit calcium excretion by stimulating reabsorption by the distal tubules. The role of calcitonin in calcium homeostasis is to decrease elevated serum calcium levels by inhibiting bone resorption.

Clinical significance of vitamin D deficiency

As a result of the addition of vitamin D to milk, deficiencies in this vitamin are rare in this country. The main symptom of vitamin D deficiency in children is ***rickets*** and in adults is ***osteomalacia.*** Rickets is characterized improper mineralization during the development of the bones resulting in soft bones. Osteomalacia is characterized by demineralization of previously formed bone leading to increased softness and susceptibility to fracture.

24.4. Vitamin E (tocopherol) as principal antioxidant in human body

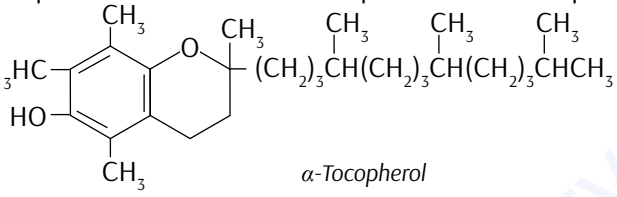
Vitamin E (α-tocopherol)

Vitamin E is a mixture of several related compounds known as ***tocoph-***

erols.

These are a family of molecules that function as antioxidants, particularly to prevent oxidation of unsaturated fatty acids and maintain the integrity of cell membranes. Deficiency can lead to reproductive function, leading to the nickname of "antisterility vitamin".

The α-tocopherol molecule is the most potent of the tocopherols.



α-Tocopherol

Major sources and storage

Major sources of vitamin E are vegetable oils, leafy green vegetables and whole grains.

Vitamin E is absorbed from the intestines packaged in chylomicrons. It is delivered to the tissues via chylomicron transport and then to the liver through chylomicron remnant uptake. The liver can export vitamin E in VLDLs. Due to its lipophilic nature, vitamin E accumulates in cellular membranes, fat deposits and other circulating lipoproteins. The major site of vitamin E storage is in adipose tissue.

Biochemical properties

The major function of vitamin E is to act as a natural antioxidant by scavenging free radicals and molecular oxygen. In particular vitamin E is important for preventing peroxidation of polyunsaturated membrane fatty acids. The vitamins E and C are interrelated in their antioxidant capabilities. Active α-tocopherol can be regenerated by interaction with vitamin C following scavenge of a peroxy free radical. Alternatively, α-tocopherol can scavenge two peroxy free radicals and then be conjugated to glucuronate for excretion in the bile.

Clinical significances of vitamin E deficiency

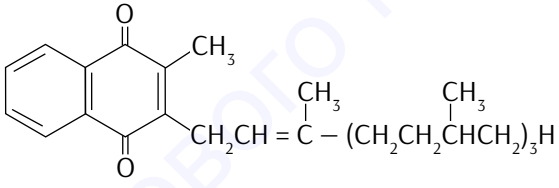
No major disease states have been found to be associated with vitamin E deficiency due to adequate levels in the average American diet. The major symptom of vitamin E deficiency in humans is an increase in red blood cell fragility. Since vitamin E is absorbed from the intestines in chylomicrons,

any fat malabsorption diseases can lead to deficiencies in vitamin E intake. Neurological disorders have been associated with vitamin E deficiencies associated with fat malabsorptive disorders. Increased intake of vitamin E is recommended in premature infants fed formulas that are low in the vitamin as well as in persons consuming a diet high in polyunsaturated fatty acids. Polyunsaturated fatty acids tend to form free radicals upon exposure to oxygen and this may lead to an increased risk of certain cancers.

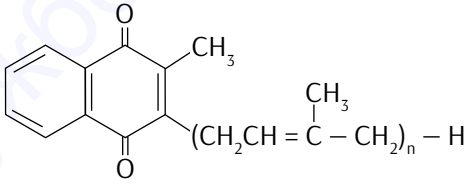
24.5. Vitamin K: structure of vitamers. Role in blood clotting

Vitamin K

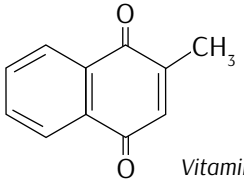
The K vitamin exist naturally in the form of two *vitamers*, and namely K_1 (*phylloquinone)* of green vegetables and K_2 *(menaquinone)* produced by intestinal bacteria. Synthetic menadione is referred to as vitamin K_3. When administered, vitamin K_3 is alkylated to one of the vitamin K_2 forms of menaquinone.



Vitamin K_1



Vitamin K_2 "n" can be 6, 7 or 9 isoprenoid groups



Vitamin K_3

Vitamin K is necessary for formation of several blood-clotting factors in the liver, and deficiency leads to bleeding disorders.

Major sources: Majority is synthesized by bacteria in the large intestine. Dietary sources include the photosynthetic (green) parts of plants.

Biochemical properties

The major function of the K vitamins is in the maintenance of normal levels of the blood clotting proteins, factors II, VII, IX, X and protein C and protein S, which are synthesized in the liver as inactive precursor proteins. Conversion from inactive to active clotting factor requires a posttranslational modification of specific glutamate (E) residues. This modification is a carboxylation and the enzyme responsible requires vitamin K as a cofactor. The resultant modified E residues are **γ-carboxyglutamate *(gla).*** This process is most clearly understood for factor II, also called **preprothrombin.** Prothrombin is modified preprothrombin. The *gla* residues are effective calcium ion chelators. Upon chelation of calcium, prothrombin interacts with phospholipids in membranes and is proteolysed to thrombin through the action of activated factor X (Xa).

During the carboxylation reaction reduced hydroquinone form of vitamin K is converted to a 2,3-epoxide form. The regeneration of the hydroquinone form requires an uncharacterized reductase. This latter reaction is the site of action of the dicumarol-related anticoagulants such as ***warfarin.***

Clinical significance of Vitamin K Deficiency

Naturally occurring vitamin K is absorbed from the intestines only in the presence of bile salts and other lipids through interaction with chylomicrons. Therefore, fat malabsorptive diseases can result in vitamin K deficiency. The synthetic vitamin K_3 is water soluble and absorbed irrespective of the presence of intestinal lipids and bile. Since the vitamin K_2 form is synthesized by intestinal bacteria, deficiency of the vitamin in adults is rare. However, long term antibiotic treatment can lead to deficiency in adults. The intestine of newborn infants is sterile, therefore, vitamin K deficiency in infants is possible if lacking from the early diet. The primary symptom of a deficiency in infants is a hemorrhagic syndrome.

Part 6

BIOCHEMISTRY OF SPECIALIZED TISSUES AND PHYSIOLOGICAL FUNCTIONS

Chapter 25.

BIOCHEMISTRY OF BLOOD. HEMOSTASIS. COAGULATION CASCADE SYSTEM

25.1. Blood: composition, biochemical functions

The blood accounts for about 8 % of the mass of the human body. It is composed of whole cells and blood plasma.

Blood plasma constitutes an aqueous solution of proteins and low molecular weight compounds. Blood cells include ***erythrocytes*** which are responsible mainly in gas transport and various types of ***leucocytes, lymphocytes*** and ***thrombocytes*** that provide manifold homeostatic and protective functions – Figure 25.1.

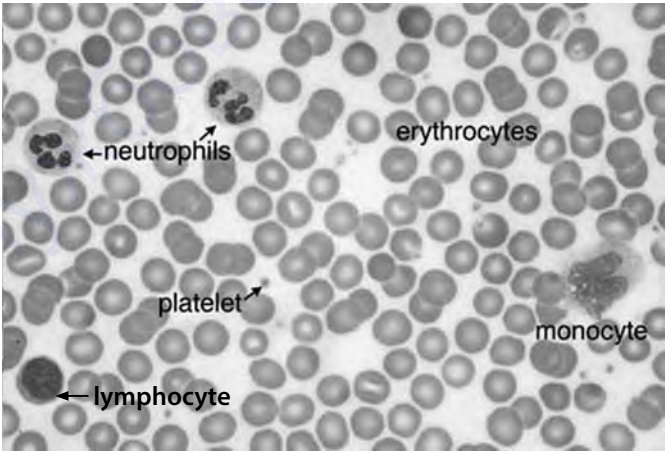


Figure 25.1. Human blood smear: a – erythrocytes; b – neutrophil; c – eosinophil; d – lymphocyte

In humans, the various cellular elements make up approximately 45 % of total volume of the blood. This percentage is referred to as the ***hematocrit***.

Blood: biochemical and physiological functions

The specialized cells and plasma constituents of the human blood perform a variety of biochemical and physiological functions vitally important for the whole organism. Among them:

- **Transport function.**

Blood is the liquid tissue that transports biologically essential molecules between different organs of the body. The molecules transported are: gases (O_2 and CO_2 as participants of tissue respiration), bioorganic molecules including, intermediates, hormones, waste metabolites etc., inorganic ions which have different regulative and catalytic properties.

- **Maintenance of acid-base homeostasis.**

The complex human organism has the amazing ability to maintain the acid-base homeostasis of its intracellular and extracellular fluids, in which function the blood plays an important role. This is realized owing to the associated action of specific enzyme systems of red blood cells, inorganic buffer systems and plasma proteins that on the whole provides the constant control of intracellular and extracellular pH in the body.

- **Immune defense.**

The blood constitutes the essential part of human immune defense system which is switched on when the invasion into the body of diverse foreign microorganisms and/or macromolecules, called ***antigens*****,** takes place. Components of immune system are blood cells, that is lymphocytes predomi-nantly, several other types of leucocytes performing phagocytic functions, as well as various plasma proteins and peptides, particularly immunoglobulins, interferons and multiple cytokins including interleukins, chemokins, cytotoxic factors and growth factors, components of complement system etc.

- **Clotting of the blood.**

It is well known in everyday life that any blood vessel injury results in bleeding followed by formation of blood clot, or ***thrombus*****,** which stops bleeding. This vitally important defense system that prevents the blood loss following damage becomes dangerous when *thrombosis* develops inside the vessel as it is in the event of severe atherosclerosis. In both cases, the

activation of *hemostatic*, or *blood clotting* (otherwise *blood coagulation*) system takes place. The principal biochemical mechanisms of blood clotting will be considered below.

25.2. Hemoglobin: transport of oxygen. Hemoglobinopathies

Hemoglobin is a hemoprotein found in erythrocytes of mammalian where it is responsible for binding oxygen in the lung and transporting the bound oxygen throughout the bloodstream to the cells where it is used in aerobic metabolic pathways.

Hemoglobin is a tetrameric protein consisting of four peptide subunits that are designated α, β, γ and δ present in different types of the most commonly occurring functional hemoglobins.

Each subunit of a hemoglobin tetramer has a heme prosthetic group identical to that of myoglobin. The structure of adult person blood hemoglobin (HbA) corresponds to the formula [α(2): β(2)], that contains two pairs of identical subunits (two α and two β) – Figure 25.2.

Although the secondary and tertiary structure of various hemoglobin subunits are similar, reflecting extensive homology in amino acid composition, the variations in amino acid composition do exist that impart marked differences in hemoglobin's oxygen carrying properties.

Oxygen saturation. Allosteric properties of hemoglobin

In addition, the quaternary structure of hemoglobin leads to physiologically important allosteric interactions between the subunits, a property lacking in monomeric myoglobin which is otherwise very similar to the α-subunit of hemoglobin.

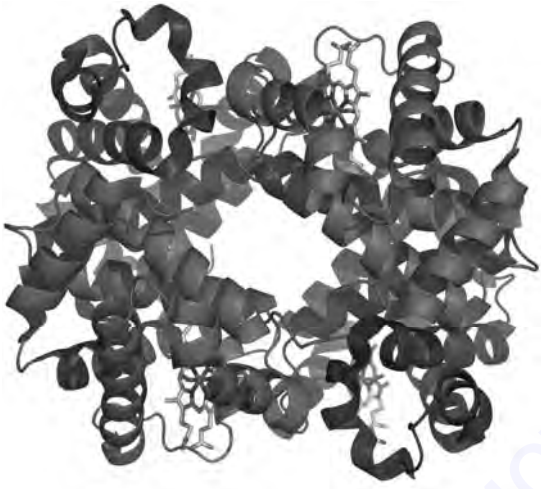


Figure 25.2. Structure of **hemoglobin**. The protein subunits are in red and blue, and the iron-containing *heme* groups in green

Comparison of the oxygen binding properties of ***myoglobin*** and ***hemoglobin*** illustrates the allosteric properties of hemoglobin that results from its quaternary structure and differentiate hemoglobin's oxygen binding properties from that of myoglobin. The curve of oxygen binding to hemoglobin is ***sigmoidal*** typical of allosteric proteins in which the substrate, in this case oxygen, is a positive homotropic effector – Figure 25.3.

The biochemical mechanism of the phenomenon is that the binding affinity of hemoglobin for oxygen is increased by the oxygen saturation of the molecule, with the first oxygens bound influencing the shape of the binding sites for the next oxygens, in a way favorable for binding.

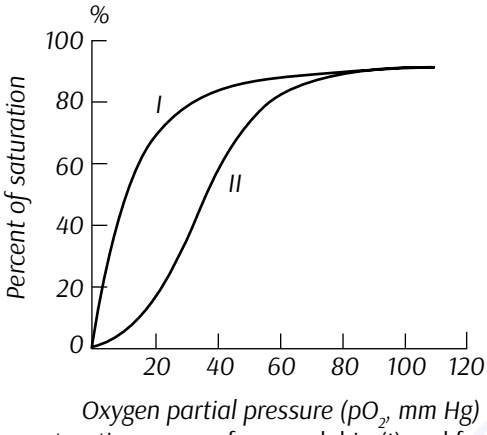


Figure 25.3. Oxygen saturation curves: for myoglobin (I) and for hemoglobin (II)

This positive cooperative binding is achieved through steric conformational changes of the hemoglobin protein complex, i.e. when one subunit protein in hemoglobin becomes oxygenated, this induces a conformational or structural change in the whole complex, causing the other subunits to gain an increased affinity for oxygen. As a consequence, the oxygen binding curve of hemoglobin is sigmoidal, or S-shaped, as opposed to the normal hyperbolic curve associated with noncooperative binding of myoglobin.

Regulation of hemoglobin affinity for oxygen

There are four primary regulators of hemoglobin affinity for O_2, each of which has a negative impact on the oxygen binding. These are CO_2, hydrogen ion (H^+), chloride ion (Cl^-), and 2,3-bisphosphoglycerate (2,3BPG).

Although they can influence O_2 binding independent of each other, CO_2, H^+ and Cl^- primarily function as a consequence of each other on the affinity of hemoglobin for O_2.

Role of 2,3-bisphosphoglycerate (2,3-BPG)

The compound *2,3-bisphosphoglycerate (2,3-BPG),* derived from the glycolytic intermediate 1,3-bisphosphoglycerate, is a potent allosteric effector on the oxygen binding properties of hemoglobin.

It is shown that when 2,3-BPG is not available, or not bound in the central cavity of the hemoglobin molecule, the latter can be converted to HbO_2 more readily. Thus, like increased hydrogen ion concentration, increased 2,3-BPG concentration decreases the amount of oxygen bound by Hb at any

oxygen concentration. That is why, the synthesis of 2,3-BPG in erythrocytes is critical for controlling hemoglobin affinity for oxygen.

Hemoglobin molecules differing in subunit composition are known to have different 2,3-BPG binding properties with correspondingly different allosteric responses to 2,3-BPG. For example, HbF (the fetal form of hemoglobin) binds 2,3-BPG much less avidly than HbA (the adult form of hemoglobin) with the result that HbF in fetuses of pregnant women binds oxygen with greater affinity than the mothers HbA, thus giving the fetus preferential access to oxygen carried by the mothers circulatory system.

Hemoglobinopathies

The overall hemoglobin composition in a normal adult is approximately 97.5 % HbA_1, 2 % HbA_2 and 0.5 % HbF.

A large number of mutations have been described in the globin genes. These mutations can be divided into two distinct types: those that cause qualitative abnormalities (e.g. sickle cell anemia) and those that cause quantitative abnormalities *(thalassemias*). Taken together these disorders are referred to as the hemoglobinopathies.

Sickle cell anemia

Of the mutations leading to qualitative alterations in hemoglobin, the missense mutation in the β-chain gene that causes ***sickle cell anemia*** is the most common. The mutation causing sickle cell anemia is a single nucleotide substitution (A to T) in the codon for amino acid 6. This change converts a glutamic acid codon (GAG) to a valine codon (GTG). The form of hemoglobin is persons with sickle cell anemia is referred to as HbS.

The underlying problem in sickle cell anemia is that the valine for glutamic acid substitution results in hemoglobin tetramers that aggregate into arrays upon deoxygenation in the tissues. This aggregation leads to deformation of the red blood cell making it relatively inflexible and unable to traverse the capillary beds. Repeated cycles of oxygenation and deoxygenation lead to irreversible sickling of erythrocytes – Figure 25.4.

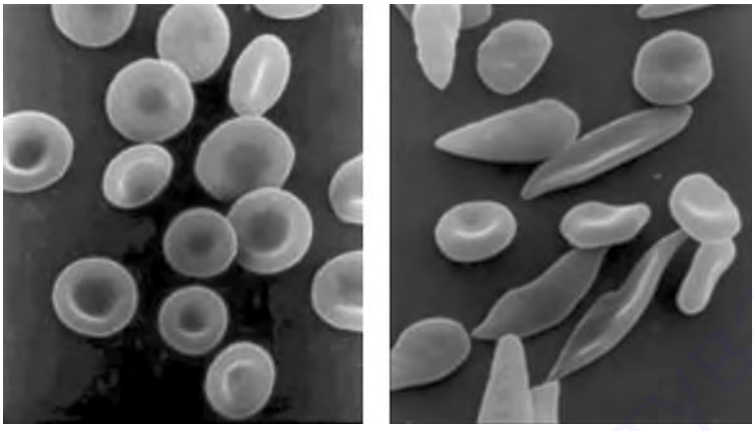


Figure 25.4. Erythrocytes from normal human blood (left) and a patient with sickle cell anemia (right)

The end result is clogging of the fine capillaries. Because bones are particularly affected by the reduced blood flow, frequent and severe bone pain results. This is the typical symptom during a sickle cell "crisis". Long term the recurrent clogging of the capillary beds leads to damage to the internal organs, in particular the kidneys, heart and lungs. The continual destruction of the sickled red blood cells leads to chronic anemia and episodes of hyperbilirubinemia.

An additional relatively common mutation at codon 6 is the conversion to a lysine codon (AAG) which results in the generation of **HbC.**

Electrophoresis of hemoglobin proteins from individuals suspected of having sickle cell anemia (or several other types of hemoglobin disorders) is an effective diagnostic tool because the variant hemoglobins have different charges. An example of this technique is shown in the Figure 25.5.

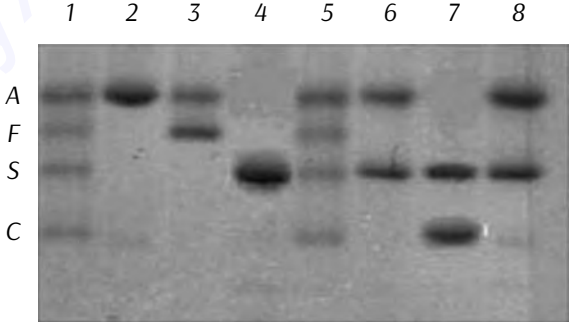


Figure 25.5. Pattern of hemoglobin electrophoresis from several different individuals. Lanes 1 and 5 are hemoglobin standards. Lane 2 is a normal adult. Lane 3 is a normal neonate. Lane 4 is a homozygous HbS individual. Lanes 6 and 8 are heterozygous sickle individuals. Lane 7 is a sickle cells disease individual

Another effective tool to identify the genotype of individuals suspected of having sickle cell disease as well as for prenatal diagnosis is to use PCR.

An interesting and common (up to 30 % of persons from Southeast Asia) hemoglobinopathy that has both quantitative and qualitative characteristics is caused by the synthesis of *hemoglobin E.* Hemoglobin E arises due to a point mutation in codon 26 that changes glutamic acid (GAG) to lysine (AAG). Individulas with this mutation make only around 60 % of the normal amount of b-globin protein. The reason for this is that the mutation creates a cryptic splice site such that 40 % of the hemoglobin E mRNA is shorter by 16 nucleotides and does not give rise to detectable b-globin protein.

There are some individuals in whom the developmental timing of globin production is altered as a consequence of mutation. Persons with hereditary persistence of fetal hemoglobin, HPFH, continue to produce HbF as adults. Because the syndrome is benign most individuals do not even know they carry a hemoglobin abnormality. Many HPFH individuals harbor large deletions of the d- and b-coding region of the cluster. There is no deletion of the fetal globin genes and by an as yet uncharacterized mechanism expression of these genes persists in adulthood.

Thalassemias

The thalassemias are the result of abnormalities in hemoglobin synthesis and affect both clusters. Deficiencies in α-globin synthesis results in the α-thalassemias and deficiencies in β-globin synthesis results in the β-thalassemias. The term thalassemia is derived from the Greek thalassa meaning "sea" and was applied to these disorders because of the high frequency of their occurrence in individuals living around the Mediterranean Sea.

In normal individuals an equal amount of both α- and β-globin proteins are made allowing them to combine stoichiometrically to form the correct hemoglobin tetramers.

In the **α-thalassemias** normal amounts of β-globin are made. The β-globin proteins are capable of forming homotetramers (β_4) and these tetramers are called **hemoglobin H, HbH.** An excess of HbH in red blood cells leads

to the formation of inclusion bodies commonly seen in patients with α-thalassemia. In addition, the HbH tetramers have a markedly reduced oxygen carrying capacity. In **β-thalassemia,** where the β-globins are deficient, the α-globins are in excess and will form α-globin homotetramers. The α-globin homotetramers are extremely insoluble which leads to premature red cell destruction in the bone marrow and spleen.

α-Thalassemias

With the a-thalassemias the level of a-globin production can range from none to very nearly normal levels which critically affects the clinical manifestations of the thalassemia. In particular, if 3 of the 4 a-globin genes are functional, individuals are completely asymptomatic. This situation is identified as the "silent carrier" state or sometimes as **a-thalassemia 2.**

In individuals of African descent with **a-thalassemia 1,** the disorder usually results from the inactivation of 1 a-globin gene on each chromosome and is designated a-/a-. This means that these individuals are homozygous for the a-thalassemia 2 chromosome. The phenotype of a-thalassemia 1 is relatively benign. The mean red cell volume (designated MCV in clinical tests) is reduced in a-thalassemia 1 but individuals are generally asymptomatic.

The clinical situation becomes more severe if only 1 of the 4 a-globin genes is functional. Because of the dramatic reduction in a-globin chain production in this latter situation, a high level of b_4 tetramer is present. Clinically this is referred to as ***hemoglobin H disease.*** Afflicted individuals have moderate to marked anemia and their MCV is quite low, but the disease is not fatal. The most severe situation results when no a-globin chains are made (genotypically designated –/–). This leads to prenatal lethality or early neonatal death. The predominant fetal hemoglobin in afflicted individuals is a tetramer of g-chains and is referred to as ***hemoglobin Bart's***. This hemoglobin has essentially no oxygen carrying capacity resulting in oxygen starvation in the fetal tissues. Heart failure results as the heart tries to pump the unoxygenated blood to oxygen starved tissues leading to marked edema. This latter situation is called *hydrops fetalis.*

β-Thalassemias

A large number of mutations have been identified leading to decreased or absent production of b-globin chains resulting in the b-thalassemias. In the most severe situation mutations in both the maternal and paternal b-globin

genes leads to loss of normal amounts of b-globin protein. A complete lack of HbA is denoted as **b^0-thalassemia**. If one or the other mutations allows production of a small amount of functional b-globin then the disorder is denoted as b^+-thalassemia.

Both **b^0-** and **b^+-thalassemias** are referred to as **thalassemia major**, also called **Cooley's anemia** after Dr. Thomas Cooley who first described the disorder. Afflicted individuals suffer from severe anemia beginning in the first year of life leading to the need for blood transfusions. As a consequence of the anemia the bone marrow dramatically increases its' effort at blood production. The cortex of the bone becomes thinned leading to pathologic fracturing and distortion of the bones in the face and skull. In addition, there is marked hepatosplenomegaly as the liver and spleen act as additional sites of blood production. Without intervention these individuals will die within the decade of life.

The primary cause of a-thalassemias is deletion, whereas, for b-thalassemias the mutations are more subtle. In b-thalassemias, point mutations in the promoter, mutations in the translational initiation codon, a point mutation in the polyadenylation signal and an array of mutations leading to splicing abnormalities have been characterized.

25.3. Hemostasis: blood clotting; fibrinolysis

Blood Coagulation

The ability of the body to control the flow of blood following vascular injury, which results in the cessation of bleeding, is extremely important to the survival of a whole organism. On the other hand the excessive blood clotting, or blood coagulation inside the vessel, leading to the formation of thrombus, as is the case in *atherosclerosis*, can be deadly dangerous for the human.

Hence, the control of **hemostasis,** that is the blood clotting and then the constant subsequent dissolution of the clot formed inside the vessel, is reckoned to as one of the paramount physiological functions of the high multicellular organism.

The whole process of hemostasis includes the coordinated actions of blood cells, mainly ***thrombocytes*** (or *platelets*), and multiple soluble com-

ponents derived from plasma, blood vessels walls and surrounding tissues which are commonly known as ***coagulation factors,*** their activators and antagonists. From the physiological point of view, hemostasis is composed of an array of successive events of which the following four are the primary.

1. The starting point of the process is vascular constriction which limits the flow of blood to the area provided with the injured vessel.
2. After that, thrombocytes become activated by ***thrombin*** and their aggregation at the site of vessel damage occurs, forming a temporary, loose *platelet plug*. Platelets aggregate by binding to collagen fibrils that become exposed following the injury of the endothelial lining of vessels.

The adhesion of platelets to the collagen exposed on endothelial cell surfaces is mediated by ***von Willebrand factor*** (vWF), which is a multimeric glycopro-tein that is produced by and stored in the α-granules of platelets. The function of vWF is to act as a bridge between a specific glycoprotein on the surface of platelets (GPIb/IX) and collagen fibrils. In addition to its role as a bridge between platelets and exposed collagen on endothelial surfaces, vWF binds to and stabilizes coagulation factor VIII that is required for normal survival of factor VIII in the circulation.

Upon activation by thrombin, platelets release adenosine-5'-diphosphate (ADP) and thromboxane A_2 (TXA A_2) which activate additional platelets, serotonin, phospholipids, lipoproteins, and other biomolecules needed for the coagulation cascade switching that follows.

3. Then, to insure stability of the initially loose platelet plug, the turning on of a coagulation cascade takes place which finally forms a *fibrin mesh* also called the ***blood clot.*** The list of primary factors of coagulation (or ***clotting factors***) is presented in the Table 25.1. Biochemically, many of the factors nu-merated are proteinases, others are regulatory molecules (activators or inhibi-tors), and the **coagulation system as a whole is a cascade consequence of enzymatic reactions** in which a series of *zymogen activations* occurs (see below).

If the clot formed contains only thrombocytes, it is termed a ***white thrombus;*** if erythrocytes are present it is called a ***red thrombus.***

4. To ensure normal blood flow after tissue repair, the clot must be dissolved. The dissolution of the blood clot is reached through the activation of **fibrinolysis** and implies the participation of the special system of proteinases.

Table 25.1. Primary Factors of Coagulation (Clotting Factors)

Factor	Common Name
I	Fibrinogen
II	Prothrombin
III	Tissue factor (thromboplastin)
IV	Calcium (Ca^{2+})
V	Proaccelerin; accelerator (Ac^{-}) globulin
VI(Va)	Accelerin
VII	Proconvertin; serum prothrombin conversion accelerator (SPCA)
VIII	Antihemophilic factor A; antihemophilic globulin (AHG)
IX	Antihemophilic factor B; Christmas factor
X	Stuart-Prower Factor
XI	Plasma thromboplastin antecedent (PTA); Rosenthal Factor
XII	Hageman Factor
XIII	FGibrin stabilizing factor (FSF); fibrinoligase

The Clotting Cascade

There are two mechanisms, or two pathways, to switch on the *clotting* (or *blood coagulation*) system and to make up the fibrin clot. These are called the ***intrinsic*** and the ***extrinsic pathways (clotting cascades)*** which converge into ***common clotting pathway*** – Figure 25.6. The pathways are sequences of enzy-mes catalyzed biochemical reactions and involve the participation of a set of numerous different proteins termed **clotting factors** (see Table 17.1).

It is accepted nowadays that the formation of a red thrombus or a clot in response to an abnormal vessel wall in the absence of tissue injury is the result of the *intrinsic pathway*. Fibrin clot formation in response to tissue injury is the result of the *extrinsic pathway* activation.

The intrinsic pathway (also called *intravascular reaction pathway*) is initiated when contact is made between blood and the endothelial cell sur-

face exposed by injury. The pathway begins with the activation of the so-called *Hageman factor* (or *Factor XII*) to make active endopeptidase, *Factor XIIa*.

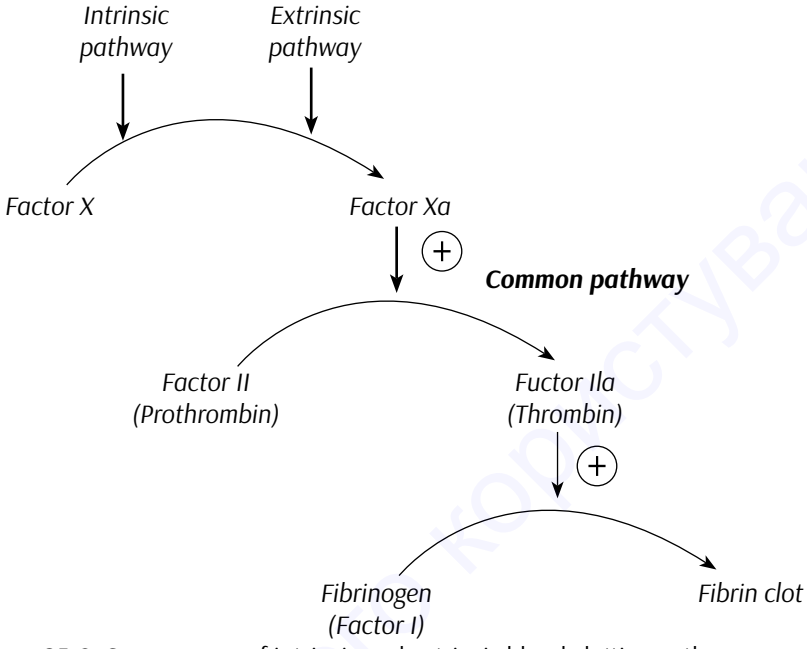


Figure 25.6. Convergence of intrinsic and extrinsic blood clotting pathways

This rather intricate pathway involves the participation of clotting factors XI, IX, VIII as well as *prekallikrein*, high-molecular weight (HMW) *kininogen*, Ca^{2+} and platelet phospholipids. The components mentioned are assembled on the surface of endothelial cell membranes which results in the proteolytic conversion of ***Factor XII to Factor XIIa.***

The development of successive biochemical events leads to the catalytically active ***Factor Xa (thrombokinase)*** appearance which is the key point of the onset of the so-called **common pathway** *of clotting cascade***.**

The extrinsic pathway (also called ***extravascular reaction pathway***) is triggered in the condition of tissue trauma when essential components for this mechanism are released, of which ***Factor III*** (*tissue factor; tissue thrombo-plastin*), a subendothelial cell-surface glycoprotein, is the initiating. Tissue factor interacts with the Factor VII to activate the latter and to promote the factor VIIa-catalyzed activation of factor X to factor Xa in a manner identical to that of the intrinsic pathway.

The common pathway of the clotting cascade

Although the **intrinsic** and **extrinsic pathways** are initiated by distinct mechanisms, the two converge on a **common pathway** that leads to fibrin clot formation and is initiated by the appearance of ***Factor Xa*** formed as a result of either of two alternative mechanisms functioning – Figure 25.7.

Activation of Prothrombin to Thrombin

The initial event of the common pathway is the catalyzed by ***Factor X***a conversion of ***prothrombin (Factor II)*** to the active proteinase ***thrombin (Factor IIa).***

Prothrombin is a 72 kD, single-chain protein. Prothrombin is cleaved at two sites by factor Xa. This cleavage generates a 2-chain active ***thrombin*** molecule containing an A and a B chain which are held together by a single disulfide bond. The activation of thrombin occurs on the surface of activated platelets and requires formation of a *prothrombinase complex*. This complex is composed of the platelet phospholipids and requires, in addition to factor Xa, phosphatidylinositol, phosphatidylserine, Ca^{2+} and factor Va.

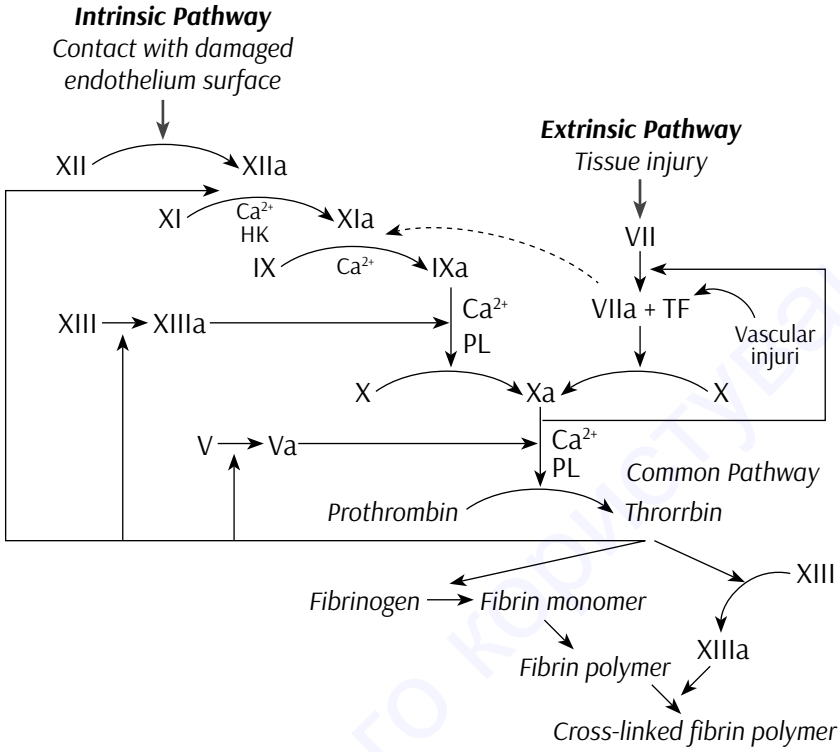


Figure 25.7. The clotting cascades: The intrinsic cascade is initiated when contact is made between blood and exposed endothelial cell surfaces. The extrinsic pathway is initiated upon vascular injury which leads to exposure of tissue factor (TF) (Factor III), a subendothelial cell-surface glycoprotein that binds phospholipid. The two pathways converge at the activation of factor X to Xa. Factor Xa has a role in the further activation of factor VII to VIIa as depicted by the green arrow. Active factor Xa hydrolyzes and activates prothrombin to thrombin. Thrombin can then activate factors XI, VIII and V furthering the cascade. Ultimately the role of thrombin is to convert fribrinogen to fibrin and to activate factor XIII to XIIIa. Factor XIIIa (also termed transglutaminase) cross-links fibrin polymers solidifying the clot. HK = high molecular weight kininogen. PK – prekallikrein. PL – phospholipid

Activation of Fibrinogen to Fibrin

After the formation of active thrombin, goes the conversion of ***fibrinogen (Factor I)*** to yield ***fibrin*** – the principal protein constituent of the blood clot.

Fibrinogen (factor I) consists of 3 pairs of polypeptides $([A\alpha][B\beta][\gamma])_2$. The 6 chains are covalently linked near their N-terminals through disulfide

bonds. The A and B portions of the Aα and Bβ chains comprise the fibrinopeptides, A and B, respectively – Figure 25.8.

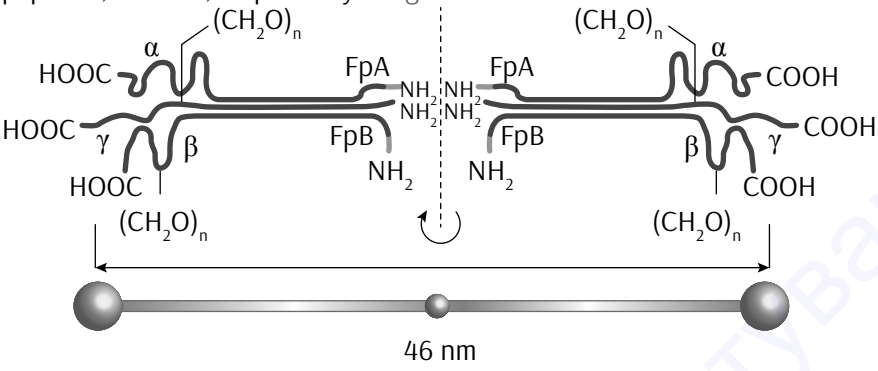


Figure 25.8. Diagrammatic representation of fibrinogen molecule. FpA – fibrinopeptide A; FpB – fibrinopeptide B; $(CH_2O)n$ – carbohydrates residues

Active thrombin is a serine protease that hydrolyses fibrinogen at four -Arg-Gly- bonds which releases the fibrinopeptides residues to generate fibrin monomers with a subunit structure $(\alpha, \beta, \gamma)_2$. The monomers formed spontaneously aggregate in a regular array, forming a fibrin mesh – Figure 25.9.

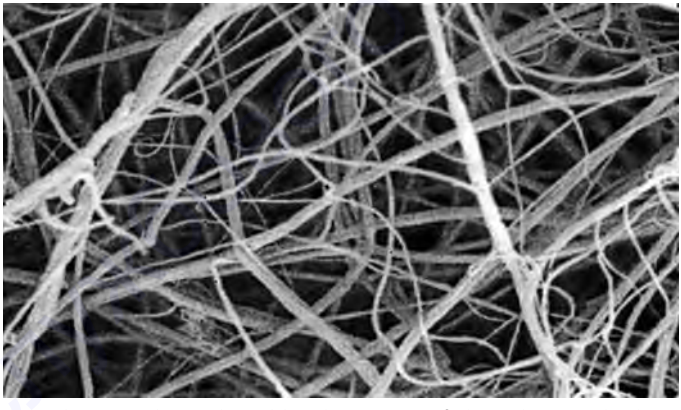


Figure 25.9. Fibrin mesh produced by the action of thrombin on the human blood plasma (after E. Lugovsky, 2003)

The role of thrombin is not only to activate fibrinogen to fibrin but also to convert ***Factor XIII*** to ***XIIIa. Factor XIIIa*** is highly specific ***transglutaminase*** that introduces cross-links composed of covalent bonds between the amide nitrogen of glutamines and ε-amino group of lysines in the fibrin monomers. Cross-linkage of fibrin polymers solidifies the fibrin clot.

Fibrinolysis

Degradation of fibrin clots is the function of ***plasmin,*** a serine protease that circulates as the inactive proenzyme, ***plasminogen.*** The process is called *fibrinolysis.*

To activate plasminogen, special serine proteases, that is ***Tissue plasminogen activator (alteplase; tPA)*** and, to a lesser degree, ***urokinase*** are released into the circulation from vascular endothelium. Active tPA cleaves *plasminogen* to *plasmin* which then cleaves the fibrin molecules to give soluble products of fibrinolysis. Thus, the fibrin clot becomes dissolved.

Clinical manifestations of hemostasis disorders

Defects in the process of hemostasis in humans, leading to bleeding disorders, have been identified at the level of the proteins of the clotting cascades, platelet activation and function etc. The various forms of hemophilia that is of disease associated with the inability to clot blood clotting are well examined. known.

Hemophilia A

Hemophilia A is an X-chromosome linked disorder resulting from a deficiency in factor VIII, a component of the coagulation cascade which is a cofactor in the activation of factor X to factor Xa in a reaction catalyzed by factor IXa.

Hemophilia A arises from a variety of gene mutations. Some 150 different point mutations have been characterized in the factor VIII gene in hemophilia A. Inheritance of the disorder occurs with a frequency of 1:5,000 to 1:10,000 males in all populations. There are severe, moderate and mild forms of hemophilia A that reflect the level of active factor VIII in the blood plasma.

Individuals with deficiencies in factor VIII suffer joint and muscle hemorrhage, easy bruising and prolonged bleeding from traumas. Treatment of hemophilia A is accomplished by infusion of factor VIII concentrates prepared from either human plasma or by recombinant DNA technology.

Hemophilia B

Hemophilia B results from deficiencies in factor IX. The prevalence of

hemophilia B is approximately one-tenth that of hemophilia A. All patients with hemophilia B have prolonged coagulation time and decreased factor IX clotting activity.

At least 300 unique factor IX mutations have been identified, including point mutations, short nucleotide deletions, insertions etc. Accordingly, like under hemophilia A, there are severe, moderate and mild forms of the disease reflecting the concentration of factor IX in patient plasma.

Haemophilia C

Haemophilia C is a mild form of haemophilia affecting both sexes. However, it predominantly occurs in Jews of Ashkenazi descent. In the USA it is thought to affect 1 in 100,000 of the adult population, making it 10 % less common than haemophillia A.

The disease is caused by a deficiency of *coagulation factor XI* (also known as Rosenthal Factor) and is distinguished from haemophilia A and B by the fact it does not lead to bleeding into the joints. The common manifestations of the pathology include prolonged bleeding from injuries, frequent or heavy nosebleeds and traces of blood in the urine. Treatment is usually not necessary, except in relation to surgical *operations*, when to prevent excessive bleeding, *fresh frozen plasma* or recombinant factor XI may be used.

von Willebrand Disease

von Willebrand disease (vWD) is due to inherited deficiency in von Willebrand factor (vWF). vWD is the most common inherited bleeding disorder of humans. Using sensitive laboratory testing, abnormalities in vWF can be detected in approximately 8000 people per million. Clinically significant vWD occurs in approximatley 125 people per million. This is a frequencey at least twice that of hemophilia A.

Deficiency of vWF results in defective platelet adhesion, causes a secondary deficiency in factor VIII and is displayed in bleeding similar to that found in hemophilia A. vWD is an extremely heterogeneous disorder that has been classified into several major subtypes, in particular type I, II and III that differ in clinical manifestations and results of biochemical laboratory tests.

Fibrinogen Disorders

There are although rare, several inherited forms of coagulation disturbances due to fibrinogen disorders. These include ***afibrinogenemia*** which is a complete lack of fibrinogen, ***hypofibrinogenemia*** (reduced levels of fibrinogen) and ***dysfibrinogenemia*** which results of the presence of dysfunctional fibrinogen.

Afibrinogenemia is characterized by neonatal umbilical cord hemorrhage, mucosal hemorrhage, internal hemorrhage, and recurrent abortion. ***Hypofibrinogenemia*** is characterized by fibrinogen levels below 100mg/dL (normal is 250–350 mg/dL) and can be either acquired or inherited. Symptoms of hypofibrinogenemia are similar to, but less severe than, afibrinogenemia. ***Dysfibrinogenemias*** are a group of heterogeneous abnormalities affecting the structure and biochemical properties of fibrinogen. Clinical consequences of dysfibrinogenemias include hemorrhage, spontaneous abortion and thromboembolism.

Chapter 26.

BIOCHEMISTRY OF SPECIALIZED CELLS AND PHYSIOLOGICAL FUNCTIONS-2. BIOCHEMISTRY OF LIVER. METABOLISM OF XENOBIOTICS

Liver is the largest organ of human body. It weighs approximately 1.5 kg which constitutes 2–3 % of the total body mass. At the same time, liver cells account for nearly 20–25 % of the whole body oxygen consumption that testifies the great intensity of metabolic reactions in hepatocytes. The liver has two main cell types. They are Kupffer cells which are phagocytes, important in immune function, and ***hepatocytes*** that realize the principal biochemical and physiological functions of the liver.

26.1. Liver: survey of major biochemical functions

Biochemical functions of the liver

1) ***The uptake, processing and disposition of nutrient molecules which are delivered from the digestive tract via the portal vein.***

During digestion in mammals, the three main classes of nutrients, and namely carbohydrates, proteins, and fats, undergo enzymatic hydrolysis into their simple constituents. After being absorbed into epithelial cells of the intestine, monosaccharides, amino acids and lipids (monoacylglycerols and some triacylglycerols) travel to the liver.

The circulatory system of the liver is unlike that seen in any other organ.

Of great importance is the fact that roughly 75 % of the blood entering the liver is venous blood from ***the portal vein.*** Only the remaining 25 % of the blood supply to the liver is arterial blood from the hepatic artery. Accordingly, all of the venous blood returning from the small intestine, stomach, pancreas and spleen converges into the portal vein. The important consequence of this is that the liver has first access to everything absorbed in the small intestine, that is all nutrients which are absorbed.

Inside hepatocytes, the syntheses of vital metabolites which can be secreted by the liver for usage in extrahepatic tissues take place. Among them are some specific plasma proteins (for example, blood coagulation factors), glycogen, lipoproteins, cholesterol and ketone bodies.

2) ***The biotransformation of harmful compounds that enter human body as xenobiotics or are produced as waste metabolites underlies the liver detoxification function.***

The molecular basis of this function is a set of enzymatic reactions through which realization multiple toxic substances including foreign chemical substances (drugs, pesticides etc.) as well as products of endogenous hormones catabolism ("waste products") are transformed into molecular structures that are less detrimental for living cells and can be eliminated from the body (see below in detail).

3) ***The excretion with the bile of harmful substances and their metabolites that is the products of enzymatic biotransformation.***

Bile is a complex fluid containing water, electrolytes and a battery of organic molecules including bile acids, cholesterol, phospholipids and bilirubin that flows through the biliary tract into the small intestine.

Now, it makes sense to remind the foundations of the biliary system architecture. As can be seen from the Figure 26.1, hepatocytes secrete bile into the ***bile canaliculi,*** which are the dilated intercellular space between adjacent hepatocytes and flow in parallel to the ***sinusoids*** that are blood vessels containing nutrient-rich blood from the *portal vein* and the oxygen-rich blood from the *hepatic artery*.

At the ends of the canaliculi, bile flows into **bile ducts,** and the latter eventually form the ***common bile duct,*** which dumps bile into the duodenum.

There are three fundamentally important functions of bile in a human organism:

- Bile contains bile acids (primarily derivarives of cholic acid) and phos-

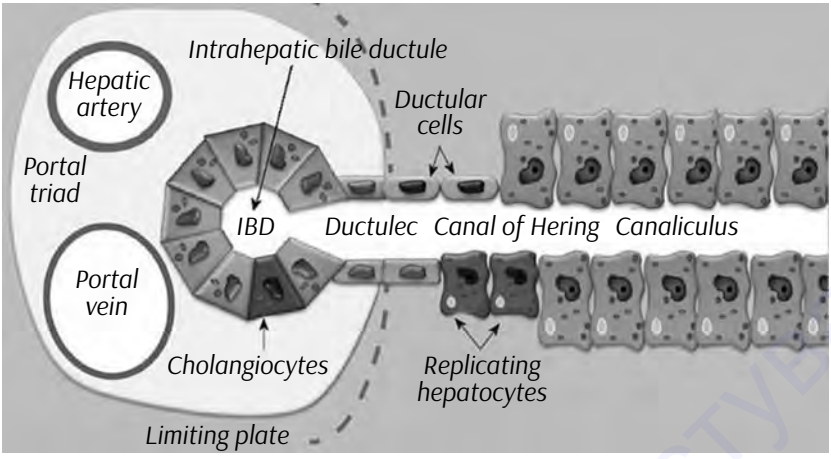


Figure 26.2. Architecture of bile canaliculi and bile duct

pholipids (mainly phosphatidylcholine), which are critical for digestion and absorption of fats and fat-soluble vitamins in the small intestine.

- Many waste products, are eliminated from the body by secretion into bile and excretion in feces. Among these are both endogenous molecules (bilirubin, which is a useless and toxic breakdown product of hemoglobin breakdown, products of steroid hormones metabolism etc.) and exogenous compounds (metabolites of drugs and other *xenobiotics*). A substantial number of these compounds are reabsorbed in the small intestine and ultimately eliminated by the kidney.
- Bile secretion is a major route for eliminating excess of cholesterol from the body. Free cholesterol is virtually insoluble in aqueous solutions, but in bile, it is made soluble by bile acids and lipids like lecithin. When chemical processes which promote bile cholesterol to precipitate develop, gallstones arise.

Carbohydrate Metabolism

Liver is the principal organ to control the glucose content in blood, and thereby to regulate the carbohydrate metabolism in the majority of extrahepatic tissues (see also Lecture 6).

Hepatocytes house many different metabolic pathways and employ dozens of enzymes that are alternatively turned on or off depending on whether

blood levels of glucose are rising or falling out of the normal range. Two alternative metabolic situations are **well-fed state (1)** and **starvation (2).**

1) After a meal excess glucose entering the blood is rapidly taken up by the liver and, after being converted into glucose-6-phosphate (G-6-P), is used predominantly for glycogen synthesis.

Later, that is in several hours, when blood concentration of glucose begins to decline, the liver activates the pathway which leads to depolymerization of glycogen **(glycogenolysis)** and export of glucose back into the blood for transport to all other tissues.

This unique liver function is due to the presence in hepatocytes of glucose-6-phosphatase which is the only enzyme that can release free glucose from G-6-P:

$$\text{Glucose-6-phosphate} + H_2O \rightarrow \text{Glucose} + P_i$$

2) When hepatic glycogen reserves become exhaused, as occurs when an animal has not eaten for several hours (after 6 to 12 hours of starvation), the metabolic route which is responsible for glucose synthesis **(gluconeogenesis)** is switched on. The new molecules of glucose, utilized by hepatocytes themselves and peripheral organs, are synthesized out of such compounds as glucogenic amino acids, pyruvate and glycerol derived from fats degradation. Thus, the maintenance of blood glucose at a level sufficient to ensure an adequate monosaccharide supply to such organs as brain, erythrocytes and medulla of suprarenal glands is achieved.

Lipid Metabolism

Various aspects of lipid metabolism are occurring inside hepatocytes, and some of them are carried out in the liver predominantly.

The characteristic features of the liver participation in the whole body metabolism of lipids are as follows:

1) The liver is the most important site of *de novo* fatty acids synthesis. The concurrent availability of glycerol provides for active triacylglycerols formation and manufacturing of lipoproteins as the transport form of fats. The VLDL (very low density lipoproteins) thus formed, are secreted into blood and carried to adipose tissue and muscles

where VLDL constituents are released, absorbed and kept as storage form of metabolic energy.

2) The liver is the unique place for ketone body production – the biochemical route that is activated during prolonged period of starvation. The ketone bodies produced by the liver, and mainly acetoacetate, are used to meet the energy requirement of other tissues during the first days of fasting. It is shown that all human tissues except the liver can utilize ketone bodies for ATP synthesis which saves glucose reserves and lessens the necessity for tissue proteins degradation.
3) The liver synthesizes large quantities of cholesterol and phospholipids. Some of these are packaged in the form of plasma lipoproteins which makes these molecules available to the rest of the body cells. The remainder of hepatic cholesterol is converted to bile acids and excreted in bile.

Protein and Amino Acid Metabolism

The most critical aspects of protein metabolism that occur in the liver are:

1) Hepatocytes are responsible for synthesis of most of the plasma proteins. Albumin, the major plasma protein, is synthesized almost exclusively by the liver. Also, the liver synthesizes many of the clotting factors necessary for blood coagulation and several other plasma enzymes.
2) The synthesis of non-essential amino acids takes place exclusively in liver. This is vitally important in supplying building material for protein biosynthesis in extrahepatic tissues.
3) Deamination and transamination of amino acids, followed by conversion of the carbon skeletons of those molecules to glucose or lipids.
4) Removal of ammonia from the body by synthesis of urea. Ammonia is very toxic and if not rapidly and efficiently removed from the circulation, will result in central nervous system disease.

26.2. Liver: metabolism of heme and bile pigments.

Bilirubin transformation. Icterus

Bilirubin metabolism and excretion

Red blood cells, or erythrocytes, are the principal cellular contrivance for delivering oxygen from the lungs to body tissues via the blood. A typical erythrocyte contains about 270 million hemoglobin molecules, with each including globin moiety and the heme consisting of four pyrrole rings with ferrous iron inserted (see Chapter 16).

The duration of life of erythrocytes is about 120 days. It means that under physiological conditions in the human body, $1–2x10^8$ erythrocytes are destroyed per hour. The main sites of erythrocytes destruction are the reticuloendothelial cells of the liver, spleen and the bone marrow.

1) Before being subjected to phagocytosis, the senescent or damaged red blood cells swell up to a sphere-like shape. In this form, erythrocytes are engulfed by phagocytes, in particular by Kuppfer cells of the liver.

Inside the phagocytic cells the plasmatic membrane of erythrocyte is lysed, whereupon globin and porphyrin parts of gemoglobin molecules are degraded.

After the polypeptide chains of globin are splitted by proteinases enzymes, the released amino acids are reused and so is the iron of the heme which enters the total iron pool of the body.

2) The heme portion of hemoglobin degraded is subjected to its own enzymatic catabolism to yield waste substances **biliverdin** and **bilirubin** which are rather toxic compounds and must be eliminated from the body. The elimination of biliverdin and bilirubin is occurring via bile excretion, and therefore the both substances are customarily named ***bile pigments*.**

The degradation of heme and the elimination of its catabolites is accomplished in a series of biochemical steps:

2.1) The catabolism of heme inside liver is carried out through the action of complex enzyme system located in the membranes of edoplasmatic reticulum of hepatocytes.

The first step of heme degradation is catalysed by ***heme oxygenase***. With the help of NADPH, the enzyme inserts oxygen atom to the methenyl bridge between pyrroles A and B of the porphyrin. As the result of enzymatic action, the heme molecule is cleaved to give the green pigment **biliverdin**

which is a tetrapyrrole consisting of four linearly connected pyrrole rings.

An iron atom which is reused and carbon monooxide release as by-products.

2.2) Then a soluble enzyme called ***biliverdin reductase*** transforms water-so-luble ***biliverdin*** to the water-insoluble orange-colored pigment **bilirubin.**

The reaction requires NADPH and consists in the reduction of the methenyl bridge between pyrrole C and pyrrrole D to yield a methylene group $-CH_2-$(Figure 26.2).

It is worthy of notice that the transformation of red hemoglobin to bilirubin can be observed in vivo, in everyday life, when the purple bruise caused by subcutaneous hematoma slowly changes its colouring due to the yellow-orange bilirubin accumulation!

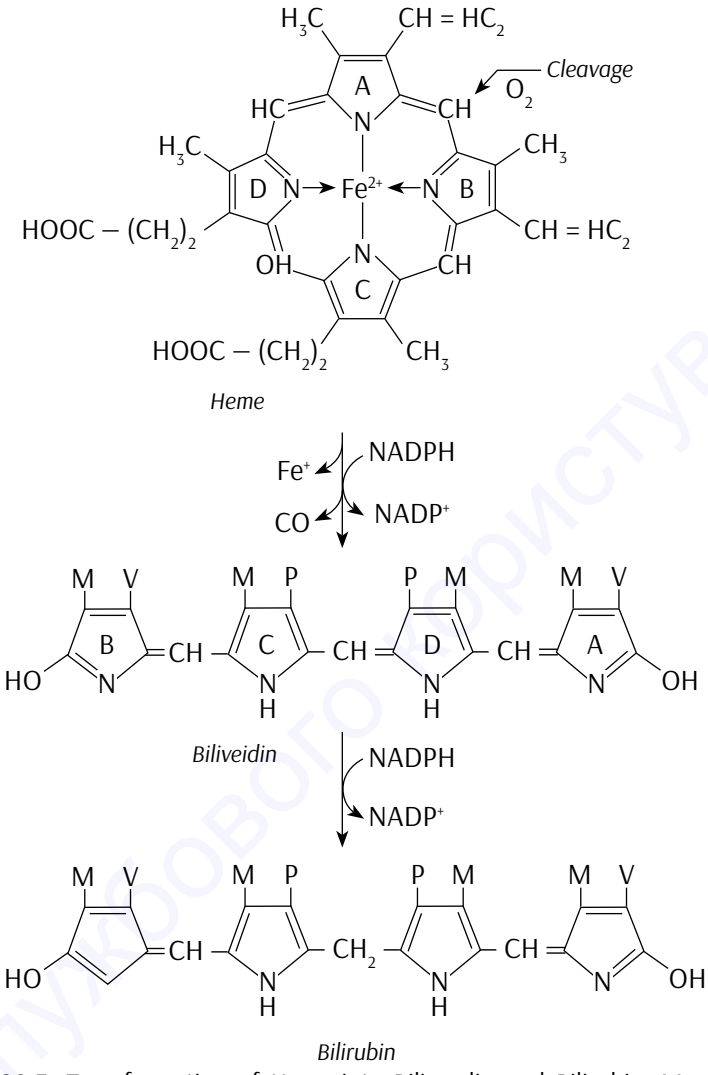
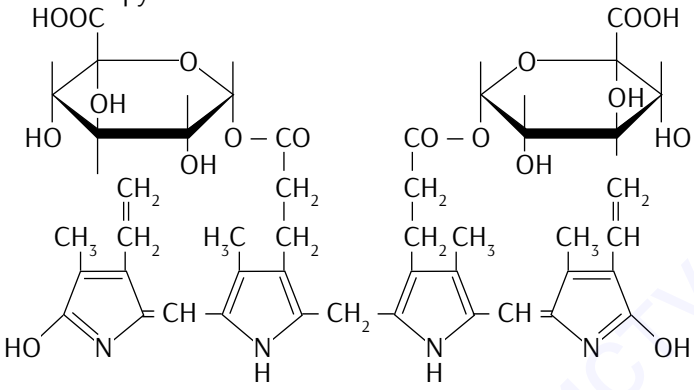


Figure 26.3. Transformation of Heme into Biliverclin and Bilirubin. M – methyl (– CH_3), P – propyl (– CH_2 – CH_2 – COOH), V – vinyl (– CH_2 = CH_2)

2.3) Bilirubin is water-insoluble substance which is rather toxic for cellular metabolism, especially neurons of the brain. That is why a special biochemical system exists which provides detoxification and elimination of the pigment.

This is achieved by the action of the special conjugating enzyme

UDP-glucuronosyltransferase. In the reaction product – ***bilirubin glucuronide,*** one or two glucuronic acid residues are attached to the propionate side chain of tetrapyrrole via ester bonds.



Conjugation with glucuronate markedly increases the water-solubility of bilirubin, and the conjugated form of the pigment can be easily transported into bile and eliminated from the body.

Plasma bilirubin

Along with intracellular bilirubin molecules, that is bilirubin located inside hepatocytes, some of the pigment, both unconjugated and conjugated fractions, is constantly present in human blood plasma.

The **unconjugated bilirubin,** which is poorly soluble in water, occurs in blood plasma being bound to albumin. It is called in clinical laboratory practice **"indirect bilirubin".** The fraction of plasma bilirubin, that is bound to *glucuronic acid* to form the **conjugated bilirubin,** is commonly called **"direct bilirubin".**

In clinical studies of jaundice, measurement of bilirubin plasma concentration is of great value. With a view of diagnostics of definite jaundice types, the van den Bergh method, founded on Ehrlich`s chemical test application, is used. The terms "direct" and "indirect" for two fractions of plasma bilirubin reflect the way by which the two types of pigment react to diazotized sulfanilic acid (Ehrlich`s diazo reagent). Conjugated bilirubin is water-soluble and reacts "directly" when the reagent is added to the blood specimen. The non-water soluble, free bilirubin does not react to the reagents until an alcohol is added to make up the reaction mixture water soluble. Therefore, the

measurement of this type of bilirubin is said to be "indirect".

The reference range for total bilirubin is 2–14 μmol/L or 0.3–1.9 mg/dL. For direct bilirubin, it is 0–4 μmol/L or 0–0.3 mg/dL, and indirect bilirubin is calculated from the total and direct bilirubin.

2.4) As the conjugated bilirubin reaches the intestine, it is hydrolyzed by bacterial enzymes – glucuronidases to remove the glucuronides residues. The released bilirubin moiety is reduced to tetrapyrrole compound called **urobilinogen.**

Urobilinogen is a colourless product, part of which is resorbed in intestines and returned to the liver (enterohepatic circulation, see above), while the rest of tetrapyrrole is excreted in the form of **stercobilinogen.** In the air, the oxidation of bile pigments occurs to give orange-brown compounds **urobilin** and **stercobilin** which mainly cause the coloring of feces.

Urobilinogen also appears in the urine which is especially characteristic for liver detoxification function impairment. Oxidation of the pigment to urobilin contributes to the normal yellow color off urine and its darkening on standing in open air.

Hyperbilirubinemia. Jaundice

When bilirubin concentration in the blood plasma is more than 1 mg/dl (17.1 μmol/l), hyperbilirubinemia exists. The causes of hyperbilirubinemia are concerned with various shifts and abnormalities in bilirubin producing, metabolism and elimination.

In cases when the elevated bilirubin level exceeds approximately 2–2.5 mg/dl, the pigment diffuses into different tissue giving them a yellow colour. Such condition is called **jaundice** or **icterus.** The coloration of the skin and mucous membranes is especially easily visible is when the white conjunctiva of the eyes is inspected.

There are more than one distinctive clinical types of jaundice. The reasons of them can be as follows:

- The superfluous formation of bilirubin because of excessive degradation of erythrocytes. This is called **"pre-hepatic"** or "**hemolytic" jaundice** which can be caused by poisoning or the hereditary red cell enzymopathy due to the ***glucose-6-phosphate dehydrogenase*** deficiency ("favism").
- The impaired processing of bilirubin in hepatocytes when biochemical processes of glucuronate conjugatiom and/or transport of conjugated

bilirubin into bile do not function correctly. These can be associated with different kinds of hepatitis due to viruses, bacterial infection or chemical intoxication – **"hepatic" jaundice.**

- The impared removal of bile from the gall bladder reasoned by gallstones or bile-duct obstruction due to tumor growth – this is called **"post-hepatic", "extrrahepatic",** or **"obstructive" jaundice.**

Enzymatic jaundices

These are caused by hereditary insufficiencies of definite enzymatic systems responsible for bilirubin metabolism or membrane transport of the substance into bile due to certain genetic defects. Among them are:

Crigler-Najjar Syndrome

The disease is characterized by congenital jaundice due to impairment of gene encoding enzyme *bilirubin-UDP-glucuronosyltransferase in liver cells. There are two types of syndrome (*Type I and Type II) differing in clinical manifestations. In more severe form, Type I Crigler-Najjar Syndrome, bilirubin concentration in plasma usually exceeds 20 mg/dl. In this case, the unconjugated bilirubin can cross the blood-brain barrier which usually results in neonatal *kernicterus* that is toxic damage of vitally important brain centers. The disease is often fatal within the first year of life.

Dubin-Johnson syndrome

The metabolic disorder is characterized with hyperbilirubinemia due to the increased level of conjugated bilirubin. The biochemical cause of abnormality is the inborn lack of protein which is in charge of bilirubin transfer from hepatocytes to *bile canaliculi*. The Pathology is manifested in early childhood and can last during adult life without any other disturbances of hepatic functions.

Gilbert syndrome – a genetic disorder of bilirubin metabolism which can result in mild jaundice, found in about 5 % of the population.

The laboratory testing shows that the pathology is caused by elevated levels of unconjugated bilirubin in the blood due to the deficiency of conjugating enzyme.

26.3. Liver detoxification function. Biotransformation of xenobiotics and endogenous waste products

Liver detoxification function

Inside the hepatocytes, there are sophisticated mechanisms that have evolved over millions of years to break down toxic substances.

Every *xenobiotic*, that is drug, pesticide and other artificial chemical is broken down (metabolized) by enzyme pathways inside the liver cells.

Many of the toxic chemicals that enter the body are fat-soluble, which means they dissolve only in fatty or oily solutions and not in water. This makes them difficult for the body to excrete. Fat soluble chemicals have a high affinity for fat tissues and cell membranes, which are made of lipid substances. In the fatty parts of the body, mainly in adipose tissue, toxins may be stored for years, being released during times of exercise, stress or fasting. During the release of these toxins, symptoms such as headaches, poor memory, stomach pain, nausea, fatigue, dizziness etc. may occur.

The body's primary defense against metabolic poisoning is carried out by the liver. The liver has two major detoxification pathways designed to convert fat-soluble chemicals into water soluble chemicals which in the form of waste products may be easily excreted from the body via watery fluids such as bile and urine. In relation to drug metabolism in human body, the reactions considered here are referred to as the ***biotransformation of drugs.***

The two major pathways of xenobiotics detoxification are commonly called the Phase I and Phase II reactions.

Phase I

Phase I reactions (also termed *nonsynthetic reactions*) may occur by oxidation, reduction, hydrolysis, cyclization, and decyclization reactions.

Oxidation involves addition of oxygen (forming a negatively charged radical) or removal of hydrogen (forming a positively charged radical). The process of oxidation takes place in the liver under participating of the ***mixed function oxidases,*** or ***monooxygenases enzymes.*** These oxidative reactions typically involve a cytochrome P-450 haemoprotein, NADPH and oxygen.

The most common reaction catalysed by cytochrome P450 is a **monooxygenase reaction,** which consists in ***oxidative hydroxylation,*** i.e. insertion of one atom of oxygen into an organic substrate (RH) while the other oxygen atom is reduced to water:

$$R - H + O_2 + 2H^+ + 2e^- \longrightarrow R - OH + H_2O$$

As regards the intracellular localization of reactions under consideration, the smooth endoplasmic reticulum (ER) of the liver cells is the principal site of cytochrome P450-catalyzed xenobiotics metabolism. At the same time, although every biological tissue has some ability to metabolize drugs and other toxic substances.

Usually, inside ER membranes, multicomponent electron transfer chains, called P450-containing systems, are formed. And the overall reaction equation which takes places is as follows:

$$NADPH + R - H + O_2 \longrightarrow NADP^+ + R - OH + H_2O$$

Cytochrome P450 (abbreviated **CYP, P450,** infrequently **CYP450**) is a diverse superfamily of hemoproteins found in Bacteria, Archaea and Eukaryotes. The name P450 refers to the "pigment at 450 nm", so named for the characteristic Soret peak formed by absorbance of light at wavelengths near 450 nm when the heme iron is reduced (with sodium dithionite) and complexed to carbon monoxide.

In human body, cytochromes P450 are involved in metabolism of a plethora of both exogenous and endogenous compounds. To perform these biologically vital functions, inside hepatocytes ER membranes, electron transport chains including NADPH-cytochrome P450 reductase, which is FAD-containing flavoprotein, P450 itself and cytochrome b_5 are formed (Figure 26.3).

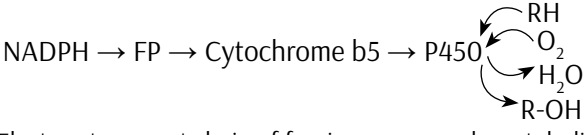


Figure 26.3. Electron transport chain of foreign compounds metabolism in hepatocytes ER membranes

These are commonly named in biochemistry as *electron transport chains*

of microsomal oxidation.

Cytochome P-450 is regarded to be the most versatile enzyme. So far at least 14 families of P450 are known to be encoded by human cells genome, and the number of enzyme isoforms discovered in human tissues counts to several tens of individual proteins.

The examples of individual oxidative reactions catalyzed by distinct cytochromes P-450

- ***Oxidative hydroxylation of aliphatic compounds:***
  - *alkanes and their derivatives*

$$R - CH_3 \rightarrow R - CH_2OH$$

  - *alkyl side radicals aromatic compounds*

$$C_6H_5 - CH_3 \rightarrow C_6H_5 - CH_2OH$$

- ***Oxidative hydroxylation of aromatic compounds:***

$$C_6H_6 \rightarrow C_6H_5 - OH$$

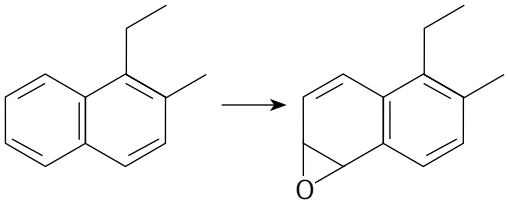
- ***Oxidative dealkylation, including:***
  - *N-dealkylation to yield amines and formaldehyde*

$$R - NH - CH_3 \rightarrow R{-}NH - CH_2OH \rightarrow R - NH_2 + HCHO$$

  - O-dealkylation to yield alcohol and formaldehyde

$$R - O - CH_3 \rightarrow R - O - CH_2OH \rightarrow R - OH + HCHO$$

- ***Epoxidation of cyclic compounds:***



The reactions examples presented play a central role in the biotransformation in human liver of multiple pharmaceutical drugs, industrially manufactured xenobiotics as well as endogenous steroids.

Phase II

This phase is called the conjugation pathway, whereby the liver cells add another substance (eg. glucuronate, methyl, acetyl, sulfate residues, cysteine, glycine or glutathione molecules) to a toxic chemical or drug.

Through conjugation, the lipophilic toxin, drug or steroid hormone becomes water-soluble, so it can then be excreted from the body via watery fluids such as bile or urine. In this way, liver renders multiple xenobiotics and products of their biotransformation much less harmful for the whole organism.

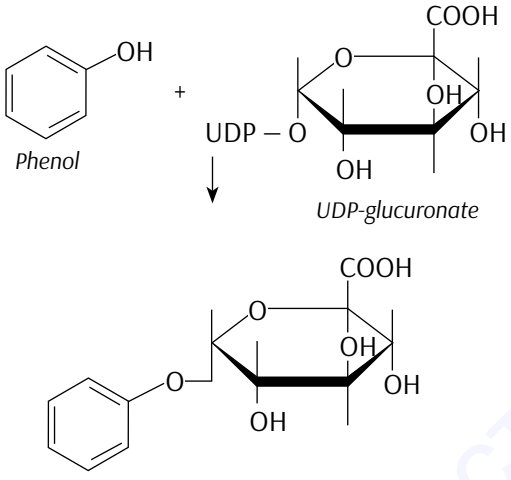
The principal Phase II pathways include glucuronide, sulfate, acetyl and glycine conjugations. The conjugation molecules are acted upon by specific enzymes to catalyse the reaction step. Individual xenobiotics and metabolites usually follow one or two distinct pathways. In some cases, phase I precedes Phase II reactions.

The examples of conjugation reactions are as follows.

- ***Glucuronidation reactions:***

$$R - OH + \text{UDP-glucuronate} \rightarrow \text{R-glucuronide} + \text{UDP}$$

UDP-glucuronic acid is the glucuronyl donor in the reaction, and a variety of glucuronyltransferases present in the liver and other human tissues are the enzymes.



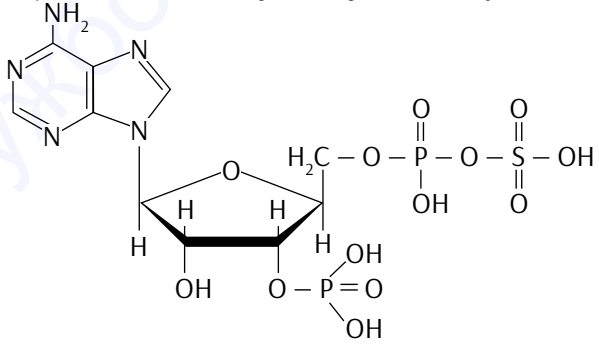
Phenol-O-glucuronide

- ***Sulfation reactions*:**

$$R - OH + 3`,5`\text{-PAPS} \rightarrow R - SO_3H + 3`,5`\text{-ADP}$$

The sulfate donor in sulfation reactions is **adenosine 3`-phosphate-5`-phosphosulfate (3`,5`-PAPS)** which is called "active sulfate".

The enzymes which transfer sulfate group from 3`,5`-PAPS to the hydroxyl group of an acceptor producing the sulfated derivative and 3`-phosphoadenosine 5`-phosphate are called ***sulfotransferases*** *(sulfokinases*).

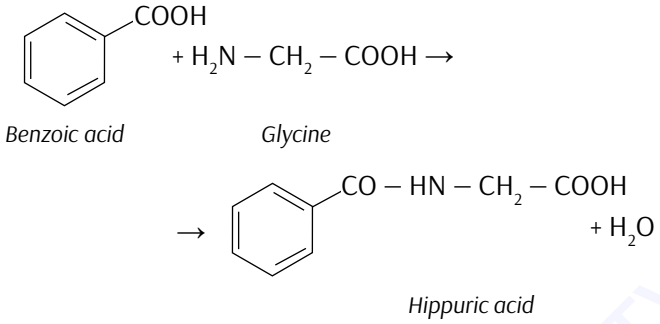


Adenosine 3`-phosphate-5`-phosphosulfate (3`,5`-PAPS)

- ***Conjugation with glycine.***

The reaction of clinical relevance is the detoxication of benzoic acid via

conjugation with glycine to give **hippuric acid** *(N-benzoylglycine)* which can be referred to as diagnostic test to estimate the activity of liver detoxification function:



This method is applicable in industrial medicine in screening liver function in workers exposed to xenobiotics such as toluene. The point of the test is loading of humans by sodium benzoate with next determination of the produced hippuric acid in urine.

The generalized scheme of liver detoxification metabolic pathways is presented in Figure 18.7.

Toxic Overload

If the Phase I and II detoxification pathways become overloaded, the excessive accumulation of toxic substances inside human body takes place. It is also noteworthy to accent that drug or other xenobiotic metabolism by cytochrome P-450 oxidative system can result in toxication or detoxication that is the activation or deactivation of the chemical. Moreover, during this process free radicals are produced which, if excessive, can additionally damage the liver cells.

As was mentioned earlier, many of xenobiotics are fat soluble substances and incorporate themselves into fatty tissues of the body where they may stay for years, if not for a lifetime. The brain and the endocrine (hormonal) glands are fatty organs, and are common sites for fat-soluble toxins to accumulate. This may result in symptoms of brain dysfunction and hormonal imbalances, such as infertility, breast pain, menstrual disturbances, adrenal gland exhaustion and early menopause. Many of these chemicals (eg. pesticides, petrochemicals) are carcinogenic and have been implicated in the rising incidence of many cancers.

Chapter 27.

BIOCHEMISTRY OF TOOTH AND SALIVA

27.1. Anatomy and physiology of human teeth

Teeth are anatomic structures of human body that are placed in the jaws and used to tear, scrape and chew food. Specifically, the bottom teeth are used more for the grinding of food and the top front teeth are mainly used for biting.

After birth, human newborns usually have 20 ***primary teeth*** (also called ***deciduous*****,** baby, or milk teeth). Among primary teeth 10 are found in the upper jaw *(lat. – maxilla)* and the other 10 in the lower jaw *(lat. – mandible)*.

Among 32 ***permanent teeth*****,** which adults have, 16 are found in the maxilla with the other 16 in the mandible.

Teeth are classified as ***incisors*****,** ***canines*****,** and ***molars*****.**

The maxillary teeth are the maxillary central incisor, maxillary lateral incisor, maxillary canine, maxillary first premolar, maxillary second premolar, maxillary first molar, maxillary second molar, and maxillary third molar.

The mandibular teeth are the mandibular central incisor, mandibular lateral incisor, mandibular canine, mandibular first premolar, mandibular second premolar, mandibular first molar, mandibular second molar, and mandibular third molar.

Third molars commonly develop in the age of human maturity and are sometimes called “wisdom teeth”. These may never erupt into the mouth or form at all.

Human tooth anatomy

The type of tooth present in humans, also in dogs, cats and pigs, con-

sists of a **crown** above the gingiva, a constricted **neck** at the gum line, and a **root** embedded in the jawbone A tooth may have just one root or multiple roots (Figure 27.1–27.2).

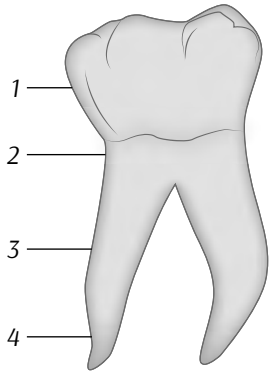


Figure 27.1. General image of human tooth: 1 – crown of the tooth (corona dentis); 2 – neck of the tooth (collum dentis); 3 – root of the tooth; 4 – apex radicis dentis

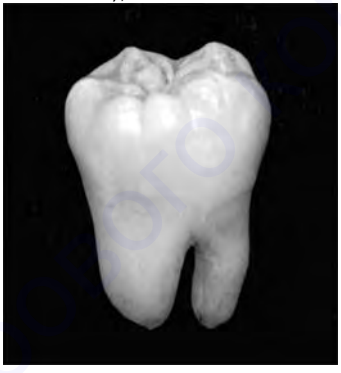


Figure 27.2. Human molar wisdom tooth

The crown of the tooth is encased in **enamel** and the root in **cementum.** Inside of a tooth there is the **pulp** (Figure 27.3).

Enamel is the hardest substance in the body being densely packed with hydroxyapatite (mineral) crystal and heavily mineralized with calcium salts.

Dentin, a bonelike material, is under the enamel and makes up most of the tooth.

Cementum is calcified connective tissue which borders with ***periodontium*** and is attached to alveolar bone.

Pulp (the pulp cavity) includes blood vessels, lymphatics and nerves.

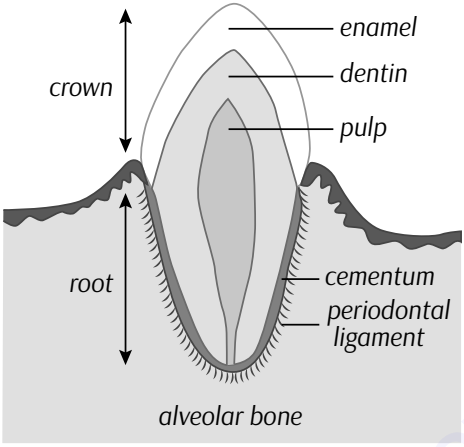


Figure 27.3. Tooth structure. Cementum, periodontal ligaments and alveolar bone are presented

27.2. Biochemical content and molecular organization of dental tissues

Biochemical organic and inorganic components of different tissues of human tooth are presented in Table 27.1.

Table 27.1. Biochemical contents of human tooth (after S. Rapoport, 1964)

Tooth tissue	Enamel	Dentin	Cementum	Pulp
% from wet tissue mass				
Water	2,3	13,2	32	30–40
Organic compounds	1,7	17,5	22	40
Inorganiccompounds	96,0	69,0	46	20–30
% from dry tissue mass				
Ca	36,1	35,3	35,5	30
Mg	0,5	1,2	0,9	0,8

Na	0,2	0,2	1,1	0,2
K	0,3	0,1	0,1	0,1
P	17,3	17,1	17,0	25,0
F	0,03	0,02	0,02	0,01

27.3. Tooth enamel: properties, biochemical contents

Tooth enamel is the hardest and most highly mineralized substance of the human body and with *dentin, cementum*, and *dental pulp* is one of the four major tissues which make up the tooth. It is the normally visible dental tissue of a tooth and is normally supported by underlying dentin.

The normal color of enamel varies from light yellow to grayish white. At the edges of teeth where there is no dentin underlying the enamel, the color sometimes has a slightly blue tone. Since enamel is semitranslucent, the color of dentin and any restorative dental material underneath the enamel strongly affects the appearance of a tooth. Enamel varies in thickness over the surface of the tooth and is often thickest at the cusp, up to 2.5 mm, and thinnest at its border, which is seen clinically as the *cementoenamel junction*.

96 % of enamel consists of mineral, with water and organic material composing the rest of the substance.

Organic components of enamel

Unlike dentin and bone, *enamel* does not contain collagen. Instead, it has two unique classes of proteins called *amelogenins* and *enamelins*. While the role of these proteins is not fully understood, it is believed that they aid in the development of enamel by serving as a framework support, among other functions.

Inorganic components of ename

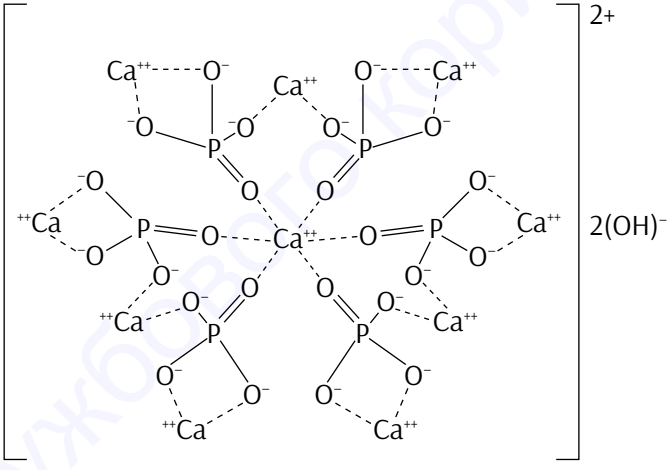
Enamel's primary mineral is *hydroxylapatite*, also called *hydroxyapatite*,

is a mineral naturally occurring in human tooth in the form of *calcium apatite* with the formula $Ca_5(PO_4)_3(OH)$. It is usually written $Ca_{10}(PO_4)_6(OH)_2$ to denote that the crystal unit cell comprises two molecules.

Apatite is one of few minerals that are produced and used by biological micro-environmental systems. A relatively rare form of apatite in which most of the OH groups are absent and containing many carbonate and acid phosphate substitutions is a large component of *bone* material.

Fluorapatite (or fluoroapatite) is more resistant to acid attack than is hydroxyapatite. For this reason, toothpaste typically contain a source of fluoride anions (e.g. sodium fluoride, sodium monofluorophosphate). Similarly, fluoridated water allows exchange in the teeth of fluoride ions for hydroxyl groups in apatite. Too much fluoride results in dental fluorosis and/or skeletal fluorosis.

Hydroxylapatite *is the hydroxyl end member of the complex apatite group. The OH^- ion inside hydroxylapatite structure can be replaced by fluoride, chloride or carbonate. It crystallizes in the hexagonal crystal system. It has a specific gravity of 3.08 and is 5 on the Mohs hardness scale. Pure hydroxylapatite powder is white. Naturally occurring apatites can however also have brown, yellow or green colorations, comparable to the discolorations of dental fluorosis.*



Molecular architectonics of tooth hydroxylapatite $Ca_{10}(PO_4)_6(OH)_2$

Carbonated-calcium deficient hydroxylapatite is the main mineral of which *dental enamel* and *dentin* are comprised. Seventy percent of bone is made up of hydroxylapatite. Hydroxyapatite crystals are also found in the small calcifications (within the pineal gland and other structures) known as *corpora arenacea* or "brain sand".

The large amount of minerals in enamel accounts not only for its strength but also for its brittleness. Tooth enamel ranks 5 on *Mohs hardness scale*.

Dentin, less mineralized and less brittle, 3–4 in hardness, compensates for enamel and is necessary as a support for enamel.

Enamel rods

The basic structural unit of enamel is called an *enamel rod*. Measuring 4–8 μm in diameter an enamel rod, formerly called an enamel prism, is a tightly packed mass of hydroxyapatite crystals in an organized pattern. In cross section, it is best compared to a keyhole, with the top, or head, oriented toward the crown of the tooth, and the bottom, or tail, oriented toward the root of the tooth.

The arrangement of the crystals within each enamel rod is highly complex. Both *ameloblasts* (the cells which initiate enamel formation) and *Tomes' processes* affect the crystals' pattern. Enamel crystals in the head of the enamel rod are oriented parallel to the long axis of the rod. When found in the tail of the enamel rod, the crystals' orientation diverges slightly from the long axis.

The arrangement of enamel rods is understood more clearly than their internal structure. Enamel rods are found in rows along the tooth, and within each row, the long axis of the enamel rod is generally perpendicular to the underlying dentin. In permanent teeth, the enamel rods near the *cementoenamel junction* (CEJ) tilt slightly toward the root of the tooth. Understanding enamel orientation is very important in restorative dentistry, because enamel unsupported by underlying dentin is prone to fracture.

The area around the enamel rod is known as *interrod enamel*. Interrod enamel has the same composition as enamel rod, however a histologic distinction is made between the two because crystal orientation is different in each. The border where the crystals of enamel rods and crystals of interrod enamel meet is called the *rod sheath*.

27.4. Dentin: structure; biochemistry. Periodontum. Cementum

27.4.1. Dentin

Dentin is a bone-like substance that makes up the protective middle layer of each tooth.

Dentin is the calcified tissue of teeth, making up the majority of the tooth weight and volume, and giving these organs much of their overall shape.

By appearance, *dentin* is the porous, yellow-hued material which is covered by enamel on the crown and cementum on the root and surrounds the entire pulp. Yellow in appearance, it greatly affects the color of a tooth due to the *translucency* of enamel.

Dentin, which is less mineralized and less brittle than enamel, is necessary for the support of enamel. Because it is softer than enamel, it decays more rapidly and is subject to severe cavities if not properly treated, but due to its elastic properties it is a good support for enamel. Its flexibility prevents the brittle enamel fracturing.

Chemically, dentin is made up of 70 % inorganic materials (mainly *hydroxylapatite* and some non-crystalline amorphous calcium phosphate), 20 % organic materials (90 % of which is *collagen* type 1 and the remaining 10 % ground substance, which includes dentine-specific proteins – see below), and 10 % water (which is absorbed on the surface of the minerals or between the crystals).

Dentinal tubules

Dentin consists of microscopic channels, called ***dentinal tubules,*** which span the entire thickness of dentin and radiate outward through the dentin from the pulp to the exterior cementum or enamel border.

From the outer surface of the dentin to the area nearest the pulp, these tubules follow an S-shaped path. The diameter and density of the tubules are greatest near the pulp. Tapering from the inner to the outermost surface, they have a diameter of 2.5 μm near the pulp, 1.2 μm in the middle of the dentin, and 0.9 μm at the *dentino-enamel junction* (DEJ). Their density is 59,000 to 76,000 per square millimeter near the pulp, whereas the density is only half as much near the enamel.

Dentinal tubules contain fluid and cellular structures. As a result, dentin has a degree of permeability which can increase the sensation of pain and the rate of tooth decay.

Within the tubules, there is an *odontoblast process*, which is an extension of an odontoblast, and dentinal fluid, which contains a mixture of albu-

min, transferrin, tenascin and proteoglycans. In addition, there are branching canalicular systems that connect to each other. These branches have been categorized by size, with major being 500–1000 μm in diameter, fine being 300–700 μm, and micro being less than 300 μm. The major branches are the terminal ends of the tubules. About every 1–2 μm, there are fine branches diverging from dentinal tubules at 45 degree angles. The microtubules diverge at 90 degree angles.

Dentinogenesis

The formation of dentin, known as *dentinogenesis*, begins prior to the formation of enamel and is initiated by the *odontoblasts* of the pulp. During dentinogenesis, odontoblasts secrete an unmineralized, type I collagen-rich extracellular matrix (ECM), termed *predentin*.

Predentin is transformed to ***dentin*** when apatite crystals are deposited. This process of *biomineralization* is dynamic, involving multiple changes that occur within the window of time that predentin is formed and it is converted to dentin.

Odontoblasts

Odontoblasts are elongated cells, which initially synthesize an uncalcified matrix, ***predentin***. This uncalcified connective tissue is then transformed into dentin at a distinct site away from the periphery of odontoblasts.

Unlike enamel, dentin continues to form throughout life and can be initiated in response to stimuli, such as tooth decay or *dental attrition*.

There are different types of dentin, differentiated by appearance and stage of development:

- *primary dentin* forms most of the tooth;
- *secondary dentin* develops after root formation is complete and forms much slower than primary dentin;
- *tertiary dentin* forms as a biological response to stimuli.

Biochemistry of Dentin Matrix Proteins

Collagen

The major component of the organic matrix of dentine is **collagen.** Aside from their role as structural components of teeth and bones, the collagen molecules of these tissues may be actively involved as heterogeneous catalysts for nucleation and deposition of hydroxyapatite. Biochemical studies showed ***dentine collagen*** to be essentially *type I*, albeit with unique post-translational modifications, notably covalent attachment of phosphate groups and increased lysine hydroxylation and intermolecular cross-linking, distinguishing it from the type I collagen of skin.

Non-collagenous proteins of dentin matrix

In addition to type I collagen, the extracellular matrix of dentin contains a number of *non-collagenous proteins* (NCPs).

Dentin NCPs include sialic acid-rich (SA-rich) proteins found both in bone and in dentin: *dentin sialophosphoprotein (DSPP), bone sialoprotein, osteopontin, dentin matrix protein 1 (DMP1)* and *bone acidic glycoprotein-75 (BAG-75)*.

Chemically, dentin NCPs are phosphorylated glycoproteins which are believed to play key biological roles in the mineralization of dentin. Some functions of these phosphorylated glycoproteins are dependent on the nature and extent of posttranslational modifications such as proteolytic processing, phosphorylation and glycosylation.

Dentin sialophosphoprotein

Dentin sialophosphoprotein is the protein encoded by DSPP gene. Soon after it is produced, the protein is cut into three smaller peptides – components of dentin extracellular matrix: ***dentin sialoprotein***, ***dentin phosphoprotein*** (also known as ***phosphophoryn***) and ***dentin glycoprotein***.

These three DSPP-derived proteins are essential for normal tooth development. *Dentin phosphoprotein* and *dentin glycoprotein* are thought to be involved in the normal hardening of collagen, the most abundant protein in dentin. Specifically, these proteins play a role in the deposition of mineral crystals among collagen fibers (mineralization).

The DSPP gene is also active at low levels in the inner ear, and may play a role in normal hearing (see below)!

Genetic disorders in the DSPP gene

Dentinogenesis imperfecta

The mutations in the DSPP gene cause inherited disease called ***dentinogenesis imperfecta.*** Dentinogenesis imperfecta affects an estimated 1 in 6,000 to 8,000 people. Most of these mutations change single protein building blocks (amino acids) in dentin sialophosphoprotein. These genetic changes are responsible for two forms of this disorder, type II and type III.

Dentinogenesis imperfecta is a disorder of tooth development. This condition causes the teeth to be discolored (most often a blue-gray or yellow-brown color) and translucent. Teeth are also weaker than normal, making them prone to rapid wear, breakage, and loss.

Three types of *dentinogenesis imperfecta* with similar dental abnormalities were discovered. Type I occurs in people who have *osteogenesis imperfecta*, a genetic condition in which bones are brittle and easily broken. Dentinogenesis imperfecta type II and type III usually occur in people without other inherited disorders. A few families with type II have progressive hearing loss in addition to dental abnormalities.

Dentin dysplasia, type II. A 16T-G transversion in the DSPP gene in a family with dentin dysplasia type II was reported. The resultant *asp6-to-tyr* (D6Y) substitution in the hydrophobic signal peptide domain causes a failure of translocation of the encoded proteins into the endoplasmic reticulum. This abnormality results in a loss of function of both dentin sialoprotein and dentin phosphoprotein.

Bone sialoprotein (alternative titles: *Bone sialopropein II, Integrin-binding bone sialoprotein, ibsp, bsp, bspII, spII).*

Bone sialoprotein (BSP) is a component of mineralized tissues such as bone, dentin, cementum and calcified cartilage. BSP is synthesized by skeletal-associated cell types, including hypertrophic chondrocytes, osteoblasts, osteocytes, and osteoclasts.

Bone sialoprotein is an acidic glycoprotein of approximately 70 kD that undergoes extensive posttranslational modifications. It constitutes approximately 8–12 % of the noncollagenous proteins in human bone and cementum.

BSP has been demonstrated to be extensively post-translationally modified, with carbohydrates and other modifications comprising approximately 50 % of the molecular weight of the native protein. These modifications, which include N- and O-linked glycosylation, tyrosine sulfation and serine

and threonine phosphorylation, make the protein highly heterogeneous

Osteopontin was detected in dentin extracts, with a relative level less than one-seventieth of that in bone.

Dentin matrix protein 1 **(DMP1)** and *bone acidic glycoprotein-75 (BAG-75)* are additional acidic proteins of dentin.

27.4.2. Periodontium

Periodontium refers to the specialized tissues that both surround and support the teeth, maintaining them in the maxillary and mandibular bones. The word comes from the Greek terms *peri-*, meaning "around" and *-odons*, meaning "tooth". The following four tissues make up the periodontium (Figure 27.4):

- *Cementum.*
- *Periodontal ligament.*
- *Alveolar bone.*
- *Gingiva or gums.*

Cementum is a specialized calcified substance (variety of connective tissue) covering the anatomic root of a tooth. Cementum anchors the *periodontal ligament* (see below) to the root of the tooth. Visually, cementum is bone-like and light yellow substance.

Cementum is excreted by cells called *cementoblasts* within the root of the tooth and is thickest at the root apex. It is softer than enamel and dentin due to being less mineralized. Its main function is to anchor the tooth by attaching it to the alveolar bone via the periodontal ligaments (Figure 27.4).

Cementum plays an important role in forming new teeth. Hence, its bottom surface is tangent to the periodontal ligaments running through the jaw (via collagen fibers), and the upper portion of the surface is firmly cemented to the dentin of the tooth.

The chemical makeup of cementum is similar to that of bone, but it lacks vascularization. Volumetrically, it is approximately 45 % inorganic material (mainly hydroxyapatite), 33 % organic material (mainly collagen type1) and 22 % water.

Periodontal ligament

The **periodontal ligament,** commonly abbreviated as the PDL is a group

of specialized connective tissue fibers that essentially attach a tooth to the alveolar bone within which it sits.

The PDLs are about 0.2 mm in width, and these dimensions decrease with age. Fibers, which constitute the PDL, are composed primarily of type I collagen, although type III fibers are also involved. Compared to most other ligaments of the body, these are highly vascularized. These fibers help the tooth withstand the substantial compressive forces which occur during chewing.

Another function of the PDL is to serve as a source of proprioception, , so that the brain can detect the forces being placed on the teeth and react accordingly. To achieve this end, there are pressure sensitive receptors within the PDL which allow the brain to discern the amount of force being placed on a tooth during chewing.

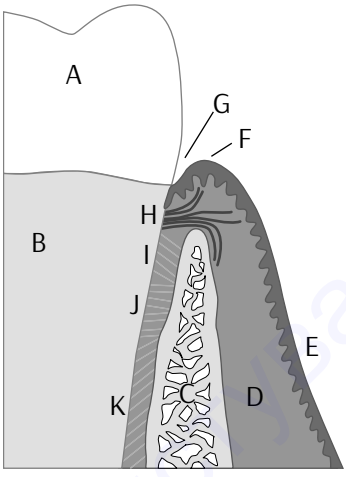


Figure 27.4. Periodontium: cementum, periodontal ligameny, alveolar bone, gingiva. The cementum is the surface layer of the tooth root (B). It is attached to the alveolar bone (C) by the fibers of the periodontal ligament and to the soft tissue of the gingiva by the gingival fibers (H)

Types of fibers in periodontal ligaments

There are several distinctive types of PDL fibers that connect the *cementum* surface of the tooth root to the adjacent cortical surface of the *alveolar bone*, and namely: *alveolar crest fibers; horizontal fibers; oblique fibers; apical fibers; interradicular fibers.*

27.5. Tooth pulp: physiology, metabolism

The dental ***pulp*** is the structure in the center of a tooth made up of living soft tissue and cells called *odontoblasts.*

Functions of the pulp:

- producing dentin (by the odonyoblasts cells);
- nutritive function: the pulp keeps the organic components of the sur-

rounding mineralized tissue supplied with moisture and nutrients;
- sensory function: extremes in temperature, pressure, or trauma to the dentin or pulp are perceived as pain;
- protective function: the formation of reparative or secondary dentin.

Biochemistry of the pulp

The human tooth pulp is characterized by highly active metabolism of carbohydrates, lipids and aminoacids.

Bioenergetic demands of pulp cells are provided both with glycolysis and aerobic oxidation of glucose. The essential source of metabolic energy for biological functions of the pulp is glycogen which content diminishes under different pathological conditions.

The interconversions of pyruvate and lactate in the course of anaerobic and aerobic glycolysis in pulp cells depend drastically on the catalytic properties of the enzyme lactate dehydrogenase (LDH) which is presented in the pulp by different isoenzymes (LDH-1, LDH-2, LDH-3, LDH-4 and LDH-5).

Intensive turnover of phospholipids, which is inherent to the pulp, is due mainly to the everlasting processes of new cellular membranes construction and renovation.

Biosynthesis of proteins as well as nucleic acids DNA and RNA occurs in pulp cells with great activity. Using the up-to-date proteomics methodology about 96 different enzymic and structurak proteins were detected in human dental pulp.

Clinical significance

An inflammation of a pulp is known as pulpitis. Pulpitis can be extremely painful and in serious cases calls for root canal therapy or the removal of the infected tooth or teeth.

27.6. Tooth pathology. Caries: biochemical aspects

Destruction of Enamel. Caries

The high mineral content of enamel, which makes this tissue the hardest

in the human body, also makes it susceptible to a *demineralization process* which often occurs as ***dental caries.***

Demineralization occurs for several reasons, but the most important cause of tooth decay is the ingestion of sugars which are metabolized to give fctive carboxylic acids, which can dissolve tooth enamel:

$$Ca_{10}(PO_4)_6(OH)_2(s) + 8H^+(aq) \rightarrow 10Ca^{2+}(aq) + 6HPO_4^{2-}(aq) + 2H_2O(l)$$

A significant role in tooth decay, and consequently in enamel destruction, play sugars from candies, soft drinks, and fruit juices. The mouth contains a great number and variety of bacteria, and when sucrose, the most common of sugars, coats the surface of the mouth, some intraoral bacteria interact with it and form lactic acid, which decreases the pH in the mouth.

Bruxism

Bruxism, also known as clenching of or grinding on teeth, destroys enamel very quickly. The wear rate of enamel, called *attrition*, is about 8 micrometers a year from normal factors. Hence, the destructive mechanical forces, as found in bruxism, can cause irreversible damage to the enamel.

27.7. Biochemical components and functions of saliva

Saliva is the watery and usually frothy substance produced in the mouths of humans from the salivary glands. Human saliva is composed mostly of water (99.5 %), and includes electrolytes, mucus, various enzymes and some antibacterial substances. At initial physiological step of food digestion, the enzymes in the saliva break down some of the starch and fats.

Saliva in digestion

Saliva is a fluid containing the active enzyme *amylase* that breaks some starches down into maltose and dextrin. Thus, digestion of food begins in the mouth. Salivary glands also secrete enzymes (salivary *lipase*) to start fat digestion.

Daily salivary output

The estimates about the amount of saliva that is produced in a healthy person per day ranges from 0.75 liters per day to 1.5 liters per day.

Biochemical Contents of Human saliva

Produced in salivary glands, human saliva is 98 % water, but it contains many important substances, including electrolytes, mucus, antibacterial compounds and various enzymes. The contents of major inorganic and bio-organic substances in human saliva is as follows:

Water – up to 98–99 %.

Electrolytes:

- 2–21 mmol/L sodium (lower than blood plasma);
- 10–36 mmol/L potassium (higher than plasma);
- 1.2–2.8 mmol/L calcium;
- 0.08–0.5 mmol/L magnesium;
- 5–40 mmol/L chloride (lower than plasma);
- 25 mmol/L bicarbonate (higher than plasma);
- 1.4–39 mmol/L phosphate.

Mucus. Mucus in saliva mainly consists of mucopolysaccharides and glycoproteins.

Antibacterial compounds – immunoglobulin A, lysozyme.

Enzymes. There are the following major enzymes in saliva:

α-amylase (EC 3.2.1.1) – enzyme starts the digestion of starch before the food is even swallowed. It has a pH optima of 7.4.

lingual lipase (EC 3.1.1.3). lingual lipase has a pH optimum ~4.0 so it is not activated until entering the acidic environment of the stomach.

Minor enzymes of saliva include salivary acid phosphatases (EC 3.1.3.2), N-acetylmuramyl-L-alanine amidase (EC 3.5.1.28), NAD(P)H-dehydrogenase (EC 1.6.99.2), salivary lactoperoxidase (EC 1.11.1.7), superoxide dismutase (EC 1.15.1.1), glutathione transferase (EC 2.5.1.18), glucose-6-phosphate isomerase (EC 5.3.1.9), tissue kallikrein (EC 3.4.21.35).

Chapter 28.

BIOCHEMISTRY OF MUSCLE. MOLECULAR PHYSIOLOGY OF MUSCLE CONTRACTION

28.1. Muscle tissue: general characteristics of functions, structure and biochemistry

Muscle is the contractile tissue of the body and is derived from the mesodermal layer of embryonic germ cells. Its physiological function is to produce force and cause motion, either locomotion or movement within internal organs.

Much of muscle contraction is necessary for the survival of the whole organism and occurs without conscious thought, like the contraction of the heart or peristalsis, which pushes food through the digestive system. Voluntary muscle contraction is used to move the body and can be finely controlled, such as movements of the finger or gross movements of the body limbs.

Three general types of muscle:

- **Skeletal muscles** or "voluntary muscles" are anchored by tendons to bone and are used to realize skeletal movement such as locomotion that is moving of the body from place to place.
- **Smooth muscles** or "involuntary muscles" is found within structures such as intestines, blood vessels etc.
- **Cardiac muscle** (*lat. – myocardium*) is also an "involuntary muscle" but it's a specialized kind of muscle found only within the heart.

Myocytes, myofibrils

Muscle is composed of multinucleate, cylindrical cells or ***myocytes,*** also known as "muscle fibers". The contractile elements of myocytes (or muscle fibers) are myofibrils which are composed of special contractile proteins, mainly *actin* and *myosin*. Actin and myosin, in complex, make up ***myofilaments*** that constitute about 60 % of total muscle fiber protein.

The plasma membrane of muscle fibers is known as the ***sarcolemma.*** The interior space of myocytes is named s the **sarcoplasm,** containing myofibrils and all the usual subcellular elements.

Skeletal muscles

The discrete skeletal muscles are formed of very long **muscle fibers** which lengths reach more than 30 cm, and comprise about 40 % of the mass of the average human body. The characteristic feature of skeletal muscle cells is the highly developed system of sarcoplasmic reticulum which is a special type of smooth ER that serves as the reservoir to store and pump calcium ions (Ca^{2+}).

Skeletal and cardiac muscle are called "striated" muscles because their myofibrils are partitioned into sarcomeres which are arranged end to end. As distinct from smooth muscle, **s**keletal and cardiac muscles are often used in short, intense bursts, whereas smooth muscles sustain longer or even near-permanent contractions.

Up to this date, the biochemical mechanisms of muscle activities are best understood for skeletal muscle, hence the discussion below is focused mainly on this type of muscle. The biochemical characteristics that differentiate smooth and cardiac muscle are reviewed shortly.

Diagrammatic presentation of a typical skeletal muscle is shown in the Figure 28.1. It can be seen how **actin thin filaments** and **myosin thick filaments** are arranged to form the myofilaments of a sarcomere, continuing with the formation of **myofibrils** from many **myofilaments**.

Muscle bioenergetics

The principal task of the muscles is to perform mechanical work at the expense of ATP.

In skeletal muscles, ATP is synthesized via oxidation of a range of different metabolites, including glucose, fatty acids and ketone bodies. During

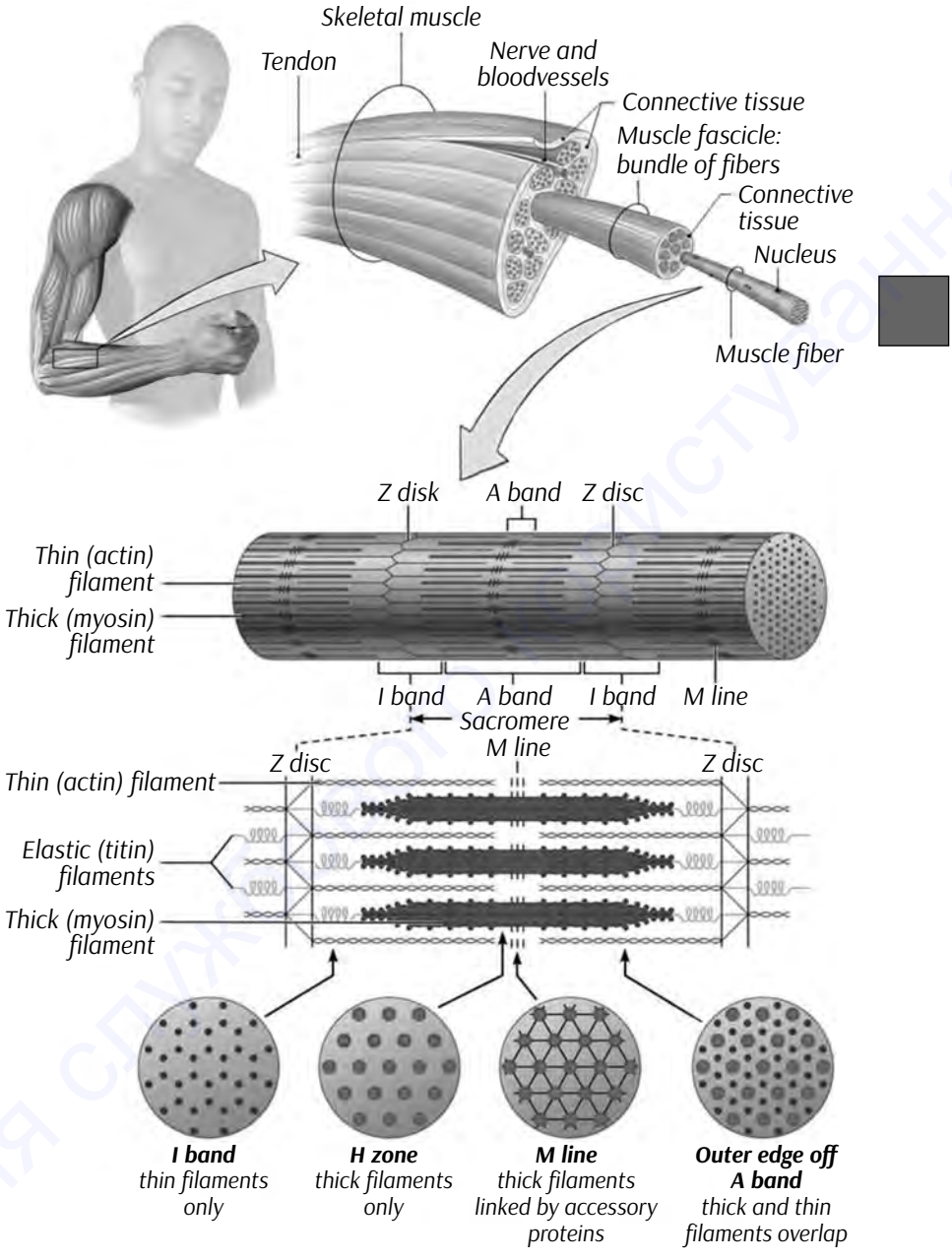
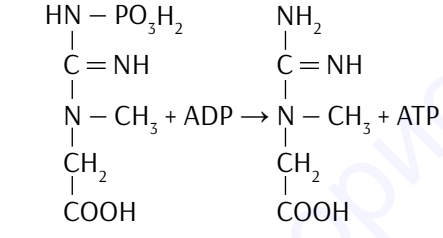


Figure 28.1. Structural organization of skeletal muscle

light exercise, these fuels are completely degraded to CO_2 and H_2O. As this process is oxygen-dependent, muscle contraction leads to a strong increase in oxygen consumption which is a characteristic feature of muscle physiology.

After the onset of contraction, the ATP present in resting muscles would be consumed in less than a second were it not for highly efficient metabolic pathways that continuously replenish ATP, among them the following:

(a) Rapid replenishment of ATP can be achieved by transferring a phosphate group from **creatine phosphate** to ADP in reaction catalyzed by enzyme ***creatine kinase.***



Creatine phosphate *Creatine*

Creatine phosphate is an energy reserve typical of muscle tissue. It contains an "energy-rich" bond between the phosphate residue and the nitrogen of the guanido group. However, this store lasts for a few seconds.

(b) Another mechanism for short-term increase in the level of ATP is conversion of ADP to ATP and AMP, catalyzed by ***adenylate kinase (myokinase):***

$$ADP + ADP \rightarrow ATP + AMP.$$

The AMP produced in this reaction can subsequently be converted to IMP by ***AMP deaminase.***

(c) The most important long-term reserve in the muscles is **glycogen**. Glycogen is synthesized from glucose in resting muscle, and can account for up to 2 % of muscle mass.

As in the liver, mobilization of glycogen in muscles occurs by phosphorolysis of polysaccharide. The glucose-1-phosphate formed is isomerized to glucose 6-phosphate, which yields ATP either via oxidative phosphorylaion or through conversion to lactate by anaerobic glycolysis (the last occurs when oxygen is in short supply).

(d) **Oxidative phosphorylation** is the most efficient pathway for the generation of ATP, and in cardiac muscle it supplies almost all of the ATP required for the continuous contraction and relaxation of the heart.

This is why the heart is extremely dependent on a constant and adequate supply of oxygen. Namely therefore *heart attacks* occur as a result of interruption of the myocardium oxygen supply.

- In *slow contracting skeletal muscles*, oxygen supply is facilitated by the presence of **myoglobin,** the hemoglobin-like protein which is able to store oxygen. The presence of myoglobin imparts the muscle tissue its characteristic red color.
- *Rapidly contracting muscles* do not have red myoglobin, and therefore appear white (see below). They show a preference for **anaerobic glycolysis** to satisfy their high ATP requirement during heavy exercise. Although they are capable of rapid contraction, these muscles can only continue to work for short periods of time, because of the relatively low ATP yield of glycolysis. After a while, the muscles become exhausted as a result of the decline in the pH of the muscle cells. The lactate that accumulates during anaerobic glycolysis is released into the blood and transported to the liver, where it is, in part, utilized for the resynthesis of glucose in the *Cori cycle.*

Molecular organization of the sarcomere and myofilaments. Contractile proteins in muscle

Structure of sarcomeres

Sarcomeres represent the minimal contractile unit of a muscle. It is the coordinated contraction and elongation of millions of sarcomeres in a muscle that underlies mechanical skeletal activity.

When examined by electron microscopy, it can be seen that sarcomeres are composed mostly from two types of overlapping structures, and namely **thick filaments** lying parallel to one another, and **thin filaments** that stretch in the spaces between the former (Figure 28.2). The thick filaments are confined to the A band, they contain the protein myosin. The thin filaments lie in the I band and extend into the A band apart from H zone. The major biochemical component of thin filaments is filamentous protein **actin**,

and the minor proteins are **tropomyosin** and **troponin.**

The proteins at the junctions between sarcomeres are attached to Z lines (or Z disks), which contain **α-actinin.** Thus, each sarcomere extends along a myofibril from one Z line to the next Z line.

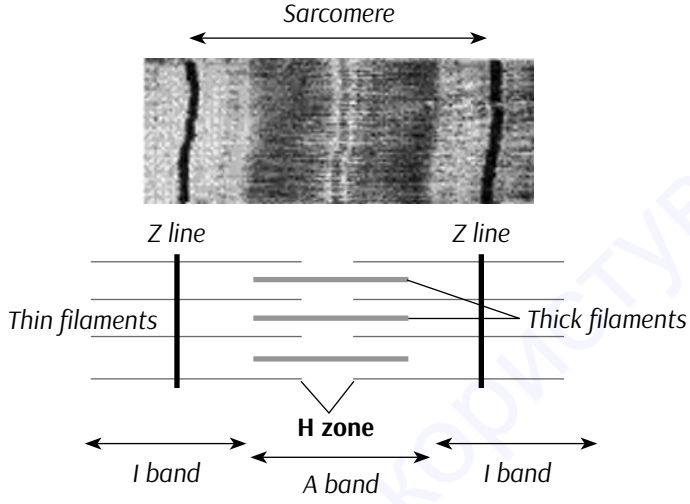


Figure 28.2. Sarcomere structure (fragment of electron microfotograph and schematic image)

As can be evident from the cross section presentation (Figure 28.3), in the A band, each thick filament is surrounded by six thin filaments, and the thin filament lies symmetrically between three thick filaments. This makes the hexagonal array of myofilaments as the basis of skeletal muscle sarcomere molecular architecture.

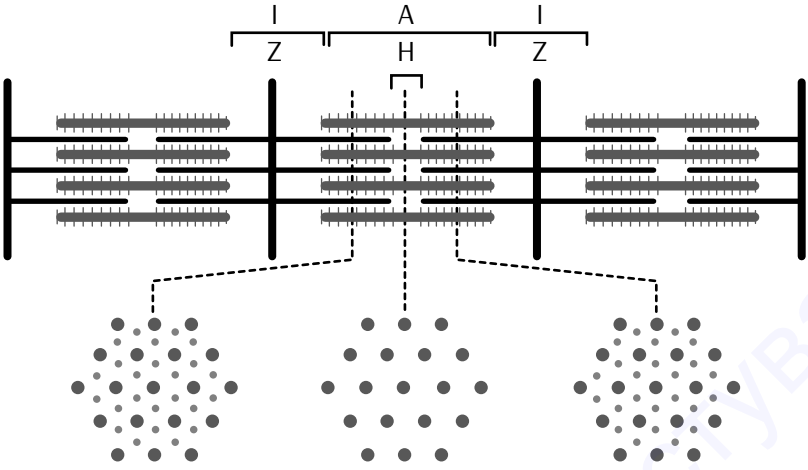


Figure 28.3. Molecular architecture of sarcomere. The secondary hexagonal array of filaments organization is shown

Contractile proteins in muscle

As was stated already, each sarcomere of the muscle cell is composed of hundreds of filamentous protein aggregates, each known as a ***myofilament.*** And two kinds of myofilaments are biochemically identifiable on the basis of protein composition, and especially:

- ***thick myofilaments*** are composed of several hundred molecules of a fibrous protein known as **myosin.**
- ***thin myofilaments*** are composed mainly of two helically interwound, linear polymers of a globular protein known as **actin.**

Myosin. Organization of myosin thick filaments

Solubilized myosin molecules are long fibrous proteins with a molecular weight of about 460 kD. The schematic image of a myosin molecule is given in the Figure 28.4.

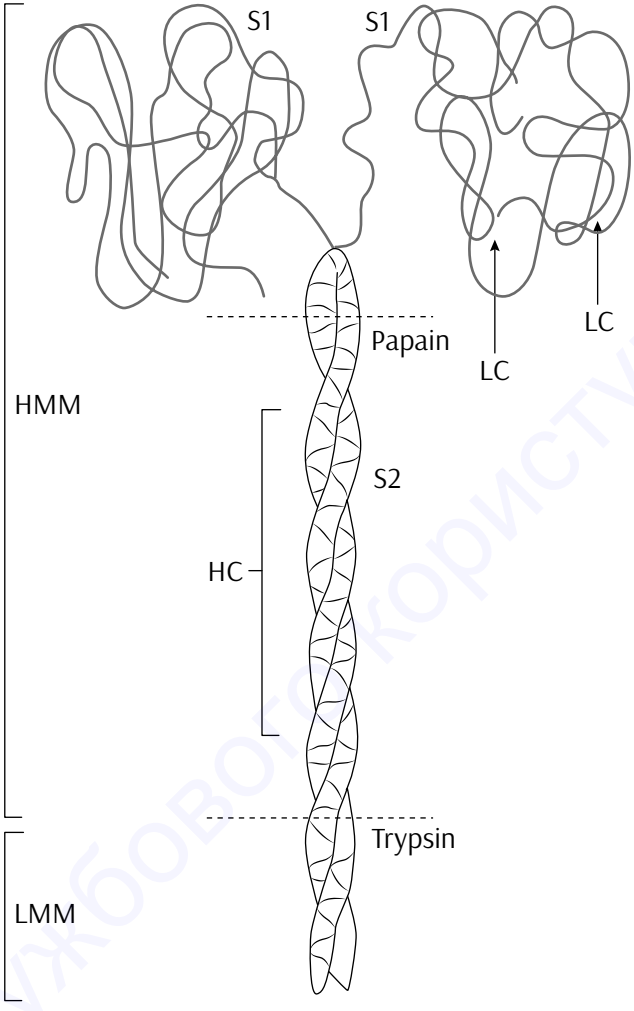


Figure 28.4. Structure of myosin molecule

Each myosin molecule is an asymmetric hexamer formed of 6 polypeptides, and namely two very large, heavy chains (HC), and two pairs of smaller, light chains (LC). The molecular mass of each heavy chain is about 200 kD, and of light chains approximately 20 kD. Thus, in a given muscle fiber two subunits identical are present, although there are different HC isoforms in different types of muscle fibers.

Heavy chains of myosin contain a long fibrous C-terminal α-helical do-

main (1,300 amino acids) and a globular N-terminal domain of about 800 amino acids. The two linear domains are helically interwound, giving the whole molecule a long, rigid superhelical structure with two globular headpieces.

Several functionally important landmarks exist on the myosin molecule. Trypsin digestion cleaves myosin into 2 portions: 1 containing both globular headpieces and some superhelical region, and the other consisting of the remaining superhelical portion of the carboxy terminus. The portion containing the headpiece is known as *heavy meromyosin* (HMM; molecular weight 350 kD). The C-terminal fragment is known as *light meromyosin* (LMM; molecular weight 125 kD).

A second proteolytic landmark is susceptible to papain. Papain cleaves a site very close to the globular headpieces; these then separate to form 2 subfragments, each known as an S 1 (for *subfragment* 1). The remaining superhelical portion of the molecule is known as S 2. The ATPase activity of the myosin is associated with the S 1 units.

The whole thick filament is composed of approximately 400 myosin molecules, 200 arrayed on either side of the central region of the bundle which is sometimes called as M band (Figure 28.5). The individual myosin molecules are maintained in bundles by a special C protein (clamp protein) and the hydrophobic interactions of the myosin molecules themselves.

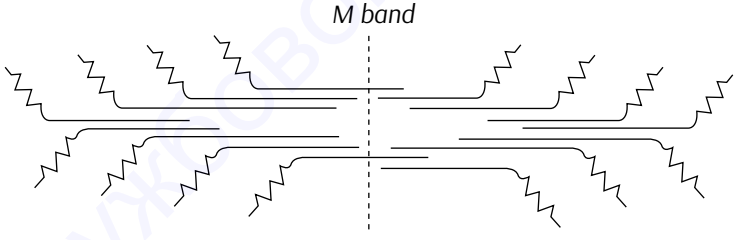
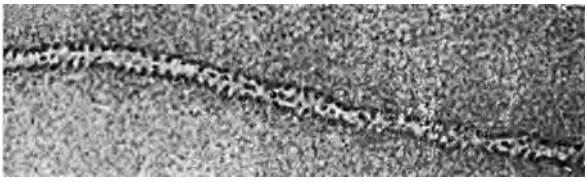


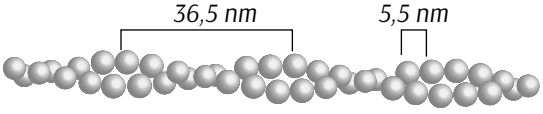
Figure 28.5. Molecular arrangement of a thick filament from myosin molecules

Actin. Organization of actin thin filaments

Thin filaments are composed of many subunits of the globular protein G-actin (42 kD) and several accessory proteins. In thin filaments, G-Actin is polymerized into long fibrous arrays known as F-actin (Figure 28.6). A pair of linear F-actin arrays is helically wound to form the backbone structure of 1 complete thin filament.



A. Electron micrograph of actin filament



B. Organization of F-actin chains from G-actin molecules

Figure 28.6. Molecular architecture of actin thin filaments

Thin and thick filaments also contain accessory proteins, which are: **tropomyosin, troponin, C protein,** and **M line protein, a-actinin**, **b-actinin.**

Tropomyosin is a long, rod-like, a,b helically-interwound heterodimer that spans a length of 7 G-actin residues. A pair of **tropomyosin** molecules is associated with every 7 pairs of G-actin residues along a thin filament, 1 tropomyosin molecule in each of the grooves of the F-actin helix.

There is a **troponin complex** associated with the thin filaments which consists of troponins T, I and C. The troponin heterotrimer is attached to one end of each tropomyosin molecule and to actin, physically linking tropomyosin to actin – Figure 28.7.

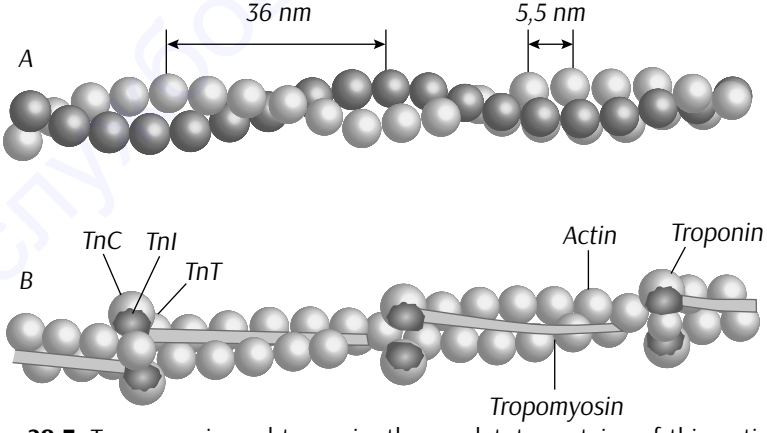


Figure 28.7. Tropomyosin and troponin, the regulatoty proteins of thin actin filaments

The troponins T and I, in cooperation with tropomyosin and calcium ions, take key role in the regulation of actin-myosin interaction that underlies muscle contraction (see below). One of the troponin subunits, troponin C (TnC), is a calmodulin-like calcium-binding protein.

The onset of muscle contractile activity involves activating of troponin complex, which serves the bridging structure that controls the spatial arrangement of tropomyosin as to actin sites which interact with S1 myosin heads. Conformational changes in the troponin complex which are induced by Ca^{2+}, are responsible for moving tropomyosin on and off myosin binding sites of actin and thus regulating muscle contraction (see below).

Proteins of the Z line, including **a-actinin,** serve as an embedding matrix or anchor for one end of the thin filaments, which extend toward the center of sarcomeres on either side of the Z line. The Z line proteins often appear continuous across the width of a muscle fiber and seem to act to keep the myofibrils within a myofiber in register. The distal end of each thin filament is free in the sarcoplasm and is capped with a protein known as **b-actinin.**

28.2. Sliding filament model of muscle contraction. Role of calcium in muscle contraction control

The organization of individual sarcomere contractile proteins peculiarities is considered to be a key feature of the **sliding filament model** of muscle contraction. The hypothesis, based on electron-microscopic, x-ray and biochemical studies, was proposed in the early 1950s by two groups of investigators and namely by Andrew Huxley and Ralph Niedergerke, and Hugh Huxley and Jean Hanson, independently.

The fundamental conceptions of muscle contraction mechanism that were put forward according to sliding filament model are as follows:

1) During muscle contraction, the length of the thick and thin filaments do not change.
2) Instead, the length of the sarcomere decreases which is due to the increase of the overlap between the two types of filaments. In other words, thick and thin filaments slide past each other during contraction.

3) The force of contraction is generated by a molecular process that actively moves one type of filament past neighboring filaments of the other type.

The structural changes in sarcomere due to the notions of the sliding filament **[cross-bridge]** model are presented in Figure 28.8.

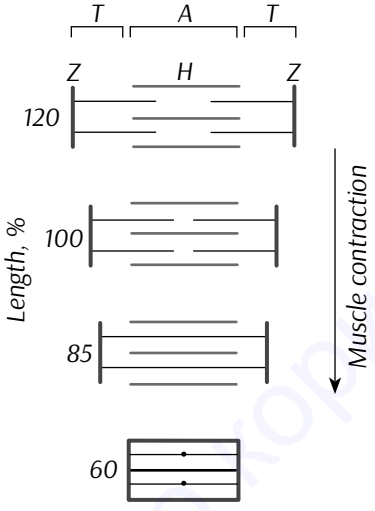


Figure 28.8. Sliding filament model of muscle contraction

- Thus, when muscle contracts, there is no change in the lengths of the thick and thin filaments, but the H zones and the I bands shorten (see legend to Figure 28.2).
- Simply speaking, to diminish the sarcomere length, the bundles of interdigitating filaments must slide past one another during contraction.
- Moreover, it was shown that cross-bridges that link thick and thin filaments generate at certain stages in the contraction cycle, and they are the molecular devices which sustain the muscular tension.

Biochemical mechanisms of muscle contraction

According to their biochemical essence, the cross-bridges that develop tension during muscle contraction, are temporary links formed between two kinds of filaments by S1 heads of myosin and specific sites of actin.

Thus, the biochemical basis of muscle activity is related to the enzymatic and physical properties of actin, myosin, and the accessory proteins that constitute the thin and thick filaments.

Myosin and the Power Stroke of Contraction

The cyclic formation and dissociation of complexes between actin and S1 myosin heads, designated above as interfilamental cross-bridges, lead to a reciprocal sliding of the thin and thick filaments. A plausible mechanism for the generation of force shown below, is thought to produce directional movement in the following way (Figure 28.9).

1) In resting muscle (Figure 28.8 position A), S1 heads are unable to interact with actin units in thin filaments because of steric interference by tropomyosin, a regulatory protein. In this state, the hydrolysis products ADP and P_i are still bound to myosin.
2) When muscle is stimulated, tropomyosin shifts position. S1 heads can then reach out from the thick filament and attach to actin units on thin filaments (position B).

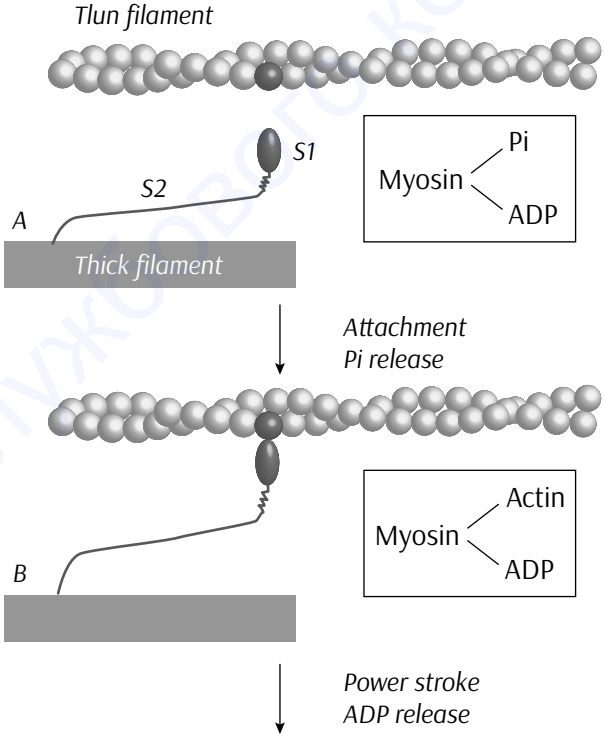


Figure 28.9. Mechanism for the force generation in muscle (A, B)

3) The binding of myosin-ADP-P_i to actin leads to the release of P_i. The

subsequent dissociation of ADP induces a major conformational change in S1 (position C). The change in orientation of S1 relative to actin constitutes the **power stroke** of muscle contraction – the thin filament is pulled a distance of about 10 nm. ADP is released from myosin at the end of the power stroke.

4) The subsequent binding of ATP to myosin leads to the rapid release of actin. The S1 head is again detached from the thin filament (position D).

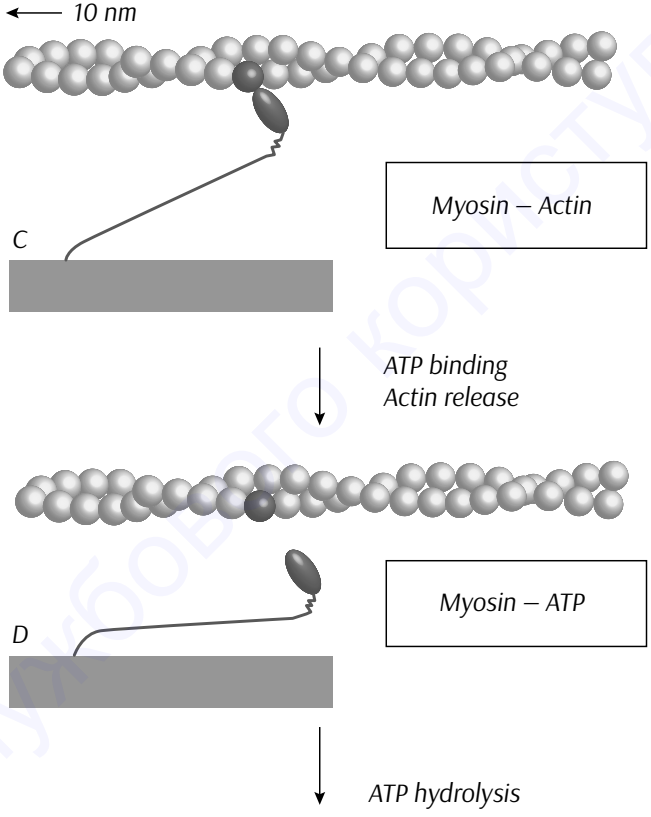


Figure 28.9. (C, D)

5) Finally, the bound ATP is hydrolyzed by the free myosin head, which resets it for the next interaction with the thin filament (position E).

Thus, the essence of the process is a cyclic change both in the shape of the myosin S1 head and its affinity for actin.

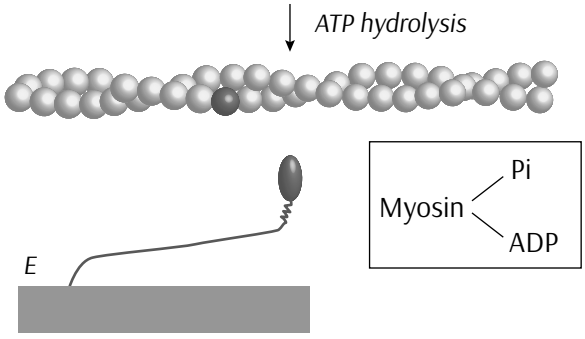


Figure 28.9. (E)

Thus, the essence of the biochemical process studied is a cyclic change both in the shape of the myosin S1 head and its affinity for actin. It leads to the reciprocal sliding of thin and thick filaments past one another, on account of which the sarcomere becomes shorter in its linear dimension, and that corresponds to the muscular contraction.

Changes in sarcomeres structure which underlie the muscle contraction are presented in the Figure 29.10.

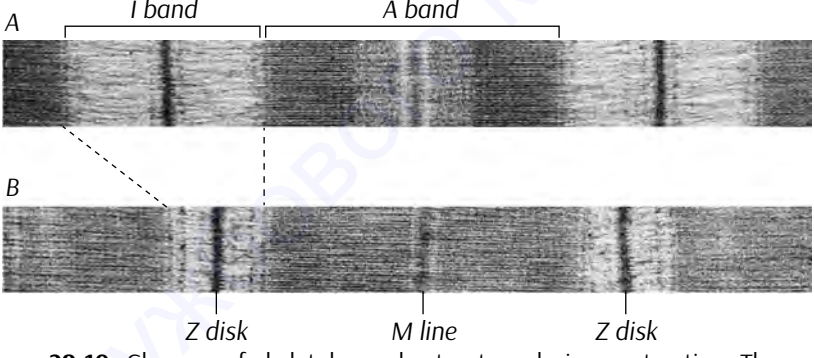


Figure 28.10. Changes of skeletal muscle structure during contraction. The organization of thick and thin filaments in the myofibril gives it a striated appearance. When muscle contracts, the I bands narrow and the Z disks come closer together, as seen in electron micrographs of (A) relaxed and (B) contracted muscle

28.3. Regulation of muscle contraction. Role of calcium in muscle contraction control

Events that stimulate muscle activity begin with neural excitation at neuromuscular junctions and lead to drastic raising of sarcoplasmic calcium (Ca^{2+}) level. Excitation of sarcolemma induces local membrane depolarization, which spreads to the associated *T tubule* system of myofibril. T tubule depolarization spreads to the sarcoplasmic reticulum (SR), that produces the opening of voltage-gated calcium channels in the SR membranes. This is followed by massive, rapid movement of cisternal calcium into the sarcoplasm close to nearby myofibrils, the key biochemical event that switches muscle contraction through subsequent Ca^{2+} interaction with troponin-tropomyosin system.

It was shown earlier (Figure 19.7) that in relaxed muscle, each tropomyosin molecule covers the myosin binding sites of seven G-actin units, thus preventing the interaction between actin and myosin and impeding muscle contyraction. When the cytosolic concentration of Ca^{2+} increases greater than about 1 to 5 micromolar, intensive calcium binding by Tn C molecules occurs. This entails conformational shifts of the whole troponin structure which moves the tropomyosin molecules away from the myosin binding sites on actin surface. This molecular occurence permits nearby myosin S1 heads to interact with adjacwent actin filaments, and contractile activity ensues.

Removing calcium from the sarcoplasm, by means of ions sequestring in SR channels and cysterns, restores the original conformational states of troponin and tropomyosin, preventing interaction between actin and myosin and leading to the relaxed state of muscle.

Smooth muscle contraction and its control

Whereas the sliding filament model adequately describes the basic structural and biochemical mechanisms of contraction in all muscle types, there are significant differences in the control of contractile activity. The thorough consideration of these differences shows that although smooth muscle lacks troponin, its contractile activity is still regulated by cytoplasmic calcium levels. The way as inside skeletal muscle, it was found the presence of Ca^{2+}/calmodulin (CaCM) binding protein known as ***caldesmon*** in smooth muscle that is involved in regulating the movement of smooth-muscle tropomyosin on and off the myosin binding sites of thin filaments.

When calcium ions are depleted, the CaCM complex dissociates and actomyosin ATPase activity is correspondingly inhibited. In essence, caldes-

mon replaces troponin as a calcium-dependent regulator of tropomyosin's location on smooth muscle thin filaments.

Chapter 29.

BIOCHEMISTRY AND MOLECULAR PATHOLOGY OF CONNECTIVE TISSUE

Connective tissue is a type of animal tissue forming a framework and support structure for the majority of body organs. Many connective tissue disorders result in severe diseases of human beings including rheumatic arthritis, scleroderma, dermatomyositis, vasculitis etc.

29.1. Connective tissue: cells and biomolecules

Connective tissue consists of **fibers, ground substance** (formed predominantly by proteoglycans supramolecular net), and a set of specialized **cells** in varying combinations and amounts. The fibers and ground substance are referred to collectively as the **extracellular matrix,** or simply **matrix.** The distinctive feature of connective tissue is a relative sparsity of cells and an abundance of the secreted, non-living, complex intercellular matrix made of fibers and ground substance.

Besides connective tissue, distinctive sorts of extracellular matrix surround cells located in various kinds of tissues and organs. In these cases the term **stroma** is used to describe the connective tissue component of an organ, and the term **parenchyma** refers to the actual functioning component of the organ, e.g., the hepatocytes of liver, or secretory epithelium of glands.

Functions of Connective Tissue

Connective tissues provides:

- mechanical support of cells and protection of soft tissues;

- physiological support:
  - connective tissue serves as a pathway for vessels and nerves**;**
  - tissue fluids of connective tissue act as a diffusion medium for exchange of metabolites between tissues and vessels;
- defence against infection;
- repair of tissue and organs injuries.

Components of connective tissue

The nature and function of the various types of **connective tissue** depend upon the proportion of definite ***cells, fibers*** and ***ground substance***. Ground substance, together with the fibers, constitutes the ***extracellular matrix*** of connective tissue.

1. Cells found in connective tissue

The major classes of connective tissue cells are ***fibroblasts, macrophages (histiocytes), adipose (fat) cells, plasma cells*** and certain of blood cells, especially ***lymphocytes, neutrophils, eosinophils, monocytes*** etc.

Fibroblasts. These cells constitute the cell type that is mostly common and characteristic for connective tissue. The principal biological functions of fibroblasts is their engagement in the production of extracellular matrix including fibers and ground substance.

Macrophages (histiocytes) – These are phagocytic cells derived from blood ***monocytes***. They may be fixed in position or may migrate into the connective tissue after tissue injury or infection.

Plasma cells – These cells synthesize antibody molecules which circulate in the blood stream. Plasma cells are commonly found in the connective tissue of the respiratory, gastrointestinal and genitourinary systems.

Lymphocytes – These blood cells are normally found in connective tissue in small numbers but produce dense tissue infiltrations during chronic inflammatory states.

Neutrophils and Eosinophils – Blood cells which infiltrate connective tissue in large numbers only during tissue inflammation. The neutrophil is characterized by a multilobed nucleus. The eosinophil contains reddish-orange granules and has a horseshoe-shaped (bilobed) nucleus.

Mast cells – Commonly occur along blood vessels. The cells are large, ovoid and densely packed with metachromatic granules containing heparin, serotonin, and histamine which can be readily released in case of inflammation and allergic reactions.

2. Fibers found in Connective Tissue

1) **Collagen Fibers** – The most common connective tissue fibers; these are long, non-branching and straight (or slightly wavy in the absence of tension). The fibers consist of bundles of fine ***collagen fibrils***, composed of repeating ***tropocollagen*** molecules. The partially overlapping, 280 nm long, tropocollagen units impart to the fibrils a 64 nm periodicity visible at the electron microscope level (Figure 29.1).

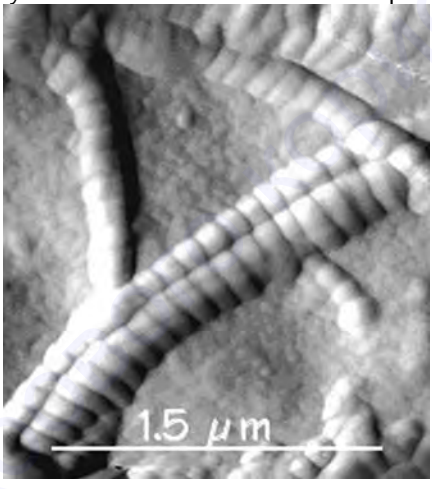


Figure 29.1. Collagen fibers of human connective tissue extracellular matrix

2) **Reticular Fibers** – These are fine ***collagen fibrils*** bound to glycoprotein components. They form delicate supporting networks in adipose and lymphoid tissue and along the basement membranes where connective tissue lies in contact with epithelial cells or muscle fibers. The fibrils have the typical 64 nm periodicity and may develop into larger diameter collagen fibers during maturation.
3) **Elastic Fibers –** They are long, thin, branching threads, exhibiting no periodicity at the electron microscopic level. The fibers are composed of tiny peripheral microfibrils and a central core of **elastin,** a protein

which contains two unusual amino acids, ***desmosine*** and ***isodesmosine.*** The additional structural protein essential for the formation of elastic fibres is **fibrillin.**

3. Extracellular matrix

Extracellular matrix of connective tissue is a complex structural entity surrounding and supporting cells that are found within mammalian tissues.

Extracellular matrix contains three major classes of biomolecules:

1) The structural proteins, namely **collagen, elastin** and **fibrillin.** These make up different kinds of connective tissue fibers.
2) The proteins with certain specialized functions, such as adhesive proteins **fibronectin** and **laminin.**
3) **Proteoglycans,** containing high molecular weight heteropolysaccharides ***(glycosaminoglycans).***

Ground substance

Ground substance is an amorphous material found in all cavities and clefts between the fibers and cells of connective tissues. Water, salts and other low molecular substances are contained within the ground substance, but its main structural constituent are proteoglycans.

Proteoglycans of connective tissue consist of 5 % protein and 95 % carbohydrate as polymers of disaccharide units that is **glycosaminoglycans**. Proteoglycans make up the amorphous material which is usually designated as the ***ground substance*** of connective tissue.

Ground substance serves as a diffusion medium in the spaces around the cells and fibers. It plays a major role in determining the physical nature of a connective tissue. In ***connective tissue proper,*** it is relatively free flowing, while in ***supportive connective tissue,*** such as bone or cartilage, it is rigid.

29.2. Structural and adhesive proteins of connective tissue. Structural proteins of extracellular matrix

Collagens

Collagen is the major structural *protein* of *connective tissue* extracellular matrix in animals and the most abundant protein in mammals, making up about 1/4 of the total. It is one of the long, fibrous structural proteins whose functions are quite different from those of globular proteins such as enzymes. It is tough and inextensible, with great tensile strength, and is the main component of cartilage, ligaments and tendons, and the main protein component of bone and teeth. Along with soft keratin, it is responsible for skin strength and elasticity, and its degradation leads to wrinkles that accompany aging. It strengthens blood vessels and plays a role in tissue development. It is present in the cornea and lens of the eye in crystalline form.

So far there have been about 12 types of collagen identified. Types I, II and III are the most abundant and form fibrils of the extracellular matrix. Type IV collagen forms a two-dimensional reticulum and is a major component of the basal lamina. Collagens are predominantly synthesized by fibroblasts but epithelial cells also synthesize these proteins.

The basic unit of collagen structure is a triple helix structure called tropocollagen. And this formation is usually referred to as entire collagen molecule (Figure 29.2). This fundamental structure of collagens is a long and thin diameter rod-like protein. For example, type I collagen entire molecule is a 300 nm long, 1.5 nm in diameter.

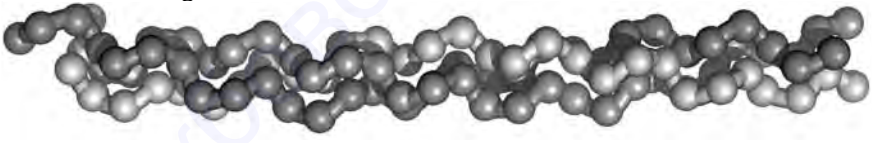


Figure 29.2. Triple helix of collagen molecule (tropocollalen)

In mature type I collagen each subunit of a triple helix is a polypeptide chain, called alpha (a) chain, containing about 1000 AA residues. These three polypeptides are slightly distinctive in their primary structure and are designated as two a1(I) chains and one a2(I) chain. Hence, the common formula $[(a1)_2a2]$ depicts the ***quaternary structure*** of collagen type I.

Primary Structure of Collagens

Analysis of the polypeptide chains reveals that **gly** and **pro** or **hydroxypro** are very prominent, almost one third of the AA are **gly** and ¼ is **pro** or

hydroxypro.

Thus, the primary structure of collagen is characterized by multiple repetitions of the typical sequence

$$(\text{glycine (Gly)} - X - Y)_n$$

where **X** is often ***proline*** (Pro) and **Y** is often ***4-hydroxyproline*** (4Hyp), that is proline to which an -OH group is added after synthesis of the procollagen polypeptide, i.e. posttranslationally. Sometimes ***3-hydroxyproline*** (3Hyp) and ***5-hydroxylysine*** (5Hyl) also occur.

The substitution of the Gly residues by any other amino acids often lead to diseases such as ***osteogenesis imperfecta***, ***Ehlers-Danlos syndrome***, and ***Alport syndrome*** as well as others (see below).

Formation of secondary, tertiary and quaternary structures of collagen

Each individual polypeptide subunit, or alpha chain is twisted into a characteristic left-handed helix of three residues per turn. Three of these alpha chains are then wound into a right-handed superhelix, forming a rod-like molecules mentioned above (Figure 29.3 *A*).

Due to the high level of **pro** or **Hpro** the polypeptide chains constituting collagen molecules cannot adopt either canonical alpha-helix or beta-sheet conformations. Instead, polypeptides assume a typical for collagen left handed helix conformation which is stretched and more open than the coiled alpha-helix.

Formation of collagen fibrils

And then, the rod-like collagen molecules, called also sometimes as ***collagen fibers,*** are assembled by lateral association of individual fibers to give more complex structures, or bundles of collagen molecules, designated as **collagen fibrils** (Figure 29.3 *B*).

Thus, collagen fibrils are formations of collagen molecules, roughly 50 nm diameter, which are organized through the lateral interactions, or "staggering" of triple helices of collagens. To achieve this, a longitudinal displacement of individual collagen molecules occurs, and this results in the formation of fibrils.

The packing of collagen is such that adjacent molecules are displaced approximately 1/4 of their length (67 nm). This staggered array produces a striated effect that can be seen in the electron microscope.

At either end of collagen fibril, the individual collagen molecules are cross-linked via modified lysine side chains. The number of such cross-links increases as the organism ages.

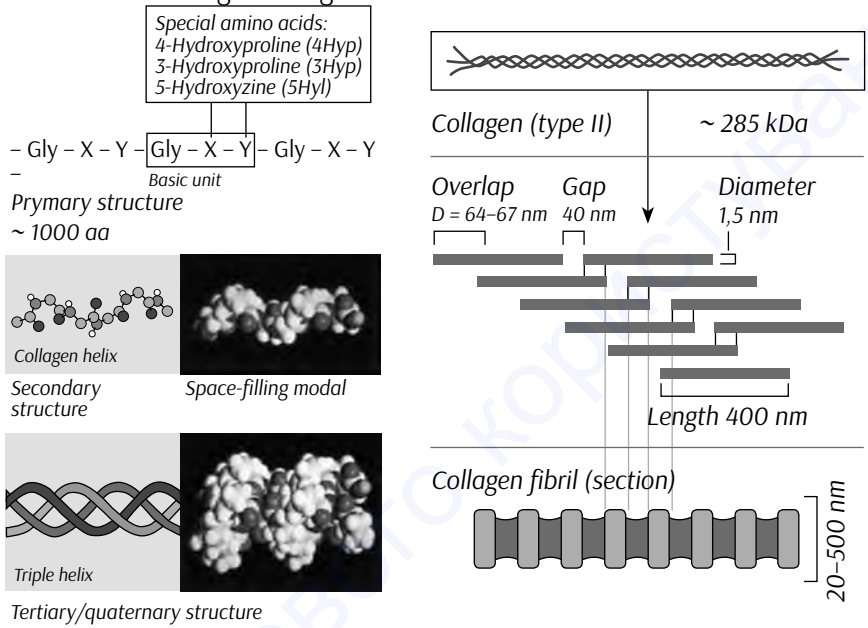


Figure 29.3. Levels of collagen molecule architecture (A) and the steps of supramolecular structures of collagen formation (B). **A** – primary, secondary and tertiary / quaternary levels of collagen molecide structure. **B** – schema tic presentation of colla gen supramolecular structures formation

In bone, entire collagen triple helices lie in a parallel, staggered array. 40 nm gaps between the ends of the tropocollagen subunits probably serve as nucleation sites for the deposition of long, hard, fine crystals of the mineral component, which is (approximately) *hydroxyapatite*, $Ca_5(PO_4)_3(OH)$, with some *phosphate*. It is in this way that certain kinds of cartilage turn into bone. Collagen gives bone its elasticity and contributes to *fracture* resistance.

The principal types of human tissues collagens are presented in the Table 29.1.

Table 29.1. Types of Collagen

Types	Chain Composition	Structural Details	Localization
I	[α1(I)]2[a(I)]	300 nm, 67 nm banded fibrils	skin, tendon, bone, etc.
II	[a1(II)]3	300 nm, small 67 nm fibrils	cartilage, vitreous humor
III	[a1(III)]3	300 nm, small 67 nm fibrils	skin, muscle, frequently with type I
IV	[a1(IV)2[a2(IV)]	390nm C-term globular domain, non-fibrillar	all basal lamina
V	[a1(V)][a2(V)][a3(V)]	390nm N-term globular domain, small fibers	most interstitial tissue, assoc. with type I
VI	[a1(VI)][a2(VI)][a3(VI)]	150 nm, N+C term. globular domains, microfibrils, 100 nm banded fibrils	most interstitial tissue, assoc. with type I
VII	[a1(VII)]3	450 nm, dimer	epithelia
VIII	[a1(VIII)]3	?, ?	some endothelial cells
IX	[a1(IX)][a2(IX)][a3(IX)]	200 nm, N-term. globular domain, bound proteoglycan	cartilage, assoc. with type II
X	[a1(X)]3	150 nm, C-term. globular domain	hypertrophic and mineralizing cartilage
XI	[a1(XI)][a2(XI)][a3(XI)]	300 nm, small fibers	cartilage
XII	a1(XII)	?, ?	interacts with types I and III

Biosynthesis of collagen

Collagens are synthesized as longer precursor proteins called ***prepro-collagens*** which are step by step transformed into **procollagen** and mature **collagen.**

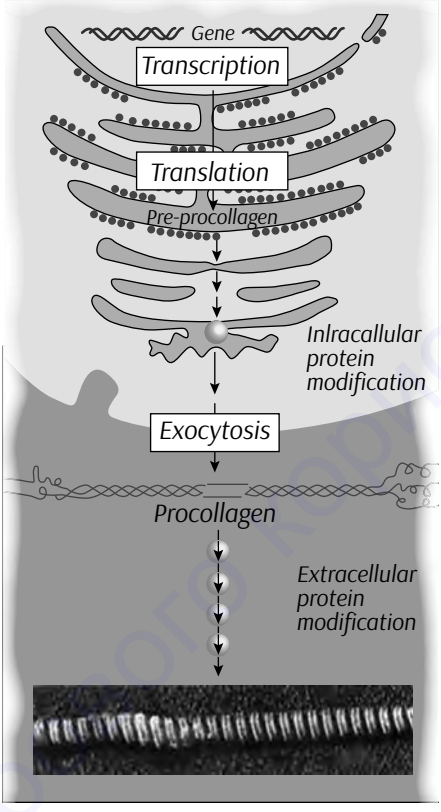


Figure 29.4. Steps of collagen biosynthesis and posttranslational modification

This process includes (Figure 29.4):

- Removal of the signal sequence which is accompanied by the numerous modifications in the collagen chains.
- The hydroxylations of proline and lysine residues of procollagen catalyzed by enzymes ***dioxygenases,*** which have iron atoms in their active centers. Hydroxylations of specific proline residues are catalyzed by ***prolyl 4-hydroxylase*** and ***prolyl 3-hydroxylase.*** Specific lysine residues also are hydroxylated by ***lysyl hydroxylase.***

Vitamin C, is essential cofactor for the maintenance of the function of these enzymes. Many symptoms of vitamin C deficiency (scurvy), e.g. skin

and vessels wall damage, loss of teeth etc. can be explained by disturbances in collagen biosynthesis.

There is also a genetic defect called **Ehlers-Danlos syndrome,** which produces a defective ***lysine hydroxylase*** resulting in poor cross-linking between fibres, so collagen is weakly held together and loose. Makes the skin very hyperextensible, very thin translucent in places, fingers are spidery, joints are hyperflexible. Caused by a lack of **hydroxy-lys** in collagen so no sugars, and no cross-linking between fibres and with cells.

- Glycosylations of O-linked type also occurs during the transit of peptide via Golgi apparatus.
- Collagen fibers begin to assemble in the endoplasmic reticulum and Golgi complexes.
- Following completion of processing, the procollagens are secreted into the extracellular space where extracellular enzymes remove the propeptides.
- Staggering of individual molecules to form fibrils then begins. Accompanying fibril formation is the oxidation of certain lysine and hydroxylysine residues by extracellular enzyme ***lysyl oxidase*** forming reactive aldehydes. These reactive aldehydes form specific cross-links between two chains thereby, stabilizing the staggered array of the collagens in the fibril.

Elastin

Elastin, is a structural *protein* in *connective tissue* that is *elastic* and allows many tissues in the body to resume their shape after stretching or contracting. Elastin helps skin to return to its original position when it is poked or pinched. Elastin is particularly abundant in large elastic blood vessels such as the *aorta*.

Elastin is primarily composed of the *amino acids* glycine, valine, alanine and proline. Elastin is made by linking many soluble *tropoelastin* protein molecules to make a massive insoluble, durable cross-linked array. In its turn, **tropoelastin** is a *water-soluble molecule* with a *molecular weight* of approximately 70 000 *daltons*. Multiple tropoelastin molecules *covalently* bind together with crosslinks to form the *protein elastin* that is very prevalent in the body.

Fibrillin

Fibrillin is a *glycoprotein*, which is essential for the formation of elastic fibres found in connective tissue.

Fibrillin-1 is a major component of the *microfibrils* that form a sheath surrounding the amorphous **elastin.** It is believed that the *microfibrils* are composed of end-to-end *polymers* of fibrillin. To date, 3 forms of fibrillin have been described. The fibrillin-1 protein was isolated by Sakai in 1986, and mutations in the gene have been linked to the *Marfan syndrome*. At present more than 100 different mutations have been described. **Fibrillin-2** was isolated in 1994 by Zhang and is thought to play a role in early elastogenesis. Mutations in the fibrillin-2 gene have been linked to ***arachnodactily*** (which is also a clinical symptom of Marfan syndrome). More recently, **fibrillin-3** was described and is believed to be located mainly in the brain.

Adhesive proteins of connective rissue

Adhesive proteins connect the different constituents of the extracellular matrix. Important examples include **fibronectin** and **laminin.** These multifunctional proteins are characterized by their ability to bind to several other matrix constituents at the same time. In particular, the adhesive proteins mediate the attachment of cells to the intracellular matrix via cell surface receptor proteins or **integrins** (see also Table 19.4).

Fibronectin

The role of fibronectins is to attach cells to a variety of extracellular matrices. Fibronectin attaches cells to all matrices except type IV that involves laminin as the adhesive molecule. Fibronectins are dimers of 2 similar peptides. Each chain is 60–70nm long and 2–3nm thick. At least 20 different fibronectin chains have been identified that arise by alternative RNA splicing of the primary transcript from a single fibronectin gene.

Fibronectins contain at least 6 tightly folded domains each with a high affinity for a different substrate such as heparan sulfate, collagen (separate domains for types I, II and III), fibrin and cell-surface receptors.

Laminin

Laminins are a family of heterotrimeric *glycoproteins* found in the *basal lamina* underlying *epithelia*. Laminin anchors cell surfaces to the basal lamina. Besides, binding of laminins to type IV *collagen* contributes to the self-assembly of the basal lamina from components secreted by cells, and their recognition by *growth cone integrins* is important to the function of the basal lamina.

29.3. Biochemistry of extracellular matrix. Glycosaminoglycans and proteoglycans of connective tissue

As was considered above (29.1), **extracellular matrix** of connective tissue includes **fibers** and **ground substance.** In its turn, the ground substance is constituted mainly by proteoglycans, which are supramolecular complexes, containing high molecular weight heteropolysaccharides ***glycosaminoglycans.***

Glycosaminoglycans

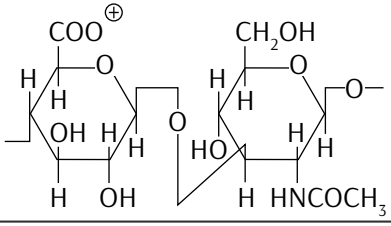
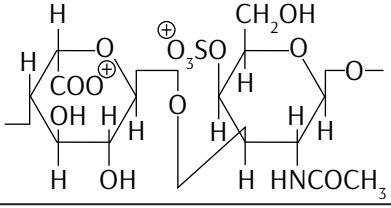
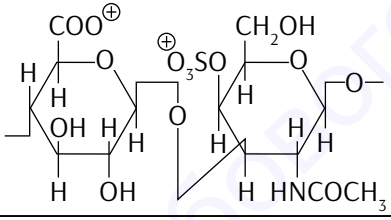
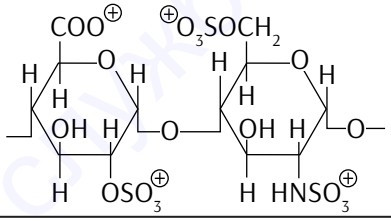
Glycosaminoglycans (GAGs), also called ***mucopolysaccharides***, are the most abundant heteropolysaccharides in the human body.

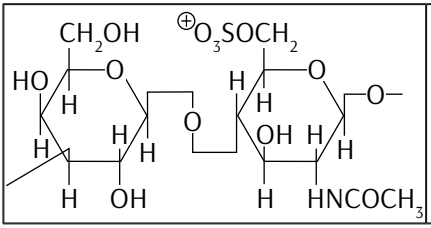
Glycosaminoglycans are long unbranched polysaccharides molecules containing a repeating disaccharide unit. The disaccharide units of GAGs are presented either of two modified sugars – N-acetylgalactosamine (GalNAc) or N-acetylglucosamine (GlcNAc) and a uronic acid such as glucuronate or iduronate. GAGs are highly negatively charged molecules, with extended conformation that imparts high viscosity to the solution. GAGs are located primarily on the surface of cells or in the extracellular matrix. Along with the high viscosity of GAGs comes low compressibility, which makes these molecules ideal for a lubricating fluid in the joints. At the same time, their rigidity provides structural integrity to cells and provides passageways between cells, allowing for cell migration.

The specific GAGs of physiological significance are **hyaluronic acid, der-**

matan sulfate, **chondroitin sulfate**, **heparin**, **heparan sulfate**, and **keratan sulfate**. Although each of these GAGs has a predominant disaccharide component (see Table 19.2), heterogeneity does exist in the sugars present in the make-up of any given class of GAG.

Table 29.2. Major glycosaminoglycans of human connective tissue

	***Hyaluronates:*** *composed of **D**-glucuronate + GlcNAc linkage is b(1, 3)*
	***Dermatan sulfates:*** *composed of **L**-iduronate (many are sulfated) + GalNAc-4-sulfate linkages is β(1, 3)*
	**Chondroitin 4- and 6-sulfates:** *composed of **D**-glucuronate + GalNAc-4- or 6-sulfate linkage is β(1, 3) (the figure contains GalNAc 4-sulfate)*
	**Heparin and Heparan sulfates:** *composed of **D**-glucuronate-2-sulfate (or iduronate-2-sulfate) + N-sulfo-**D**-glucosamine-6-sulfate linkage is α(1, 4) (heparans have less sulfate than heparins)*



Keratan sulfates:
composed of galactose + GlcNAc-6-sulfate linkage is α(1, 4)

Hyaluronic acid is the dominant glycosaminoglycan in connective tissues. It is also a major component of the synovial fluid and the vitreous body of the eye.

The molecular weight of hyaluronic acid is very high (~1.000.000 D). Hyaluronic acid is unique among the GAGs in that it does not contain any sulfate and is not found covalently attached to proteins as a proteoglycan. It is, however, a component of non-covalently formed complexes with proteoglycans in the ECM.

With a length of about 2.5 μm hyaluronic acid is **very large molecule**. It serves as a "backbone" for the assembly of other glycosaminoglycans in connective and skeletal tissue, which results in even larger molecule complexes (MW 30,000,000–200,000,000). Hyaluronic acid polymers can displace a large volume of water. This property makes them excellent lubricators and shock absorbers.

The remaining four major glycosaminoglycans are ***chondroitin sulfate***, ***dermatan sulfate***, ***keratan sulfate*** and ***heparan sulfate*** (Table 19.3). These glycosaminoglycans attach via core- and link-proteins to a backbone formed by the hyaluronic acid. The coiled arrangement of the hyaluronate and other attached glucosaminoglycans fills a roughly spherical space with a diameter of ~0.5 μm. This space is called a *domain*. Neighbouring domains overlap and form a more or less continuous three-dimensional *molecular sieve* in the interstitial spaces of the connective tissues.

Table 29.3. Characteristics of GAGs

GAG	Localization	Comments
Hyaluronate	synovial fluid, vitreous humor, ECM of loose connective tissue	large polymers, shock absorbing

Chondroitin sulfate	cartilage, bone, heart valves	most abundant GAG
Heparan sulfate	basement membranes, components of cell surfaces	contains higher acetylated glucosamine than heparin
Heparin	component of intracellular granules of mast cells lining the arteries of the lungs, liver and skin	more sulfated than heparan sulfates
Dermatan sulfate	skin, blood vessels, heart valves	
Keratan sulfate	cornea, bone, cartilage aggregated with chondroitin sulfates	

The large polyanionic carbohydrates of the glycosaminoglycans bind large amounts of water and cations. The bound water in the domains forms a medium for the diffusion of substances of low molecular weight such as gases, ions and small molecules, which can take the shortest route, for example, from capillaries to connective tissue cells. Large molecules are excluded from the domains and have to find their way through the spaces between domains.

The restricted motility of larger molecules in the extracellular space inhibits the spread of microorganisms through the extracellular space. A typical bacterium (0.5 × 1 μm) is essentially immobilised in the meshwork formed by the domains. The pathogenicity of a bacterium is indeed to some extent determined by its ability to find its way through the mesh, and some of the more invasive types produce the enzyme ***hyaluronidase*,** which depolymerises hyaluronic acid.

Proteoglycans are composed of a protein core to which is attached long chains of repeating disaccharide units termed of **glycosaminoglycans** (GAGs) forming extremely complex high molecular weight components of the extracellular matrix.

The GAGs extend perpendicularly from the core in a brush-like structure. The linkage of GAGs to the protein core involves a specific trisaccharide composed of two galactose residues and a xylulose residue (GAG-Gal-GalXyl – O – CH_2-protein) – Figure 19.5.

The trisaccharide linker is coupled to the protein core through an O-glycosidic bond to a S residue in the protein. Some forms of keratan sulfates

are linked to the protein core through an N-asparaginyl bond. The protein cores of proteoglycans are rich in S and T residues, which allows multiple GAG attachments.

Proteoglycans are responsible for the highly viscous character of the ground substance. Proteoglycans consist of proteins (~ 5 %) and polysaccharide chains (~ 95 %), which are covalently linked to each other. The polysaccharide chains belong to one of the five types of glycosaminoglycans, which form the bulk of the polysaccharides in the ground substance.

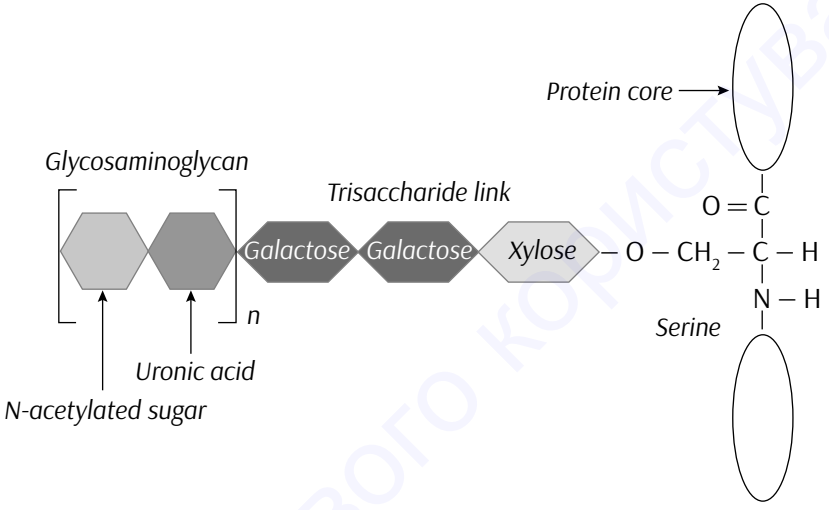


Figure 29.5. Structure of the GAG linkage to protein in proteoglycans

Different types of extracellular matrix according to various kinds of collagen, proteoglycan nature, ancor proteins and cell-surface receptors is presented in Table 19.4.

Table 29.4. Representative matrix types produced by vertebrate cells

Collagen	Anchor	Proteoglycan	Cell-Surface Receptor	Cells
I	fibronectin	chondroitin and dermatan sulfates	integrin	fibroblasts

II	fibronectin	chondroitin sulfate	integrin	chondrocytes
III	fibronectin	heparan sulfate and heparin	integrin	quiescent hepatocytes, epithelial; assoc. fibroblasts
IV	laminin	heparan sulfate and heparin	laminin receptors	all epithelial cells, endothelial cells, regenerating hepatocytes
V	fibronectin	heparan sulfate and heparin	integrin	quiescent fibroblasts
VI	fibronectin	heparan sulfate	Iitegrin	quiescent fibroblasts

29.4. Molecular pathology of connective tissue

Clinical Significance of Collagen Disorders

Alterations in collagen structure resulting from abnormal genes or abnormal processing of collagen proteins results in numerous diseases, e.g. *Ehlers-Danlos syndrome, Osteogenesis imperfecta* and *Marfan`s syndrome* (Table 29.5).

Ehlers-Danlos syndrome is actually the name associated with at least ten distinct disorders that are biochemically and clinically distinct yet all manifest structural weakness in connective tissue as a result of defects in the structure of collagens.

Osteogenesis imperfecta also encompasses more than one disorder. At least four biochemically and clinically distinguishable disorders have been identified all of which are characterized by multiple fractures and resultant bone deformities.

Marfan's syndrome manifests itself as a disorder of the connective tissue and was believed to be the result of abnormal collagens. However, recent evidence has shown that Marfan's results from mutations in the extracellular protein, *fibrillin,* which is an integral constituent of the non-col-

lagenous microfibrils of the extracellular matrix.

Table 29.5. Molecular pathology of human collagens

Disorder	Collagen Defect	Symptoms
Ehlers-Danlos IV	Decrease in type III	Arterial, intestinal and uterine rupture, thin easily bruised skin
Ehlers-Danlos V	Decreased cross-linking	Skin and joint hyperextensibility
Ehlers-Danlos VI	Decreased hydroxylysine	Poor wound healing, musculo-skeletal deformities, skin and joint hyperextensibility
Ehlers-Danlos VII	N-terminal pro-peptide not removed	Easily bruised skin, hip dislocations, hyperextensibility
Oseteogenesis imperfecta	Decrease in type I	Blue sclerae, bone deformities
Scurvy	Decreased hydroxyproline	Poor wound healing, deficient growth, capillary weakness

Clinical significance of proteoglycans and GAGs pathology

Proteoglycans and GAGs perform numerous vital functions within the body, some of which still remain to be studied. One well-defined function of the GAG heparin is its role in preventing coagulation of the blood. Heparin is abundant in granules of mast cells that line blood vessels. The release of heparin from these granules, in response to injury, and its subsequent entry into the serum leads to an inhibition of blood clotting, in the following manner. Free heparin complexes with and activates antithrombin III, which in turn inhibits all the serine proteases of the coagulation cascade. This phenomenon has been clinically exploited in the use of heparin injection for anti-coagulation therapies.

Several genetically inherited diseases, for example the lysosomal storage diseases, result from defects in the lysosomal enzymes responsible for the metabolism of complex membrane-associated GAGs. These specific diseases, termed **mucopolysaccharidoses** (in reference to the earlier term, ***muco-***

***polysaccharide*,** used for glycosaminoglycans) lead to an accumulation of GAGs within cells. There are at least 14 known types of lysosomal storage diseases that affect GAG catabolism; some of the more commonly encountered examples are *Hurler's syndrome, Hunter's syndrome, Sanfilippo syndrome, Maroteaux-Lamy syndrome* and *Morquio's syndrome*. All of these disorders, excepting Hunter's syndrome, are inherited in an autosomal recessive manner.

Table 29.6. Diseases of glycosaminoglycan (mucopolysaccharide) metabolism

Type: Syndrome	Enzyme Defect	Affected GAG	Symptoms
MPS I H: Hurler's	*α-L-iduronidase*	dermatan sulfate, heparan sulfate	corneal clouding, dystosis multiplex, organomegaly, heart disease, dwarfism, mental retardation; early mortality
MPS I H Scheie's	*α-L-iduronidase*	dermatan sulfate, heparan sulfate	corneal clouding; aortic valve disease; joint stiffening; nor-mal intelligence and life span
MPS I H/S: Huler/Scheie`s	*α-L-iduronidase*	dermatan sulfate, heparan sulfate	intermediate between I H and I S
MPS II: Hunter's	L-iduronate-2-sulfatase	dermatan sulfate, heparan sulfate	mild and severe forms, only X-linked MPS, dystosis multiplex, organomegaly, facial and physical defor-mities, no corneal clouding, mental retardation, death before 15 except in mild form then survival to 20 – 60
MPS III A: Sanfilippo A	Heparan N-sul-fatase	heparan sulfate	profound mental deterioration, hyperactivity, skin, brain, lungs, heart and skeletal muscle are affected in all 4 types of MPS-III

Type: Syndrome	Enzyme Defect	Affected GAG	Symptoms
MPS III B: Sanfilippo B	a-N-acetyl-D-glucosaminidase	heparan sulfate	phenotype similar to III A
MPS III C: Sanfilippo C	AcetylCoA: a-glucosamini-de-acetyltrans-ferase	heparan sulfate	phenotype similar to III A
MPS III D: Sanfilippo D	N-acetylglucosa-mine-6-sulfatase	heparan sulfate	phenotype similar to III A
MPS IV A: Morquio A	Galactose-6-sulfatase	keratan sulfate, chondroitin 6-sulfate	corneal clouding, odontoid hypoplasia, aortic valve disease, distinctive skeletal abnormalities
MPS IV B: Morquio B	b-Galactosidase	keratan sulfate	severity of disease similar to IV A
MPS V a designation no longer used			
MPS VI: Maroteaux-Lamy	N-acetylgalactosamine-4-sulfatase also called arylsulfatase B	dermatan sulfate	3 distinct forms from mild to severe, aortic valve disease, dystosis multiplex, normal intelligence, corneal clouding, coarse facial features
MPS VII: Sly syndrome	b-Glucuronidase	heparan sulfate, dermatan sulfate, chondroitin 4-, 6-sulfates	hepatosplenomegaly, dystosis multiplex, wide spectrum of severity, hydrops fetalis

Chapter 30.

BIOCHEMISTRY OF SPECIALIZED CELLS AND PHYSIOLOGICAL FUNCTIONS-4. BIOCHEMISTRY OF NERVE TISSUE AND NEUROTRANSMITTERS

30.1. Nerve tissue: general characteristics of structure and functions

Nervous System is divided into the Central Nervous System and the Peripheral Nervous System.

The Central Nervous System of a human is divided into two parts: the brain (cerebrum) and the spinal cord (Figure 30.1). The average adult human brain weighs 1.3 to 1.4 kg (approximately 3 pounds). The spinal cord is about 45 cm long in adult men and 43 cm long in adult women and weighs about 35–40 grams.

Neurons

The brain contains about 100 billion nerve cells, or **neurons,** and trillions of "supporting cells" called **glia (neuroglia).** Three cell types of neuroglia are known: (1) astrocytes with their two varieties, protoplasmic and fibrous; (2) oligodendroglia; and (3) microglia.

A **neuron** is a nerve cell, that provides the major physiological functions of the nervous system. The brain is made up of approximately 100 billion neurons.

Neurons are similar to other cells in the body, that is neurons are sur-

rounded by a membrane, they have a nucleus that contains genes, cytoplasm, mitochondria and other organelles.

However, neurons differ from other cells in the body in some ways such as:

- Neurons have specialized projections called **dendrites** and **axons.** Dendrites bring information to the cell body and axons take information away from the cell body (Figure 30.1).
- Axons are typically surrounded by **Schwann cells,** which form a myelin-containing sheath of a nerve. This sheath is responsible for the electrical insulation of the neuron, and it increases the rate of signal transmission.
- Neurons communicate with each other through a special electrochemical process which is realized by means of specialized connections called **"synapses".**

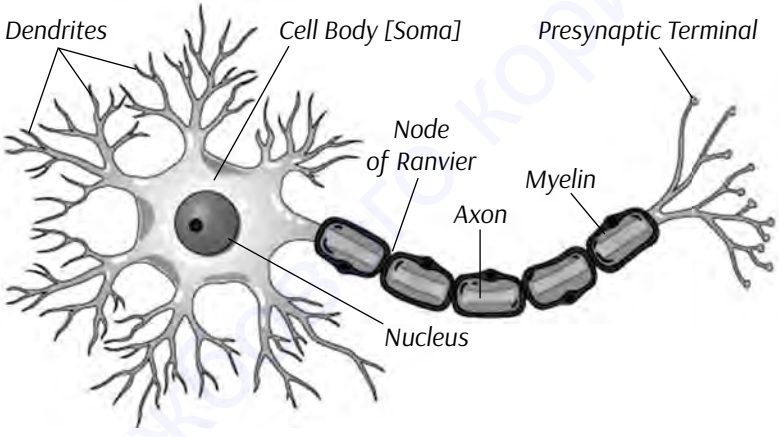


Figure 30.1. Structure of typical neuron

- Neurons produce special chemicals called **"neurotransmitters"** that are released at the synapse and serve to transmit a signal to another nerve or to a muscle and gland.

Synaptic Transmission. Synapses

Synaptic transmission refers to the propagation of nerve impulses from one nerve cell to another. This occurs at a specialized cellular structure known as the **synapse** (Figure 30.3) – a junction at which the axon of the

presynaptic neuron terminates at some location upon the postsynaptic neuron.

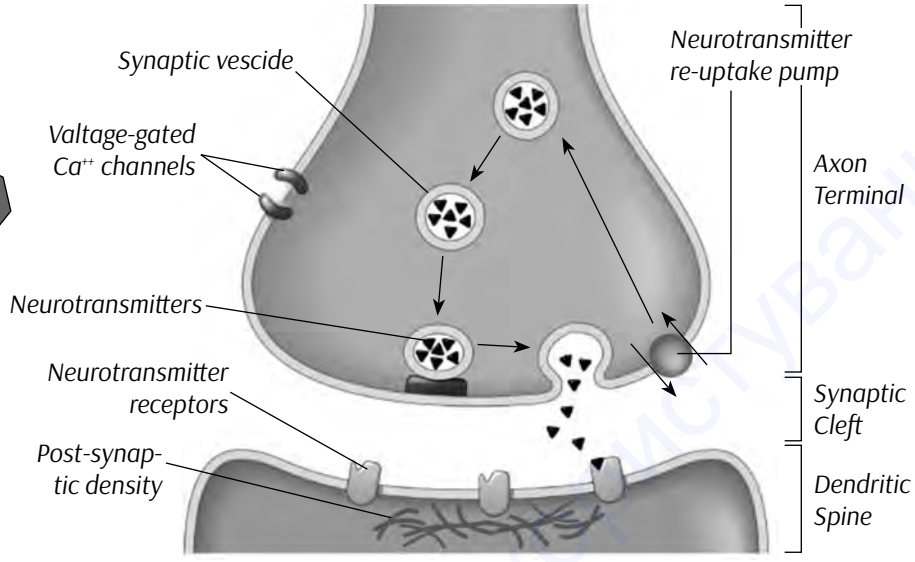


Figure 30.3. Structure of typical synapse

The word "synapse" comes from "synaptein" which Sir Charles Scott Sherrington and his colleagues coined from the Greek "syn-" meaning "together" and "haptein" meaning "to clasp".

Charles S. Sherrington (1857–1952)

Sir Charles Scott Sherrington (November 27, 1857 – March 4, 1952) was

a British scientist known for his contributions to physiology and neuroscience. He shared the 1932 Nobel Prize in Physiology or Medicine with Edgar Douglas Adrian for their work with neurons. Sherrington is considered one of the fathers of neuroscience.

Synapses allow nerve cells to communicate with one another through axons and dendrites, converting electrical signals into chemical ones. In other words, owing to the existence of synapses cells of the nervous system signal to one another and to non-neuronal cells such as muscles or glands. A synapse between a motor neuron and a muscle cell is called a neuromuscular junction.

Moreover, synapses allow the neurons of the central nervous system to form interconnected neural circuits. They are thus crucial to the biological computations that underlie perception and thought. They also provide the means through which the nervous system connects to and controls the other systems of the body.

The human brain contains a huge number of synapses, with young children having about 1,000 trillion. This number declines with age, stabilizing by adulthood. Estimates for an adult vary from 100 to 500 trillion synapses.

30.2. Peculiarities of brain biochemical content and metabolism

The typical characteristic of brain biochemical content is the predomination of lipids over other major biomolecules.

Nerve cells, both neurons and neuroglia, have a high lipid content, which may account for about 50 % of their dry weight. The lipids of nerve tissue are presented mainly by complex polar lipids, in particular phospholipids, glycolipids and sphingolipids which play key roles in the molecular architecture of nerve cells membranes.

Metabolism of the brain

Bioenergetics. The brain is well supplied with blood, and has an extremely potent energy metabolism. Although human brain accounts for only about 2 % of the body mass, it consumes near 20 % of the **oxygen** and 60 % of the **glucose** consumed by the whole body.

In the cells of the nervous system and especially in the brain the bulk of glucose is fully oxidized to CO_2 and H_2O by means of glycolysis and subsequent citric acid cycle. Fatty acids that are present in blood plasma cannot reach the nerve cells because of the **blood-brain barrier**. And the carbon skeletons of free amino acids either cannot be used for ATP generation in neurons on account of the lack in the brain of gluconeogenesis enzymes.

Thus, glucose is practically the only energy-yielding fuel used by the brain. It is only in the condition of prolonged starvation that the brain reorganizes it metabolic abilities so that to use ketone bodies as an additional energy sources.

As far as the glycogen reserves of nerve cells are insignificant, the dependence of the brain on glucose supplied by blood is absolute! Hence, the biochemical consequences of the blood glucose level decline for brain metabolism and function are drastic. Hypoglycemia causes severe clinical manifestations associated with brain disfunction which are life-threatening and can lead to coma and death. The example of the situation is the overdosage of insulin to patients suffering from diabetes mellitus!

Amino acids of nerve tissue. The free amino acids intracellular concentrations inside the brain are much higher than in most other tissue, including the liver. This phenomenon reflects the active involvement of neurons in amino acid metabolism.

Particularly abundant in brain tissue are dicarboxylic amino acids **glutamate** and **aspartate.** These amino acids are formed by transamination of the citric acid cycle metabolites, namely 2-oxoglutarate and oxaloacetate, and function as essential metabolites in brain cells physiology. In certain neurons, amino acids, including **glutamate, aspartate, γ-aminobutyrate** (GABA; 4-aminobutyrate) and **glycine** serve as neuro-transmitters of excitatory or inhibitory action, which are stored in specialized synapses and released under the influence of the **action potential** (see below).

30.3. Neurotransmitters: classification, receptors, representatives

Nerve impulses are transmitted at synapses by the release of chemicals called **neurotransmitters.**

Neurotransmitters are stored in the nerve cell's bulbous ends (axons).

When a nerve impulse, or **action potential,** travelling along the nerve reaches the axon, the neurotransmitter is released into the synaptic space. The interaction of neurotransmitter with the receptor on postsynaptic membrane either prompts or inhibits continued electrical impulses along the nerve.

There are more than 300 known neurotransmitters. They constitute a diverse group of chemical compounds ranging from simple amines such as *dopamine* and amino acids such as γ-*aminobutyrate* **(GABA),** to polypeptides such as the *endorphins* and *enkephalins*. The representatives of neurotransmitters of nonpeptide nature are presented in the Table 30.1.

Table 30.1. Major classes of neurotransmitters

Transmitter Molecule	Derived From	Site of Synthesis
Acetylcholine	Choline	CNS, parasympathetic nerves
Serotonin 5-Hydroxytryptamine (5-HT)	Tryptophan	CNS, chromaffin cells of the gut, enteric cells
GABA	Glutamate	CNS
Glutamate		CNS
Aspartate		CNS
Glycine		spinal cord
Histamine	Histidine	hypothalamus
Epinephrine synthesis pathway	Tyrosine	adrenal medulla, some CNS cells
Norpinephrine synthesis pathway	Tyrosine	CNS, sympathetic nerves
Dopamine synthesis pathway	Tyrosine	CNS
Adenosine	ATP	CNS, peripheral nerves
ATP		sympathetic, sensory and enteric nerves
Nitric oxide, NO	Arginine	CNS, gastrointestinal tract

Neuropeptides

Many other neurotransmitters are derived from precursor proteins, the so-called *peptide neurotransmitters*. As many as 50 different peptides have been shown to exert their effects on neural cell function. Several of these peptide transmitters are derived from the larger protein *pro-opiomelanocortin (POMC)*. Neuropeptides are responsible for mediating sensory and emotional responses including pain and pleasure, hunger, thirst, sex drive etc.

The biochemical mechanisms by which neurotransmitters elicit responses in both presynaptic and postsynaptic neurons are rather diverse depending on the chemical nature of the substance itself and the kind of receptor.

Neuromuscular Transmission

A different type of nerve transmission occurs when an axon terminates on a skeletal muscle fiber, at a specialized structure called the *neuromuscular junction*. An action potential occurring at this site is known as *neuromuscular transmission*. At a neuromuscular junction, the axon subdivides into numerous terminal buttons that reside within depressions formed in the **motor end-plate.** The particular transmitter in use at the neuromuscular junction is **acetylcholine.**

Neurotransmitter Receptors

Once the molecules of neurotransmitter are released from a cell as the result of the firing of an action potential, they bind to specific **receptors** on the surface of the postsynaptic cell. In all cases in which these receptors have been cloned and characterized in detail, it has been shown that there are numerous subtypes of receptors for any given neurotransmitter. As well as being present on the surfaces of postsynaptic neurons, neuro-transmitter receptors are found on presynaptic neurons. In general, presynaptic neuron receptors act to inhibit further release of neurotransmitter.

According to their molecular architecture and mode of action, there are different kinds of receptors for neurotransmitters. These can be roughly divided into two major classes:

Firstly, the receptors which are associated with ion channels. The activation of these receptors which takes place as the result of bonding of neurotransmitter with the receptor protein, leads to the opening of trans-

membrane ion channel – presumably Na^+ (or K^+) channel. Hence, the ionic channels under consideration are called **ligand-gated ion channels.** The influx of Na^+ ions into the postsynaptic nerve or muscle raises the resting potential of the membrane which denotes the excitation of the corresponding cell. The example of such receptor entity is the subclass of nicotine-sensitive receptors of acetylcholine.

Secondly, there are receptors whose stimulation results in the intracellular production of second messengers, particularly cGMP, cAMP or components of phosphoinositide system. They are usually called ***metabotropic receptors.*** In this case, the transmission of chemical signal from the ligand-associated receptor protein to the cellular metabolism is carried out with the help of **G-proteins** (GTP-binding proteins-transducers) which conformation changes result in the switching of intracellular enzyme cascades.

According to their molecular organization, the majority of metabotropic receptors belong to a class of proteins known as the ***serpentine receptors.*** This class exhibits a characteristic transmembrane structure which spans the cell membrane, not once but seven times. The examples of serpentine receptors are receptors for catecholamines and muscarine-sensitive receptors of acetylcholine (see below).

Acetylcholine

Acetylcholine **(ACh)** is a simple molecule synthesized from choline and acetyl-CoA through the action of *choline acetyltransferase:*

$$(CH_3)_3 \equiv \overset{+}{N} - CH_2 - CH_2 - OH + CH_3 - \overset{O}{\overset{\|}{C}} - CO\text{-}S\text{-}CoA \rightarrow$$

$$\rightarrow (CH_3)_3 \equiv \overset{+}{N} - CH_2 - CH_2 - O - \overset{O}{\overset{\|}{C}} - CH_3 + HS - CoA$$

Neurons that synthesize and release ACh are termed *cholinergic neurons.* When an action potential reaches the terminal button of a presynaptic neuron a voltage-gated calcium channel is opened. The influx of calcium ions, Ca^{2+}, stimulates the exocytosis of presynaptic vesicles containing ACh, which is thereby released into the synaptic cleft. Once released, ACh must be removed rapidly in order to allow repolarization to take place; this step, hydrolysis, is carried out by the enzyme, ***acetylcholinesterase.*** The ***acetylcholinesterase*** found at nerve endings is anchored to the plasma mem-

brane through a glycolipid.

Two main classes of ACh receptors have been identified on the basis of their responsiveness to the toadstool alkaloid, *muscarine*, and to nicotine, respectively: the *muscarinic receptors* and the *nicotinic receptors*.

Nicotinic acetylcholine receptor is the polypeptide complex composed of four different polypeptide subunits arranged in the form **[(α_2)(β)(γ)(δ)]** (Figure 30.4). The receptors of this class are selectively stimulated by **nicotine,** which is alkaloid of the plant *Nicotiana tabaccum*.

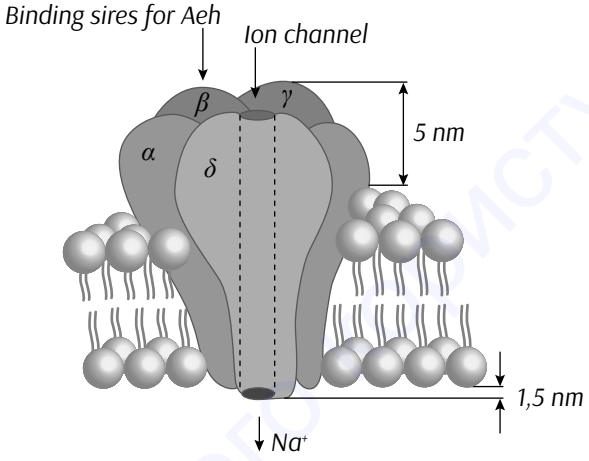


Figure 30.4. Intramembrane architecture of nicotinic acetylcholine receptor

According to the mechanism of functioning, it is one of **ligand-gated ion channels** which contains both the acetylcholine binding sites and the ion channel that is activated by acetylcholine binding.

Following receptor activation, nicotinic acetylcholine receptors increase postsynaptic membrane permeability for cations Na^+, K^+ or Ca^{2+} that results in the propagation of the membrane excitation wave in the neuron or muscle cell.

Muscarinic acetylcholine receptor. Muscarinic cholinergic receptors were named this way because of their specific ability to be stimulated by *muscarine* that is the alkaloid of poisonous mushroom *Amanita muscarina* (Figure 30.5).

Figure 30.5. The mushroom Amanita muscarina, which is the source of alkaloid muscarine exerting unfavorable action upon human CNS

This receptor variety belongs to a class of **metabotropic receptors** which ligand-induced activation promotes the cellular metabolic changes associated with the production of second messengers. By their intramembrane organization, these are **seven-helix receptors,** or receptors of "serpentine type" (Figure 30.6).

Muscarinic sensitive AcH receptors are G proteins-associated receptors which stimulation is accompanied with the activation of ***guanylyl cyclase*** and the generation of cGMP as the intracellular second messenger. By now, five subtypes of muscarinic receptors have been cloned, that is M1-M5.

Both receptor classes (n- and m-cholinergic) are abundant in the human brain. Nicotinic receptors are further divided into those found at neuromuscular junctions and those found at neuronal synapses. The activation of nicotinic receptors by the binding of ACh leads to an influx of Na^+ into the cell and an efflux of K^+, resulting in a depolarization of the postsynaptic neuron and the initiation of a new action potential.

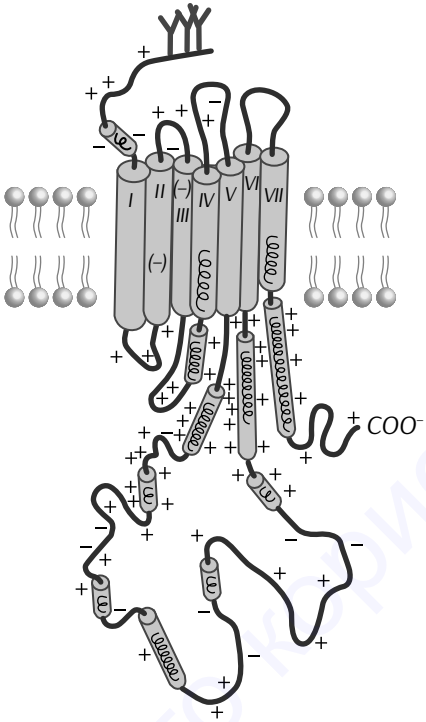


Figure 30.6. m-Cholinergic receptor of "serpentine type"

Cholinergic Agonists and Antagonists

Numerous compounds have been identified that act as either **agonists** or **antagonists** of cholinergic neurons – Table 30.2.

The principal action of cholinergic agonists is the excitation or inhibition of autonomic effector cells that are innervated by postganglionic parasympathetic neurons and as such are referred to as *parasympatho-mimetic agents. T*he cholinergic agonists include choline esters (such as ACh itself) as well as protein- or alkaloid-based compounds. Several naturally occurring compounds have been shown to affect cholinergic neurons, either positively or negatively.

The responses of cholinergic neurons can also be enhanced by administration of ***cholinesterase*** (ChE) inhibitors. ChE inhibitors have been used as components of nerve gases but also have significant medical application in the treatment of disorders such as *glaucoma* and *myasthenia gravis* as well as in terminating the effects of neuromuscular blocking agents such as

atropine.

Table 30.2. Natural Cholinergic Agonist and Antagonists

	Source of Compound	Mode of Action
Agonists		
Nicotine	Alkaloid prevalent in the tobacco plant	Activates nicotinic class of ACh receptors, locks the channel open
Muscarine	Alkaloid produced by *Amanita muscaria* mushrooms	Activates muscarinic class of ACh receptors
α-Latrotoxin	Protein produced by the black widow spider	Induces massive ACh release, possibly by acting as a Ca^{2+} ionophore
Antagonists		
Atropine (and related compound Scopolamine)	Alkaloid produced by the deadly nightshade, *Atropa belladonna*	Blocks ACh actions only at muscarinic receptors
Botulinus Toxin	Eight proteins produced by *Clostridium botulinum*	Inhibits the release of ACh
γ-Bungarotoxin	Protein produced by *Bungarus* genus of snakes	Prevents ACh receptor channel opening
d-Tubocurarine	Active ingredient of curare	Prevents ACh receptor channel opening at motor end-plate

Catecholamines

The principal catecholamines are ***norepinephrine***, ***epinephrine*** and ***dopamine***. These compounds are formed from phenylalanine and tyrosine (Figure 30.7). Tyrosine is produced in the liver from phenylalanine through the action of *phenylalanine hydroxylase*. The tyrosine is then transported to catecholamine-secreting neurons where a series of reactions convert it to dopamine, to norepinephrine and finally to epinephrine.

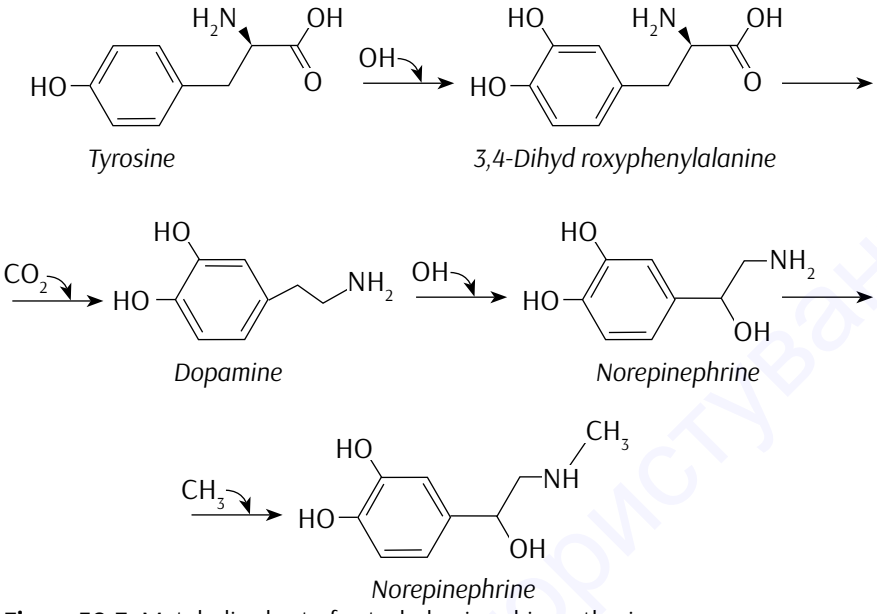


Figure 30.7. Metabolie chart of catecholamines biosynthesis

Catecholamines exhibit peripheral nervous system excitatory and inhibitory effects as well as actions in the CNS such as respiratory stimulation and an increase in psychomotor activity. The excitatory effects are exerted upon smooth muscle cells of the vessels that supply blood to the skin and mucous membranes. Cardiac function is also subject to excitatory effects, which lead to an increase in heart rate and in the force of contraction. Inhibitory effects, by contrast, are exerted upon smooth muscle cells in the wall of the gut, the bronchial tree of the lungs, and the vessels that supply blood to skeletal muscle.

In addition to their effects as neurotransmitters, norepinephrine and epinephrine can influence the rate of metabolism (Lecture 15). This influence works both by increasing the rate of glycogenolysis and fatty acid mobilization and by modulating endocrine function such as insulin secretion.

The catecholamines bind to two different classes of receptors termed the *α*- and *β-adrenergic receptors*. The catecholamines therefore are also known as *adrenergic neurotransmitters*; neurons that secrete them are *adrenergic neurons*. Norepinephrine-secreting neurons are *noradrenergic*. The adrenergic receptors are classical serpentine receptors that couple to intracellular G-proteins. Some of the norepinephrine released from presynaptic

noradrenergic neurons recycles in the presynaptic neuron by a reuptake mechanism.

Adrenergic receptors and mechanism of action

The cardinal physiologic effects of epinephrine and norepinephrine are mediated by their binding to adrenergic receptors on the plasma membranes of target cells.

There are multiple types of adrenergic receptors which are differentially expressed in different tissues and cells – Table 30.3. The α- (alpha) and β- (beta) adrenergic receptors and their subtypes were originally defined by differential binding of various agonists and antagonists and, more recently, by analysis of molecular clones.

Table 30.3. Subtypes of adrenergic receptors

Receptor	Effectively Binds	Effect of Ligand Binding
Alpha1	Epinephrine, Norepinphrine	Increased cytosolic calcium
Alpha2	Epinephrine, Norepinphrine	Decreased cyclic AMP
Beta1	Epinephrine, Norepinphrine	Increased cyclic AMP
Beta2	Epinephrine	Increased cyclic AMP

Catecholamine Catabolism

Epinephrine and norepinephrine are catabolized to inactive compounds through the sequential actions of *catecholamine-O-methyltransferase* (COMT) and *monoamine oxidase* (MAO) – Figure 30.8.

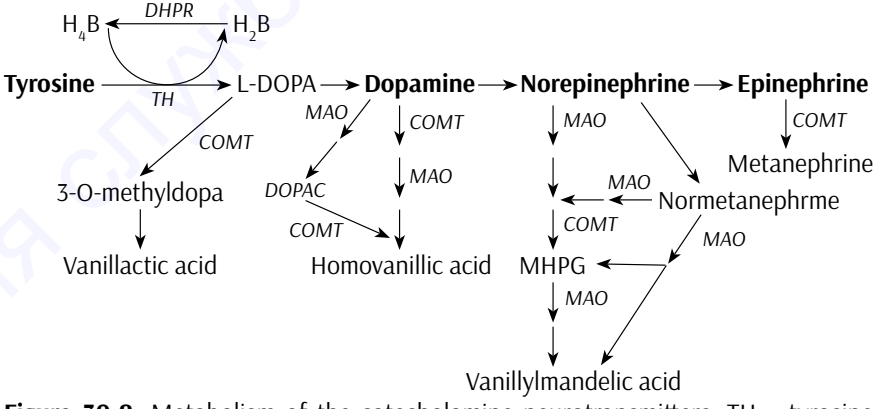


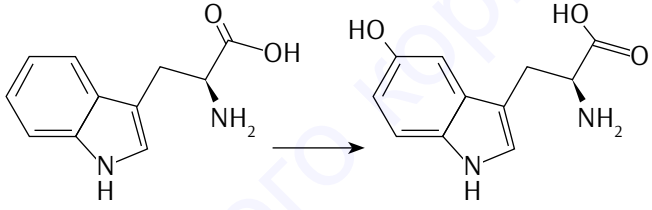
Figure 30.8. Metabolism of the catecholamine neurotransmitters. TH = tyrosine

hydroxylase, DHPR = dihydropteridine reductase, H2B = dihydrobiopterin, H4B = tetrahyrobiopterin, MAO = monoamine oxidase, COMT = catecholamine-O-methyltransferase, MHPG = 3-methoxy-4-hydroxyphenylglycol, DOPAC = dihydroxyphenylacetic acid

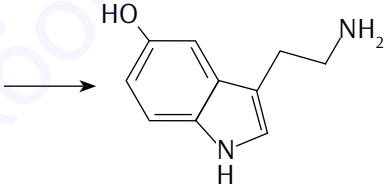
Compounds that inhibit the action of MAO have been shown to have beneficial effects in the treatment of clinical depression, even when tricyclic antidepressants are ineffective. The utility of MAO inhibitors was discovered serendipitously when patients treated for tuberculosis with isoniazid showed signs of an improvement in mood; isoniazide was subsequently found to work by inhibiting MAO.

Serotonin

Serotonin (5-hydroxytryptamine, 5-HT) is formed by the sequential hydroxylation and decarboxylation of tryptophan:



Tryptophan *5-Hydroxytryptophan*



5-Hydroxytryptamine (Serotonin)

The greatest concentration of 5-HT (90 %) is found in the enterochromaffin cells of the gastrointestinal tract. Most of the remainder of the body's 5-HT is found in platelets and the CNS. The effects of 5-HT are felt most prominently in the cardiovascular system, with additional effects in the respiratory system and the intestines. Vasoconstriction is a classic response to the administration of 5HT.

Neurons that secrete 5-HT are termed *serotonergic*. Following the release

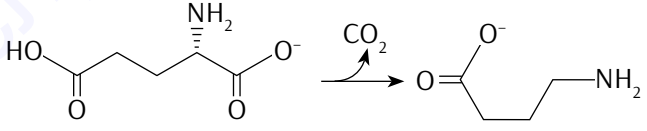
of 5-HT, a portion is taken back up by the presynaptic serotonergic neuron in a manner similar to that of the reuptake of norepinephrine.

The function of serotonin is exerted upon its interaction with specific receptors. Several serotonin receptors have been cloned and are identified as $5HT_1$, $5HT_2$, $5HT_3$, $5HT_4$, $5HT_5$, $5HT_6$, and $5HT_7$. Within the $5HT_1$ group there are subtypes $5HT_{1A}$, $5HT_{1B}$, $5HT_{1D}$, $5HT_{1E}$, and $5HT_{1F}$. There are three $5HT_2$ subtypes, $5HT_{2A}$, $5HT_{2B}$, and $5HT_{2C}$ as well as two $5HT_5$ subtypes, $5HT_{5a}$ and $5HT_{5B}$. Most of these receptors are coupled to G-proteins that affect the activities of either ***adenylate cyclase*** or ***phospholipase Cγ*.** The $5HT_3$ class of receptors are ion channels.

Some serotonin receptors are presynaptic and others postsynaptic. The $5HT_{2A}$ receptors mediate platelet aggregation and smooth muscle contraction. The $5HT_{2C}$ receptors are suspected in control of food intake as mice lacking this gene become obese from increased food intake and are also subject to fatal seizures. The $5HT_3$ receptors are present in the gastrointestinal tract and are related to vomiting. Also present in the gastrointestinal tract are $5HT_4$ receptors where they function in secretion and peristalsis. The $5HT_6$ and $5HT_7$ receptors are distributed throughout the limbic system of the brain and the $5HT_6$ receptors have high affinity for antidepressant drugs.

GABA

As was mentioned already, several amino acids have distinct excitatory or inhibitory effects upon the nervous system. The amino acid derivative, *γ-aminobutyrate* (GABA), also called 4-aminobutyrate, is a well-known inhibitor of synaptic transmission in the CNS, and also in the retina. The formation of GABA occurs by the decarboxylation of glutamate catalyzed by *glutamate decarboxylase:*



Glutamate *4-Aminobutyrate (GABA)*

The entire metabolic pathway to produce and break down GABA in nerve tissue occurs as the bypass of citric acid cycle (TCA). This enzymatic reac-

tions consequence is known as *GABA shunt* (Figure 30.9).

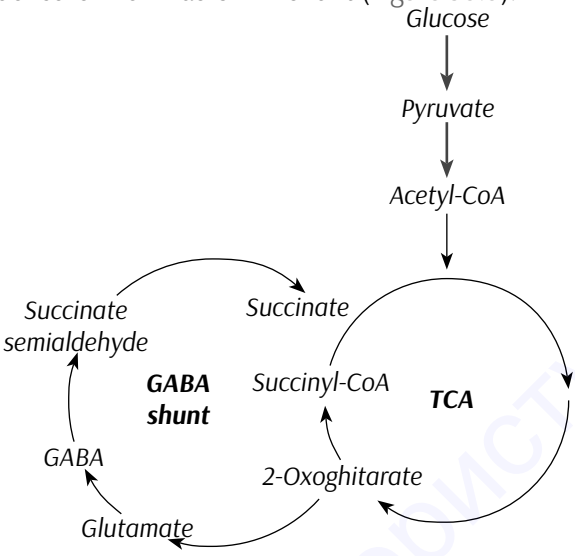


Figure 30.9. 4-Aminobutyrate (GABA) metabolism in the brain and GABA shunt

Neurons that secrete GABA are termed *GABAergic. GABA* exerts its effects by binding to two distinct receptors, **GABA-A** and **GABA-B.** The GABA-A receptors form a Cl^- channel. The GABA-b receptor is a G-protein related receptor, that is of metabotropic type. The binding of GABA to GABA-A receptors increases the Cl^- conductance of postsynaptic membrane and makes the inhibitory effect as to the excitability of corresponding neurons – Figure 30.10.

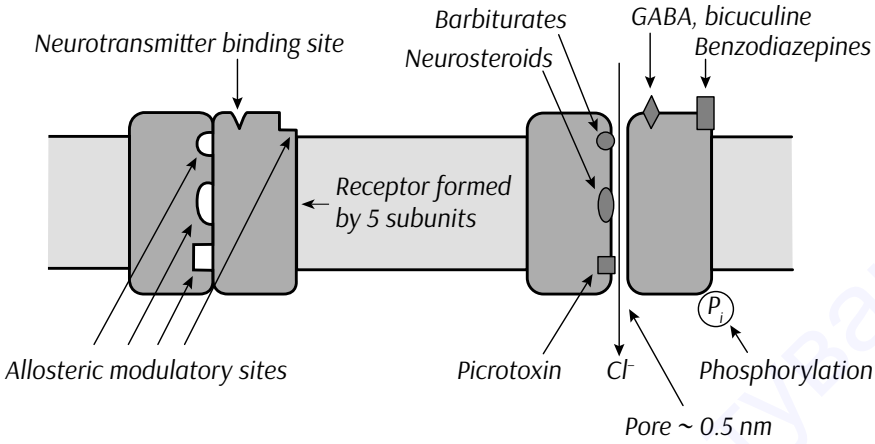


Figure 30.10. The interaction of ligands with the GABA-A receptor

The anxiolytic drugs of the **benzodiazepine** family exert their soothing effects by potentiating the responses of GABA-A receptors to GABA binding. The GABA-B receptors are coupled to an intracellular G-protein and act by increasing conductance of an associated K^+ channel.

Opiate peptides. Endorphin

Opiate peptides constitute the subclass of ***neuropeptides,*** having an amazing ability to interact with membrane receptors which possess high affinity to a family of psychoactive drugs called ***opiates*** (or ***opioids***), that includes **morphine** and related substances (see 20.4).

Opiate peptides are neurotransmitters, that take part in transmitting signals of pain and modulate the development of some highly specialized psychic and emotional human feelings. These neuropeptides are commonly divided into:

- **Endorphins,** that are polypeptides built of from 16 to 19 amino acid residues. The term comes from the abbreviation for "endogenous morphine", and it designates the naturally occurring substance that binds to opioid receptors in the brain.

There are ***α-, β-, γ-*** and ***δ-endorphins*** which are neurotransmitters found in the brain that have pain-relieving properties similar to morphine.

- **Enkephalins,** which are presented by two pentapeptides ***met-enkephalin*** and ***leu-enkephalin.***

Met-enkephalin (Tyr-Gly-Gly-Phe-Met).
Leu-enkephalin (Tyr-Gly-Gly-Phe-Leu).
Dynorphins, of which A- and B-dinorphins are representatives.

Opiate receptors. The receptors for opiate peptides are divided into **μ-(mu), δ-(delta), κ-(kappa), σ-(sigma) and ε-(epsilon)** subtypes.

Endorphins interact with the specialized **opiate receptor** neurons that contain **mu (μ) receptors.** These G-protein-coupled receptors are located on the postsynaptic membrane of neurons involved in the transmission of pain signals. Thus, **the physiological action of endorphins results in reducing the intensity of pain.**

Many painkilling drugs, such as morphine and codeine, act like endorphins and actually activate opiate receptors (see below). Besides behaving as a pain regulator, endorphins are also thought to be connected to physiological processes including euphoric feelings, appetite modulation, and the release of sex hormones. Prolonged, continuous exercise contributes to an increased production and release of endorphins, resulting in a sense of euphoria that has been popularly labeled "runner's high".

30.4. Drugs, neurotransmitters and synapses

Many drugs that alter mental state achieve at least some of their effects by acting at synaptic receptors.

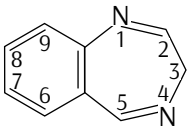
GABA Receptors

It was shown before that $GABA_A$ receptor is a ligand-gated chloride channel. Activation of the receptors increases the influx of chloride (Cl^-) ions into the postsynaptic cell raising its membrane potential and thus inhibiting it (Figure 20.11).

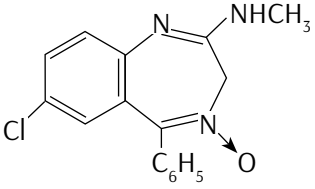
A number of drugs bind to the $GABA_A$ receptor. They bind at sites different from the spot where GABA itself binds, but increase the strength of GABA's binding to its site. **Thus they enhance the inhibitory effect of GABA in the CNS.**

These drugs include:

- **sedatives** like phenobarbital;
- beverage **alcohol** (ethanol);
- **anti-anxiety drugs** like Valium, Librium, Halcion (all members of a group called benzodiazepines).



1,4-Benzodiazepine



Chlorodiazepoxide (Elenium. Librium)

In view of their common action, it is not surprising that they act additively; taken together (e.g., alcohol and Valium) these drugs can produce dangerous overdoses.

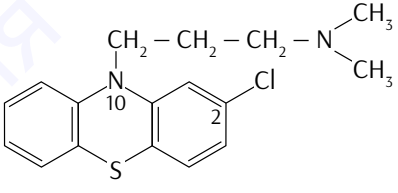
Catecholamine and serotonin synapses

Many antidepressant drugs (the so-called tricyclic antidepressants like amitriptyline ["Elavil"]) interfere with the **reuptake** of **noradrenaline** and **serotonin** from their synapses and thus enhance their action at the synapse.

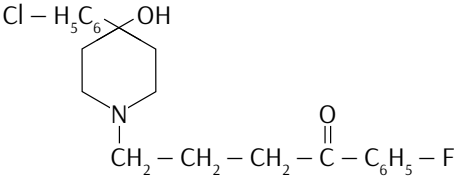
Another popular antidepressant fluoxetine **("Prozac"),** blocks the reuptake of serotonin by the presynaptic serotonin receptors.

Dopamine synapses

One class of dopamine receptors is bound by such drugs as chlorpromazine (Thorazin, Aminazine) and Haloperidol (Haldol). These drugs are called **tranquilizers** (also **neuroleptics**, or ***antipsychotic medications***), and their binding with D_2-receptors counteracts the physiological effects of dopamine which eases some of the clinical symptoms of **schizophrenia.**



Aminazin



Haloperidol

Synapses blocking pain signals

As was stated already, at synapses on the neurons involved in transmitting pain signals back to the brain the **endorphins, enkephalins *(met-enkephalin, leu-enkephalin)*** and **dinorphines** are released.

The ability to perceive pain is vital. However, faced with massive, chronic, intractable pain, it makes sense to have a system that decreases its own sensitivity. Opiate peptides synapses provide this intrinsic pain suppressing system.

What are opiates?

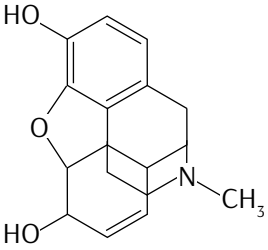
These are substances isolated from the **opium poppy** or synthetic relatives. (They are also called *opioids*).

Figure 30.11. Papaver somniferum flower

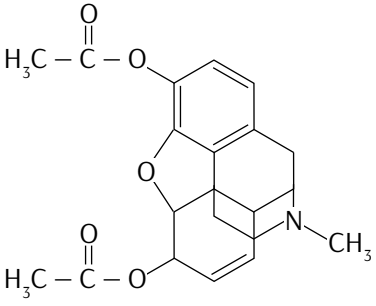
"Opium" is a Greek word for *juice*, and from here, for the drug obtained out of the juice of the plant "opium poppy" (*Papaver somniferum*) – Figure 30.11. Opium is not a pure chemical substance, it is a mixture that contains over 20 distinct alkaloids.

The examples of well-known opiates are:

- morphine;
- codeine;
- heroin;
- methadone;
- oxycodone.



Morphine



Heroin

Opiates depress nerve transmission in sensory pathways of the spinal cord and brain that signal pain. **The biochemical basis of opiates function is their binding to opiate receptors, that is receptors which have intrinsic affinity to natural edorphines and enkephalins.** Thus, opiates enhance the pain-killing effects of the natural neuropeptides. This explains why opiates are such effective pain killers.

Opiates also inhibit brain centers controlling coughing, breathing, and intestinal motility. Both morphine and codeine are used as pain killers, and codeine is also used in cough medicine.

But, unfortunately, opiates are exceedingly addictive, they quickly produce **tolerance,** that is the need for higher doses to achieve the prior effect, and **dependence.** The dependence signifies that if use of the drug ceases, the now relatively insensitive synapses respond less well to the soothing effects of the intrinsic opioids, and the **painful symptoms of withdrawal appear.**

Hence, although heroin is even more effective as a painkiller than morphine and codeine, it is so highly addictive that its use is illegal!

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