Міністерство охорони здоров'я України
Харківський національний медичний університет

Л.Д. Попова, Г.В. Полікарпова

БІОХІМІЯ

Навчально-методичний посібник
для студентів вищих навчальних закладів
IV рівня акредитації та лікарів-інтернів

L.D. Popova, A.V. Polikarpova

BIOCHEMISTRY

Manual for medical students and interns

Рекомендовано
Міністерством освіти і науки України
як навчальний посібник для студентів
вищих навчальних закладів

Х а р к і в 2011

УДК 577.1(075.8)=111
ББК 28.072.я7
В 60

Рекомендовано Міністерством освіти і науки України
(08.02.2011)

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В 60 Біохімія: Навч.-метод. посібник для студентів та лікарів-інтернів/ Л.Д. Попова, Г.В. Полікарпова – Харків: ХНМУ, 2011. – 539 с.
ISBN

В 60 Biochemistry: Manual for medical students or interns/ L.D. Popova, A.V. Polikarpova – Kharkiv: KNMU, 2011. – 539 p.
ISBN

The manual summarizes current knowledge relevant to the static, dynamic, and functional biochemistry. Structure and metabolism of basic classes of biomolecules (proteins, amino acids, nucleic acid, nucleotides, carbohydrates, lipids) are considered. Modern conceptions of molecular biology and genetics, biochemical bases of physiological functions and neurohumoral regulation are elucidated. Biochemistry of blood, kidney, muscle, liver, immune, nervous, connective tissue is presented. Molecular mechanisms of metabolic disorders of different classes of substances and pathways of their correction are discussed. The book is dedicated to the medical students and interns.

УДК 577.1(075.8)=111
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CONTENTS

LIST OF ABBREVIATIONS

A	adenine	CDP	cytidine diphosphate
ACAT	acyl-CoA:cholesterol acyltransferase	CSF	colony stimulating factor
		Cys	cysteine
ACP	acyl carrier protein		
ACTH	adrenocorticotropic hormone	DOPA	dihydroxyphenylalanine
ADH	antidiuretic hormone	DAG	diacylglycerol
ADP	adenosine diphosphate	DAP	dihydroxyacetone phosphate
AIDS	aquired immunodeficiency syndrome	DNA	deoxyribonucleic acid
Ala	alanine	E_a	activation energy
AMP	adenosine monophosphate	E_0	redox potential value
APC	antigen presenting cell	EF	elongation factor
apo	apoprotein		
Arg	arginine	FAD	flavin adenine dinucleotide
Asn	asparagine	$FADH_2$	reduced form of FAD
Asp	aspartic acid	FFA	free fatty acid
ATP	adenosine triphosphate	FH_2	dihydrofolate
		FH_4	tetrahydrofolate
C	cytosine	fMet	formylmethionine
cAMP	cyclic adenosine monophosphate	FMN	flavin mononucleotide
		FSH	follicle-stimulating hormone
cGMP	cyclic guanosine monophosphate	G	guanine
		G^0	standard free energy
CoA	coenzyme A	GABA	γ-aminobutyric acid
CoQ	coenzyme Q, ubiquinone		

GAG	glycosaminoglycan	G-1-P	glucose-1-phosphate
Gal-1-P	galactose-1-phosphate	G-6-P	glucose-6-phosphate
GAP	glyceraldehyde-3-phosphate		
Gln	glutamine	K_a	acid dissociation constant
Glu	glutamic acid	K_{eq}	equilibrium constant of reaction
Gly	glycine	K_m	Michaelis constant
Hb	hemoglobin	α-KG	α-ketoglutarate
HbO_2	oxyhemoglobin		
HDL	high-density lipoprotein	LCAT	lecithin:cholesterol acylthransferase
HGPRT	Hypoxantine-guanine phosphoribosyl transferase	LCK	light chain kinase
His	histidine	LDL	low-density lipoprotein
HIV	human immunodeficiency virus	Leu	leucine
		LH	luteinizing hormone
HMG-CoA	3-hydroxy-3-methylglutaryl-CoA	Lys	lysine
Hsp	heat shock protein	MHC	major histocompatibility complex
I	inosine	mRNA	messenger RNA
IDL	intermediate-density lipoprotein	N	unspecific base of nucleotide
IF	initiation factor		
IFN	interferon	NAD	nicotinamide adenine dinucleotide
IG	immunoglobulin		
IGF	insulin-like growth factor	NADH	reduced form of NAD
IL	interleukine	NADP	nicotinamide adenine dinucleotide phosphate
Ile	isoleucine		

NADPH	reduced form of NADP	S	"synthesis" phase of cell cycle
NCAM	nerve cell adhesion molecule	SAM	S-adenosyl methionine
		Ser	serine
Ⓟ	high-energy phosphate	STH	somatotropic hormone
P450	cytochrome P450		
P_i	inorganic phosphate	T	thymine
PAF	platelet activation factor	T_3	triiodothyronine
PEP	phosphoenolpyruvate	T_4	thyroxine
PG	prostaglandin	TAG	triacylglycerol
Phe	phenylalanine	TCA	tricarboxylic acid cycle
PK	proteinkinase	TF	transcription factor
PKU	phenylketonuria	Thr	threonine
PLP	pyridoxal phosphate	TNF	tumor necrosis factor
PP_i	inorganic pyrophosphate	TPP	thiamine pyrophosphate
Pro	proline	tRNA	transfer RNA
PRPP	5-phosphoribosyl-1-pyrophosphate	Trp	tryptophan
		TSH	thyroid stimulating hormone
		Tyr	tyrosine
Q	ubiquinone		
		U	uracil
RNA	ribonucleic acid	UDP	uridine diphosphate
RNase	ribonuclease		
rRNA	ribosomal RNA	Val	valine
		VLDL	very low-density lipoprotein
S	Svedberg unit		

FOREWORD

Biochemistry holds a key position in training medical students, and is one of the basic preclinical science subjects in medical universities. Through knowledge og biochemistry by medical students is very important for the understanding and maintenance of health and for the understanding and effective treatment of disease.

Biochemistry is being transformed with astonishing rapidity. The material contained in the manual reviews the major advances in the static, dynamic and functional biochemistry.

The material of manual is presented in 20 chapters. It includes structure and metabolism of basic classes of biomolecules (proteins, amino acids, nucleic acids, nucleotides, carbohydrates, lipids), regulation of metabolism and physiologic functions, biochemistry of enzymes, vitamins, blood, kidney, muscle, liver, immune, nervous, connective tissues. Modern conceptions of molecular biology and genetics, other complex topics are presented in brief and accessible form.

Elucidation of molecular mechanisms of different classes of substances metabolism disorders, development of different diseases increases the clinical orientation of manual. It is imperative for understanding the causes and rational treatment of many diseases, two major areas of interest to physicians and other health care workers.

Chapter 1 STRUCTURE OF PROTEINS AND AMINO ACIDS

Proteins are bioorganic high molecular nitrogen containing compounds, heteropolymers, which consist of amino acids residues combined by peptide bonds.

Proteins are mostly prevalent from all the classes of biomolecules. They are involved to all cell components of microorganisms, plants, animals and inerstitial structures.

1.1 Functions of Proteins

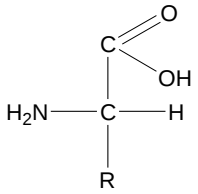
- *Catalytic function*. Biological catalysts are proteins.
- *Structural function*. Proteins are included to biomembrane structure, form the base of cytoskeleton, intercellular matrix and certain specialized tissues.
- *Receptor function*. Receptors are proteins.
- *Regulatory function*. A lot of bioregulators (hormones, mediators, modulators) are proteins.
- *Transport function*. Proteins provide intercellular and intracellular transfer of different substances. Also they carry chemical compounds in blood.
- *Contractive function*. Proteins realize contraction of muscles, flagellums, cilia etc.
- *Protective function*. Proteins provide the immune protection, prevent bleeding and intravessel thromb formation.

1.2 Proteinogenic Amino Acids.

Proteins are high molecular substances, their molecular weight varies from some thousands till some millions atomic mass units or Daltons. A protein is the polymer in which the monomer units are amino acids.

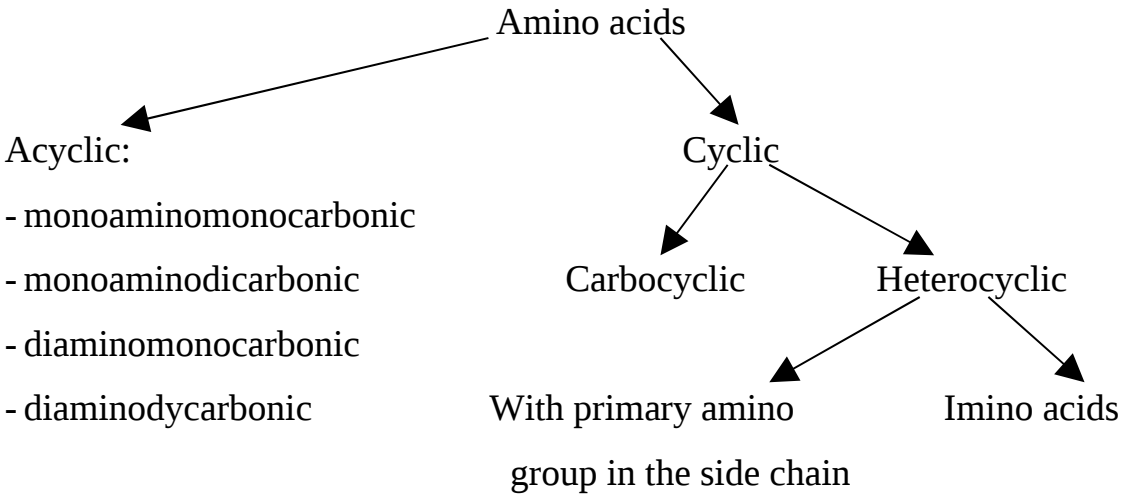
Amino acid is an organic compound that contains both an amino ($-NH_2$) and a carboxyl (-COOH) groups. The amino acids found in proteins are always α-amino acids - that is, amino acids in which the amino group is attached to the α- carbon

atom of the carboxylic acid carbon chain. The general structural formula of an α-amino acid is:



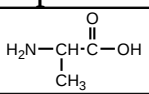
The R-group present in α-amino acid is called the amino acid side chain. The nature of these side chains distinguishes α-amino acids from each other. Side chain varies in size, shape, charge, acidity, functional group present, hydrogen-binding activity and chemical reactivity.

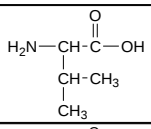
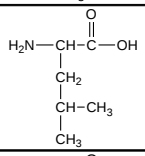
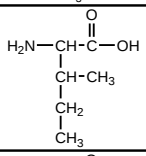
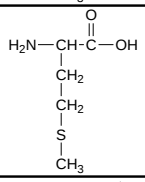
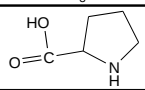
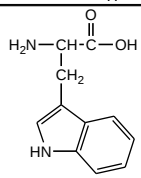
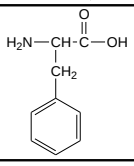
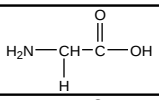
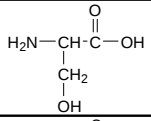
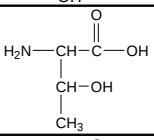
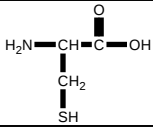
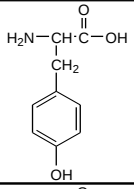
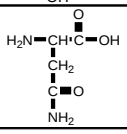
Over 700 different naturally occurring amino acids are known, but only 20 of them, called standard amino acids, are normally present in proteins. Standard amino acid is one of the 20 α-amino acids normally found in proteins. These amino acids are called proteinogenic.

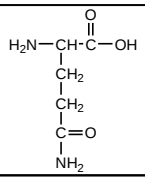
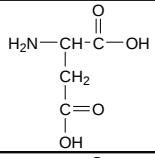
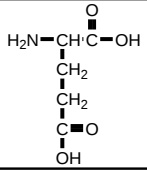
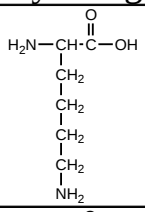
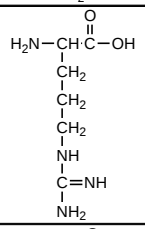
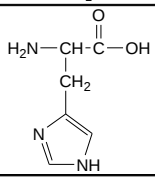


Besides their classification by side-chain functional group amino acids are also classified by side-chain polarity. In this system there are four categories: (1) nonpolar amino acids, (2) polar neutral amino acids, (3) polar negatively charged amino acids, (4) polar positively charged amino acids (table 1.1).

Table 1.1 Proteinogenic Amino Acids

Name	International symbol	Structural formula	pI
Amino acids with non polar R			
Alanine	Ala (A)		6.02

Valine	Val (V)		5.97
Leucine	Leu (L)		5.98
Isoleucine	Ile (I)		6.02
Methionine	Met (M)		5.75
Proline	Pro (P)		6.10
Tryptophan	Trp (W)		5.88
Phenylalanine	Phe (F)		5.98
Amino acids with polar neutral R			
Glycine	Gly (G)		5.97
Serine	Ser (S)		5.68
Threonine	Thr (T)		6.53
Cysteine	Cys (C)		5.02
Tyrosine	Tyr (Y)		5.65
Asparagine	Asn (N		5.41

Glutamine	Gln (Q)		5.65
Amino acids with negatively charged R			
Aspartic acid	Asp (D)		2.97
Glutamic acid	Glu (E)		3.22
Amino acids with positively charged R			
Lysine	Lys (K)		9.74
Arginine	Arg (R)		10.76
Histidine	His (H)		7.58

- ***Nonpolar amino acids*** contain one amino group, one carboxyl group and a nonpolar side chain. They are generally found inside of proteins, where there is limited contact with water.
- ***Polar neutral amino acids*** contain one amino group, one carboxyl group, and a side chain that is polar but neutral.
- ***Polar negatively charged amino acids*** contain one amino group and two carboxyl groups, the second carboxyl group being part of the side chain. In solution at physiological pH the side chain of a polar acidic amino acid has a negative charge; the side chain carboxyl group loses its acidic hydrogen atom.
- ***Polar positively charged amino acids*** contain two amino groups and one carboxyl group, the second amino group being part of the side chain. In

solution at physiological pH the side chain of a polar basic amino acid has a positive charge; the side chain amino group accepts a proton.

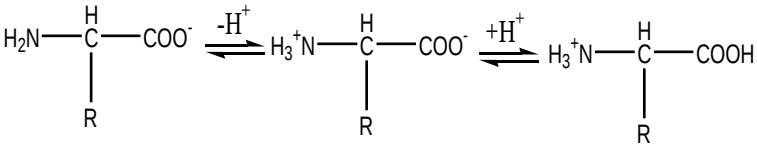
Properties of proteinogenic amino acids.

- *Chirality of amino acids.* Four different groups are attached to one α-carbon atom in all of the standard amino acids except glycine, where the R group is a hydrogen atom. This means that the structure of 19 from 20 standard amino acids posess a chiral center. With few exceptions (in some bacteria), the amino acids found in nature and in proteins are L-isomers.

Two of the 19 chiral standard amino acids, isoleucine and threonine, posess two chiral centers (table 1.1). With two chiral centers present, four stereoisomers are possible for these amino acids. However, only one of the L-isomers is found in proteins.

- *Acid-base properties.* In neutral solution carboxyl groups have a tendency to lose protons (H^+), producing a negatively charged species and amino groups have a tendency to accept protons (H^+), producing a positively charged species.

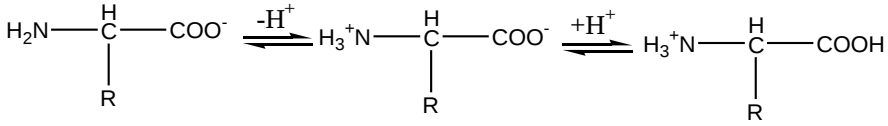
In neutral solution the –COOH group of an amino acid donates a proton to the $–NH_2$ of the same amino acid and amino acid molecules have a structure:



Such a molecule is known as ***zwitterion***, from the German term meaning “double ion”. A zwitterion is a molecule that has a positive charge on one atom and the negative charge on another atom. The net charge on a zwitterion is zero even though parts of the molecule carry charges.

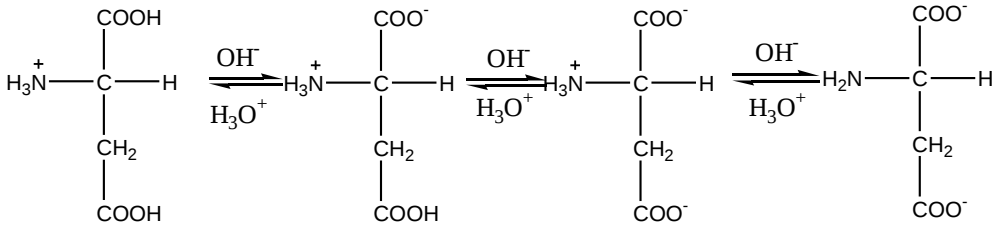
Zwitterion structure changes when the pH of a solution containing an amino acids is changed from neutral either to acidic (low pH) by adding an acid such as HCl or to basic (high pH) by adding a base such as NaOH. In an acidic solution, the zwitterion accepts a proton (H^+) to form a positively charged ion. In basic solution, the $–NH_3^+$ of the zwiterion loses a proton and a negatively charged species is formed.

Thus, in solution three different amino acid forms can exist (zwitterions, negative ion, positive ion). The three species are actually in equilibrium with each other, and the equilibrium shifts with pH change. The overall equilibrium process can be represented as follows:



In acidic solution, the positively charged species on the right predominates; nearly neutral solutions have the middle species (the zwitterion) as the dominant species; in basic solution, the negatively species on the left predominates.

Because of the extra site that can be protonated or deprotonated, acidic and basic amino acids have four charged forms in solution.



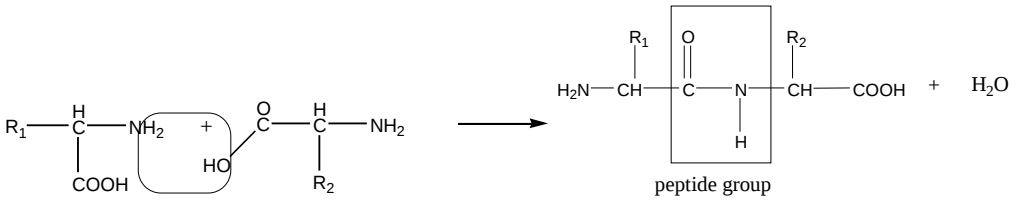
low-pH form (+1 charge) | moderately-low-pH form (zwitterion) | neutral-pH form (-1 net charge) | high-pH form (-2 net charge)

The existence of two low-pH forms for aspartic acid results from the two carboxyl groups being deprotonated in different times. For basic amino acids, two high-pH forms exist because deprotonation of the amino groups does not occur simultaneously. The side-chain amino group deprotonates before the α-amino groups.

Under the equilibrium of negative and positive charges amino acid molecule shows the isoelectric state. Individual for each amino acid pH level, under which amino acid has summary zero charge is called *isoelectric point.*

- *Ability to form amide bonds*. Removal of the elements of water from the reacting carboxyl and amino groups leads to the formation of the amide bond.

In amino acid chemistry, amide bonds that link amino acids together are given the specific name of peptide bond. A ***peptide bond*** is a bond between the carboxyl group of one amino acid and the amino group of another amino acid.

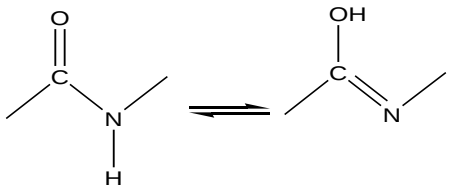


Short to medium-sized chains of amino acids are known as peptides. A ***peptide*** is a sequence of amino acids up to 50 units in which the amino acids are joined together through amide (peptide) bonds.

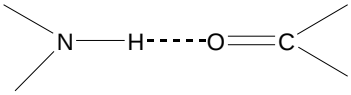
In all peptides, the amino acid at one end of the amino acid sequence has a free amino group, and the amino acid at the other end of the sequence has a free carboxyl group. The end with a free amino group is called the N-terminal end, and the end with a free carboxyl group is called the C-terminal end. By convention, the sequence of amino acids in a peptide is written with the N-terminal end amino acid at the left. The individual amino acids within a peptide chain are called amino acid residues.

Peptide Bond Properties

- *Complanarity.* Four atoms which form peptide bond (-CO-NH-) are situated in the same geometric flatness.
- Oxygen of carbonyl group and hydrogen of NH- group are situated in *trans*-position.
- *Length* of bond between carbon from CO group and nitrogen from NH is equal 1,032 nm. Peptide bond is intermediate between single and double bond (that is this bond has the partial double bond character, therefore rotation around this bond is impossible). These restriction facilitate the specific configuration of chains.
- *Keto-enol thautomery.* There are two conformations of peptide bond – ketone and enol. The resonance structure formation is provided by coupling free p-electrone pair of double bond C=O.



- Peptide bond is able to form hydrogen bonds.



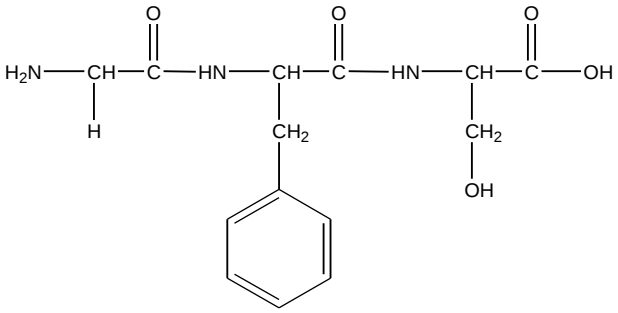
1.3 Levels of Protein Structure

Proteins are polypeptides that contain more than 50 amino acid residues.

Primary structure is the unique linear sequence of amino acid residues in a polypeptide chain.

The name of a peptide is made up of the name of the first N-terminal amino acid bearing a free NH_2-group (with the ending -yl) and of the names of amino acids added in succession (likewise each with ending -yl); the formulation ends the full name of the C-terminal amino acid with a free COOH group.

For example: glycyl-phenylalanyl-serine or Gly-Phe-Ser:



Primary structure is very stable. It provides the stability of protein molecules. It predetermines all the other levels of protein organization.

- *Secondary structure* is the configuration of a polypeptide chain due to formation of hydrogen bonds between components of peptide bonds. Polypeptide chain strives to configuration with maximum possible number of hydrogen bonds. But possibilities of spatial packing of polypeptide chain are limited by partial double bond character of peptide bond and impossibility of rotation around of this bond. Therefore polypeptide chain has specific conformation.

The following types of secondary structure are known:

- α-coil or α-helix;
- β-pleated sheet;
- helix of collagen (triple helix);
- irregular conformations (disordered regions).

- α-Helix is the conformation which is generated at the space rotation of polypeptide chain by the hydrogen bonds, which are formed between C=O and NH groups distant one to another at four amino acid residues. Hydrogen bonds of α-helix are directed parallelly to the molecular axis. The direction of α-helix rotation in natural proteins is right.

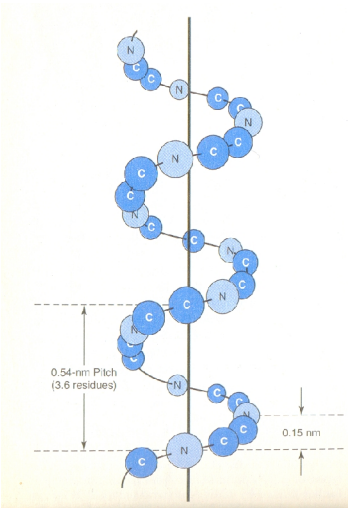


Figure 1.1 The α-helix

Geometric parameters of α-helix: radius – 0,25 nm, step – 0,54 nm, length of displacement to one amino acid residue – 0,15 nm, one revolution of α-helix is equal to 3,6 amino acid residues.

Several amino acids such as Pro, Gly, Glu, Asp, Arg counteract of α-helix formation or destabilize it.

- β-Pleated sheet is the structure similar to the folded lay. It is formed from zigzag-liked unwrapped nearly situated polypeptide chains. β-Pleated sheet structure is also formed by the hydrogen bonds, which are formed between C=O and NH groups of neighbouring chains.

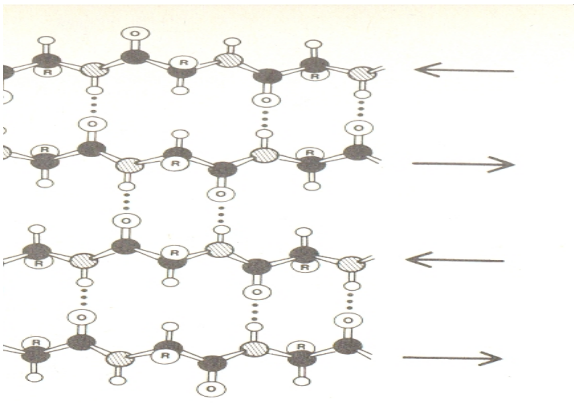


Figure 1.2. β-pleated sheet

- The triple helix is another secondary structure for proteins. This structure involves three coiled polypeptide chains wound around each other about a common axis to give a rope-like arragement. The interwining of the three polypeptide chains and some cross-linking between chains involving covalent bonds hold the triple helix together.

Collagen, the structural protein of connective tissue, which contains 33% of glycine and 21 % of hydroxyproline, has a triple helix structure.

Super-secondary structure. The α-helix and β-pleated sheets are connected together by unstructured polypeptides. The existence for some proteins of super-secondary structure has been found by means of X-ray crystallography methods.

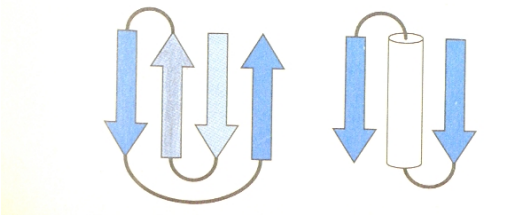


Figure 1.3. Supersecondary structure.

These proteins are called ***domain proteins***. These proteins contain to a conciderable extent isolated globules-domains. These globules in domain proteins are formed by the same single polypeptide chain. Tertiary structure of domain proteins is the packing different domains each to another. Domains usually perform different functions. Functional properties of domain proteins are like to those of oligomeric ones.

- *Tertiary structure* is the overall three-dimentional shape that results from the attractive forces between amino acid side chains (R groups) that are widely separated from each other within the chain.

Four types of attractions contribute to the tertiary structure of a protein: covalent disulfide bonds, ionic bonds (salt bridges), hydrogen bonds and hydrophobic interactions.

- Disulfide bonds (-S-S-), the strongest of the tertiary structure interactions, result from –SH groups two cysteine molecule residues, which are included to the same or different polypeptide chains. This type of interaction is the only one of the four tertiary-structure interactions that involves a covalent bond.

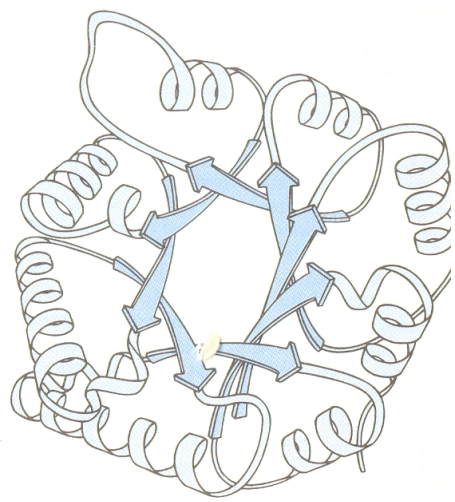
- Ionic bonds, also called salt bridges, always involve amino acids with charged side chains. These amino acids are the acidic and basic amino acids. The two R-groups, one acidic and one basic, interact through ion-ion interactions.

- Hydrogen bonds can occur between amino acids with polar R groups. A variety of polar side chains may be involved especially those that possess the following functional groups: -OH, $-NH_2$, $-\overset{\overset{O}{\|}}{C}-OH$, $-\overset{\overset{O}{\|}}{C}-NH_2$.

Hydrogen bonds are relatively weak and are easily disrupted by changes of pH and temperature.

- Hydrophobic interactions result when two nonpolar side chains are close to each other. Anthough hydrophobic interactions are weaker than hydrogen and ionic bonds, they are a significant force in some proteins because there are so many of them.

Depending on the shape and features of three-dimensional shape there are globular and fibrillar proteins.



Globular proteins have roughly spherical shape. The ratio between long and short axes varies from 1:1 till 50:1. Globular proteins are built from one or some polypeptide chains, which are tightly packed.

Figure 1.4 Tertiary structure.

In aqueous solution, globular proteins usually have their polar R groups outward toward the aqueous solvent (which is also polar), and their non polar R groups inward (away from the polar water molecules). The non polar R groups then interact with each other.

- Fibrillar proteins show the linear shape. They usually form multimolecular complexes – fibrils, which consist of some parallel polypeptide chains. Fibrillar proteins are structural components of connective and other tissues. A lot of fibrillar proteins are formed by superspiralization.

- *Quaternary structure* is the highest level of protein organization. It is found in proteins that have two or more polypeptide chains. These multichain

proteins are often called *oligomeric proteins*. Quaternary structure is formed by aggregation of some polypeptide chains or protomers, each of them shows characteristic ordered conformation. Subunits are combined by non covalent bonds. This provides easy dissociation under change of physico-chemical properties of medium.

Physico-chemical Properties of Proteins

The most characteristic physico-chemical properties inherent in proteins are: high viscosity in solution; low diffusion; pronounced swelling ability; optical activity; mobility in electric field; low osmotic and high oncotic pressures; ability ro absorb UV light at 280 nm wave-length (this property which is attributable to the occurence of aromatic amino acids in proteins, is used of for protein quantitative determination).

The most of physico-chemical properties of proteins are provided by their acid-base properties and high molecular mass.

- *Acid-base properties.* Due to the presence of high amount of ionogenious groups proteins are amphoteric electrolytes and form amphions in the water solutions, which charge depends on the amino acid composition and pH of medium.

Presence of charge provides the protein mobility in the electric field. Electrophoretic activity depends on the charge and molecular mass and allows the using electrophoresis to separate protein mixture.

pH changing can cause the state, when summary charge of protein molecule is zero (isoelectric state). The level of pH, when summary charge of protein molecule is zero is called isoelectric point (pI).

At the isoelectric point, the proteins are the least stable in solution and are prone to an easy precipitation.

- *Solubility* of proteins in different physico-chemical mediums depends on the presence of polar or non polar amino acid residues. The increase of metal cations or ammonium concentrations in the solution leads to dehydration and sedimentation of protein.

- *Denaturation* is the loss of spatial conformation, characteristic to the native protein molecule with the resultant loss of their native properties. Denaturation occurs under action of hard physical and chemical factors. The mechanism of such factors influence is based on destruction of weak bonds, which stabilize the spatial conformation.

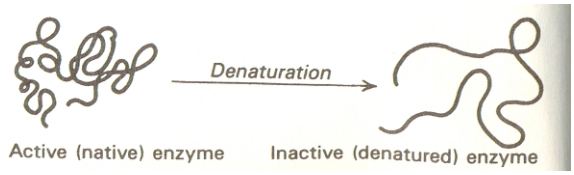


Figure 1.5. Protein denaturation.

- *Interaction with different chemical ligands*. The presence on molecular surface of different active functional groups provides the protein ability to combine with various chemical ligands (low and high-molecular compounds). The binding with ligands can be the step of transport, regulatory or catalytic functions realizing.

1.4 Protein Classification

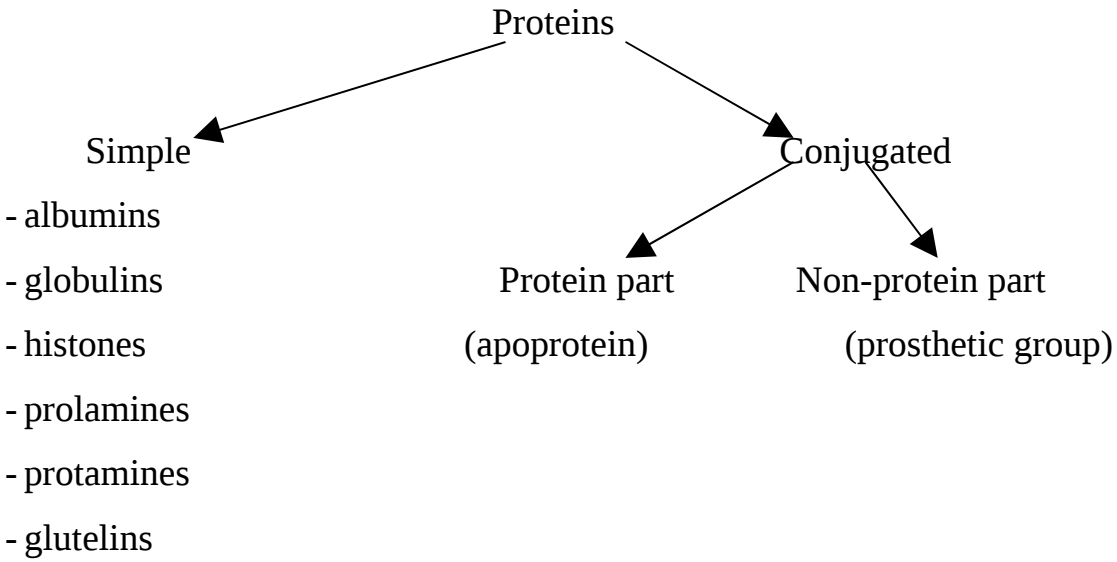


Table 1.1 Simple Proteins

Group	Localization	Biological role	Features of structure and composition
Histones. Molecular mass is 11 – 22 kDa	Cell nuclei (deoxyribo-nucleo-proteins)	They provide the formation and function of nuclear chromatin, regulation of genetic information transduction	Bacis amino acids are prevalent: Lys, Arg, His
Albumins. Molecular mass ~ 70 kDa.	In organs and tissues: blood, muscles.	They keep oncotic pressure, transport hormones, fatty acids, bilirubine, biogenic elements, drugs, form protein reserve, play plastic, detoxification roles.	Acidic amino acids are prevalent: Glu, Asp. They contain 15% of Leu, 1% of Gly.
Globulins.	Blood,	They perforn transport,	Contain 3% of Gly.

Molecular mass is 150 kDa.	muscles, lymph	protective functions, take part in blood clotting	Similar to albumin, but contain low amount of Asp, Glu.

The distinctive definitions “albumins and globulins” have been suggested in reference to the respective solubility or insolubility of these proteins in distilled water and half-saturated aqueous $(NH_4)_2SO_4$ solution. Globulins are soluble only in dilute saline solutions and insoluble in other media. It should be noted that some classical globulins of blood serum (for example, β-lactoglobulin) are soluble in 50% $(NH_4)_2SO_4$ solution.

The different solubility of blood serum albumins and globulins is widely used in clinical practice for their fractionation and quantitive determination.

Conjugated Proteins

Conjugated proteins consist of protein part, which is called apoprotein, and non-protein part or prosthetic group. Apoprotein and prosthetic group can be combined together by covalent or noncovalent bonds.

According to the chemical nature of proshtetic group conjugated proteins are divided to:

- ***Glycoproteins***. The prosthetic group in glycoproteins is represented by carbohydrates and their derivatives bound quite tightly to the protein moiety of glycoprotein molecules. Protein complexes with high molecular heteropolysaccharides are called proteoglycans.
- ***Lipoproteins*** are complex proteins, which protein part is combined with lipids.
- ***Nucleoproteins*** are proteins combined with nucleic acids (DNA or RNA). Nucleoproteins are supramolecular complexes, which form cell organells (ribosomes), chromatin.
- ***Chromoproteins*** are proteins with colored prosthetic group.

Table 1.2 Chromoproteins

Groups	Biological role	Prosthetic group
Flavoproteins (FAD, FMN)	Participation in the biological oxidation, oxido-reductive processes	Isoalloxazine derivatives

Rhodopsin	Protein of visual purple, participation in visual act	Retinal
Hemoproteins: hemoglobin, cytochromes, catalase, peroxidases, myoglobin	Respiratory, transport, electron transfer, catalytic functions	Iron-containing protoporphyrines (heme)

- ***Metaloproteins*** contain metal, which is not involved to the metaloporphyrine complex.
- ***Phosphoproteins*** are proteins, which contain phosphate residue combined by phosphodiesteric bond with hydroxyl group of serine, threonine or tyrosine of polypeptide chain.

Tests for Self-control

1. In proteins the α-helix and β-pleated sheet are examples of:
A. Primary structure
B. Secondary structure
C. Tertiary structure
D. Quaternary structure
E. None of the above mentioned
2. Which amino acids contain negatively charged R-group?
A. Leucine and asparagine
B. Glutamine and glycine
C. Glutamic and aspartic
D. Arginine and valine
E. Glycine and serine
3. Which amino acids contain positively charged R-group?
A. Leucine and asparagine
B. Glutamine and glycine
C. Glutamic and aspartic
D. Arginine and valine
E. Arginine and histidine
4. Which chemical feature is not characteristic for peptide bond?
A. Complanarity
B. Keto-enol thautomery
C. Ability to form hydrogen bonds
D. Mutarotation
E. Intermediate length between single and double bonds
5. Denaturation is:
A. The loss of spatial conformation, characteristic to the native protein molecule
B. Destruction of peptide bonds
C. Replace of C-end
D. Replace of N-end
E. Deamination of amino acids
6. Protein part of the complex protein is called:
A. Lipoprotein
B. Apoprotein
C. Prosthetic group
D. Phosphoprotein
E. Peptide

Chapter 2 CARBOHYDRATES AND THEIR DERIVATIVES

Carbohydrates are bioorganic substances, which by their chemical structures are aldehydo- or ketoderivatives of polyatomic alcohols or polyhydroxyaldehydes and polyhydroxyketones.

Carbohydrates, which are cannot be hydrolyzed are simple carbohydrates or monosaccharides. Carbohydrates, which are hydrolyzed with monosaccharides formation, are called polysaccharides or oligosaccharides.

Carbohydrates have an empirical formula $(CH_2O)_n$. But many polysaccharides, however, especially those which have a structural role, do not satisfy this formula in all respects because their monomer components are modified and include amino sugars, deoxy sugars and sugar acids. Nonetheless, they are called carbohydrates.

In plants, glucose is synthesized from carbon dioxide and water by photosynthesis and stored as starch or is converted to the cellulose of the plant framework. Animals can synthesize some carbohydrates from non carbohydrates compounds, but the bulk of animal carbohydrate is derived ultimately from plants.

2.1 Monosaccharides and Their Derivatives

Monosaccharides, the simplest carbohydrates, are aldehydes or ketones that have two or more hydroxyl groups.

According to the carbon atoms amount monosaccarides are divided to trioses, tetroses, pentoses, hexoses, heptoses etc. Hexoses and pentoses are most prevalent monosaccarides as participants of metabolism or components of other biomolecules.

The smallest ones, for which n=3, are glyceraldehyde and dihydroxyacetone. They are trioses. Glyceraldehyde is an aldose because it contains an aldehyde group, whereas dihydroxyacetone is a ketose because it contains a keto group.

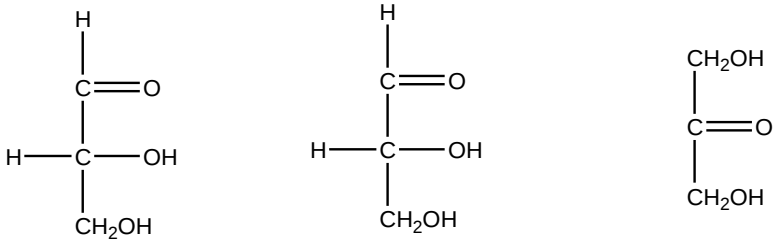
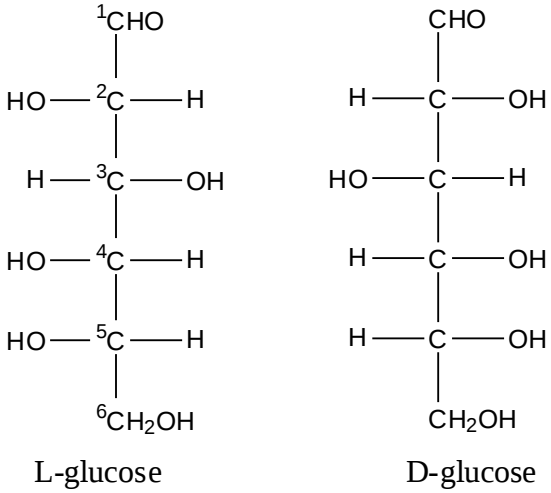


Figure 2.1 D-glyceralsehyde (left), L-glyceralsehyde (middle), dihydroxyacetone (right)

Glyceraldehyde has a single asymmetric carbon. Thus, there are two stereoisomers: D-glyceraldehyde and L-glyceraldehyde. The prefixes D and L designate the absolute configuration. They do not differ in physical properties, but each rotates the plane of polarized light in the opposite direction of each other. The direction of rotation cannot be inferred from the absolute configuration (symbols D and L), and is recorded by the symbols “plus” and “minus”, or some times by symbols d and l, which are confused since they have no relation to the D and L forms of the molecule.

Sugars with 4, 5, 6 and 7 carbon atoms are called tetroses, pentoses, hexoses and heptoses. Two common hexoses are D-glucose (an aldose) and D-fructose (a ketose). For sugars with more than one asymmetric carbon atom, the symbols D and L refer to the absolute configuration of the asymmetric carbon farthest from the aldehyde or keto group.



L-glucose D-glucose

Figure 2.2 D- and L-glucose.

In general, a molecule with n asymmetric centers has 2^n stereoisomeric forms. For aldotrioses n is equal to 1, and so there are 2 stereoisomers, D- and L-glyceraldehyde. They are enantiomers of each other, that is, so called “mirror images”.

The six-carbon aldoses have four asymmetric centers, and so there are 16 stereoisomers and 8 of them belong to the D-series. D-Glucose, D-mannose and D-

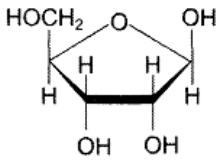
galactose are abundant six-carbon aldoses. D-glucose and D-mannose differ only in configuration at C-2. D-Sugars differing in the configuration a single asymmetric center are epimers. Thus, D-glucose and D-mannose are epimers at C-2; D-glucose and D-galactose are epimers at C-4.

The predominant forms of glucose and fructose in solution are not open-chains. Rather, the open-chain forms of these sugars form rings. The C-1 aldehyde in the open-chain form of glucose reacts with the C-5 hydroxyl group to form an ***intramolecular hemiacetal***. The resulting sixmembered sugar ring is called ***pyranose***.

The C-2 keto group in the open-chain form of fructose can react with the C-5 hydroxyl group to form an ***intramolecular hemiketal***. This fivemembered sugar ring is called ***furanose***.

An aditional asymmetric center is created when glucose ***cyclizes***. Carbon-1, the carbonyl carbon atom in the open-chain form, becomes an asymmetric center in the ring form. Two ring structures can be formed, namely: α-D-glucopyranose and β-D-glucopyranose. The designation α means that the hydroxyl group attached to C-1 is below the plane of the ring; α means that it is above the plane of the ring. The C-1 carbon is called the anomeric carbon atom, and so the α and β forms are anomers. Sugars containing free hydroxyl group of anomeric carbon atom are the reducing sugars. They reduce indicators such as cupric ion (Cu^{2+}) complexes to the cuprous form (Cu^{+}).

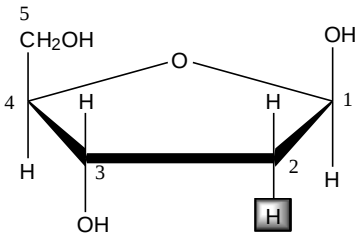
- *Pentoses.* Biologically important are:



  - aldopentoses: ribose, 2-deoxyribose, arabinose, xylose;
  - ketopentoses: ribulose, xylulose.

Figure 2.3 β-D- ribofuranose

Ribose is aldopentose, which in β-furanose form is included to nucleotide structure, coenzymes (NAD, NADP, FAD, FMN), glycosides, antibiotics, RNA.

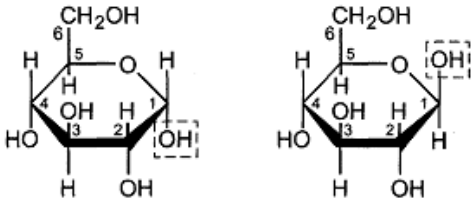


2-deoxy-D-ribose differs from ribose by the absence of oxygen atom in the second position. It is involved to the deoxiribinucleotides structure.

Figure 2.4. 2-Deoxy D-β-ribofuranose.

Xylose is the source for synthesis of alcohol xylit, which is used in medicine.

- *Hexoses.* They are divided to:
  - aldohexoses, for example glucose, galactose, mannose, fucose etc.;
  - ketohexoses, for example fructose.



Glucose (grapes sugar, dextrose) is very frequent monosaccharide in the nature. It is the structural component of disaccharides and homopolysaccharides.

Figure 2.5 α- (left) and β-(right)-D-glucose.

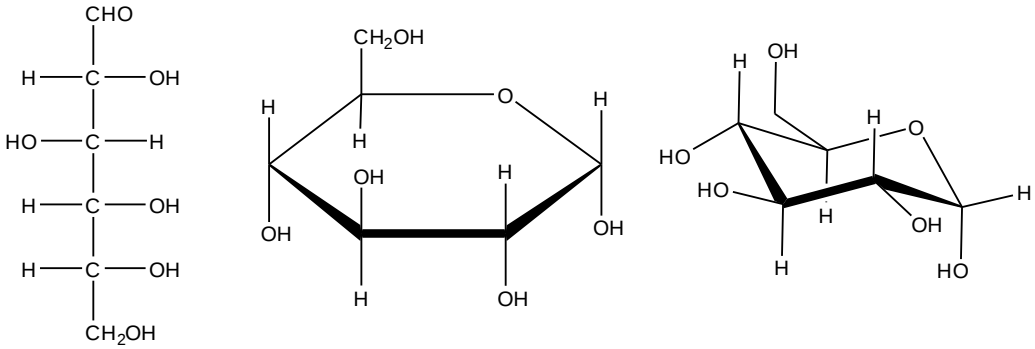


Figure 2.6 α-D-glucose.

It can exist in cyclic and linear forms. Position of H and OH groups around the fifth carbon atom causes D- or L- isoforms generation. Namely D-glucose is situated in the living been. Cyclic form can exist in α- or β- isoforms due to the position of H and OH groups around the first carbon.

Galactose (milk sugar) is involved to the disaccharide lactose structure, which can be taken from the milk. Galactose is part of hetepolysaccarides (glycosaminoglycans or mucopolycaccharides) of animal tissues.

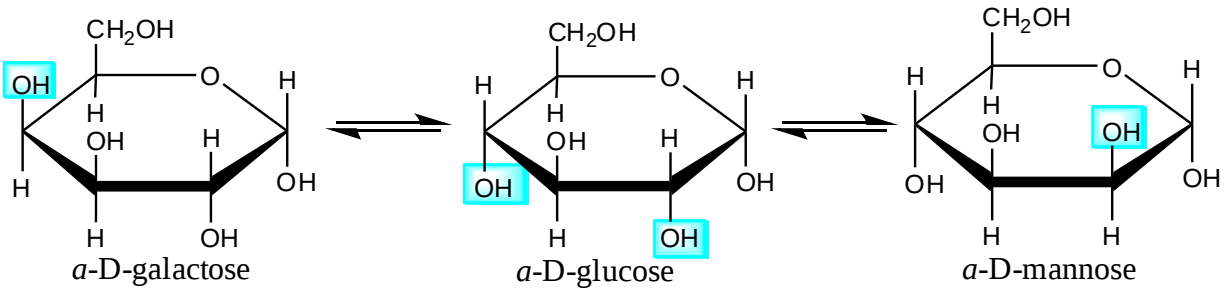


Figure 2.7 Epimerisation of glucose.

Mannose is the structural component of polysaccharide mannan, which is the component of plant glycoproteins. In the animal organism mannose is involved to oligosaccharide part of glycolipids and glycoproteins of membrane and biological liquids. Product of mannose reduction – six-atomic alcohol mannitol (mannit) is used in medicine as osmotic diuretic and for replace of sucrose in the diet of patients with diabetes mellitus.

Fructose is involved to disaccharide sucrose structure. It is monomer of polysaccharide inulin.

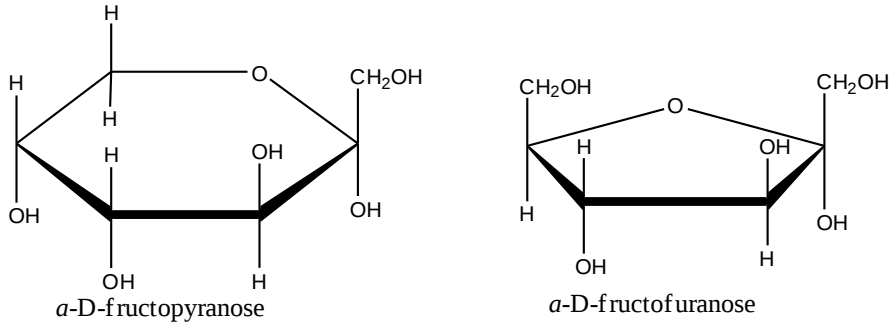


Figure 2.8 Fructose.

Sugars are joined to alcohols and amines by glycosidic bonds. The crucial biological importance of N-glucosidic linkage is evident in such central biomolecules as nucleotides, RNA and DNA. N-glycosidic linkages in virtually all naturally-occuring biomolecules have the β -configuration.

- ***Phosphorylated sugars*** are another important class of derivatives. They are key intermediates in energy generation and biosyntheses. In fact, one of the strategies of glycolysis is to form three-carbon intermediates that can transfer their phosphate groups to ADP to achieve an ATP synthesis.

Phosphorylation also serves to make sugars anionic. They can have strong electrostatic interactions with the active site of an enzyme. The negative charge

contributed by phosphorylation also prevents these sugars from spontaneously crossing lipid bilayer membranes. Phosphorylation helps to retain biomolecules inside cells. Many times phosphorylated derivative of ribose plays a key role in the biosyntheses of purine and pyrimidine nucleotides as well.

- *Amino derivatives of monosaccharides.* The mostly prevalent are two amino derivatives of hexoses – D-glucose and D-galactose – hexosamines.

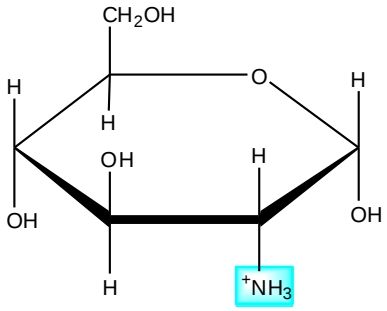


Figure 2.9. Glucosamine.

N-acetyled derivatives of hexosamines are frequently observed into heteropolysaccharides composition.

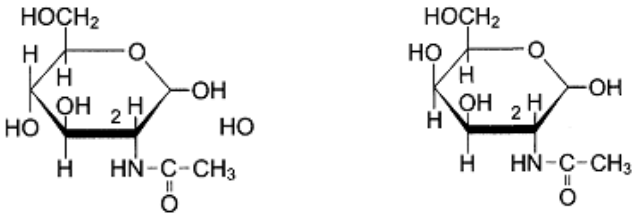
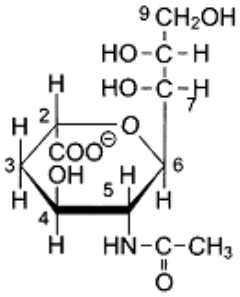


Figure 2.10. N-acetyl-D-glucosamine (left) and N-acetyl-D-galactosamine (right).

- *Neuraminic and sialic acids.* Neuraminc acid is biological important substance. It is derivative of the monosaccharide ketononose (nonulose). In organism neuraminic acid is present as N- and O-acyl-derivatives known as sialic acids.



Neuraminic and sialic acids are structural components of membrane glycolipids, glycoproteins, and proteoglycans of biological fluids, mucus, connective tissue.

Figure 2.11. N-acetyl-neuraminic acid.

- *Aldonic acids* are the products of aldehyde carbon oxidation of aldose. For example, gluconic acid, which as phosphate ester is formed in pentose phosphate pathway.

- *Uronic acids* are the products of C-6 hydroxyl group oxidation of aldose. Acids, which are formed under glucose and galactose oxidation (glucuronic and galacturonic acids), are structural components of heteropolysaccharides. L-Iduronic acid is the structural component of heparin, dermatansulfates of connevtive tissue.

Free glucuronic acid plays very important role in detoxification in animal organism.

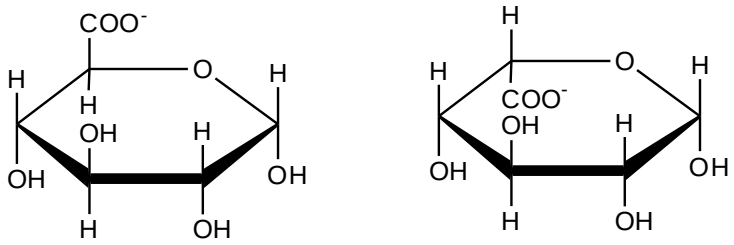


Figure 2.12. α-D-glucuronate (left) and β-L-iduronate (left).

- *Glycosides* are the product of monosaccharide condensation with alcohols or phenols. They are formed by interaction of hydroxyl group of hemiacetal carbon atom of monosaccharide with OH group of alcohol (phenol).

Plant steroid-containing glycosides show cardiotonic action and are used in medical practice as heart glycosides.

2.2 Oligosaccharides

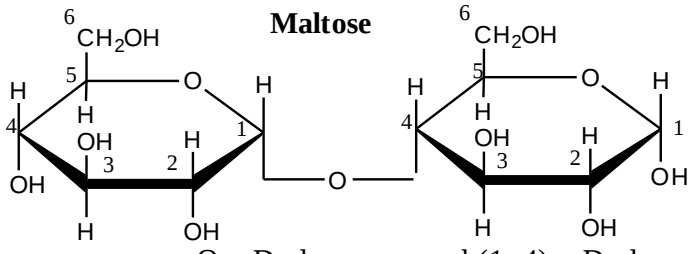
Oligosaccharides are carbohydrates that contain from two to ten monosaccharide units. Disaccharides are the most common type of oligosaccharides.

Disaccharides consist of two monosaccharides joined by an O-glycosidic bond. Three highly abundant disaccharides are sucrose, lactose and maltose.

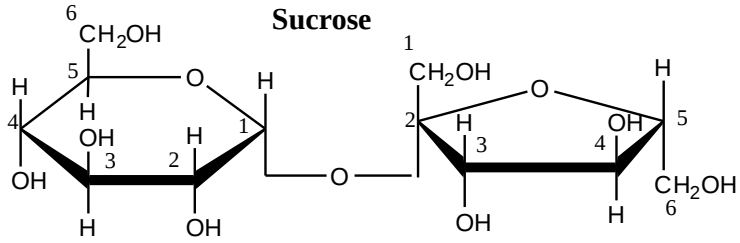
Lactose (β-D-galactosyl-1.4-α-D-glucose) is milk sugar, consists of galactose and glucose residues, combined by 1.4 glycosidic bond. Lactose is important nutrient for human.

Sucrose (α-D-glucosyl-1.2-β-D-fructose) is one from the most spread in the nature and practically important disaccharide, which is situated in the plant stalks, roots, tubers.

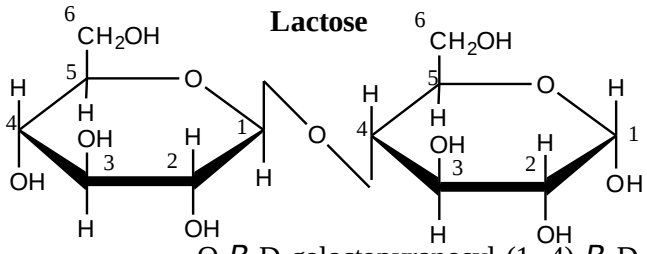
Maltose (α-D-glucosyl-1.4-α-D-glucose), malt sugar is disaccharide, which consists of two glucose molecules residues. Maltose is formed in the digestive channel from starch.



O-*a*-D-glucopyranosyl-(1--4)-*a*-D-glucopyranose



O-*a*-D-glucopyranosyl-(1--2)-*B*-D-fructofuranose



O-*B*-D-galactopyranosyl-(1--4)-*B*-D-glucopyranose

Figure 2.13. Most important disaccharides.

Plant products contain trisaccharide raffinose, tetrasaccharide stachyose.

2.3 Polysaccharides

Polysaccharides are carbohydrates made up of many monosaccharide units.

Polysaccharides

Homopolysaccharides	Heteropolysaccharides
consist of one type of monosaccharides	consist of two and more types of monosaccharides

Homopolysaccharides

- Starch is the plant polysaccharide, which consists of two fractions – amylose and amylopectin (15 – 25 and 80 – 85 % of starch mass respectively).

Amylose is linear polysaccharide, with 200 – 1000 monomers (glucose residues), which are combined by α-1,4 glycosidic bonds (molecular mass ~ 40 – 160 kDa). Amylose homopolymers form spiral structures, each revolution involves six glucose molecules.

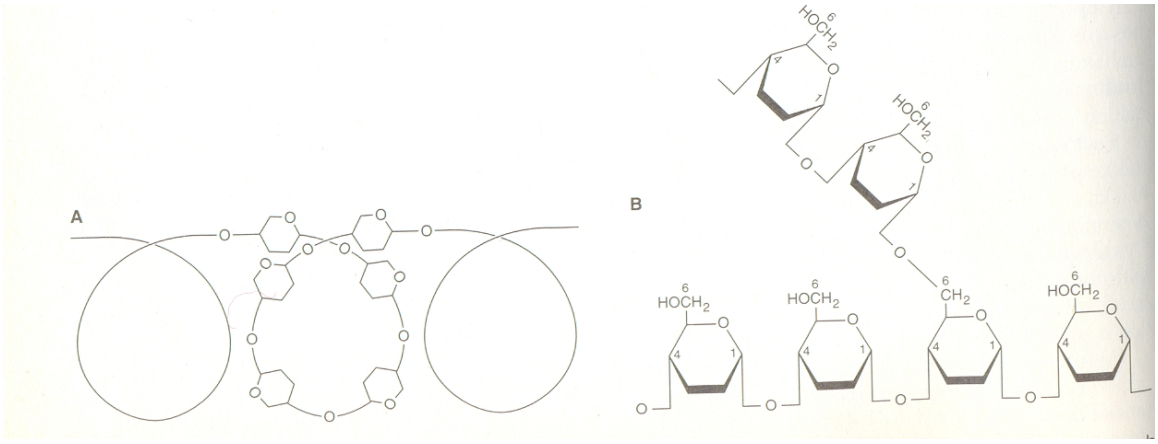


Figure 2.14. Starch structure

Amylopectin is the branched polysaccharide with molecular weight ~ 1 – 6 millions Da. The main chain of amylopectin is formed by α-1.4 glycosidic bonds; branching is generated by α-1.6 glycosidic bonds. Between branching points 20 – 30 glucose residues are situated.

The sources of starch are bread, potato, beans.

- Glycogen is animal homopolysaccharide with molecular weight ~ 100 millions Da. The chemical structure of glycogen is the similar to starch amylopectin, but has more branched molecules. The linear parts of main chain contain 6 – 12 glucose residues, combined by α-1.4 glycosidic bonds, branching is formed by α-1.6 glycosidic bonds.

Glycogen forms intracellular granules.

- Cellulose is homopolysaccharide, which is the main structural component of plant cell wall. Cellulose molecules are unbranched chains, which consist of glucose residues combined by β-1.4 glycosidic bonds.

- Dextran is branched polysaccharide of yeasts and bacterias. The main type of bond is α-1.6 glycosidic one. Branching is formed by α-1.2, α-1.3 or α-1.4 bonds. Dextrans are used in medicine as plasma and blood changers (Polyglucin, Reopolyglucin).

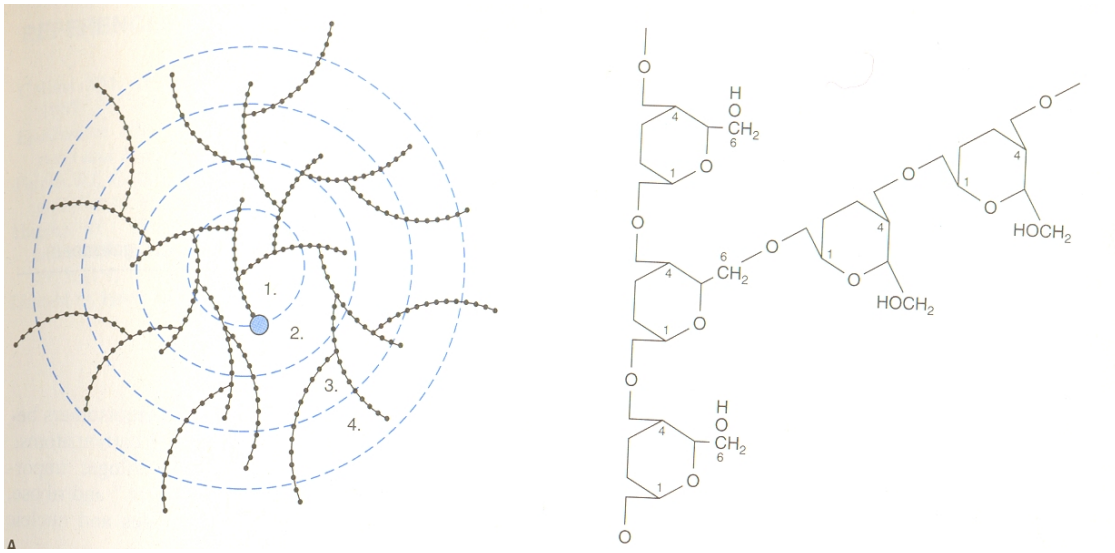


Figure 2.15. Glycogen structure.

- Inuline is plant polysaccharide, which consists of β-D-fructose residues, combined by β-1.2 glycosidic bonds, molecular mass is not more than 6 kDa.
- Pectin is polygalacturonic acid derivative, which consists of α-D-galacturonic acid residues, combined by α-1.4 glycosidic bonds.

Heteropolysaccharides

Heteropolysaccharides consist of two and more types of the different monomers. Glycosaminoglycans are the most important heteropolysaccharides in the physiology.

Glycosaminoglycans are polymers, which form interstial matrix of connective tissue. They are polyanion molecules. Some monosaccharide components contain acidic group – carboxyl or sulfate group, which provides high hydrophility.

All glycosaminoglycans make their functions in the complex with proteins. Covalent complexes are called proteoglycans.

Table 2.1 Structural Components of Glycosaminoglycans

Glycosaminoglycans	Composition of disaccharide unit
Hyaluronic acid	D-glucuronate + N-acetylglucosamine
Chondroitin sulfate	D-glucuronate + N-acetylgalactosamine sulfate

Dermatan sulfates	D-iduronate (or D-glucuronate)+ N- acetylgalactosamine sulfate
Keratan sufate	D-galactose + N-acetylglucosamine sulfate
Heparin and heparan sulfate	D-glucuronate (or D-iduronate) + N-acetylglucosamine sulfate

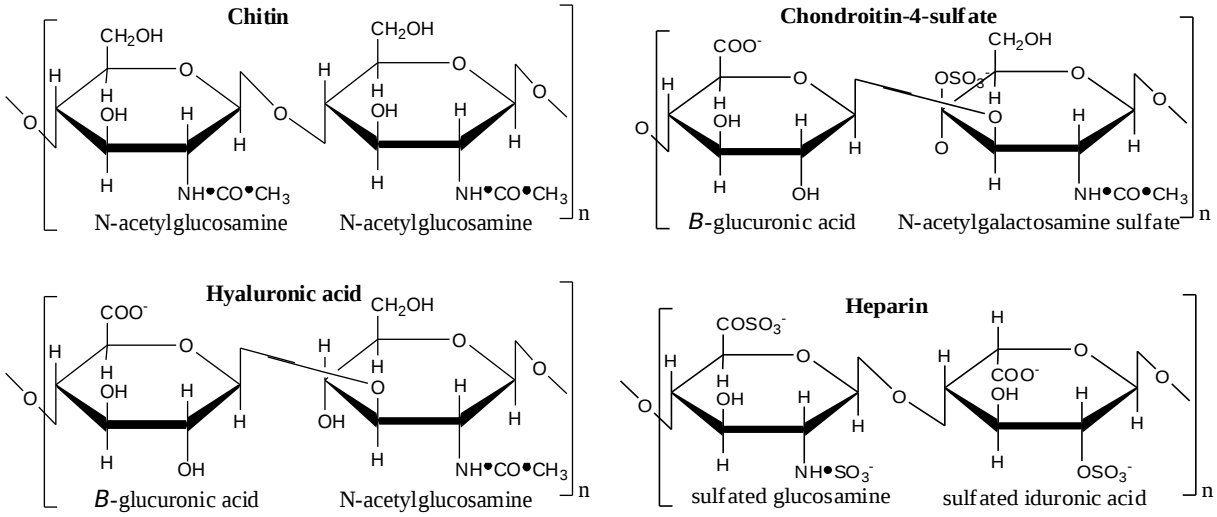


Figure 2.16. The main heteropolysaccharides.

- Hyaluronic acid is linear polysaccharide, where D-glucuronic acid and N-acetylglucosamine are combined by β-1.3 glycosidic bonds; some single fragments are recombined by β-1.4 glycosidic bonds.

Hyaluronic acid has the highest molecular weight in comparison with other glycosaminoglycans (10^5 – 10^7 Da). High amount of COO^- forms the negative charge of molecule, provides water and Na^+ ions keeping.

Hyaluronic acid is present in bacteria and is widely distributed among various animal's tissues, including synovial fluid, the vitreous body of eye, cartilage, and loose connective tissue.

The molecules coil and entwine to make a very firm gel at a very low concentration (0,1 %). The gell excludes other large molecules and also microorganisms, so that the rate of spread of bacterial infection is hindered. Many microorganisms secrete a hyaluronidase, which by shortening the average chain length of the polymer, greatly reduces the viscosity of the gel, and the secretion of hyaluronidase by certain tumour cells may correlate with the ability of these cells to metastasize.

- Chondroitin sulfates are important structural components of cartilages. Molecular mass is about 10 – 60 kDa. Disaccharide fragments consist of D-glucuronate and N-acetylgalactosamine sulfate combined by β-1.3 glycosidic bonds.
- Keratan sulfates I and II consist of repeating galactose-N-acetylglucosamine or occasionasly of galactose. Type I is abundant in cornie and type II is found along with chondroitin sulfate attached to hyaluronic acid in loose connective tissue.
- Dermatan sulfate is widely distributed in animal tissues. It consists of disaccharide units which consist of D-iduronic or D-glucuronic acids (iduronic acid is prevalent, combined with N-acetyl galactosamine).
- Heparan sulfates are glycosaminoglycans, which are present on the surface of animal cells. They consist of D-glucuronic or D-iduronic acids combined with N-acetylglucosamine sulfate by β-1.4 glycosidic bonds.
- Heparin is glycosaminoglycan, which is synthesized by connective tissue cells, and counteracts blood clotting (anticoagulant).

Similarly to the heparan sulfates heparin chains contain disaccharide units, which consist of D-glucuronic or D-iduronic acids (iduronic acid is prevalent) combined with N- or O-glucosamine sulfate or N-acetylglucosamin by β-1.4 glycosidic bonds.

Heparin and heparan sulfates have the common precursor as not sulfatized polysaccharide chain of heparin proteoglycan.

Functions of Proteoglycans

- *Structural function.* They are found in every tissue of the body, mainly in the extracellular matrix of “ground substance”. They are associated with each other and also with other major structural components of the matrix collagen and elastin. Some of them interact with certain adhesive proteins, such as fibronectin and laminin, which play the important role in the adhesion of cells to the extracellular matrix, fixation of basal membrane etc.

- *Regulation of glomerular filtration.* GAG heparin and heparan sulfate are components of basal membrane of renal glomerulars. Basal membrane regulates the passage of large molecules across the glomerulus into the renal tubule. The pores in the glomerular membrane are large enough to allow molecules up to about 8 nm to pass through. Albumin is smaller than this pore size, but it is prevent from passing through easily by the negative charges of heparan sulfate. These negative charges repel albumin which is negatively charged at the pH of blood.
- *Regulation of water-salt metabolism.* The GAGs present in the proteoglycans are polyanions and hence bind polycations and cations such as Na^{+}. This latter ability attracts water by osmotic pressure into the extracellular matrix.
- *Supporting the turgor of extracellular matrix.*
- *Protective function.* GAGs form gel at relatively low concentration, therefore they restrict the passage of large macromolecules into the extracellular matrix.
- *Regulation of permeability* (hyaluronic acid).
- *They provide the cell migration* during morphogenesis and wound repair (hyaluronic acid).
- *They provide compressibility*.
- *They participates in osteogenesis* (chondroitin sulfate).
- *They play a critical role in corneal transparency* (heparan sulfate and dermatan sulfate).
- *Heparin is important anticoagulant.*
- *Heparin activates lipoprotein lipase.*
- They are components of synaptic vesicles.

2.4 Functions of Carbohydrates

- *Energy function.* Carbohydrates provide 60% of energy which is needed to organism. Oxidation of 1 gram of carbohydrates leads to liberation of 17,2 kJ (4,1 kcal).
- *Reserve function.* Glycogen is a major storage form of carbohydrates in animals. Liver contains ~ 100g of glycogen, muscles – 200 – 250 g.

- *Structural function.* They are included into lipids, DNA, RNA, cell membrane, receptors.
- *Protective function.* Participation of carbohydrate components of immunoglobulins in supporting of immunity.
- They participate in *performing of nervous impulse* (glycolipids gangliosides).
- They *provide cell recognition, adhesion.*
- Some of them are *anticlotting* factors.

Tests for Self-control

1. Examples of hexoses are:
A. Glucose, fructose, galactose
B. Glucose, galactose, arabinose
C. Ribose, erythrose, fructose
D. Mannose, fructose, xylose
E. Glucose, allose, ribose
2. Milk sugar consists of:
A. Two glucose residues
B. Glucose and fructose
C. Galactose and fructose
D. Galactose and glucose
E. Two galactose residues
3. Which monosaccharide is included in the structure of DNA?:
A. Glucose
B. Xylulose
C. Ribose
D. Deoxyribose
E. Ribulose
4. Which chemical bond presence provides the branching of starch and glycogen molecules?
A. α-1.4 glycosidic
B. α-1.3 glycosidic
C. α-1.6 glycosidic
D. β-1.4 glycosidic
E. β-1.6 glycosidic
5. Which of the below mentioned carbohydrates is heteropolysaccharide?
A. Starch
B. Glycogen
C. Maltose
D. Heparin
E. Cellulose

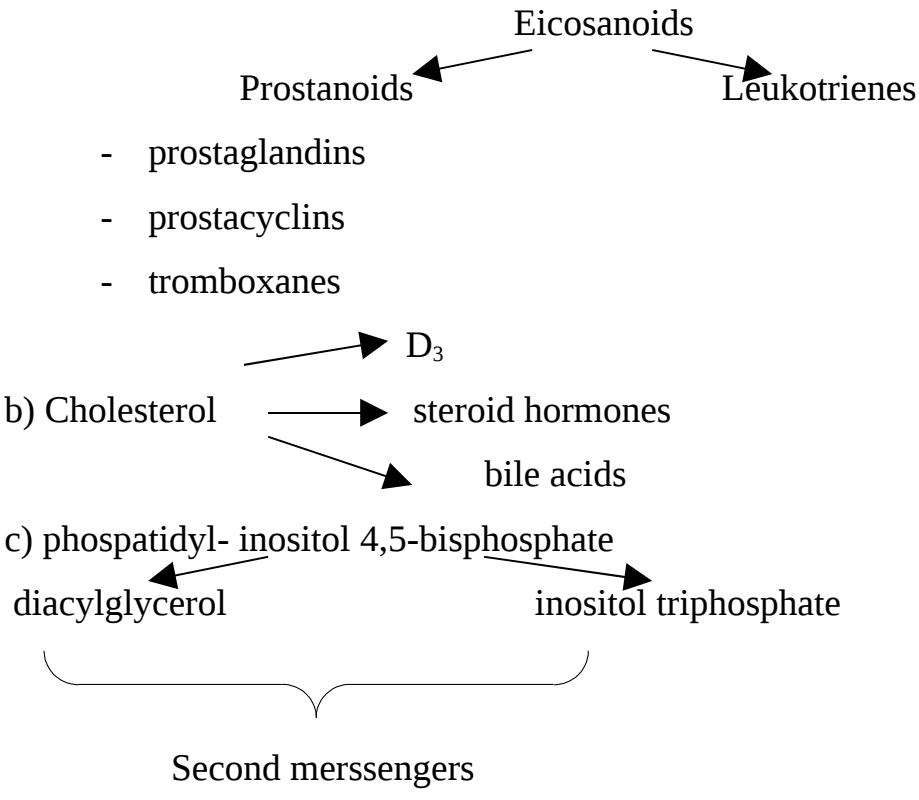
Chapter 3. STRUCTURE AND FUNCTIONS OF LIPIDS

Lipids are the chemical substances, which are insoluble in water and other polar solvents and soluble in non polar (hydrophobic) liquids.

3.1 Functions of Lipids

- *Energy function* (1g – 9,3 kcal, 38,9 kJ).
- *Reserve function.* Lipids form the energy reserve (10 kg – 30 – 40 days).
- Fats serve as *thermal insulators* in subcutaneous tissues and around certain organs.
- Nonpolar lipids act as *electrical insulators* allowing rapid propagation of depolarization waves along myelinated nerves.
- They are *included in the membrane structure* therefore they *influence on the membrane permeability and transmission of nervous impulse*.
- They are necessary for *absorption of fat-soluble vitamins*.
- They perform the *protective* function.
- They are *precursors of important biological active substances*:

a) polyunsaturated fatty acids are precursors of



- They are important source of *endogenious water* (100 g lipids → 107 ml of water).

3.2 Common Characteristic of Lipids. Fatty Acids.

By their chemical structure the main part of lipids is the complex esters of highest carbonic acids and alcohols. Some classes of lipids also involve phosphates, nitrogen compounds, carbohydrates etc.

- *Fatty acids*. Fatty acid composition of lipids is the main sign, which provides their physico-chemical and biological properties. The carbon atom amount and the length of chain, range of saturation of fatty acids determine the consistency (liquid, solid) and surface activity, namely, the ability to form complexes with proteins and finally micelle, bilay, matrix of biological membranes.

Usually lipids of human organism involve to their structure fatty acids with even number of carbon atoms, which consist of from 12 till 24 carbon atoms, prevalently from 16 C till 20 C, most commonly, an unbranched chain.

Palmitic acid is prevalent saturated acid and oleic acid is prevalent unsaturated fatty acid. The high amount of oleic acid in lipids determines the liquid state of human body fats, melting temperature of which is about 10 -15^{o}C.

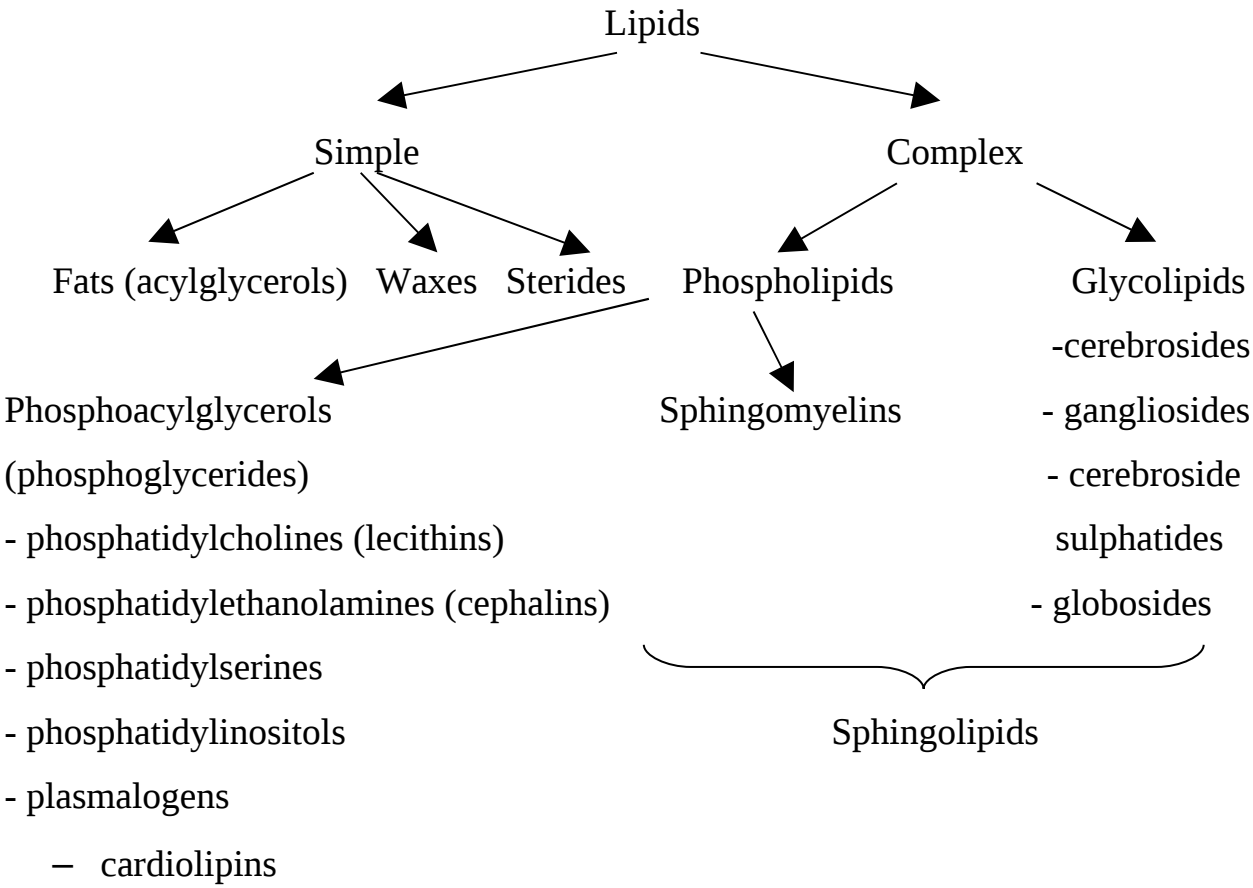
Role of Polyunsaturated Fatty Acids:

- they are precursors of important biological active substances;
- they support the liquid state of cell membranes;
- they normalize the cholesterol metabolism;
- they prevent the fatty liver;
- antiradiation effect.

Table 3.1 The Main Fatty Acids

Code index	Structure	Systematic name	Trivial name
Saturated fatty acids			
$C_{12:0}$	$CH_3(CH_2)_{10}COOH$	n-Dodecanic	Lauric
$C_{14:0}$	$CH_3(CH_2)_{12}COOH$	n-Tetradecanic	Myristic
$C_{16:0}$	$CH_3(CH_2)_{14}COOH$	n-Hexadecanic	Palmitic
$C_{18:0}$	$CH_3(CH_2)_{16}COOH$	n-Octadecanic	Stearic
$C_{20:0}$	$CH_3(CH_2)_{18}COOH$	n-Eicosanic	Arachidic
$C_{22:0}$	$CH_3(CH_2)_{20}COOH$	n-Docosanic	Behenic
$C_{24:0}$	$CH_3(CH_2)_{22}COOH$	n-Tetracosanic	Lignoceric
Monounsaturated fatty acids			
$C_{16:1}$	$CH_3(CH_2)_5CH=CH(CH_2)_7COOH$	cis-9-Hexadecenoic	Palmitooleic
$C_{18:1}$	$CH_3(CH_2)_7CH=CH(CH_2)_7COOH$	cis-9-Octadecenoic	Oleic
$C_{20:1}$	$CH_3(CH_2)_7CH=CH(CH_2)_{11}COOH$	cis-13-Docosenoic	Erucic
Polyunsaturated fatty acids			
$C_{18:2}$	$CH_3(CH_2)_4(CH=CHCH_2)_2(CH_2)_6COOH$	cis-cis-9,12-Octadecadienoic	Linoleic
$C_{18:3}$	$CH_3CH_2(CH=CHCH_2)_3(CH_2)_6COOH$	all-cis-9,12,15-Octadecatrienoic	Linolenic
$C_{20:4}$	$CH_3(CH_2)_4(CH=CHCH_2)_4(CH_2)_2COOH$	all-cic-5,8,11,14-Eicosatetraenoic	Arachidonic

Classification of Lipids



3.3 Simple Lipids

Simple lipids form the alcohol and fatty acids after hydrolysis.

- *Acylglycerols* consists of glycerol and fatty acids. Acylglycerols, namely triacylglycerols are also called neutral fats. They are the main composed part of adipocytes as molecular form of fatty acid storage. Natural tryacylglycerols are mixed lipids, because they contain different fatty acid residues.

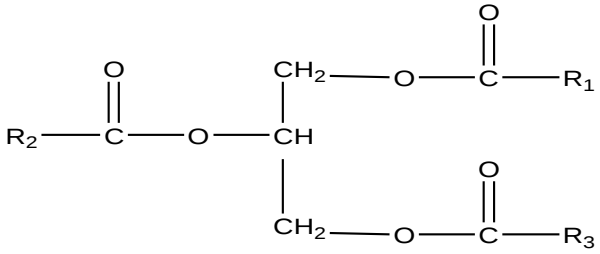
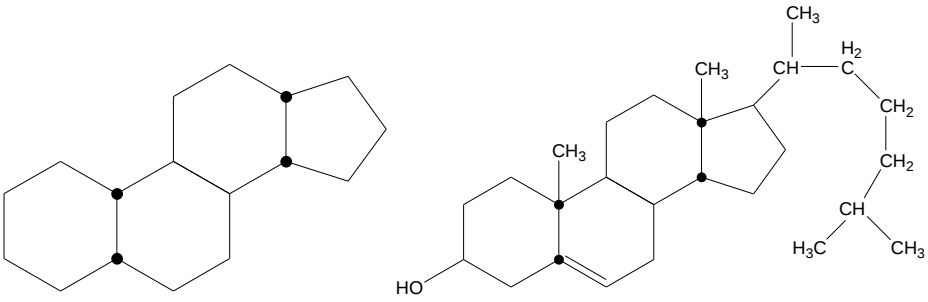


Figure 3.1 Triacylglycerol structure

- *Sterides* are esters of cyclic alcohols (sterols) and fatty acids. Sterols are 3-hydroxyderivatives of cyclopentanperhydrophenantren. Most prevalent sterol of

animal origin is cholesterol, which is involved as structural lipid to the plasmic membranes structure and is precursor of vitamin D_3, steroid hormones and the bile acids.



1 2

Figure 3.2 Cyclopentanperhydrophenantren (1) and cholesterol (2).

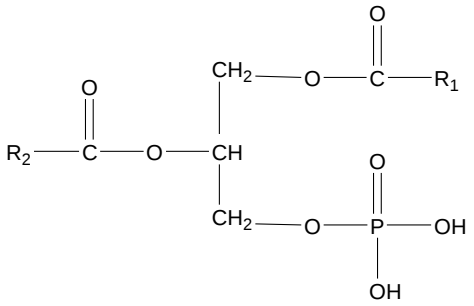
- *Waxes* are simple lipids, which are the esters of highest fatty acids and high molecular alcohols. From waxes of animal origin there are bee wax, spermaceti, lanolin, which are used for cream production.

3.4. Complex Lipids

Complex lipids have not only alcohol and fatty acids, but also additional conponent (alcohol, phosphate, nitrogen compounds, carbohydrates). Complex lipids are polar, amphypathic substances and main part of them implements structural function by involving to membrane composition.

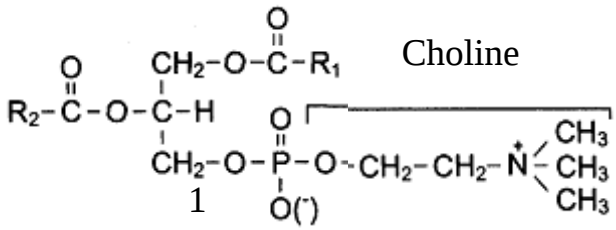
- ***Phospholipids***. Phospholipids are divided to the glycerophospholipids and sphingophospholipids due to the alcohol, which is involved in their structure.

- *Glycerophospholipids* are esters of glycerol and highest fatty acids. They are derivatives of phosphatidic acid esterified by amino alcohols choline, etanolamine, serine.



phosphatidic acid

Phosphodiesteric bond in glycerophospholipids is formed by hydroxyl group of choline (phosphatidylcholine or lecithin), ethanolamine (phosphatidylethanolamine or cephalin) or serine (phosphatidylserine).



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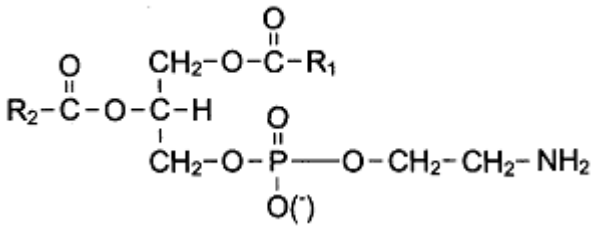

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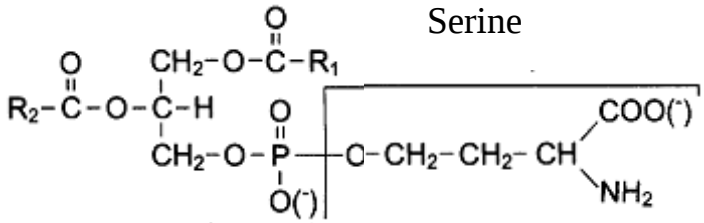

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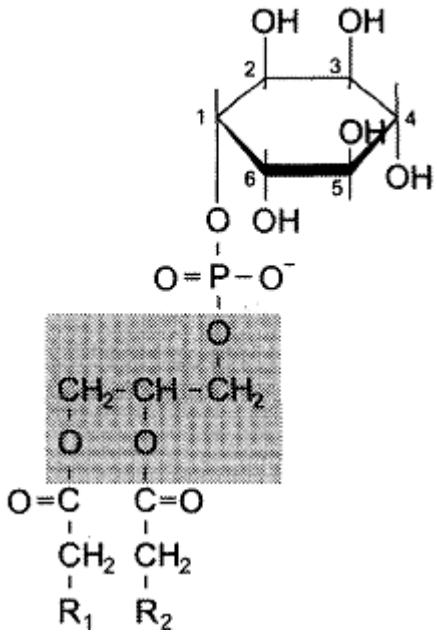


4

Figure 3.3 Glycerophospholipids: phosphatidylcholine (1), phosphatidylethanolamine (2), phosphatidylserine (3), phosphatidyl inositol (4).

In phosphatidyl inositols phosphotidic acid is linked with the six- membered cyclic alcohol inositol. Phosphatidyl inositol diphosphates are the precursors of second messengers (inositol triphosphate and diacylglycerol).

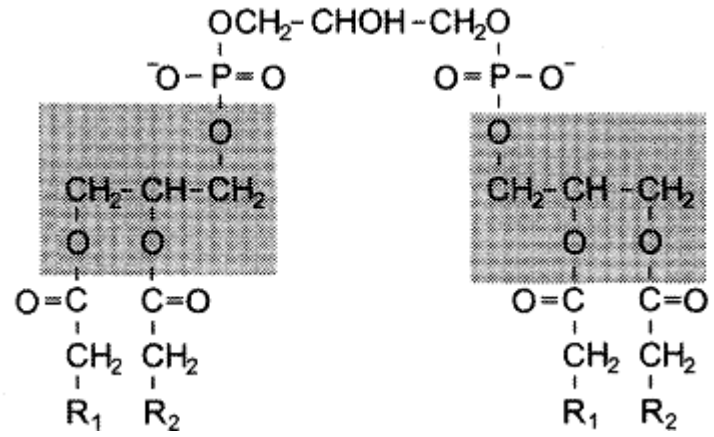


Figure 3.4 Cardiolipin (diphosphatidylglycerol).

- *Plasmalogens* differ from above phospholipids in that they, in peace of a higher fatty acid residue, contain a residue of α, β-unsaturated alcohol linked through an ether bond to the glycerol hydroxyl group at position C-1. There are three types of plasmalogens: phosphatidal cholines, phosphatidal serines, phosphatidal ethanolamines. Plasmalogens constitute 10 % of the cell membranes, they are also involved to myelin covers of nerve cells. Some of them show the strong biological activity. Much of the phospholipid in mitochondria consists of plasmalogen.

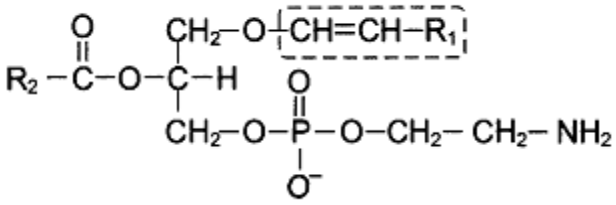


Figure 3.5 Plasmalogen (phosphatidal ethanolamine.)

Platelet activating factor (PAF) has been identified as 1-alkyl-2-acetyl-sn-glycerol-3-phosphocholine. It is formed by many blood cells and other tissues and aggregates platelets at concentrations as low as 10^{-11} mol/L. It also has hypotensive

and ulcerogenic properties and is involved in a variety of biologic responses including inflammation, chemotaxis, and protein phosphorylation.

- *Sphingophospholipids* consist of one molecule of dibasic unsaturated amino alcohol sphingosine, one molecule of highest fatty acid, one molecule of nitrogen compound (most commonly, choline) and one molecule of phosphoric acid. N-acyl derivatives of sphingosine and fatty acids are called ceramides.

Sphingophospholipids are phosphate esters of ceramides and choline, ethanolamine or serine. The nerve tissue is especially abundant in sphingomyelins (N-acylsphingosyl phosphocholines).

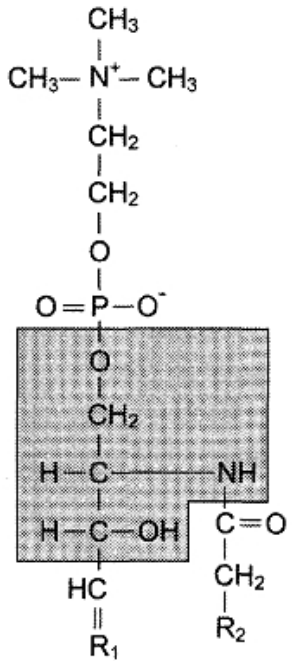


Figure 3.6 Sphingomyelin.

- ***Glycolipids*** are the complex esters of highest fatty acids and sphingosine and contain the carbohydrate component (namely glucose, galactose or their derivatives or oligosaccharide group).

- *Glycosphingolipids* are combination of ceramide with one or more sugar residues.

According to the carbohydrate part of molecule structure glycosphingolipids are divided to the several classes: cerebrosides, gangliosides, sufatides, globosides.

a) Cerebrosides include a hexose, linked through an aster bond to the hydroxyl bond of sphingosine. Galactosyl ceramide and glucosyl ceramide are prevalent. Cerebrosides are especially abundant in the nervous all membranes (in the myelin sheath). Galactosyl ceramide is the major glycosphingolipid in brain

and other nervous tissue. Glucosyl ceramide is the predominant glycosphingolipid of extraneuronal tissues, but it also occurs in the brain in small amount.

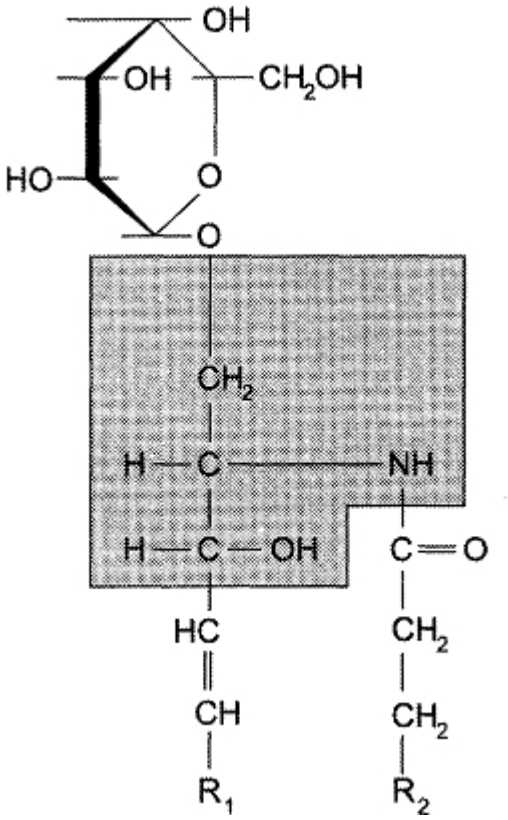


Figure 3.7 Galactosyl cerebroside.

b) Sulfatides are sulfate derivatives of cerebrosides, the most prevalent from them is galactocerebroside sulfate. Sulfatides are found in the white substance of brain. They are acidic, negatively charged substances.

c) Globosides are oligosaccharide derivatives (oligohexosides) of ceramides. Oligosaccharide residue of globosides contains most frequently galactose, glucose or N-acetylgalactosamine.

Cerebrosides and globosides are neutral glycosphingolipids, because they do not contain any charged groups.

d) Gangliosides are the similar to cerebrosides, but they have oligosaccharide instead of galactose residue. Oligosaccharide consists of monosaccharide (glucose, galactose) and a sialic acid, usually N-acetylnauraminic acid.

The highest amount of gangliosides is observed in the membranes of ganglionar neurons.

- ***Lipoproteins*** are complexes of lipids with proteins. Lipoproteins are formed by non covalent bonds and various physico-chemical interactions.

Lipoproteins of blood plasma are transport forms of lipids. Other lipoproteins are integral components of biological membrane.

Tests for Self-control

1. Which of below mentioned fatty acids are unsaturated?
A. Palmitic and stearic
B. Oleic and palmitic
C. Linoleic and oleic
D. Linoleic and arachidic
E. Behenic and myrictic
2. All the below mentioned lipids contain glycerol except:
A. Neutral fats
B. Cardiolipins
C. Plasmalogenes
D. Lecithins
E. Cerebrosides
3. Choose the simple lipid:
A. Cerebroside
B. Lecithin
C. Waxe
D. Kephaline
E. Ganglioside
4. Lipids perform all the below mentioned functions except:
A. Reserve
B. Energy
C. Protective
D. Transport
E. Structural
5. Polyunsaturated fatty acids are:
A. Palmitic and stearic
B. Oleic and palmitic
C. Linoleic and oleic
D. Linoleic and arachidic
E. Linolenic and arachidonic

Chapter 4. STRUCTURE OF NUCLEOTIDES AND NUCLEIC ACIDS

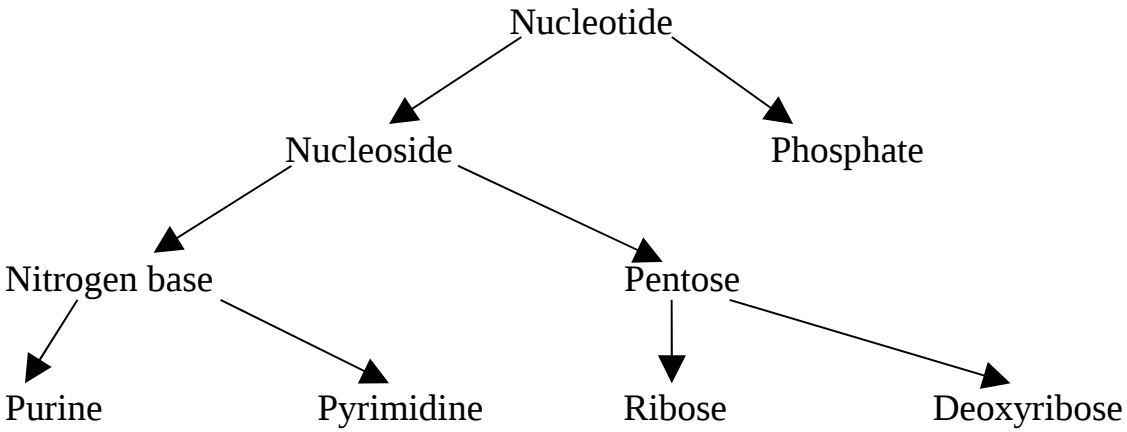
Nucleic acids – deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) are polynucleotides, which consist of the monomer units – mononucleotides.

Nucleotides are the three-component compounds, which consist of nitrogen base, pentose residue and phosphate.

4.1 Functions of Nucleic Acids and Nucleotides

- *Genetic function.* Nucleic acids play the main role in the conservation, transmission and realizing of hereditary information.
- *Cofactor function.* Free nucleotides are involved to the enzyme structure as cofactors. For example: NAD, NADP, FAD, FMN take part in oxido-reductive processes;
- *The formation of active intermediates* of carbohydrates (UDP-glucose, UDP-galactose) and lipids (CDP-choline).
- Participation in *detoxification* processes (UDP-glucuronic acid, PAPS).
- *Energy function.* Nucleoside triphosphates are high- energy substances, which accumulate the energy in macroergic bonds.
- *Modulator functions.* Nucleotides can be allosteric modulators of several enzymes.
- cAMP and cGMP are *second messengers* of hormones.

4.2 Structure of Nucleotides.



Nucleotides consist of nucleosides and phosphates.

Nucleosides are two-component molecules which contain nitrogen base and monosaccharide (pentose) molecule. Chemically nucleosides are N-glycosides of pentose and nitrogen base. N-glycoside bond is formed by N-1 of pyrimidine and C-1 of pentose in pyrimidine nucleotides and N-9 of nitrogen base and C-1 of pentose in purine nucleotides.

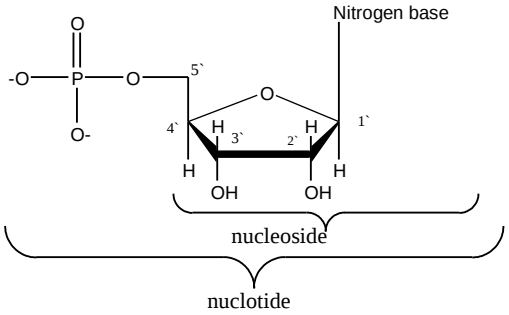
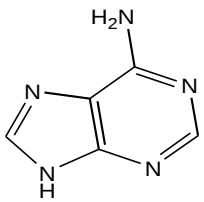


Figure 4.1 Nucleotide structure.

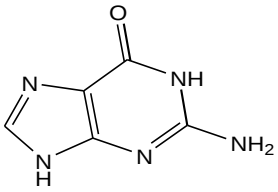
Phosphorylation of the certain hydroxyl groups of pentose leads to the nucleotide (nucleoside phosphate) formation. Phosphorylation can occur at fifth, third or second carbon. In the nucleic acids there are prevalently nucleoside 5`-phosphates.

- *Pentoses*, which are involved to the nucleotide structure, are D-ribose (nucleotides, which contain ribose, are called ribonucleotides) or 2-deoxy-D-ribose (in deoxyribonucleotides).
- *Nitrogen bases* are aromatic heterocyclic structures. According to chemical structure they are divided into two groups: purines and pyrimidines.

Purine bases are adenine and guanine.

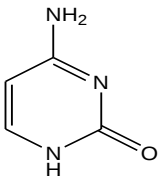


adenine

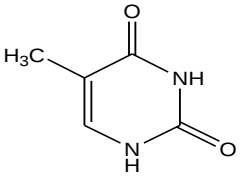


guanine

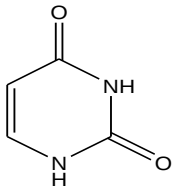
Pyrimidine bases are cytosine, thymine, uracil.



Cytosine



Thymine



Uracil

An important property of hydroxyl-containing nitrogen bases is their ability to exist in the two thautomeric forms – lactam and lactim depending on the medium pH. All the hydroxy- derivatives of purine and pyrimidine, constituents of natural nucleic acids, exist in a lactam form. It gives the possibility to generate intermolecular hydrogen bonds between purine and pyrimidine bases.

Minor nucleotides. Besides 5 main nitrogen bases some nucleic acids contain the additional (minor) nitrogen bases in very small amounts. Minor bases are methylated derivatives of the usual nitrogen bases, for example: 1-methyladenine, 2-methyladenine, 6-dimethyladenine, 1-methylguanine, 7-methylguanine, 1-methyluracil, 5-hydroxymethyluracil, 3-methylcytosine etc. Nucleoside with unusual structure is pseudouridine, where the ribose is combined with uracil in the 5 position.

The most amount of minor nucleotides is involved to the tRNA content. Human DNA contains N-methylcytosine, mRNA – N-methylderivatives of adenine and guanine.

Table 4.1 The nomenclature of nucleotides and nucleosides

Nitrogen base names		Nucleosides	Nucleotides	Brief nucleotide names
Full	Brief			
RNA				
Purine				
Adenine	A	Adenosine	Adenyl acid (adenosine-5`-monophosphate)	AMP
Guanine	G	Guanosine	Guanyl acid (guanosine-5`-monophosphate)	GMP
Pyrimidine				
Cytosine	C	Cytidine	Cytidyl acid (cytidine-5`-monophosphate)	CMP
Uracil	U	Uridine	Uridyl acid (uridine-5`-monophosphate)	UMP
DNA				
Purine				
Adenine	A	Deoxy-	Deoxyadenyl acid (deoxy-	dAMP

		adenosine	adenosine-5`-monophosphate)	
Guanine	G	Deoxy-guanosine	Deoxyguanyl acid (deoxy-guanosine-5`-monophosphate)	dGMP
Pyrymidine				
Cytosine	C	Deoxy-cytidine	Deoxycytidyl acid (deoxy-cytidine-5`-monophosphate)	dCMP
Thymine	T	Deoxy-thymidine	Deoxythymidyl acid (deoxy-thymidine-5`-monophosphate)	dTMP

4.3 Structure of Nucleic Acids

All nucleic acids are high-molecular polymers. The primary structure of nucleic acids is polynucleotide chain, which consists of monomers – nucleotides.

The single nucleotides are combined together to polynucleotide chain by phosphodiesteric bonds, which are formed between of 3` and 5` hydroxyl groups of pentoses of neighbour nucleotides.

Table 4.2 The features of nucleic acid primary structure

Nucleic acids	Pentoses	Nitrogen bases	
		purines	pyrimidines
DNA	2`-deoxyribose	Adenine, guanine	Cytosine, thymine
RNA	ribose	Adenine, guanine	Cytosine, uracil

Nucleotide polarity. In the polynucleotide chain of DNA and RNA there are two ends: 5` end, which contains free 5` pentose hydroxyl; and 3` end which contains free 3` pentose hydroxyl. In the natural nucleic acids 5`-end (5`-hydroxyl of the ending ribose or deoxyribose) is usually phosphorylated, 3` end contains free OH group. Such nucleic acid is supposed to be polar and has the direction 5`→ 3`.

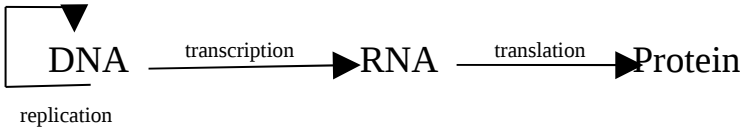
DNA Structure, Properties and Functions.

Biological role

- *Conservation of hereditary information.*
- *Transmission of hereditary information to the descendants.* The DNA duplication and transmission of the copies of parent molecule to the descendants is

the base of hereditary conservatism, keeping through the generations main biological signs of species.

- *Realizing genetic information.* This biological function is existed by the transmission of information, coded in DNA, to the molecules of messenger RNA and following deciphering of this information during the protein synthesis. The complex of these actions is called the central dogma of molecular biology:



Molecular mass and size of DNA molecules

Molecular mass of deoxyribonucleic acids is essentially various in the different biological objects: viruses, prokaryotes, eukaryotes.

- *Viral and prokaryotic DNA.* Viral DNA has the lowest size and molecular mass. In the prokaryotic cells the DNA amount is essentially higher than in viruses. Prokaryotic DNA is covalently closed double-chain ring. According to the increase of biological organization range the amount of nucleotide pairs in the double-chain DNA molecules increases. Prokaryotic DNA is one molecule, which is situated in the special part of cytoplasm – nucleoid.
- *Eukaryotic DNA.* Eukaryotic DNA is situated in the nucleus and is involved to the polymorphic structure – chromatin. In the period of preparing to mitosis DNA duplication occurs and following chromatin condensation with formation of cytological structures – chromosomes. Each chromosome of eukaryotic cell contains two fully identical DNA molecules. The chromosome amount is specific for each biologic species. The molecular mass of human DNA is $1{,}6 \cdot 10^{11}$Da, which is equal $2{,}4 \cdot 10^{9}$ nitrogen bases pairs.

There is direct correlation between the increase of evolutional level, range of biological organization of living been and the amount of genetic material, which is manifested in the nucleotide pairs quantity and molecular mass of DNA.

Features of primary structure

- Deoxyribose is involved to the nucleotide structure.
- Pyrimidine nucleotides are cytosine and thymine (5-methyluracil).

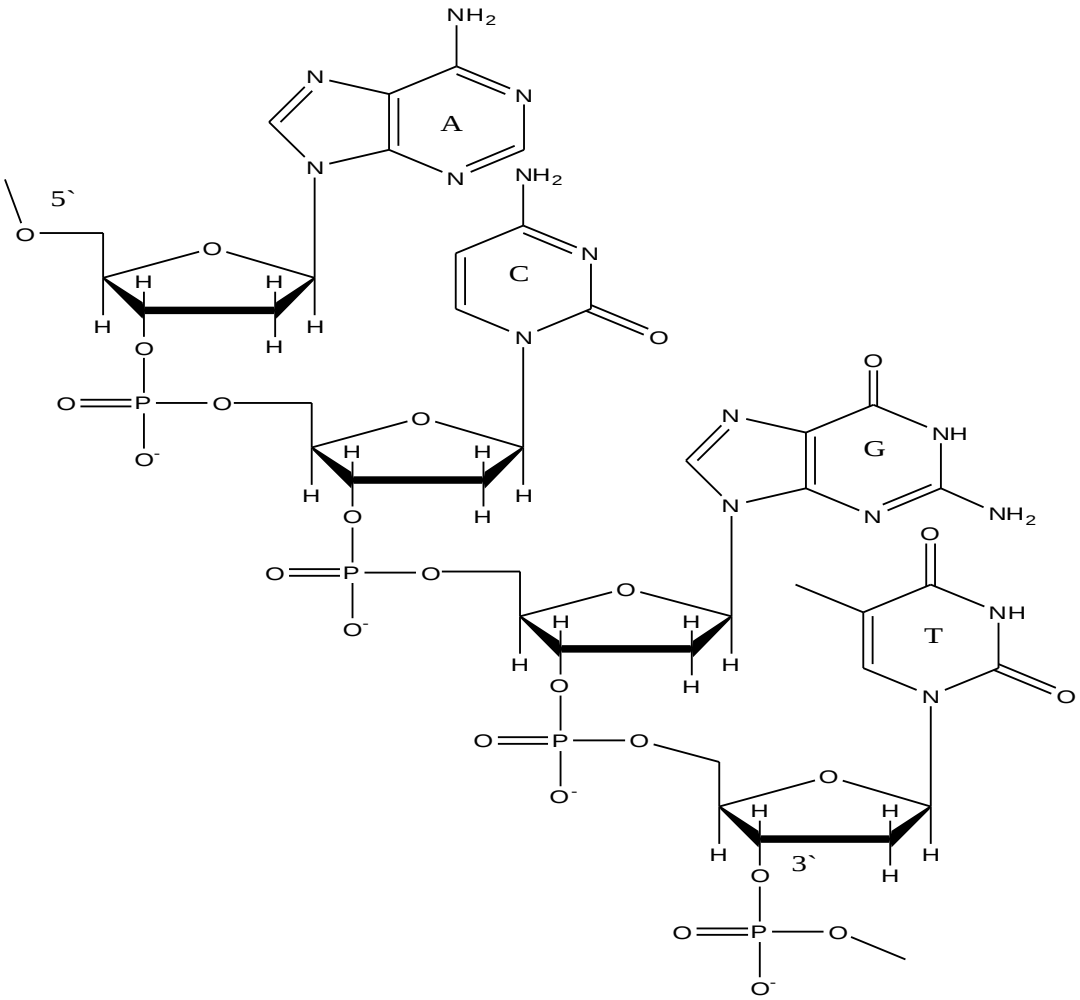


Figure 4.2 The Primary structure of DNA

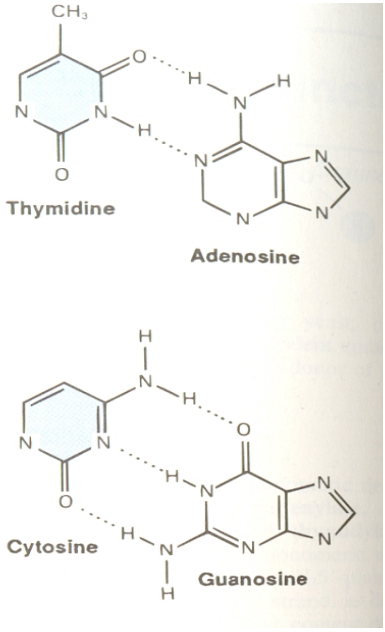
Secondary structure

All DNA molecules have the certain correlations between purine and pyrimidine nucleotides content. According to Chargaff rules:

- sum of purine bases is equal to the sum of pyrimidine bases:

$$A + G = T + C;$$

- the amount of 6-amino groups is equal to the amount of 6-ketogroups;
- adenine content is equal to the thymine content, guanine amount is equal to the cytosine amount:

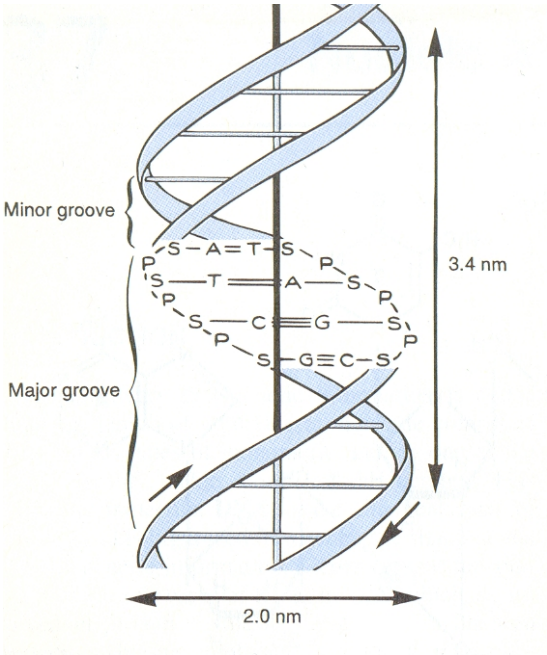


A = T, G = C.

The model of secondary structure of DNA has been proposed by J.Watson and F.Crick. The DNA molecule consists of two chains, which form right-handed helix, where both chains are coiled around the common axis. Each strand has an opposite polarity to the other. They are antiparallel.

Figure 4.3 The hydrogen bonds between complementary nitrogen bases.

The double chain stabilization occurs by the hydrogen bonds, which are formed between oppositely situated complementary nitrogen bases (adenine and thymine, cytosine and guanine), which explain the empiric Chargaff rules.



The complementary base pairs lie inside the helix, perpendicular to the sugar-phosphate backbone, which lies outside the helix. The base pairs inside the helix are stacked one above the other. The hydrogen bonds of the base pairs and the van der Waals interactions of the stacked base pairs provides the stability of double helix.

Figure 4.4 The scheme of DNA double helix (B-form).

Structural features of double helix: diameter – 2,0 nm, distance between nitrogen bases along helix axis – 0,34 nm, spiral structure is repeated with 3,4 nm interval or each 10 nucleotides pairs.

All above mentioned properties characterize B-form of DNA. But according to the interaction with different amount of cations and water molecules, DNA forms other structural shapes: A, C or Z, which can characterize the certain physiological conditions of DNA interaction with proteins of nuclear chromatin.

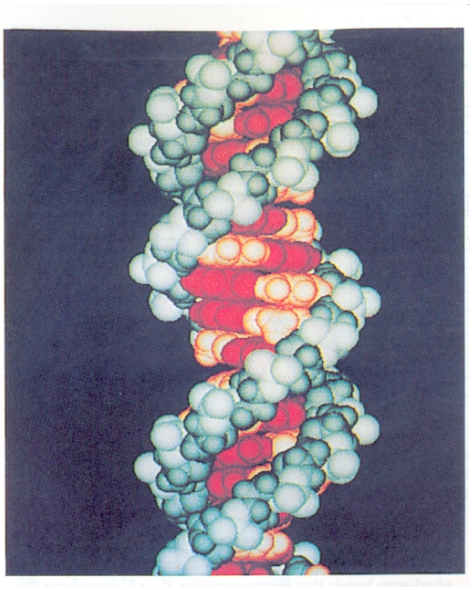


Figure 4.5 The molecular model of DNA (B-form).

Tertiary structure

In the living cell the double helix does not show the unfolding structure, but is additionally folded in the space, and forms tertiary structures – superspirals.

At the superspiralized state DNA molecules in the complex with certain proteins are involved to the nuclear chromatin structure in eukaryotic cells. The supercoiled long DNA molecules form the compact structures, for example, nuclear chromosomes.

Physico-chemical properties

- *Acid-base properties.* All polynucleotides are the strong multibasic acids with low level of pK. DNA acidity is predetermined by the secondary phosphate groups, which are fully ionizated under pH < 4.

Due to acidic properties and the presence on the surface of negative charges DNA molecules under physiological meaning of pH form the complexes with cations: polyamines (spermidine, spermine), alkaline proteins (histones, protamines), metals (Ca^{2+}, Mg^{2+}, Fe^{2+}).

- *Viscosity and optical activity.* High molecular mass and large length predetermine the high viscosity. The DNA viscosity depends on its conformation and essentially changes under denaturation and renaturation.

Due to ordered secondary structure DNA molecules are optically active and able to rotate the polarized light flatness.

- *Absorption in the ultraviolet region.* Nitrogen compounds, which are involved to the nucleic acids structure, have the ability to absorption of the ultraviolet light at 260 nm.

After polynucleotides formation the mutual influence of parallelly situated along the DNA molecules nitrogen bases pairs leads to the decrease of ultraviolet absorption. The DNA absorption at 260 nm is lower by 40% than the summary absorption of nitrogen bases - hypochromic effect. Denaturated DNA shows the higher level of absorption – hyperchromic effect.

- *Denaturation.* DNA denaturation is the destruction of the native double helix conformation of DNA with formation of disordered single strands. Renaturation is the returning to native secondary DNA conformation. The denaturated nucleic acids lose their biological properties.

The molecular basis of denaturation is destruction of hydrogen bonds between complementary nitrogen bases A-T and C-G.

Denaturation of DNA is caused by:

- sharp pH change to acidic or alkaline side;
- heating of DNA solution.

The thermal DNA denaturation is called melting. Each type of DNA is characterized by specific temperature of denaturation (T_m) due to the ratio of G-C and A-T pairs.

The ratio between G-C and A-T pairs is important index of nucleotide composition of DNA molecules from different biological objects. As between guanine and cytosine three hydrogen bonds are formed the thermal dissociation of G-C pair require higher amount of energy than A-T pair, combined together by two hydrogen bonds. That’s why the melting temperature of DNA molecule is directly proportional to the G-C pairs amount in nucleic acid.

The Structure, Properties and Functions of RNA

Ribonucleic acids are polyribonucleotides, which are divided into the main classes: messenger RNA (mRNA), transport RNA (tRNA), ribosomal RNA (rRNA).

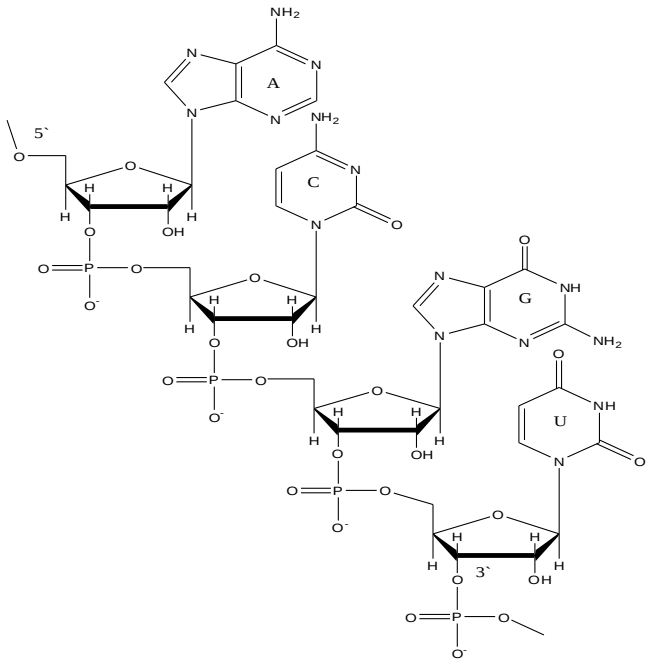
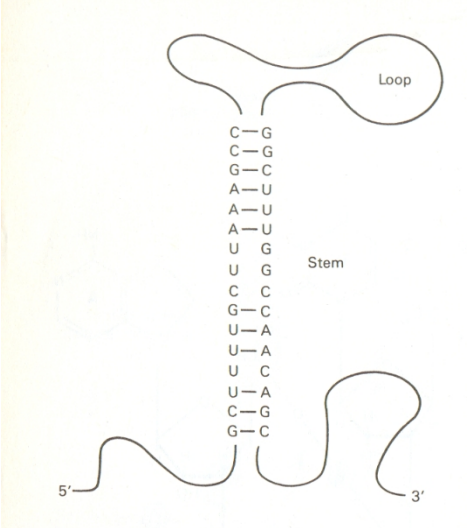


Figure 4.6 The primary structure of RNA.

- *Primary structure.*

- ribose is involved to the nucleotide structure;
- pyrimidine nucleotides are cytosine and uracil;
- there are minor bases into the molecule.

In contrast to DNA, RNA molecules are single chain polynucleotides (exception RNA of RNA-containing viruses).



- *Secondary structure* of the single chain eukaryotic polyribonucleotides is characterized by the presence of parts with double helix structure. These parts of molecule are called hairpins and are formed by complementary base sequences with opposite polarity.

Figure 4.7. The hairpin formation in the secondary structure of RNA

Such coiled regions contain 20 – 30 nucleotide pairs and alternate with unspiralized parts.

The messenger RNA

This RNA class constitutes 2 – 5% from common amount of cellular RNA. mRNA are the carriers of genetic information from the genome to protein-synthesizing system. Each mRNA serves as a template, which determines amino acid sequences in the polypeptide molecules, which are synthesized on ribosomes.

mRNA shows metabolic unstability and highest heterogeneity of molecular mass and molecular size (from $25 \cdot 10^3$ till $1 - 2 \cdot 10^6$) with sedimentation constancy from 6 till 25 S.

Messenger RNAs, particularly in eukaryotes, have some unique chemical characteristics.

The 5` terminal of mRNA is “capped” by 7-methylguanosine that is linked to an adjacent 2`-O-methyl ribonucleotide at its 5-hydroxyl through the three phosphates.

The cap is probably involved in the recognition of mRNA by ribosomes, and it stabilizes the mRNA by preventing the attack of 5`-exonucleases.

The other end of most mRNA molecules, the 3`-hydroxyl terminal, has attached a polymer of adenylate residues 20 – 250 nucleotides in length. It maintains the intracellular stability of mRNA by preventing the attack of 3`-exonucleases.

Secondary structure of mRNA is characterized a multiply intrachain double-spiral parts, which contain 40 – 50% of nucleotide composition.

Transport RNA

tRNA constitutes 10 – 20% of cellular RNA. Their molecules are polyribonucleotide chains, which consist of 70 – 90 nucleotides. Molecular mass is 23 – 28 kDa, sedimentation constant – 4 S. There are at least 20 types of tRNA in the cell according to the amount of natural L-amino acids, which interact with tRNA during the translation.

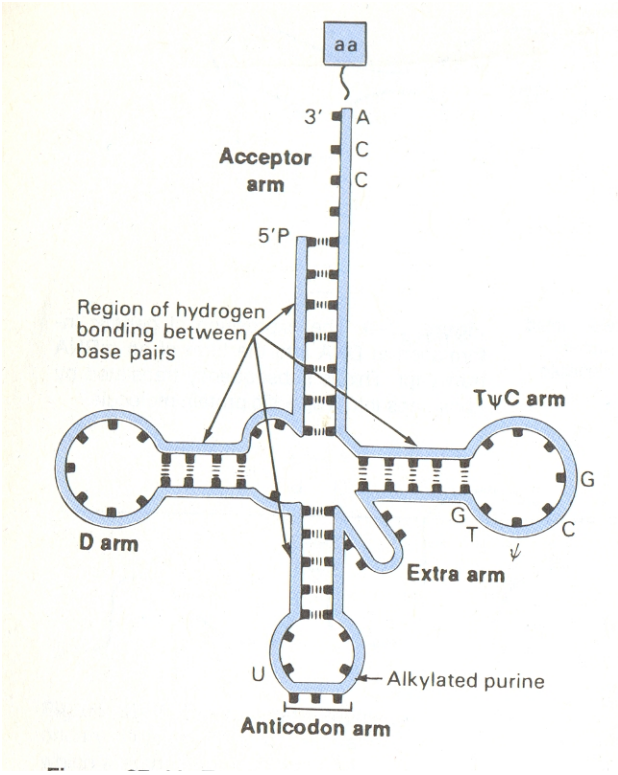


Figure 4.8 tRNA structure.

- *Primary structure* of tRNA contains a high amount of minor nucleotides (methylated nitrogen bases, pseudouridine and dihydrouridine residues).
- *Secondary structure* looks like the clover leaf which is formed by the specific interaction of complementary nitrogen bases along polyribonucleotide chain. Unpaired nucleotide sequences form specific for tPNA structural elements:

- Acceptor arm is 3` end of molecule which contains terminal nucleotide sequence CCA. The ending adenosine accepts the amino acid by 3` hydroxyl of ribose during translation.
- Dihydrouridine arm (D arm) consists of 8 – 12 nucleotides and contains 1 – 4 dihydrouridine residues.
- Anticodon arm contains the nucleotide sequence to be complementary to triplet (codon) within mRNA. This loop provides the tRNA interaction with the mRNA during translation.
- Extra arm.
- Pseudouridine arm contains obligatory nucleotide sequence – 5`-TΨC-3`. This loop is seems to be necessary for interaction of tRNA with ribosome.

- *Tertiary structure.* Characteristic for tRNA structure “clover leaf” can form more compact space conformation. Usually tertiary structure of tRNA looks like to Latin letter “L”.

Ribosomal RNA

Ribosomal RNA (rRNA) is the class of cellular RNAs, which are involved to prokaryotic and eukaryotic ribosome composition. rRNAs constitute 90% of total amount of cellular RNA.

Ribosomal RNAs together with specific proteins form the basis of ribosomal structure and functions. Ribosomes take part in the translation, that is biosynthesis of polypeptide chain on the template of mRNA. Ribonucleic acids of this type are metabolically stable molecules. By interaction with ribosomal proteins they perform the function of structural framework for organization of all intracellular protein synthesizing system.

Minor bases amount in rRNA composition is essentially lower than in tRNA. But ribosomal RNAs are also highly methylated polyribonucleotides, where methyl groups are combined with or nitrogen bases or 2`-hydroxyl groups of ribose.

The secondary structure of rRNA is characterized by a high amount of short double-spiral regions, which look like to the hairpins or sticks.

Besides the above mentioned RNA classes in the mammal nuclei there are pibonucleic acids with different molecular mass, known as heterogeneous nuclear RNAs (hnRNA). Their molecular weight can be higher than 10^7. hnRNAs are the immediate products of gene transcription, they are processed to generate the RNA molecules.

Tests for Self-control

1. Purine bases are:
A. Adenine, guanine
B. Uracil, thymine
C. Cytosine
D. Pseudouridine
E. All the above mentioned
2. Pyrimidine bases are:
A. Adenine, guanine
B. Guanine, thymine
C. Uracil, tymine, cytosine
D. Pseudouridine
E. All the above mentioned
3. Biologic functions of DNA are:
A. Preservation of hereditary information
B. Transfer of genetic information to descendents
C. Realization of genetic information
D. Preservation and transference of information
E. All the above mentioned
4. Nowadays about 50 minor bases have been found in the t-RNA structure besides the main four nitrogene bases. Choose the minor nitrogenous base:
A. Cysteine
B. Dihydrouracil
C. Uracil
D. Cytosine
E. Adenine
5. Which statement about the base content of DNA is incorrect?
A. A = T
B. G = C
C. A + T = G + C
D. A + G = T + C
E. All the above mentioned answers are correct

Chapter 5. ENZYMES

5.1 Structure and role.

General principles of catalysis:

● Catalysts change the velocity of a chemical reaction and are not consumed during the reaction.

● Catalyst is removed from reaction without changing.

● Catalyst can cause the reactions which correspond to thermodynamic laws.

● Catalyst can not change the reaction direction and the state of equilibrium of reactions

● Catalyst decreases the energy of activation of reacting molecules (that is energy barrier).

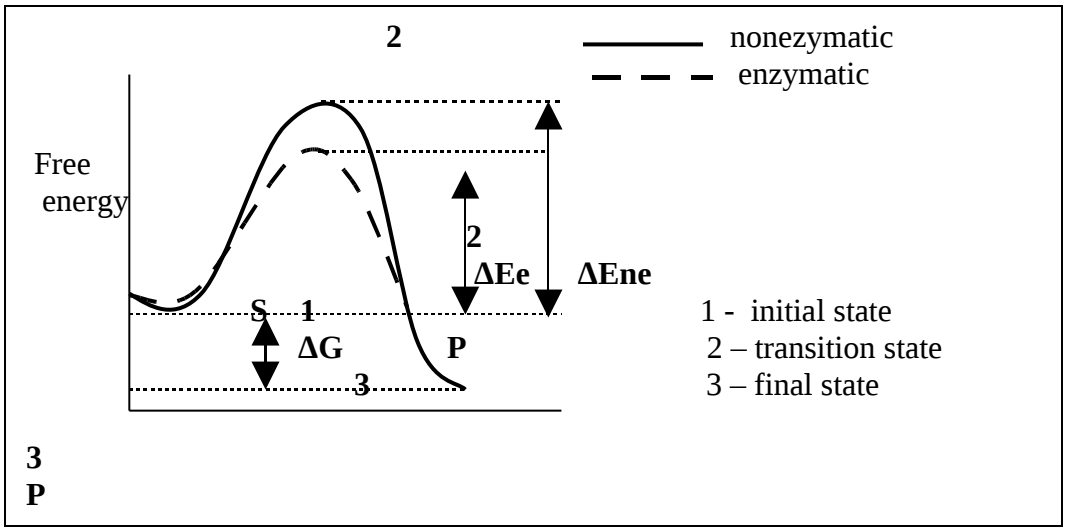


Figure 5.1. Energy scheme for enzymatic and nonenzymatic chemical reactions

● The concentrational effect is characteristic for catalysis.

Biological catalysts are **enzymes**.

Enzymes are specific proteins, which perform catalytic function.

They have all the physico-chemical properties of proteins.

Catalytic RNAs

Certain ribonucleic acids (RNAs) exhibit higher substrate – specific catalytic activity. These RNAs are termed **ribozymes**. Although, the substrates of ribozymes are limited to the phosphodiester bonds of RNAs. Ribozymes catalyze transesterification and hydrolysis of phosphodiester bonds in RNA molecules.

Ribozymes play key role in the intron splicing events essential for the conversion of pre- mRNAs to mature mRNAs.

Because enzymes are proteins they have specific properties.

Specific properties of organic catalysts, or differences between enzymes and inorganic catalysts:

● High efficiency of action.

● High specificity of action.

● They act under physiological conditions (as so called soft conditions): 37°C - temperature of body; physiological pH; normal pressure.

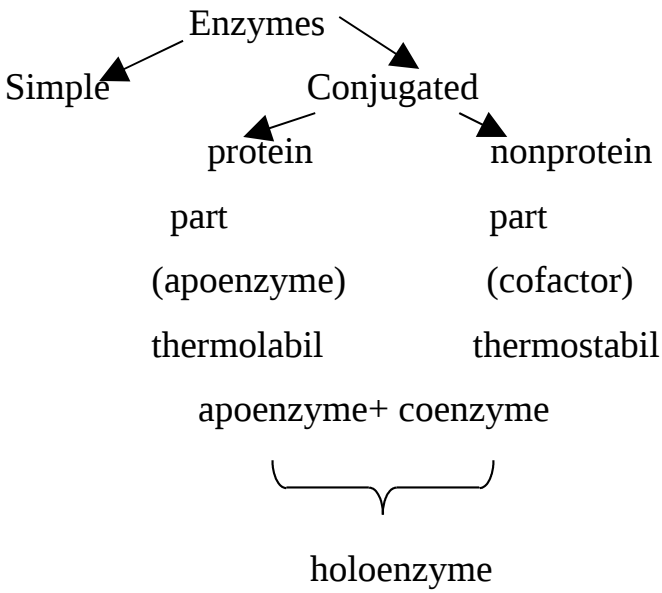
●Cooperative effect.

● The oriental effect is typical for enzymes.

● They practically do not form side products.

● Enzymes are regulated.

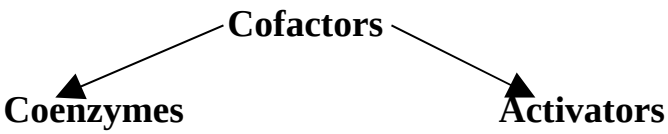
Structure of enzymes



Apoenzyme provides the specificity of enzyme (its selective interaction with substrate).

Cofactor determines a type of reaction. The same cofactor may be included into the structure of different enzymes.

For example: pyridoxal phosphate is cofactor both aminotransferases and decarboxylases of amino acids and other enzymes.



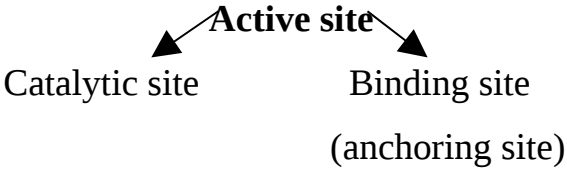
Coenzymes are defined as heat-stable, low-molecular weight organic compounds required for the activity of enzymes. Most coenzymes are linked to enzymes by noncovalent forces. Those which form covalent bonds to enzymes may also be termed *prosthetic qroups.*

Activators are not included in enzymes as integral structure components. They support enzymes in catalytically active state (metal ions, reductive substances)

Classification of coenzymes depending on the chemical structure:

- derivatives of vitamins (TPP, pyridoxal phosphate, HSCoA, FH_4);
- dinucleotides (NAD, FAD);
- nucleotides (UTP, ATP, CTP);
- complexes of porphyrins with metal ions.

Active site of enzyme is a unique combination of amino acid residues in the enzyme molecule that provides a direct interaction of enzyme with the substrate molecule and its immediate involvement in the act of catalysis.



Catalytic site is directly involved in chemical interaction with the substrate and its conversion. Residues of Arg, His, Lys, Asp, Glu, Ser, Cys, Tyr are usually included in catalytic site.

Anchoring site is responsible for the specific affinity of enzyme for substrate and the formation of an enzyme-substrate complex.

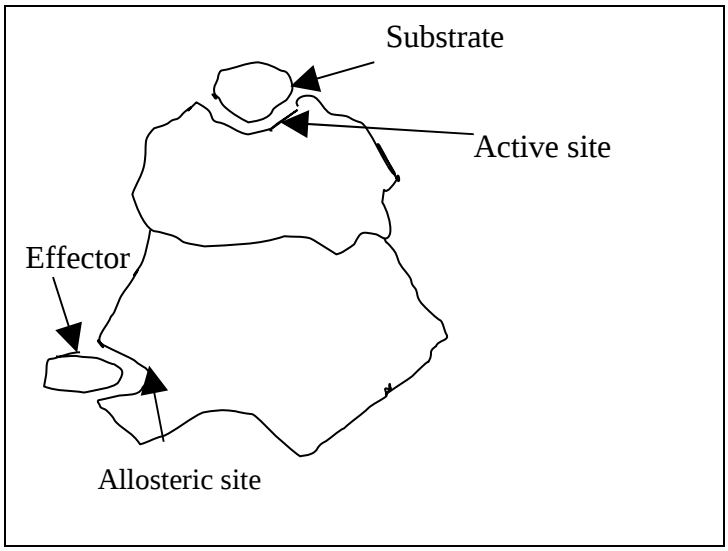
Allosteric site is a site on the enzyme molecule that serves for binding low - molecular – weight compounds (effectors, or modifiers), whose molecules are structurally dissimilar from the substrate molecules.

The attachment of an effector to allosteric site produces a change in the tertiary and quaternary structures of enzyme molecule with the resulting change of

active site configuration, which leads to the changing enzymatic activity. These enzymes are named **allosteric enzymes**. They are usually oligomeric proteins.

Isoenzymes are multiple forms of an enzyme. They catalyze the same reaction, but differ from each other by the primary structure and physico–chemical properties, affinity to substrate, maximal rate of the reaction, regulatory properties.They are oligomeric molecules with dissimilar protomers.

For example: Lactate dehydrogenase consists of four protomers of two types



(H and M). Only the tetrameric molecule possesses catalytic activity.

HHHH HHHM HHMM HMMM MMMM

i_1 (LDH_1) i_2 (LDH_2) i_3 (LDH_3) i_4 (LDH_4) i_5 (LDH_5)

In norm each tissue is characterized by specific isoenzyme spectrum. H_4 form is predominant in the heart; while M_4 form – in skeletal muscle and liver. These findings are widely used in the clinical practice, for differential diagnosis of organic and functional lesions of tissues and organs.

Enzyme specificity to substrate

Absolute specificity (1E→1S)	Relative (or group) specificity	Stereochemical specificity
For example: arginase	Enzyme acts on the group of the substrates which have a similar structure (for example: lipase)	Enzyme differs stereoisomers of the same substrate (for example:oxidases of D-amino acids and oxidases of L-amino acids)

5.2 Mechanism of Enzyme Action

Michaelis and Menten (1913) established the theory of formation of an enzyme-substrate complex and worked out the kinetics of such complex formation and its subsequent dissociation into products.

According to Michaelis-Menten theory the enzyme combines with the substrate to form an enzyme-substrate complex. This transient complex has a lower energy of activation than that of substrate alone. This results in acceleration of the reaction. Then enzyme-substrate complex is broken down into enzyme and products of the reaction.

$$E+S \rightarrow ES \longrightarrow ES^{*} \longrightarrow E+P$$

The different hypotheses of enzyme-substrate complex formation have been postulated. Early theory was postulated by **Emil Fisher**. According to this theory the catalytic site of enzyme is presumed to be preshaped to fit of the substrate. This hypothesis is called "**lock and key" or rigid template.** The next hypothesis has been proposed by **Koshland** in the late 1950 s. This hypothesis was called "induced fit". According to this theory a catalytically active configuration of enzyme molecule and that of the active site can be induced only at the moment of attachment of the substrate molecule that is the substrate induces a conformational change in the enzyme.

At the present time model of “induced fit” is modified. Binding the substrate with enzyme leads not only to the conformational change of the protein molecule, but also to a geometrical and electron-topographic rearrangement of the substrate molecules. **The modern “induced fit” hypothesis presumes the existence between the enzyme and the substrate of not only a spatial or geometrical complementarity, but also electrostatic charge coplementarity.**

Enzymes enhance reactant proximity and local concentration. The concentration of substrate on the enzyme molecule may by thousand times greater than concentration in the medium. This phenomenon of approximation enhances the rate of reaction by several thousand times.

The effect of orientation is also characteristic for biological catalysis as many of functional groups participate in binding the substrate. Binding the substrate with the active site of enzyme results in a **destabilization of certain chemical bonds of the substrate (effect of destabilization)** and facilitates a breakdown of these bonds.

Reaction groups can function either or **electrophiles or nucleophiles**. **Electrophiles** are electron-dificient substances which attack electron-rich substances. **Nucleophiles** are electron-rich substances which attack electron-dificient substances. **Catalyst** makes a potentially reactive site more **electrophilic** or more **nucleophilic**.

5.3 Kinetics of Enzymatic Catalysis

Enzymatic kinetics studies the influence of enzyme concentration, substrate concentration, pH, temperature, action of activators and inhibitors on the velocity of enzymatic reaction.

Effect of enzyme concentration on enzymatic reaction rate

The initial rate of enzymatic reaction is directly proportional to enzyme concentration.

$V = K \cdot [E]$

Effect of substrate concentration on enzymatic reaction rate

● At low concentration of substrate the velocity of reaction is proportional to concentration of substrate.

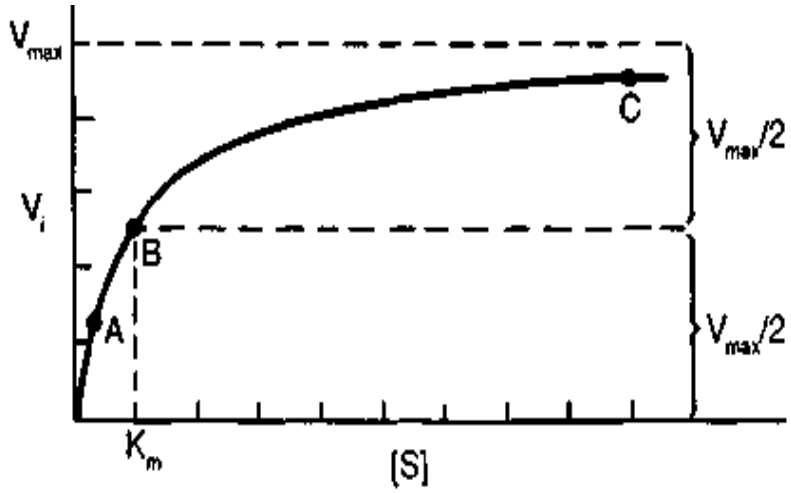


Figure 5.2. Effect of substrate concentration on the velocity of an enzyme-catalyzed reaction

● At high concentration of substrate the effect of enzyme saturation is achived, that is velocity does not depend on the substrate concentration.

Equation of V dependence on [S], or Michaelis-Menten equation

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

When $V = \frac{1}{2} V_{max}$: $\frac{V_{max}}{2} = \frac{V_{max} \cdot [S]}{K_m + [S]}$

$K_m = [S]$

K_m is the substrate concentration at which the velocity of reaction is equal half of maximal. K_{max} quantetively expresses the affinity of enzyme to substrate.

Because of the difficulty in determining Vmax from a hyperbolic curve, the Michaelis-Menten equation was transformed by Lineweaver and Burk into an equation for a straight line:

$$\frac{1}{V} = \frac{K_m}{V_{max}} \cdot \frac{1}{[S]} + \frac{1}{V_{max}}$$

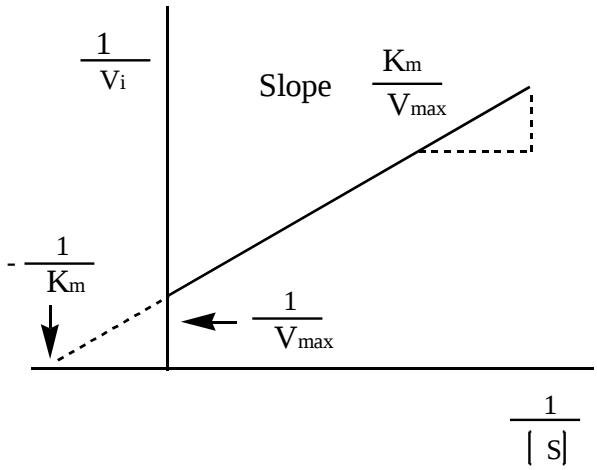


Figure 5.3. Double reciprocal or Lineweaver-Burk plot of $1/v_i$ versus 1/[s] used to evaluate K_m and V_{max}
The intercept on the $^1/_v$ axis equals $^1/_{Vmax.}$
The intercept on the $^1/_{[s]}$ axis equals $^1/_{Kmax.}$
The slope of the line equals $^{Km}/_{Vmax}$.

The influence of temperature on enzymatic activity

● Since the rates of chemical reactions are temperature-dependent, the enzyme-catalyzed reactions are also sensitive to temperature changes.

● The rate of a chemical reaction increases two fold if temperature is raised by 10°C.

● However owing to the protein nature of enzymes, the heat denaturation produced by temperature rise, will diminish the effective enzyme concentration.

● Thus, a rise in temperature not exceeding 40-50°C will make the reaction rate increase according to the classical theory of chemical kinetics.

● At a temperature above 50°C, the reaction rate is greatly influenced by heat denaturation of the enzyme which ultimately results in a complete cessation of the enzymatic process.

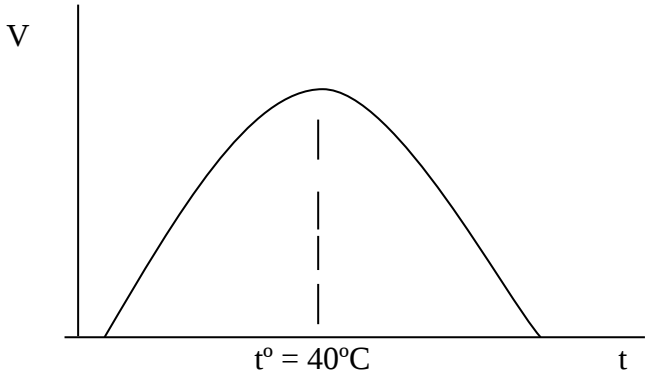


Figure 5.4. Effect of temperature on the velocity of enzyme-catalyzed reaction.

Effect of pH on enzymatic activity

Activity of enzymes depends on pH of medium.

The pH, at which the rate of biochemical reaction is maximal is called **optimal pH.**

Optimal pH is specific for every enzyme, but most of enzymes have highest activity within a narrow range of hydrogen ion concentration, which corresponds to the physiological medium pH values of 6,0-8,0

Examples of opt pH:

Salivary amylase – 6,8-7,0

Urease – 7,0-7,2

Trypsin – 7,5-8,5

However a few enzymes are active at pH values considerably outside this range. For example, pepsin has optimal pH 1,0-2,0. The optimal pH for arginase lies within a strongly alkaline interval (10,0-11,0).

Hydrogen ion concentration influences an ionization degree of acidic and basic groups of active site of enzyme.

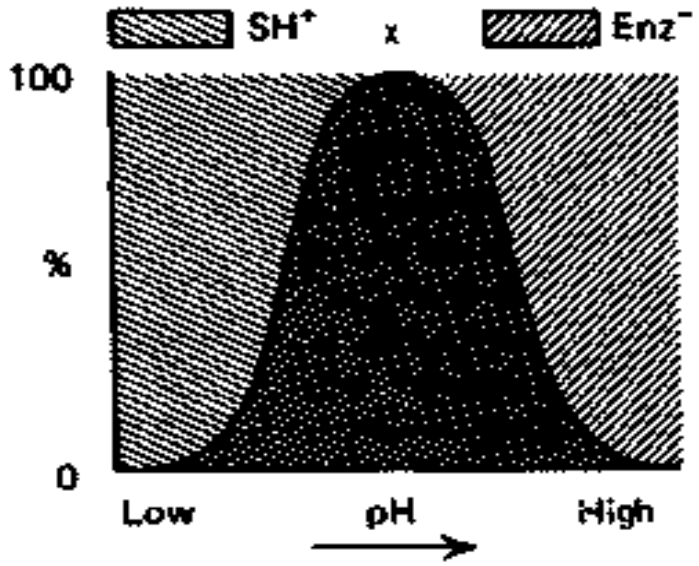
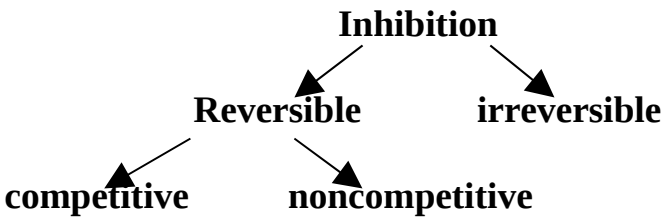


Figure 5.5. Effect of pH on enzyme activity.

The influence of Inhibitors on The Rate of Enzymatic Reactions

Enzymes undergo the action of both exogenous (xenobiotics) and endogenous substances which inhibit their activity.



Irreversible inhibitors bind tightly to the enzyme and inactivate it. The complex of inhibitor with enzyme is not able to dissociation.

Examples:

· *Iodoacetylamide* is linked with catalitically active SH-groups.

· *Diisopropyl ftoride phosphate* is irreversibly interacted with OH-groups in active site of acetylcholine esterase.

· *Cyanic acid, cyanides* inhibit cytochrome oxidase.

Reversible inhibitors. The complex of inhibitor with enzyme is able to dissociation.

Reversible inhibitors are divided into competitive and noncompetitive ones.

Competitive inhibitors. The structure of competitive inhibitor is similar to the structure of the substrate.

• Competitive inhibitor competes with the substrate for the active site of the enzyme.

• Competitive inhibition may be reversed by increasing the substrate concentration.

• In competitive inhibition V_{max} remains the same, but the apparent K_m increased.

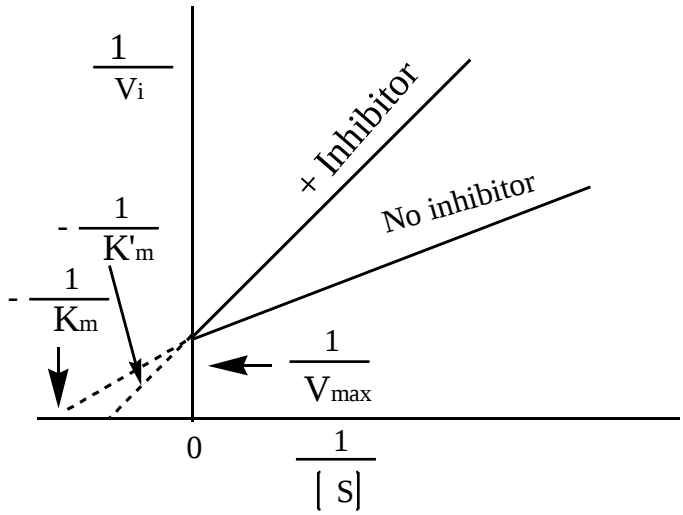


Figure 5.6. Lineweaver-Burk plot of classic competitive inhibition. Note the complete relief of inhibition at high [S] (low 1/[S]).

Examples:

• *Malonate is* inhibitor of succinate dehydrogenase.

• *Sulfanylamides* are competitive inhibitors of the bacterial enzymes, which participate in the synthesis of folic acid from paraaminobenzoic acid. Therefore they lead to inhibition of bacterial growth.

Noncompetitive inhibitors are structurally dissimilar with the substrate. They bind to the enzyme or the enzyme-substrate complex at a site different from the active site, decreasing the activity of the enzyme. The noncompetitive inhibitor decreases V_{max} but it does not influence on K_m.

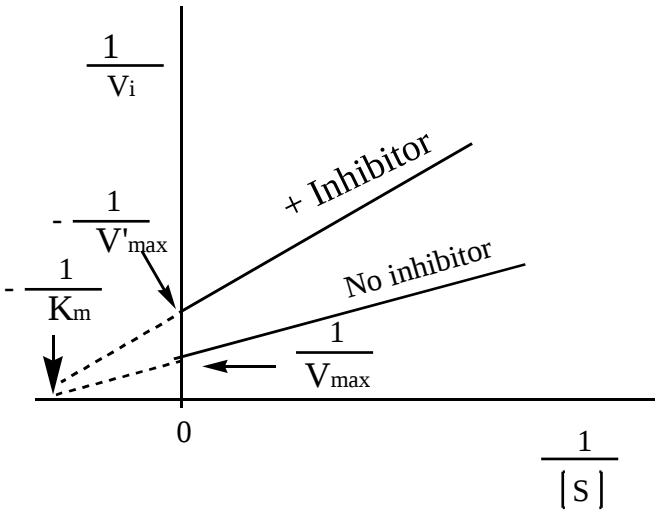


Figure5. 7. Lineweaver-Burk plot for reversible non competitive inhibition.

Example:

• Ethylene amine tetraacetate (EDTA) reversibly interacts with metal ions (Mg).

The quantitative estimation of enzyme concentration is based on measuring the enzyme-catalyzed reaction rate under definite, accepted for standard, conditions.

At optimal temperature, pH medium and full saturation of enzyme with substrate, the rate of enzyme-catalyzed reaction is directly proportional to the enzyme concentration.

The rate of enzymatic reaction is monitored either by the substrate depletion rate, or by the rate of the product formation.

Units of expression of activity of enzymes

1. ***Unit (U) of*** activity of a enzyme is the amount of enzyme, required to convert 1 micromole of substrate per minute (micromole/min)

2. The international system of Units (SI)

Katal – catalytic activity at which the reaction of substrate conversion is carried out at a rate of 1 mol of substrate per second (1 mol/s)

1 U = 16.67nkat

3. ***Specific activity*** *is given in U/mg of protein.*

5.4 Regulation of Enzymatic Processes

Regulation of enzymatic activity is performend on 3 levels:

I. The change of absolute quantity of enzyme presents (stimulation or inhibition of the enzyme synthesis). It is mechanism of prolonged regulation. Rates of synthesis and degradation determine enzyme quantity. Enzyme levels in mammalian tissues may be altered by a wide range of physiologic, hormonal, or dietary factors. For example, glucocorticoids stimulate the synthesis of key enzymes of gluconeogenesis.

The concentrations of substrates, coenzymes and possibly ions in cells may influence the rates at which specific enzymes are degraded. For example, tryptophan lowers the rate of tryptophan dioxygenase degradation by stabilizing the oxygenase against proteolytic enzymes.

Enzymes of microorganisms are divided into constitutive and inducible. Enzyme induction also occurs in eukaryotes. Examples of inducible enzymes in

animals include tryptophan dioxygenase, threonine dehydrase, tyrosine-α-ketoglutarate transaminase, enzymes of the urea cycle, β-hydroxymethylglutaryl–CoA reductase and cytochrome P450.

II. The altering the catalytic efficiency of the enzyme

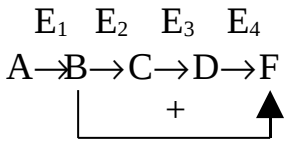
1. *The conversion of enzymes into active form by limited proteolysis*. Activation of enzymes by means of limited proteolysis is the mechanism of irreversible transformation of enzyme into catalytically active state. This regulatory mechanism functionates in the formation of the most proteolytic enzymes of gastro-intestinal tract and active proteinases which are components of clotting and fibrinolytic systems of blood. Proenzymes facilitate rapid mobilization of an activity in response to physiologic demand.

2. *Covalent modification of enzymes* (phosphorylation-dephosphorylation, methylation, ADP-ribosylation). Reversible, covalent modification regulates key mammalian enzymes. Reversible modulation of the catalytic activity of enzymes can occur by covalent attachment of a phosphate group to one or more Ser, Thr, Tyr residues. Phosphorylation and dephosphorylation of proteins are catalyzed by a variety of protein kinases and protein phosphatases respectively. The activity of both protein kinases and protein phosphatases is under hormonal and neural control.

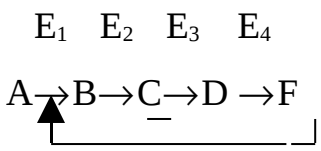
3. *Allosteric regulation.* The catalytic activity of certain regulatory enzymes is modulated by low-molecular-weight allosteric effectors that generally have little or no structural similarity to the substrates or coenzymes for the regulated enzyme.

Allosteric enzymes are enzymes which activity at the catalytic site may be modulated by the presence of allosteric effectors at an allosteric site. Allosteric enzymes typically exhibit sigmoidal substrate saturation kinetics in the presence of inhibitor. The sigmoid character of the V versus [S] curve in the presence of an allosteric inhibitor reflects the phenomenon of cooperativity.

a) Activation by precursor. For example, activation of pyruvate kinase by fructose-1,6-bisphosphate.



b) Inhibition by final product (feed-back regulation). Feed-back inhibition refers to the inhibition of the activity of an enzyme in biosynthetic pathway by an end product of that pathway. For example, heme allosterically inhibits the activity of δ-aminolevulinate synthase.



c) Allosteric regulation by other effectors. For example, phosphofructokinase is inhibited by citrate and ATP.

4. *Regulation by cyclic nucleotides* (cAMP, cGMP).

For example, cAMP activates cAMP-dependent protein kinase by means of dissociation of regulatory subunits.

$C_2R_2 + 4\ cAMP \longrightarrow C_2 + R_2 - (cAMP)_4$

5. *Regulation by regulatory proteins.*

For example, Ca^{2+}-calmodulin complex activates many enzymes such as phosphodiesterase of cyclic nucleotides, myosin light-chain kinase, adenylyl cyclase etc.

III. The altering the pool size of reactants other than enzymes

1. *The change of membrane permeability.* Local concentrations of substrates coenzymes and cations can regulate enzyme activity.

2. *Enzyme compartmentation.* Localization of specific metabolic processes in the cytosol or in subcellular organelles facilitates regulation of these processes. The translocation of metabolites across compartmental barriers is achived via shuttle mechanisms that convert the metabolite to a form permeable to the compartmental barrier followed by transport and conversion back to the original form on the other side of the barrier.

3.*Occurrence of multimolecular enzyme systems.* Organization of a set of enzymes that catalyze a sequence of metabolic reactions into a macromolecular complex channels intermediates along a metabolic path, facilitates transfer of product between enzymes without prior equilibration with metabolic pools. In addition, conformational changes in one component of the complex may be transmitted by protein-protein interactions to other enzymes of the complex. Amplification of regulatory effects thus is possible.

5.5 Classification and Nomenclature of Enzymes

The type of catalyzed chemical reaction together with the name of substrate constitute the basis for the systematic nomenclature of enzymes.

According to the modern classification the enzymes are divided into six main divisions:

I. Oxydoreductases catalyze oxidation-reduction reactions (for example: lactate dehydrogenase – full name: lactate: NAD^+ - oxidoreductase)

II. Transferases catalyze reactions involving the intermolecular transfer of atoms, atomic groups and radicals. Aminotransferases, methyltransferases, acyltransferases, phosphatetransferases belong to this class.

III. Hydrolases catalyze the cleavage of intramolecular bonds of organic compounds through the assistance of water molecules. Amylases, lipases, proteolytic enzymes belong to this class.

IV. Lyases catalyze reactions for cleavage of various bonds (-C-C, -C-O, C-N) without water. Decarboxylases, aldolases, dehydrases belong to this class.

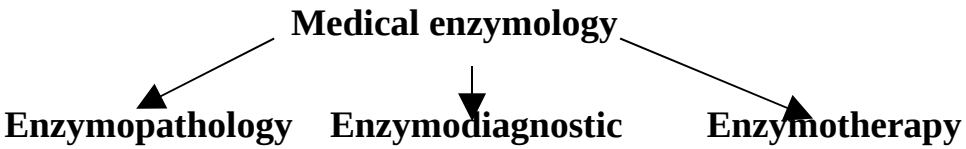
V. Isomerases catalyze isomerization reactions of different types. Epimerases, mutases belong to this class.

VI. Ligases (Synthetases) catalyze the organic synthesis from two initial compounds using the energy of ATP cleavage. Examples: carbamoyl phosphate synthetase, DNA-ligases.

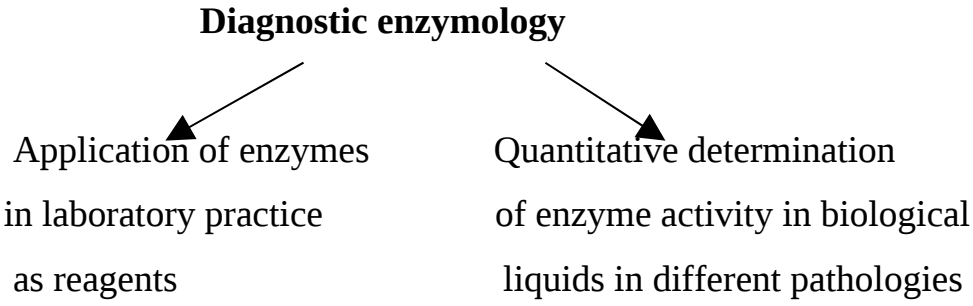
Every enzyme has the code number. The code number for each enzyme is made up of four parts separated by a dot from each other and is composed according to the following principle. The first number includes the class to which

the given enzyme belongs. The second number indicates the sub-class, which characterizes the major substrate species involved in the given type of chemical conversions. The third place in the enzyme code is the sub-sub-class number. The fourth position is ordinal number of the enzyme.

5.6 Medical Enzymology



Enzymopathology. Many inherited diseases are due to defect of enzymes. It is known about 150 hereditary enzymopathies.



This is a very important area for the use of enzymes within medicine. Most cells will go through a normal cycle division and cell death, releasing intracellular enzymes into extracellular fluids and blood. However, in certain pathologic states, there is an accelerated rate of cell death and, thus, an increase in the loss of intracellular enzymes.

Table 5.1 Diagnostic Spectrum or Plasma Enzymes.

Enzymes	Disease
Creatine kinase (CK) MB isoenzyme (CK-MB) MM isoenzyme (CK-MM)	 Myocardial infarction Various pathologic states of skeletal muscle (e.g., Duchenne's muscular dystrophy)
Lactate dehydrogenase LDH_1	Myocardial infarction
Aspartate aminotransferase (AsAT)	Myocardial infarction

Alanine aminotransferase (AlAT)	Viral hepatitis
Amylase	Acute pancreatitis
Lipase	Acute pancreatitis
Acid phosphatase (AP)	Prostate cancer
Alkaline phosphatase (ALP*)	Bone disorders, liver diseases

**Different isoenzymes of ALP can be used to distinguish bone and liver disease.*

Quantitave determination of enzyme activity in biological liquids is necessary for:

- early diagnosis;
- differential diagnosis;
- prognostic purpose;
- estimation of treatment efficiency;
- monitoring disease.

The following enzymes are most important in clinical diagnostic: aminotransferases; lactate dehydrogenase; amylase; γ-glutamyl transferase; creatine kinase; phosphatases; cholinesterase.

Aminotransferases. In spite of the absence of organ specificity the determination of alanine aminotransferase (AlAT) and aspartate aminotransferase (AsAT) has big diagnostic significance. Activity of AsAT increases in blood serum in *myocardial infarction* 4-6 hours after damage and normalizes 3-7 days. *Acute hepatitis* is accompanied by the sharp increase of AlAT activity.

Creatine kinase (CK). The increase of CK activity in blood serum may be due to damage of myocardium or skeletal muscles. Creatine kinase is widely used as early indicator of myocardial infarction and is more specific than AsAT. About 6 hours after the myocardial infarction, the activity of creatine kinase rises in the blood and peaks at about 36 hours. It is very important to determine isoenzymes because MB-form of CK is high specific for myocardium. Damage to skeletal muscle will result in increased levels of the MM dimer in blood, and this is also seen with certain muscle diseases, such as Duchenne's muscular dystrophy.

Lactate dehydrogenase (LDH). Lactate dehydrogenase is present in red blood cells; thus, it is desirable to prepare plasma or serum from blood without

excessive delay to prevent hemolysis. Activity of LDH increases in blood serum in many diseases. It is important to determine isoenzymatic spectrum of LDH. In myocardial infarction LDH_1 (H_4) increases. Its activity is enhanced to 24 hours after damage and normalizes more slowly versus to CK an AsAT. In acute hepatitis LDH_5 (M_4) is sharply increased.

The increase of ***α-amylase*** (>10 fold) in blood serum indicates the *acute pancreatitis.*

Usually, plasma or serum samples derived from blood are used in diagnosis. It may also be possible to assess blood cells for enzyme levels, for example, red and white blood cells or platelets. Urine may be used for enzyme determination as may other fluids, such as amniotic fluid or gastric juice, although these are not as easily accessible. It is also possible to take biopsy of tissue, homogenize a portion to break open the cells, and assay the liberated enzymes.

Enzyme therapy.

Enzymes are used as replacement therapy (pepsin, festal, pancreatinum). They have proteolytic and depolymerizing action (crystallic trypsin, collagenase, ribonuclease), increase the tissue permeability (lydasum, hyaluronidase) etc. Urokinase, streptokinase are used in stroke or myocardial infarction. They activate the plasma protease plasmin, which can dissolve thrombi blocking the cerebral or coronary vessels, thereby restoring blood flow and ending ishemia. Inhibitors of enzymes are also used as drugs. For example, inhibitors of monoamine oxidases are used as antidepressants; inhibitors of cyclooxygenase (aspirin, indomethacin) as antiinflammatory substances; inhibitors of dihydrofolate reductase (methotrexate) and thymidylate synthetase (5-fluorouracil) are used as chemotherapeutic agents to inhibit the proliferation of cancer cells.

Tests for Self-control

1. Point out a substrate, which is degraded by hydrolases:
A. Fatty acids
B. Proteins
C. Glucose
D. Pyruvate
E. Carbon dioxide

2. Enzyme urease is able to rupture only the structure of urea. Point the type of its specificity:
A. Stereochemical
B. Absolute
C. Absolute group
D. Relative group

3. Enzymes, which catalyze the same reaction, but differ of one another by the primary structure and physico-chemical properties are called:
A. Isoenzymes
B. Holoenzymes
C. Zymogens
D. Cofactors
E. Apoenzymes

4. Which of the below mentioned properties is characteristic only for biologic catalysts?
A. Increase a velocity of reaction, but are not consumed and aren't irreversibly changed
B. Increase a velocity of reaction, decreasing energy of activation
C. Don't change the state of equilibrium of chemical reaction
D. Ability to regulation

5. Enzymes increase the velocity of reaction, because they:
A. Change free energy of reaction
B. Decrease the velocity of reverse reaction
C. Decrease the energy of activation
D. Change the state of equilibrium of chemical reaction

6. Point out the activator of salivary amylase:
A. Sodium chloride
B. Ammonium sulfate
C. Copper sulfate
D. Magnesium chloride
E. Calcium gluconate

7. Point out the enzyme, which activity should be determined in patient's urine in acute pancreatitis:
A. Amylase
B. Protein kinase
C. Cholinesterase
D. Leucine aminopeptidase

E. Alkaline phosphatase

8. Point out the enzyme, the activity of which is determined in blood plasma of patients with pathology of bone tissue:

A. Pepsin
B. Trypsin
C. Amylase
D. Acid phosphatase
E. Alkaline phosphatase

9. The degree of the liver parenchyma lesion is estimated by the determination of:

A. Concentration of isoforms LDH_1 (H_4) and LDH_2 (H_3M) of blood plasma
B. Concentration of isoforms LDH_4 (HM_3) and LDH_5 (M_4) of blood plasma
C. Activity of amylase of urine
D. Activity of acid phosphatase
E. Concentration of isoform LDH_3 (H_2M_2) of blood plasma

10. Which mechanism of folic acid synthesis inhibition by sulfanylamides?

A. Competitive
B. Irreversible
C. Denaturation of enzyme
D. Noncompetitive
E. Binding with allosteric site of enzymes

CHAPTER 6 VITAMINS

6.1. General Characteristic. Classification. Antivitamins

Vitamins are the group of organic substances with the different chemical structures and the physico-chemical properties but all the vitamins are absolutely necessary and essential for living organism as cofactors and regulators of metabolism.

And the very absence of vitamins in organism has led to their discoveries.

Signs of vitamins:

1. All the vitamins are organic substances with low molecular masses.
2. They are essential nutritional factors.
3. Vitamins are the metabolism regulators.
4. They show the biological activity in very low concentrations.
5. They aren't plastic material in tissue structure.
6. Vitamines aren't used by organism as energy source.
7. Deficiency or absence of vitamins leads to development of hypo- or avitaminoses.

Vitamin classifications

There are different vitamin classifications. One of them is that of based on physico-chemical properties (namely, on the solubility).

The group of all the vitamins

true vitamins — vitamin-like substances

fat-soluble	water-soluble	· choline (B_4)
· retinol (A)	· thiamine (B_1)	· inositol (B_8)
·calciferol (D)	· riboflavin (B_2)	· paraaminobenzoic acid (B_{10})
· tocopherol (E)	· pantothenic acid	· orotic acid (B_{13})
· naphthoquinone (K)	· nicotinic acid (PP)	· pangamic acid (B_{15})
	· pyridoxine (B_6)	· S-methyl-methionine (U)

· biotin (H,B_7) · carnitine

· folic acid (B_9) · lipoic acid

· cobalamin (B_{12}) · ubiquinone (Q)

· ascorbic acid (C) · polyunsaturated fatty acids (F)

· bioflavonoids (P)

Vitamin-like substances don't respond to all the requirements for the vitamins:

- Some of them are synthesized in organism in sufficient amount.
- Some of them perform plastic and energy functions.
- A deficiency of these substances doesn't cause avitaminoses.

Some vitamins are synthesized in organism but in insufficient amount.

They are:

- ***Vitamin PP (coenzymic form is NAD)***

Essential amino acid tryptophan is transformed into coenzymic form of vitamin PP (during this metabolic process vitamin B_6 is necessary).

$$\text{Tryptophan} \xrightarrow{B_6} \text{NAD}$$

(coenzymic form of v. PP)

- ***Vitamin D_3***

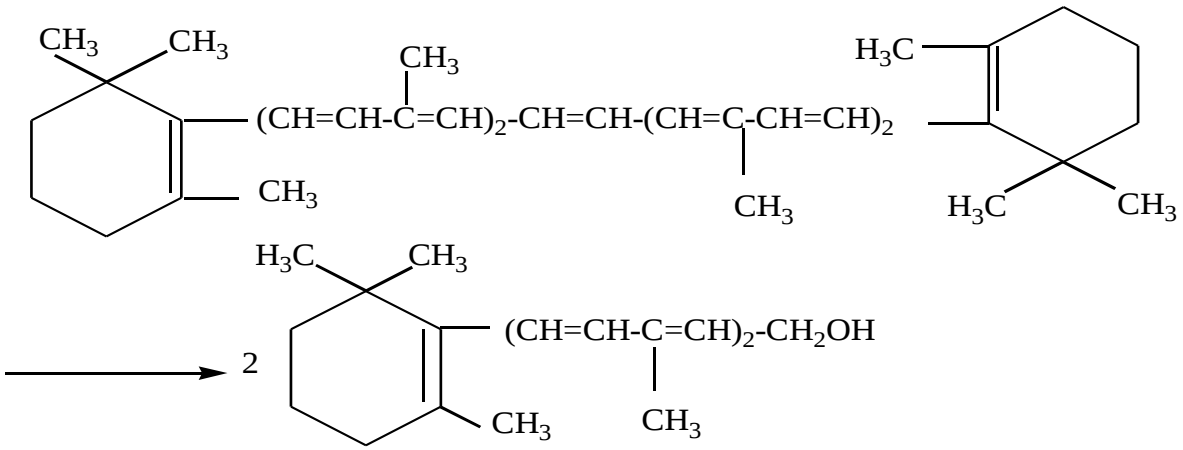
Acetyl-CoA is converted into 7-dehydrocholesterol

$$\text{Acetyl- CoA} \longrightarrow \text{7-dehydrocholesterol} \xrightarrow{\text{UV irradiation}} D_3$$

Such vitamins as ***K, B_6, B_7, B_9, B_{12}*** are synthesized by intestinal microflore.

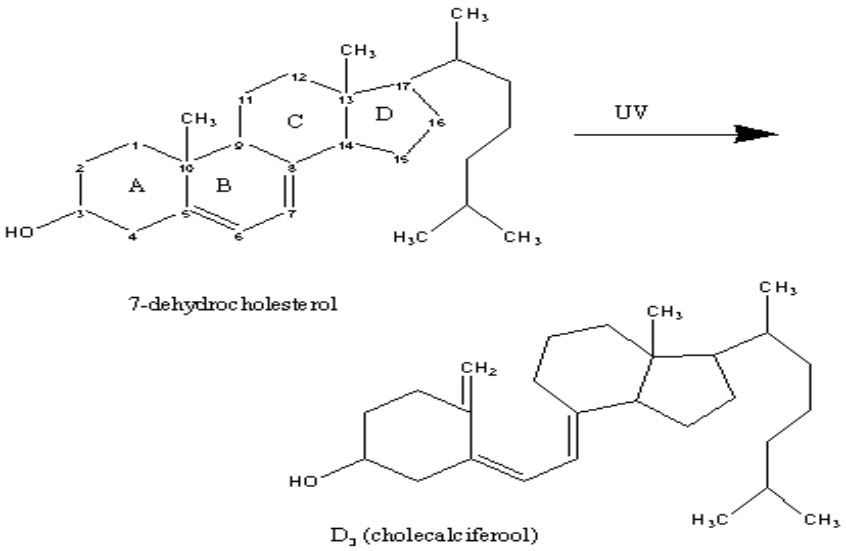
Some vitamins are supplied to the organism as provitamins (they are ***vitamins A and D***).

1. Carotenes are provitamins of vitamin A. Carotenes are converted into vitamin A under the influence of β-carotene-dioxygenase which is present in intestinal lumen and liver.



β-carotene-dioxygenase

2. Ergosterol and 7-dehydrocholecterol are provitamins of vitamins D_2 and D_3. Ultraviolet irradiation spontaneously cleaves the B ring of ergosterol or 7-dehydrocholecterol. Both ergosterol and 7-dehydrocholecterol are converted into vitamins D_2 and D_3 respectively.



7-dehydrocholesterol

D_3 (cholecalciferool)

The vitamins are divided into 3 groups depending on their functions:

1. Vitamins performing cofactor function.
2. Vitamins performing regulatory function.
3. Antioxidants.

Cofactor function of vitamins

1. ***Cofactors of common metabolic pathways.*** They are:

vitamin B_1 → coenzyme form is TPP

vitamin pantothenic acid → coenzyme form is HSCoA

2. ***Cofactors of tissue respiration.*** They are:

B_2 → coenzyme forms are FMN, FAD

PP → coenzyme forms are NAD, NADP

3. ***Cofactors of microsomal oxidation.*** (Vitamin C)

4. ***Cofactors of specific metabolic pathways.***

B_6, B_7, B_9, B_{12}, K

Vitamins A and D are considered to perform the regulatory function.

Vitamins E, A, C are also antioxidants. They eliminate reactive oxygen species (ROS).

Daily vitamin requirement varies widely: from 2 - 3μg of B_{12}, to 50 – 70 mg of vitamin C.

A food deficiency and bad assimilability of vitamins result in hypovitaminoses and absolute absence of vitamins or absolute lack of assimilability result in avitaminoses, which are divided into two groups: *exogeneous* and *endogeneous*.

Avitaminoses

exogeneous (or primary)	**endogeneous** (or secondary)
The cause is any vitamin lack in food	The causes of these avitaminoses may be: - enhanced requirement of the organism (during pregnancy, lactation, psycho-emotional stress and in different pathological states); - increased degradation of vitamin in intestine; - disturbed absorption (in gastro-intestinal, hepatic and pancreatic diseases); - disturbed transport of vitamins to target organs; - a disturbance of vitamin transformation into active forms;

- a disturbance of apoenzyme synthesis.

Avitaminoses may be also divided into:

Vitamin-dependent **Vitamin-resistant**

Vitamin-dependent states may be checked by megavitamin therapy.

Vitamin-resistant states can not be curatively treated even by administering large vitamin doses.

Surplus or deficiency of any vitamin can cause secondary deficiency of the other one, that's why it is necessary to keep the optimal relation between proteins, lipids and carbohydrates in food and optimal relation between different vitamins.

Now scurvy, pellagra, rickets and other avitaminoses disappeared as mass diseases. Nevertheless the problem of vitamin deficiency exists untill now. Hypovitaminoses can be latent for a long time without showing any symptoms but they may be harmful for a person health. That's why the classification of vitamins according to clinico-physiological effects has the great importance.

Classification of vitamins according to the clinico-physiological effects

	Group of the vitamins according to their therapeutic-profilactic effects	Brief clinico-physiological characteristics	Basic vitamins
1.	Vitamins raising organism reactivity	These vitamins regulate the state of central nervous system, metabolism and tissue trophism.	B_1, B_2, PP, B_6, A, C
2.	Antihemorrhagic vitamins	These vitamins provide normal blood-vessels penetration and their stability. They rise the blood coagulation.	C, P, K
3.	Antianemic vitamins	They normalize and stimulate the hemopoesis.	B_9, B_{12}, C
4.	Antiinfectious vitamins	They increase the organism stability to infection, phagocytosis and protective properties of epithelium, stimulate	B_9, B_{12}, C, A

		antibody production.	
5.	Vitamins of vision regulation	They increase the vision sharpness and expand the color vision field.	A, B_2, C

Antivitamins are the substances depressing biochemical utilization of vitamins in animal tissues.

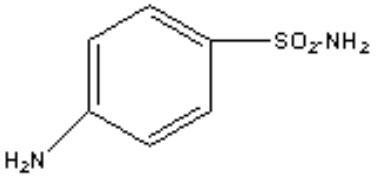
Antivitamins

specific nonspecific

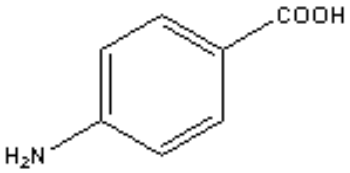
Specific antivitamins are structurally analogic to native vitamins and are competitively antagonistic to them.

For example:

Sulfanilamides are the antivitamins of paraaminobenzoic acid (PABA)



Sulfanilamide



PABA

Nonspecific antivitamins difficult absorption, transport of vitamins and their transformation into active forms etc.

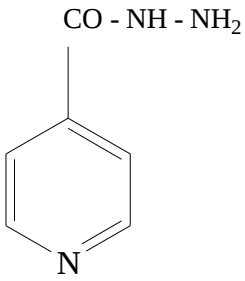
For example:

1. *Avidin* which is in egg white combines with biotin very tightly and prevents its absorption from intestine.

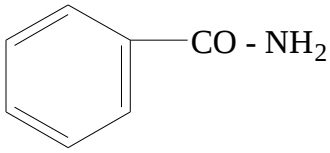
2. *Thiaminases I* and *II* cause the breaking down the vitamin B_1 molecules

Isoniazid is specific antivitamin to vitamin PP and nonspecific antivitamin to vitamin B_6.

Isoniazid (that is isonicotinic acid hydrozide) is structurally analogic to vitamin PP.



Isoniazid



Nicotinamide

In addition to this isoniazid inhibits pyridoxal kinase and therefore prevents a transformation of vitamin B_6 into active form (pyridoxal phosphate). Isoniazid can form a hydrazone with pyridoxine which blocks this enzyme. The content of pyridoxal kinase in mycobacteria is very low, therefore the pyridoxal kinase inhibition depresses their growth. It should be noted that pyridoxal phosphate is necessary for vitamins PP synthesis.

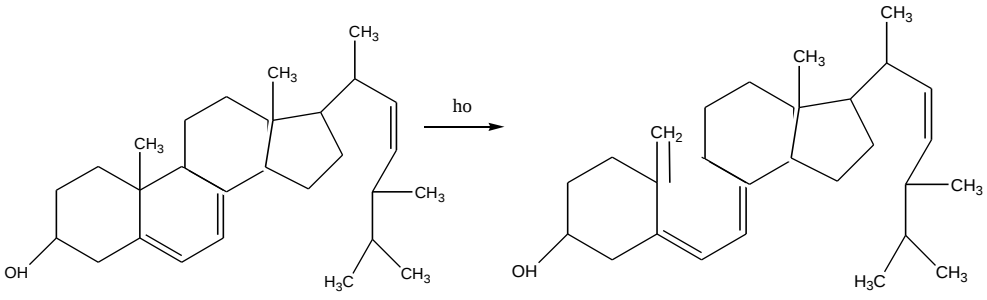
Application of antivitamins in medicine

1. *Dicoumarol* (antivitamin of vitamin K) is used as anticoagulant drug.
2. *Sulfanilamides* are used for the treatment of infectious diseases.
3. *Isoniazid* is one of the most effective drugs for the treatment of tuberculosis.
4. *Antivitamins of B_9 and B_{12}* are antitumor drugs (methotrexate).

6.2. Fat – soluble Vitamins

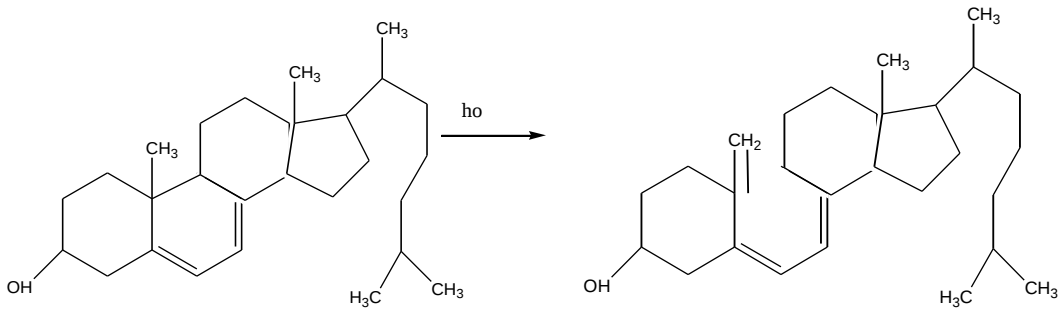
Group of D Vitamins (Calciferol, Antirachitic Vitamin)

***Structure**:*



ergosterol

ergocalciferol (D_2)



7-dehydrocholesterol cholecalciferol (D_3)

(can be synthesized in human organism)

Biological role. It regulates the calcium and phosphate metabolism.

Biologically active forms are hydroxylated derivatives (1,25-dihydroxycholecalciferol and 24,25-dihydroxycholecalciferol).

The action mechanism of 1,25-dihydroxycholecalciferol resembles that of steroid hormones. ***The name of hydroxylated derivatives of vitamin D_3 is calcitriol.***

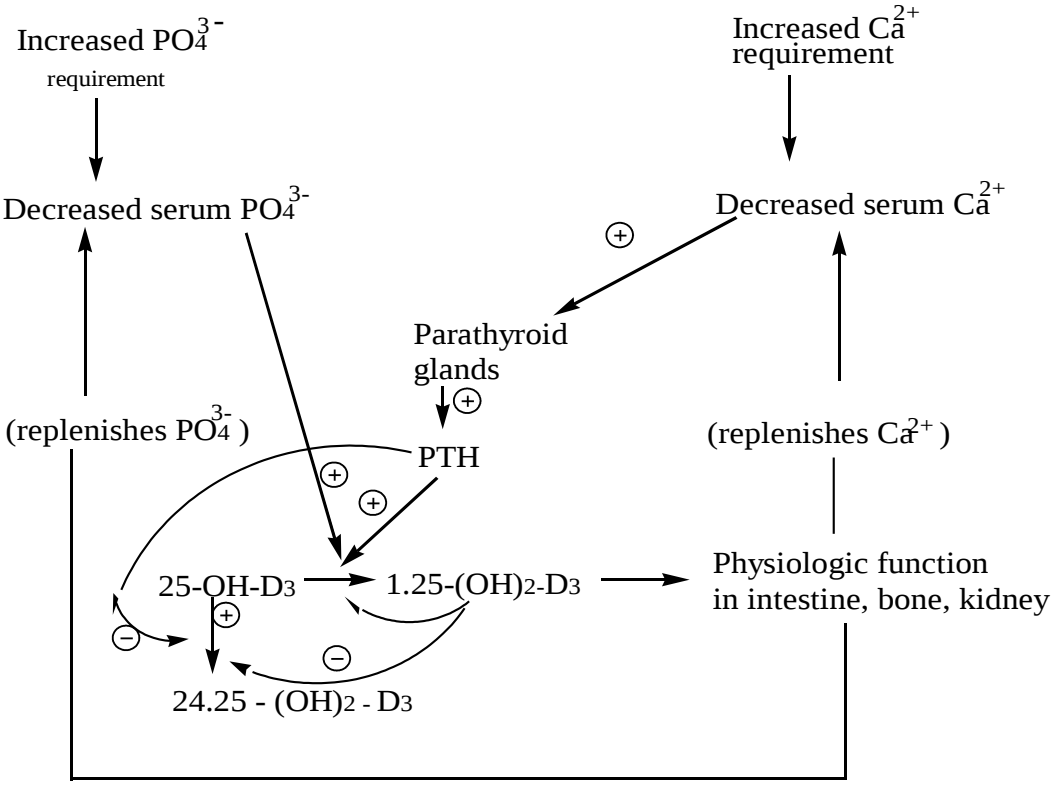
The target tissues include the intestine, bone, kidney.

- 1,25-$(OH)_2$ – D_3 induces synthesis of the intestinal calcium binding protein necessary for intestinal calcium absorption. It stimulates the absorption of phosphate too.
- 1,25-$(OH)_2$ – D_3 stimulates renal tubular reabsorption of phosphate and calcium.
- Calcitriol states optimal relation between Ca and P in biological fluids. At present there are findings supporting the influence of vitamin D on the basic components metabolism of organic bone matrix. 1,25-$(OH)_2$–D_3 is suggested to regulate the resorption process. And 24,25–$(OH)_2$–D_3 promotes normal bone mineralization and the synthesis of hydroxyapatite, which is the major form of mineralized bone and cartilage.

Hypovitaminosis. Vitamin D deficiency causes rickets, during which the bones continue to grow but mineralization is impaired. Therefore osteomalacia develops. The bones become soft, the legs unable to support the body weight, and the flat bones deform. The rib cage may become deformed as well (so called “pigeon breast”). Muscular hypotonia is also observed. The fontanelles do not

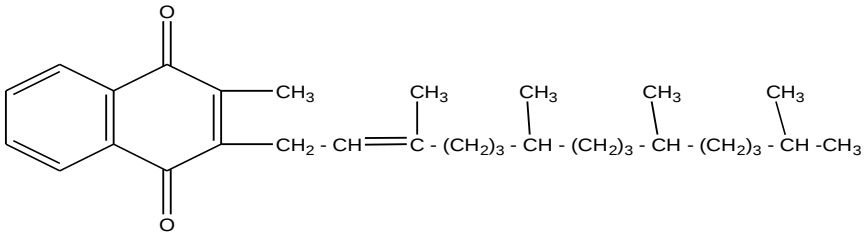
close properly. Cerebral cranium develops faster than visceral cranium. Therefore the child's head seems to be big. Teething is delayed, teething sequence is broken. In adults, vitamin D-deficiency can cause osteoporosis, leading to bone fractures.

Summary of regulation of vitamin D_3 hydroxylation.

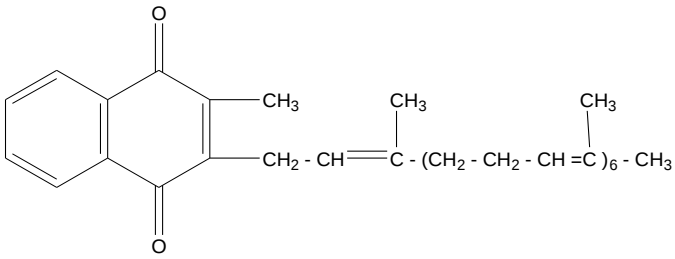


Occurrence in nature and daily requirement: 1) Ingestion in the diet with the animal food (butter, egg yolk, liver, coet-liver oil) and plants food (oil) and yeast. 2) Photolysis of 7-dehydrocholesterol in skin. Daily requirement for children is ≈ 10-25 μg. For adults it is considerably less.

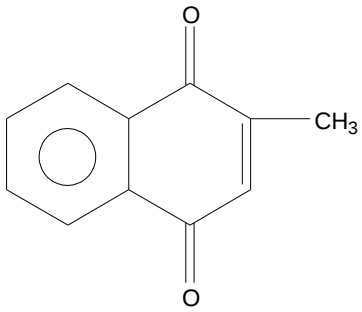
Group K Vitamins (Naphthoquinone, Antihaemorrhagic)



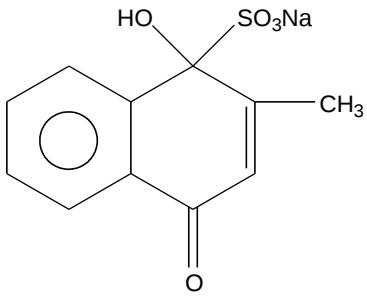
vitamin K_1(phylloquinone)



vitamin K_2 (menaquinone)



vitamin K_3



vicasol

Biological role. It is involved in the formation of γ-carboxyglutamate, several residues wich occur in the Ca^{2+}- binding sites of prothrombin (factor II), proconvertin (factor VII), Christmas factor (IX), and Stuart-Prower factor (X). It was shown that γ-carboxylation of glutamic acid residue in the protein molecules proceeds post-translationally with vitamin K – mediated participation of γ-glutamylcarboxylase. In this reaction, vitamin K appears to exercise a cofactor function. It is oxidized in the process and regenerated by reductants such as lipoic acid.

Vitamin K deficiency leads to spontaneous parenchymatous and capillary hemorrhage (nasal bleeding or internal hemorrhage). In humans, avitaminosis K is of less frequent occurrence as compared to other avitaminoses. This is due to two major reasons: firstly, the mixed diet is rich enough in vitamin K; secondly, the quantities of vitamin K synthesized by the intestinal microflora are enough for preventing the avitaminosis. The avitaminosis K is commonly concomitant with an impaired fat absorption in the intestine.

Antivitamins of vitamin K are warfarin and dicoumarol, which are used as inhibitors of blood clotting in the treatment of thrombosis.

Occurrence in nature and daily requirement

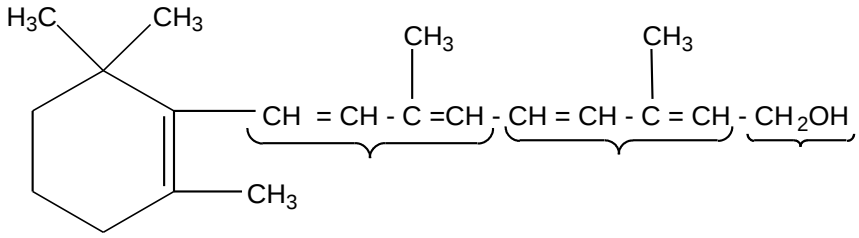
- Vitamin K is plentiful in plants, especially in the green leaves of chestnut, nettle and lucerne.

- Among the vegetable products rich in vitamin K cabbage, spinach, pumpkin, green tomato, arachis oil and ashberry.

- Vitamin K practically never occurs in animal products except pig liver.

The daily requirement in vitamin K for humans is not known exactly, since in the organism, it is produced by the intestinal microflora; the universally accepted dose of about 200-300 μg is believed to be sufficient.

Group A Vitamin (Retinol, Antixerophthalmic Vitamin)



It is composed of six-membered ring (β-ionone), two isoprene residues, and a primary hydroxyl group.

Biological role: Most target cells for vitamin A are capable to metabolize the retinol to retinal and retinoic acid. Retinol and retinal are interconverted in the presence of NAD – or NADP – requiring dehydrogenases present in many tissues. But retinoic acid cannot be reduced back to retinal or to retinol. Therefore retinol can satisfy all the requirements for vitamin A, but retinoic cannot replace retinal in its role in vision or retinal it its support of the reproductive system.

Each of the 3 major retinoids - retinol, retinal and retinoic acid - appears to have its own unique biologic functions.

- Retinol supports normal function of the reproductive system acting probably as a hormone.
- Retinal is important in the visual process. Retina contains two types of receptor cells. 1) Cones, which are specialized for color and detail vision in bright light. 2) Rods which are specialized for visual activity in dim light (night vision). Rods contain “visual purple” rhodopsin, and cones contain iodopsin. Rhodopsin and iodopsin are conjugated proteins, which consist of

different protein parts and common prosthetic group (11-cis-retinal). When rhodopsin is exposed to light it dissociates and forms trans-retinal and opsin. This reaction leads to conformational change that induces a **calcium ion** channel in the membrane of the rod cell. The rapid influx of calcium ions triggers a nerve impulse.

In the dark, a reverse process, the synthesis of rhodopsin, takes place.

- Retinoic acid acts like steroid hormones. It is probably involved in the control of the expression of certain genes. Retinoic acid supports the processes of growth and differentiation. Retinoic acid participates in the synthesis of glycoproteins as a sugar carrier in glycosylation. This may be the biochemical basis for the influence of vitamin A on the barrier function of the skin and mucous membranes, the permeability of cell membranes.

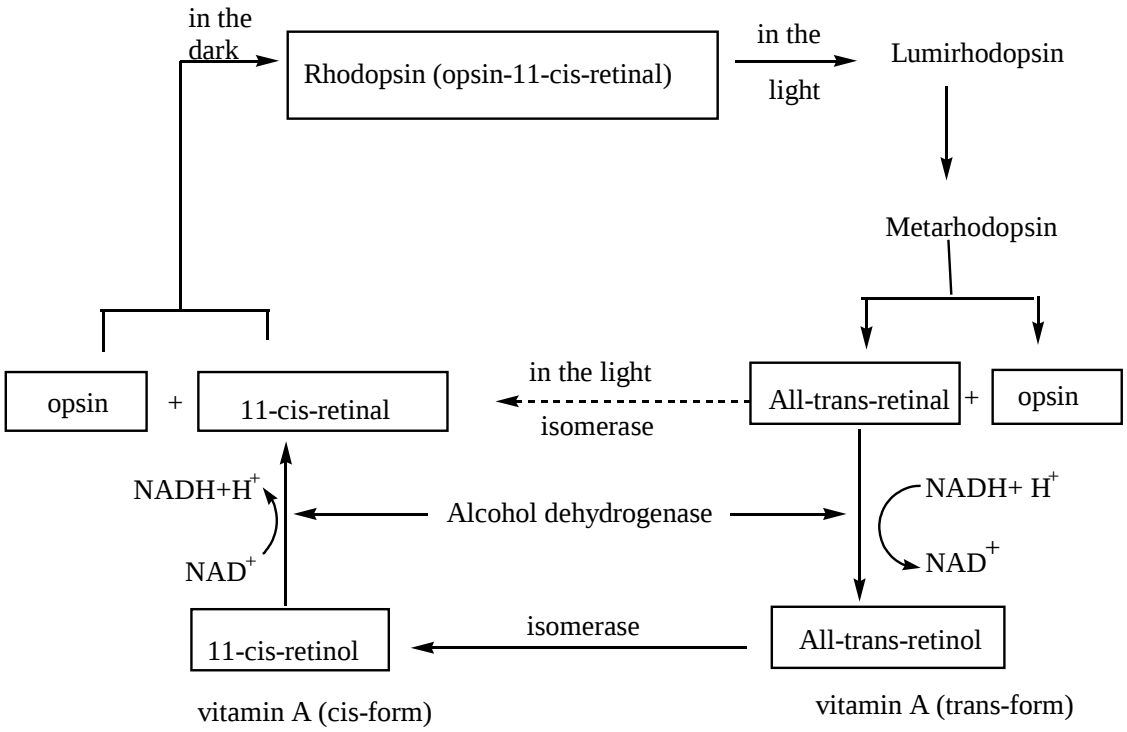


Figure 6.1 Participation of retinal in visual proces

- Vitamin A may take part in redox reactions – it is antioxidant.

Hypovitaminosis. Night blindness, or nyctalopia is an early and specific symptom of avitaminosis A. It is shown by failure or imperfection of vision at night, although during daylight hours, the patients see well.

The full syndrome of vitamin A deficiency includes xerodermia, xerophthalmia, keratomalacia, severe growth retardation (including that of nervous system), glandular degeneration, and sterility.

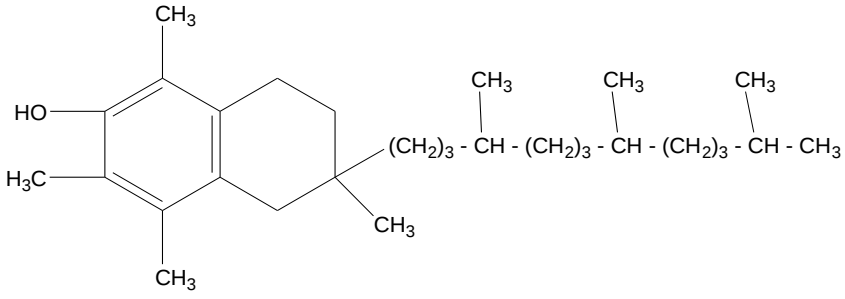
Pathologic proliferative keratinization of skin is manifested. The skin becomes dry. This pathologic state initiates secondary purulent and putrefactive processes. The mucous epithelium of gastrointestinal tract, urogenital system, and respiratory apparatus is also affected. Characteristic is also the lesion of the globe of the eye, xerophtalmia (that is dryness of the conjunctiva and cornea), which is caused by the occlusion of the lacrimal duct. The decay and softening of the cornea are caused by a progressive purulent process since putrefactive organisms, in the absence of antiseptic lacrimal fluid, quickly proliferate on the corneal surface.

Hypervitaminosis of vitamin A is caused by excessive dietary intake of the liver from polar bear, seat etc. Symptoms: inflammation of the eye, hyperkeratosis, loss of hair, general physical wasting, loss of appetite, headache, dyspeptic effects, insomnia.

Occurrence in nature*: b*ovine and pig liver; egg yolk; whole milk and cream; red flesh vegetables (vitamin A occurs as provitamins - carotenes): carrot, tomato, pepper.

The daily requirement 1,5-2,5 mg of vitamin A or 3-5mg of β-carotene.

Group E Vitamins (Tocopherol, Fertility Vitamin)



α - Tocopherol

At the present time seven natural compounds exhibiting vitamin E biological activity are known. They are: α, β, γ, δ- tocopherols, etc, and 8-methyltocopherol.

Biological role: Vitamin E is related to the tissue respiration through the feed forward mechanism, and to the extent of lipid oxidation, through the feedback mechanism.

- Vitamin E is readily oxidized and reduced and it is probable that all of its beneficial effects are due to its protection of lipids, particularly membrane lipids, from oxidation. Vitamin E is the most active and, probably, most important natural fat-soluble antioxidant.
- Tocopherols play a specific role in the selenium metabolism. The functions of vitamin E and of selenium, especially in reproduction, are closely associated: a deficiency of one increases the need of the other. This is because both of them prevent peroxidation of lipids in membranes (selenium because it is a component of glutathione peroxidase).
- In addition, vitamin E participates in the regulation of gene transcription.

Vitamin E deficiency. The human diet usually provides an adequate daily intake from plant sources. Vitamin E deficiency is rare but can occur in disorders of fat absorption and sometimes in premature infants.

- In experimental animal vitamin E deficiency produces disturbances in embryogenesis and degenerative alterations of the reproductive organs, with the development of sterility.
- In females, the placenta becomes affected to a greater extent over the ovary; the process of fertilization proceeds normally, but the nascent embryo is viable only within a short period of time.
- In males, the gonadal atrophy is observed, resulting in a partial or complete sterility.
- Specific manifestations of vitamin E deficiency are also muscular dystrophy, fat infiltration of the liver, cerebrospinal degeneration.

Vitamin E preparations are used to prevent spontaneous (or habitual) abortions in human females.

Occurrence in nature and daily requirement.

- For humans, the chief sources of vitamin E are vegetable oils as well as lettuce, cabbage and cereal grains; of the products of animal origin, vitamin E is found in meat, butter egg yolk, and pig liver.

The accepted estimate is a dose of about 10-20 mg per day.

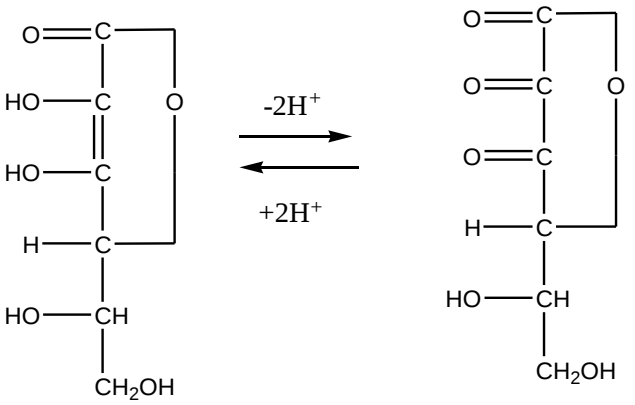
6.3 Water - soluble Vitamins

Vitamin C (Ascorbic Acid, Antiscorbutic Vitamin)

It is necessary for the human organism in the greatest amount. Vitamin C can be synthesized in a variety of plants and in all animals studded except primates and the guinea pigs. Humans are unable to synthesize ascorbic acid and they require ascorbic acid in their diet.

In its chemical properties it is quite strongly acidic, owing to ionization of the two hydroxyl groups separated by double bond.

And it is readily oxidized to semidehydroascorbate and dehydroascorbate and is the cosubstrate in various hydroxylation reactions.



L ascorbic acid

L-dehydroascorbic acid

Ascorbic acid has exclusively wide spectrum of action and that's why it is used both by healthy and sick people.

Biological role:

- Vitamin C takes part in proline and lysine hydroxylation and that’s why it is necessary for conversion of procollagen to collagen.
- It is important component of biological antioxidant system, which is linked with glutathione and tocopherol.
- It plays an important role in microsomal oxidation of a number of endogenous substances and xenobiotics in human organism.

- It increases cholesterol conversion into bile acids. This fact can explain antiatherosclerotic effect of ascorbic acid.
- Ascorbic acid participates in cyclic amino acid hydroxylation:

tryptophan → 5-hydroxytryptophan

phenylalanine → tyrosine

tyrosine → DOPA

dopamine → noradrenaline

- Vitamin C favours phagocytosis.
- Ascorbic acid takes part in synthesis of the corticosteroid hormones and hormones of adrenal medulla, hence ascorbic acid influences carbohydrate metabolism.
- Ascorbic acid elevates antibody synthesis.
- It promotes folic acid conversion into coenzymic form, that is, into tetrahydrofolate.
- Ascorbic acid shows antianemic effect. The latter is linked with folic acid conversion into tetrahydrofolate and with the Fe metabolism, as vitamin C influences the Fe reduction. This plays an important role in the absorption process and Fe mobilization by organism for hemopoiesis.
- Ascorbic acid increases dehydrogenation of NADH.
- It elevates the glucose oxidation by pentose phosphate pathway.
- Vitamin C shows protective effect to Hb inhibiting its oxidation. On other hand, vitamin C participates in Hb degradation.
- Ascorbic acid protects the SH-groups of enzymes from oxidation and therefore it protects the enzymes from inactivation.
- Vitamin C is necessary for normal Ca metabolism.

Vitamin C avitaminosis. The disease resulting from vitamin C deficiency is scurvy.

There are the typical signs:

- Increased permeability and vascular fragility, especially capillary one, because of proline and lysine conversion into hydroxyproline and hydroxylysine is disturbed. This conversion is necessary for formation of collagen, which determines vascular wall density. This results in spontaneous hemorrhages of gums, skin, joints, pericardium, lungs, kidney etc.
- Stomatitis and necrosis of the gums develop which lead to the loss of teeth. Disposition to plural caries is thought to be probably linked with disturbance of processes of odontoblastes differentiation and dentine matrix formation in which ascorbic acid takes active role. Edema of lower extremities and the pains in walking are seen.

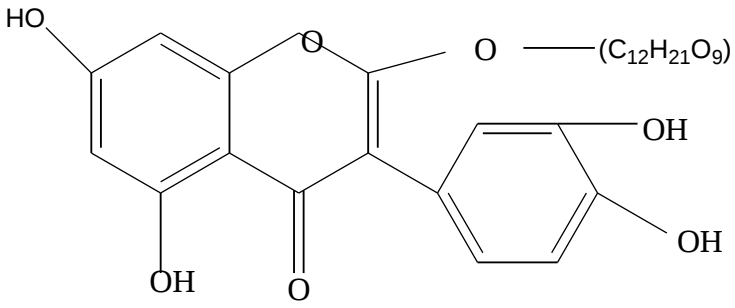
Sources. The best sources of vitamin C are citrus fruits, berries, melons, tomatoes, green peppers, raw cabbage.

Daily dose of vitamin C is 50-70 mg. Ascorbic acid is partially metabolized into oxalate, which can form urinary stones, therefore high intake of vitamin C isn't advisable.

Vitamin P (Rutin, Citrin, Permeability Factor, Common Name "Bioflavonoids").

Biological role.

- The major function of bioflavonoids is stabilization of the cement substance of connective tissue by inhibiting the hyaluronidase activity. A close link exists between vitamin C and vitamin P.
- Vitamin P decreases activity of ascorbase.
- Vitamin P protects dehydroascorbic acid from degradation
- Vitamin P inhibits histidine decarboxylase limiting toxic action of histamine.



Rutin

Hypovitaminosis. The dietary deficiency or lack of bioflavonoids causes an increase of blood vessels permeability, attended by hemorrhages.

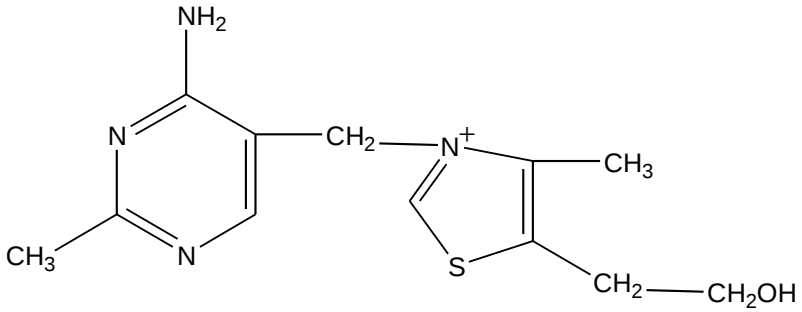
The major sources of vitamin P are products of vegetable origin rich in vitamin C.

Daily requirement of vitamin P hasn't been established.

Vitamin B_1 (Thiamine, Aneurin)

Thiamine was the first vitamin obtained in the form of crystal by Polish biochemist K. Funk in 1911.

Thiamine consists of a substituted pyrimidine joined by a methylene bridge to a substituted thiazole.



Vitamin B_1 is stable in acid medium (in acid medium it stands high temperature) and it is rapidly destroyed in neutral and basic medium. The absence of free HCl in gastric juice results in thiamine destruction.

Biological role.

Active form of vitamin B_1 is thiamine pyrophosphate. Thiamine pyrophosphate serves as a coenzyme in the following reactions:

- in oxidative decarboxylation of α-ketoacids (α-ketoglutarate and pyruvate);
- in transketolase reaction.

Moreover vitamin B_1 demonstrates neurotropic effect. These effects are probably linked with thiamine influence on acethylcholine metabolism, phosphorylation and dephosphorylation of membrane proteins and transport of Na^+ through membrane.

Vitamin B_1 avitaminosis is called **beri-beri**. The specific symptoms of B_1 deficiency are connected with disturbance of cardio-vascular and nervous systems and intestine.

The symptoms of B_1 deficiency are:

- Disturbance of motor and secretory intestine functions.
- Psychic changes (hallucinations, amnesia on recent events).
- Peripheral nervous system affection (disorders of sensibility, prickling sensation, numbness, and pain along the course of nerves). These disturbances lead finally to contractures, atrophy, and paralysis of the lower, and then upper, extremities.
- Cardiovascular changes.
- Negative nitrous balance.
- α-Ketoacid accumulation in blood (pyruvate and α-ketoglutarate).

Beri-beri has three forms, which should be distinguished:

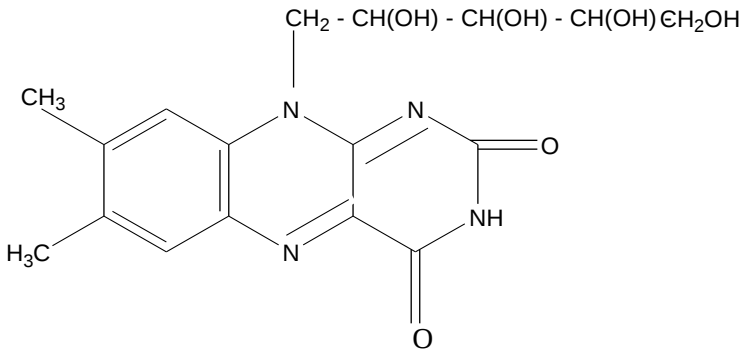
1) Dry or polyneuritis form. In this case the nervous system disturbance is displayed in a greater extent.
2) Edema form is characterized by cardio – vascular system disturbance and polyneuritis events.
3) Pernicious or cardiac form. This form leads to lethal end in result of acute cardiac deficiency.

The sources of vitamin B_1 are yeast, unrefined cereal grains, soy bean, haricot, potato, carrot, cabbage, liver, kidney, brain.

Daily dose of vitamin B_1 is 1.5-2.0 mg. Prevalence of carbohydrate diet increases a need of organism in vitamin; fats sharply decrease this necessity.

Vitamin B_2 (Riboflavin, Growth Vitamin)

The structural basis of riboflavin molecule is heterocyclic compound, isoalloxazine (a fusion of benzene, pyrazine, and pyrimidine rings) to which a pentabasic alcohol, ribitol, is attached at position 9.



Biological role. Riboflavin makes part of flavin coenzymes, in particular flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD), which are, in turn, prosthetic groups of enzymes (flavoproteins).

Flavin – dependent dehydrogenases are divided into 2 groups:

1. ***Anaerobic dehydrogenases*** which transport protons and electrons from the initial substrate (for example succinate dehydrogenase) or reduced pyridine coenzymes to components of respiratory chain. The enzymes of this type play a major role in biological oxidation.
2. ***Aerobic dehydrogenases.*** Reduced coenzymes of these enzymes are oxidized directly by oxygen. L- and D- amino acid oxidases, glycine oxidase, xanthine oxidase and some other enzymes belong to this type.

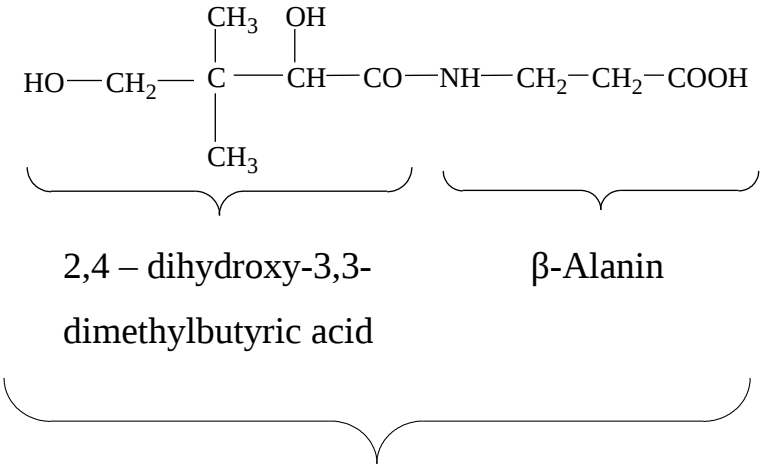
Hypovitaminosis. Apart from growth retardation and loss of hair (alopecia), other features specific of avitaminosis B_2 are inflammations of the tongue (glossitis), lips (especially at the corners of the mouth) and skin epithelium. Quite characteristic are also lesions inflicted upon the eyes: keratitis, inflammation and progressive vascularization of the cornea of the eye, and cataract (opacity of the crystalline lens of the eye) General myasthenia and cardiac weakness develop.

Occurrence in nature and daily requirement. Riboflavin is widespread enough in nature. Coarse bread, cereal grains, milk, meat and fresh vegetables are rich in this vitamin. The daily requirement in riboflavin for the adult human is **1,8-2.6 mg**

Pantothenic Acid

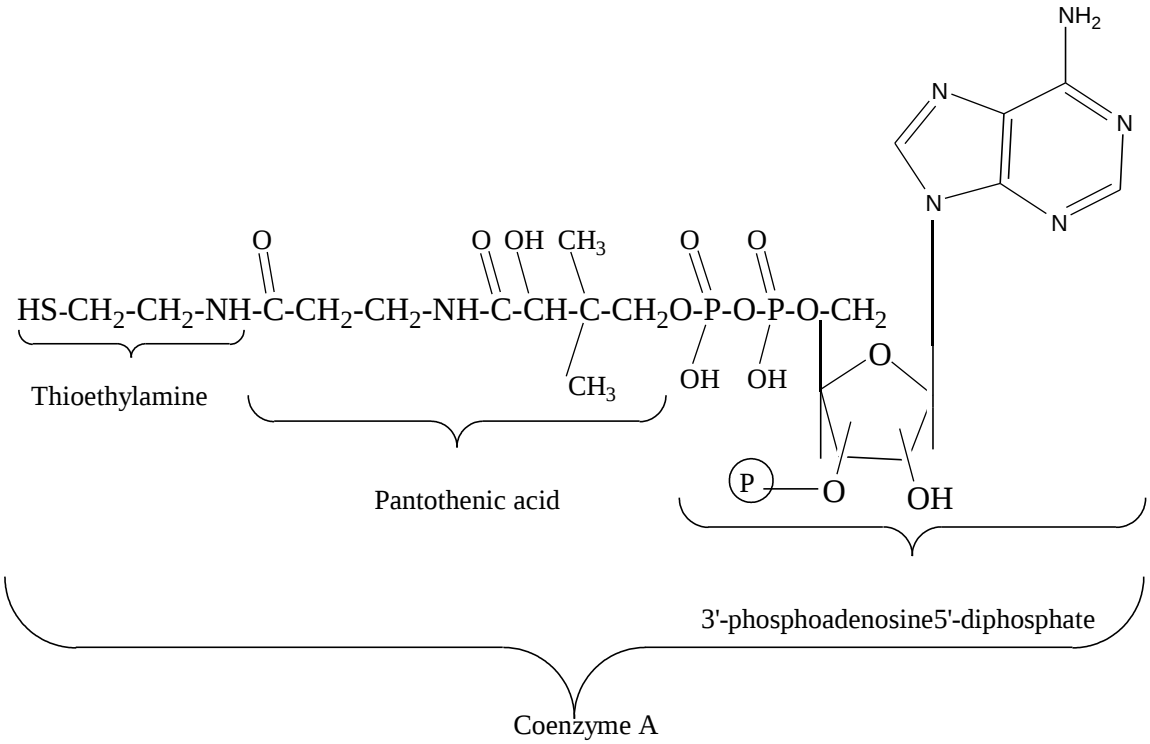
Biological role. It is part of coenzyme A (the coenzyme of acylation). CoA activates and transports acyl residues. Acyl CoA once formed elicits the activation of carboxylic acid to a higher energy level thereby providing its thermodynamically advantageous use in endergonic reactions.

The almost importance of CoA in the metabolism is evidenced by direct participation of CoA in major chemical processes – oxidation and biosynthesis of higher fatty acids, oxidative decarboxylation of α-ketoacids (pyruvate and α-ketoglutarate), biosynthesis of neutral fats, phosholipids, steroid hormones, hemoglobin, acetylcholine, hippuric acid and others.



Pantothenic acid

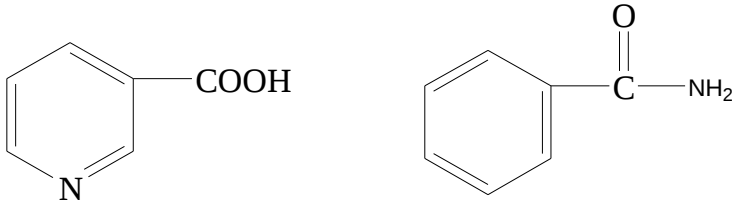
Hypovitaminosis. In humans and animals, deficiency or lack of pantothenic acid leads to dermatitis, lesions of mucous membranes, dystrophic alterations of endocrine glands (in particular, adrenal glands) and renal alterations, hair depigmentation, growth retardation, loss of appetite, general physical wasting, alopecia.



Occurrence in nature and daily requirement. It is widely distributed. The major dietary sources of pantothenic acid for humans are liver, yolk, yeast and green vegetables. In humans intestine little amount of pantothenic acid is synthesized by microflora. For the adult human, the daily requirement in pantothenic acid is **5-10 mg.**

Vitamin PP (Nicotinic Acid, Nicotinamide, Niacin, Antipellagra Factor).

Vitamin PP has also been given the name pellagra preventive factor hence its notation PP from the initials of “pellagra preventive”.



Nicotinic acid Nicotinamide

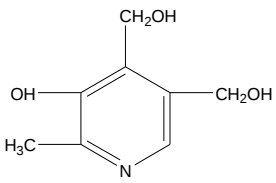
Biological role. Vitamin PP makes part of NAD and NADP, which are coenzymes for a large number of dehydrogenases involved in reversible oxidation – reduction reactions. In the course of biological oxidation NAD and NADP perform the function of intermediate carriers of protons and electrons between the substrate to be oxidized and the flavin enzymes.

Hypovitaminosis. Deficiency of this vitamin leads to a disease called pellagra. The most characteristic signs of avitaminosis PP, that is, of pellagra (from the Italian pell, skin + agro, rough) are inflammated skin (dermatitis), affected gastrointestinal tract (diarrhea), and psychic disturbances (dementia).

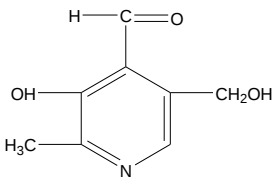
Pellagral dermatitides are most common by symmetric and occur on the portions of the body exposed to direct sunlight: hands, neck, and face. The affected skin turns red, then brown and rough to touch. The symptoms of affected intestine are anorexia (loss of appetite), nausea, abdominal pains, and diarrhea, the latter pathologic state leads to dehydration of the organism. The mucous membrane of the large intestine initially inflames and then ulcerates. Specific for pellagra are stomatitis, gingivitis, swelled tongue, and cracked glossal mucus. Other symptoms, including mental ones, are headache, vertigo, irritability, depression as well as psychoses, psychoneuroses, hallucinations, and other aberrations. The pellagra symptoms are particularly pronounced in patients with nutritional protein deficiencies. This is due to the deficiency in tryptophan, which is a precursor to nicotinamide, in part synthesized in the human and animal tissues.

Occurrence in nature and daily requirement. For humans, the main sources of nicotinic acid and nicotinamide are rice, bread, potato, meat, liver, kidney and carrot. The recommended daily intake for the adult human is **15-25 mg**

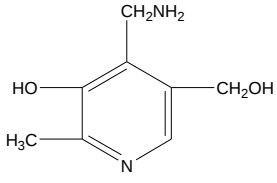
Vitamin B_6 (Pyridoxine, Antidermatic Vitamin)



Pyridoxin



Pyridoxal



Pyridoxamine

Biological role

Coenzyme forms are pyridoxal phosphate and pyridoxamine phosphate. The synthesis of pyridoxal phosphate is catalyzed by pyridoxal kinase, which is the most active in the brain tissue. Interconversions of pyridoxal phosphate and pyridoxamine phosphate have been shown to occur in animal tissues.

It should be noted, the studies by A.E. Braunstein, S.R. Mardashev, E. Snell, D. Metzler, A. Meister and other scientists have contributed substantially to the elucidation of the biological role of vitamin B_6 and pyridoxal phosphate in the nitrogen metabolism. To date, over 20 pyridoxal enzymes are known that catalyze the key reactions of nitrogen metabolism in all living organisms. For example, pyridoxal phosphate has been shown to act as a prosthetic group for the *aminotransferases*, which catalyze the reversible transfer of amino group from amino acid to α-ketoacid and for the *amino acid decarboxylases* involved in an irreversible cleavage of CO_2 from the amino acid carboxyl group with the resultant production of biogenic amines.

Besides, pyridoxal phosphate participates in enzymatic reactions of nonoxidative deamination of serine and threonine, oxidation of tryptophan and kynurenine, conversion of sulphur containing amino acids, interconversions of serine and glycine as well as in the synthesis of δ-aminolevulenic acid, a precursor of the heme molecule of hemoglobin, and other processes.

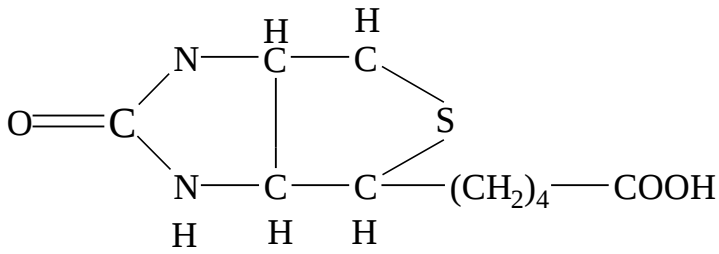
Hypovitaminosis. Deficiency of vitamin B_6 has been studied on rats; the characteristic symptom is acrodynia, or specific dermatitis with the prevalent skin lesions of the paws, the tips of ears and nose and the tail. Other signs are marked skin desquamation, loss of hair, with the progressive development of the gangrene of extremities.

In humans, vitamin B_6 deficiency is not a very specific condition, and occasional pellagra-like dermatitis, resistant to nicotinic acid treatment, is easily curable by administering pyridoxine. Dermatitis and a lesion of nervous system (also manifested by epilepform fits), caused by pyridoxine deficiency in the artificial food, have been described in infants. Pyridoxin insufficiency has been often recorded in tuberculous patients. In treating this disease, the patients are prescribed *isoniazid*, which has been found to be antagonistic to vitamin B_6.

Occurrence in nature and daily requirement. For humans, the major sources of vitamin B_6 are bread, pea, haricot beans, potato, meat, kidney and liver.

Pyridoxine is synthesized by the intestinal microflora. Recommended dose of vitamin B_6 for an adult human is about **2-3 mg** per day.

Biotin (Vitamin H, Antiseborrhea Vitamin)



The biotin molecule is a cyclic urea derivative carrying a valeric acid residue in its side chain.

Biological role. The currently known biotin enzymes act as catalysts for reactions of two types:

1. Carboxylation reactions involving the degradation of ATP, for example the reaction of the malonyl – CoA synthesis from acetyl-CoA, and the reaction of the oxaloacetate synthesis from pyruvate.
2. Transcarboxylation reactions (proceeding without involvement of ATP), in which the substrates exchange a carboxyl group.

It the organism carboxylation and transcarboxylation reactions play a crucial role in the synthesis of higher fatty acids, proteins, purine nucleotides, and, accordingly, nucleic acids.

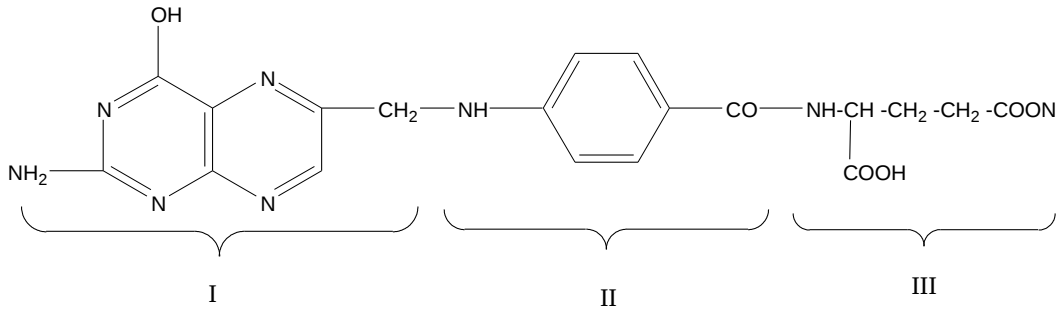
Hypovitaminosis. Clinical signs of biotin deficiency in humans have been little studied. This is explained by the fact that intestinal bacteria are capable to synthesize biotin in sufficient amounts.

The human deficiency of biotin is manifested by inflammation of the skin (dermatitis), attended by hypersecretory activity of sebaceous glands, onychosis; frequent symptoms are also muscle pain, fatigue, somnolence, depression, also anorexia and anemia.

Occurrence in nature and daily requirement. Liver, kidney, milk, and egg yolk are rich in this vitamin. In vegetable products (potato, onion, tomato and spinach), biotin occurs both in a free – and in bound state. For humans and

animals, an important supply is the biotin synthesized by intestinal microflora. The daily biotin requirement for the adult human is about 0,25 mg.

Folic Acid (B_c, B_9, Antianemic Vitamin, Pteroylglutamic Acid)



Folic (pteroylglutamic) acid molecule is made up of three structural units: pteridine residue (I), paraaminobenzoic acid residue (II) and L-glutamic acid (III).

Biological role. Coenzyme form of folic acid is tetrahydrofolic acid, that is, reduced pteridine derivative of the vitamin. The reduction is affected via rupture of two double bonds and the attachment of four nitrogen atoms at positions 5,6,7 and 8 to produce tetrahydrofolic acid (FH_4).

Coenzyme functions of FH_4 are directly related to the transport of active one carbon units, namely, formyl **(-CHO)**, methyl **(-CH_3),** methylene **(-CH_2-),** methenyl **(-CH=),** hydroxymethyl **(-CH_2OH),** and formimine **(-CH=NH)** groups. Primary sources of these units in the human organisms are the β-carbon of serine, α-carbon of glycine, the carbon of methyl groups in methionine and choline, the carbon atom at position 2 of tryptophan indole ring, the carbon at position 2 of histidine imidazole ring.

FH_4 derivatives take part in the transport of one-carbon units in **the biosynthesis of methionine and thymine** (transfer of formyl group), serine (transfer of hydroximethyl group), formation of purine nucleotides (transfer of formyl group). Therefore the folic acid deficiency leads to disturbances of the biosynthesis of proteins and nucleic acids.

Antivitamins of folic acid (for example methotrexate) are used as antitumor drugs.

A deficiency of folic acid is difficult to elicit even in experimental animals without the preliminary complete inhibition of microbial growth in the intestine, where it is synthesized in the necessary amounts.

In humans macrocytic anemia is observed, in its clinical picture being quite similar to the pernicious anemia elicited by vitamin B_{12} deficiency. Occasionally, diarrhea is observed. There has been provided evidence that folic acid deficiency impairs DNA biosynthesis in the marrow cells which are normally responsible for erythropoesis. As a consequence, immature cells-megaloblasts with a decreased DNA content arise in the peripheral blood.

Occurrence in nature and daily requirement. Folic acid is abundant in green plant leaves and yeast, and is also found in liver, kidney, meat and other products.

In animals and humans, many of the intestinal microorganisms are capable of synthesizing folic acid in the amounts that are sufficient for the organism's requirement in this vitamin. The daily dose of free folic acid for the adult human is **200-400µg**.

Vitamin B_{12} (Cobalamin, Antianemic Vitamin)

Vitamin B_{12} is the only vitamin whose molecule contains a metal atom. In the vitamin B_{12} molecule, the central cobalt atom is ligated to the nitrogen atom of four reduced pyrrole rings (interlinked to form a porphyrin – like corrin ring system) and to the nitrogen atom of 5,6 –dimethylbenzimidazole. The central cobalt – containing portion of the vitamin molecule has a planar configuration; perpendicular to its plane, a nucleotide ligand is positioned, composed of 5,6-dimethylimidazole, a ribose moiety, and a phosphate residue at carbon 3 position. The whole entity has been named cobalamin.

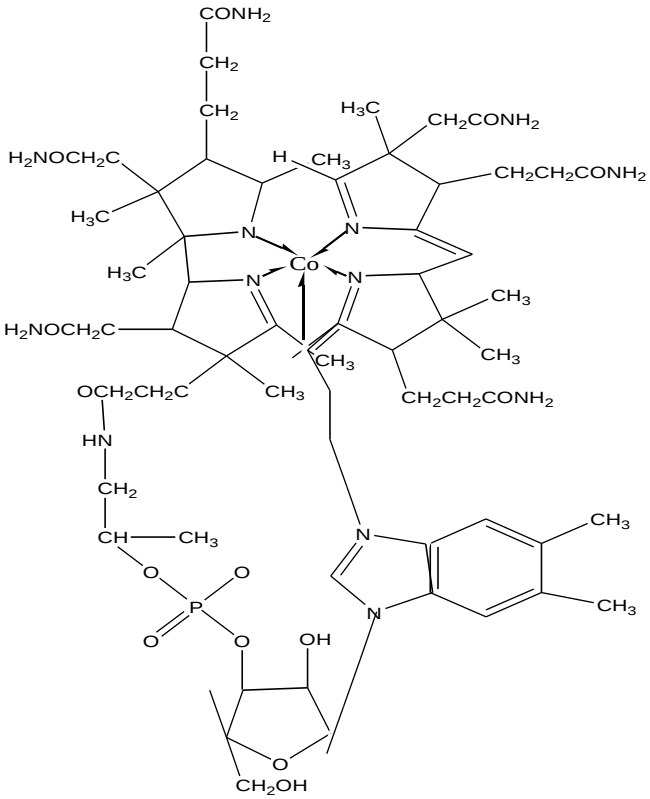
Biological role. Coenzyme forms of vitamin B_{12} are methylcobalamin (ligand – methyl group) and deoxyadenosylcobalamin (ligand – 5'- deoxyadenosyl group).

Methylcobalamin is coenzyme of homocysteine methyl transferase which forms methionine by means of transfer of methyl group from N^5-methyl – FH_4 for homocysteine. Methylcobalamin participates in conversion of folic acid

derivatives, which are necessary for synthesis of nucleotides – precursors of DNA and RNA.

5-Deoxyadenosylcobalamin is coenzyme of methyl-malonyl-CoA-mutase which catalyzes a reaction of conversion of methyl-malonyl-CoA to succinyl-CoA. Therefore *5-deoxyadenosylcobalamin* participates in metabolism of odd-numbered fatty acids and amino acids with branched- chain.

Structure:



Vitamin B_{12} deficiency. As has been found, a necessary condition for the active intestinal uptake of vitamin B_{12} is the occurrence in the gastric juice of a special protein, gastromucoprotein (*transcorrin*), commonly referred to as the *intrinsic Castle's factor*. The function of this factor is to bind specifically with vitamin B_{12} to form a complex. It is only in this transcorrin-bound form that vitamin B_{12} can be absorbed in the intestine. Therefore, an impaired synthesis of the intrinsic factor in the intestinal mucosa leads to avitaminosis $B_{12,}$ even when the dietary supply of cobalamin is normal.

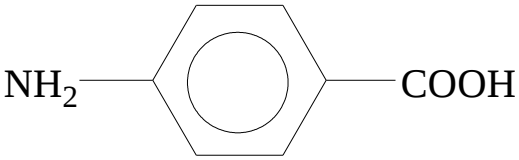
In humans and animals, vitamin B_{12} deficiency leads to pernicious macrocytic megaloblastic anemia. Alongside the impaired hematopoietic function,

specific of avitaminosis B_{12} is also disordered activity of the nervous system. Vitamin B_{12} is used in the clinic for the treatment of both pernicious anemia and its forms - megaloblastic with neurological complications, usually not curable by other vitamins in particular, folic acid.

Occurrence in nature and daily requirement. Vitamin B_{12} is synthesized exceptionally by microorganisms. Both animal and vegetal tissues lack this capacity. For humans, the major sources of vitamin B_{12} are meat, beef liver, kidney, fish, milk and eggs. It is synthesized by intestinal microflora. The adult human requires 2-5 μg (micrograms) of vitamin B_{12} per day.

6.4. Vitamin – like Substances (Vitaminoids)

Paraaminobenzoic acid

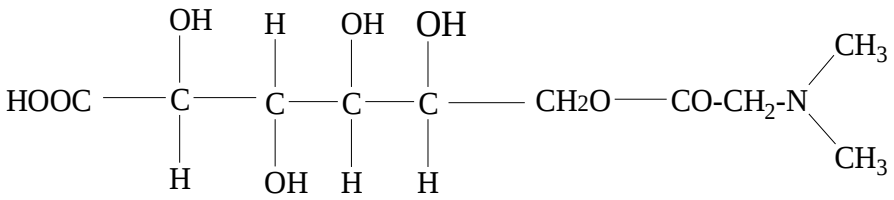


Paraaminobenzoic acid is part of folic acid. It has been reported that paraaminobenzoic acid is essential for the normal process of pigmentation of hair, feather, and skin. This vitaminoid has been shown to be capable of producing a stimulating action on tyrosinase, the key enzyme in the biosynthesis of melanins, which impart normal color to the skin.

Antivitamin. Sulfanilamides are antivitamins of paraaminobenzoic acid. Sulfanilamides can act as its competitive replacers in the systems of microorganisms and check thereby their growth and multiplication.

Sources: liver, kidney, meat, yeast and, to a lesser extent, milk, hen's eggs, potato, bread, spinach, and carrot.

Vitamin B_{15} (pangamic acid)



Pangamic acid

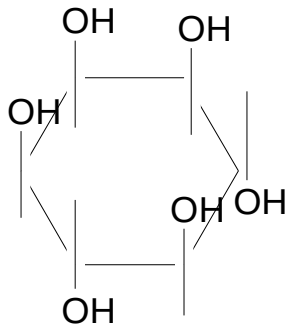
Pangamic acid is an ester of gluconic acid and dimethylglycine.

The biological role of vitamin B_{15} has been little studied. There have been reports on its involvement as a source of methyl groups in the biosynthesis of choline, methionine and creatine.

Pangamic acid preparations are effective in the treatment of fatty degeneration of the liver and certain forms of oxygen deficiency.

Sources: liver, plant seeds, and yeast.

Inositol

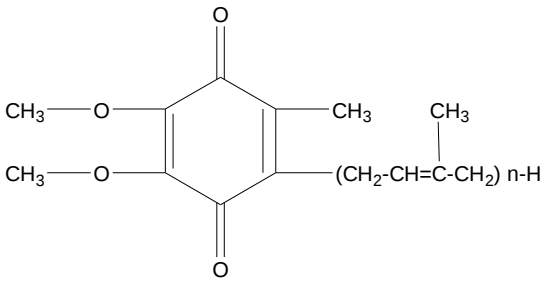


Inositol is found in the cerebral lipids. The biological role of inositol is presumably associated with phosphoglyceride metabolism. This explains the lipotropic effect of inositol.

The dietary lack of inositol leads along with growth retardation, to a specific loss of hair and to fatty infiltration of the liver with cholesterol deposition.

Sources*:* liver, meat, milk, coarse bread, fruits, and vegetables.

Coenzyme Q (Ubiquinone)

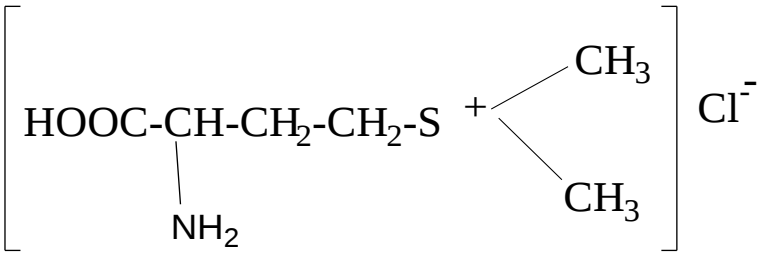


2,3-methoxy-5-methyl-1,4-benzoquinone with an isoprene chain at position 6.

Ubiquinone, as found in mitochondria of human and animal cells, contains 10 isoprene units. Coenzyme Q is a necessary component of the mitochondrial respiratory chain, engaged in the transport of electrons from membrane

dehydrogenases to cytochromes. It the human organism, CoQ can be synthesized from mevalonic acid and from metabolized product of phenylalanine and tyrosine, however, in certain pathologic states, CoQ becomes an essential factor. For example, in children confined to protein-deficient diet, anemia develops defying the known curative means of vitamin therapy (cobalamin, folic acid and others). In such cases, CoQ-based preparations produce a good result. CoQ has also proved to be an effective means in treating muscular dystrophy (its genetic form included) and cardiac insufficiency.

Vitamin U (S-methylmethionine, Ulcer-preventive Factor).



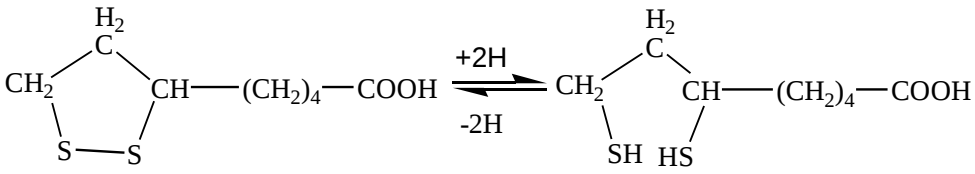
methylmethionine - sulphonium chloride

Vitamin U is known to meet completely the requirement in methionine (an essential al amino acid) in rats; its involvement in the synthesis of methionine, choline, and creatine has also been established.

It is used for treatment of peptic ulcer.

Sources: fresh cabbage, parsley, turnip, carrot, onion, pepper, green tea, bananas, fruits and fresh milk.

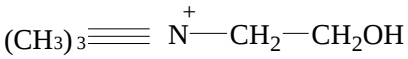
Lipoic acid



Lipoic acid as a component of multienzyme system plays a crucial role in the oxidation and transport of acyl groups. Its chief function is an immediate involvement in the oxidative decarboxylation of α-ketoacids (pyruvic and α-ketoglutaric acids) in the tissues. Lipoic acid serves as a prosthetic group, along

with thiamine pyrophosphate and CoA, for pyruvate – and ketoglutarate – dehydrorgenase multienzyme systems.

Choline



Choline was shown to be a structural component of a more complex organic phosphorus-containing compound, phosphatidylcoline, or lecithin. Choline in the human animal organisms is synthesized in sufficient amounts and cannot be therefore regarded as a food factor. However under certain conditions, for example, in dietary protein deficiency, the secondary choline deficiency may develop in the organism. In the synthesis of choline, the donors of methyl groups are methionine, or serine and glycine. Therefore in deficiency of proteins (which may be due to a deficiency of proteins - alimentary or endogenous), the symptoms of choline deficiency often manifest themselves, i.e. fatty infiltration of the liver, hemorrhagic dystrophy of the kidney, impaired blood coagulation (due to a disordered synthesis of coagulation factor V, proaccelerin). Choline is primarily a constituent of the biologically active acetylcholine, it participates in the biosynthesis of methionine, purine and pyrimidine nucleotides, phospholipides and other compounds.

Tests for Self-control

1. Complex of vitamins with vitamin E is recommended to old persons. Which is the main function of this vitamin?

A. Antiscorbutic
B. Antihemorrhagic
C. Antioxidant
D. Antineuritic
E. Antidermatic

2. Which hypovitaminosis is manifested by disturbance of reproductive function of organism and muscular dystrophy?

A. B_1
B. A
C. K
D. D
E. E

3. Vicasol (synthetic analogue of vitamin K) is recommended to 6-years old boy to prevent the post-operational bleeding. Which posttranslational changes of blood clotting factors are activated by vicasol?

A. Carboxylation of glutamic acid
B. Phosphorylation of serine radicals
C. Limited proteolysis
D. Polymerization
E. Glycosylation

4. Patient has dermatitis, diarrhea and dementia. Which vitamin lack is the cause of this state?

A. Folic acid
B. Ascorbic acid
C. Nicotinamide
D. Biotin
E. Rutin

5. Dermatitis appears in a patient after eating raw eggs. What avitaminosis is developed?

A. Folic acid
B. Biotin
C. Pantothenic acid
D. Paraaminobenzoic acid
E. Inositol

6. Concentration of pyruvate is increased in a patient's blood. Its large amount is excreted with urine. Which avitaminosis is observed in the patient?

A. Avitaminosis B_2
B. Avitaminosis E
C. Avitaminosis B_3
D. Avitaminosis B_6
E. Avitaminosis B_1

7. Pernicious hyperchromatic anemia (Birmer's disease) appears as a result of deficiency of vitamin B_{12}. Which bioelement is included into this vitamin?

A. Iron
B. Molibdenum
C. Zink
D. Cobalt
E. Magnesium

8. After resection of 2/3 stomach, a quantity of erythrocytes in blood is decreased, their volume is increased, hemoglobin level is decreased. Which vitamin deficiency results in such changes in blood?

A. PP
B. C
C. P
D. B_6
E. B_{12}

9. The structural analogue of vitamin B_2 acrichin is prescribed to patients with enterobiosis. What enzyme synthesis is disturbed by this substance?

A. Cytochrome oxydases
B. FAD-dependent dehydrogenases
C. Peptidases
D. NAD-dependent dehydrogenases
E. Aminotransferases

10. Which vitamin is synthesized in human's organism from tryptophan?

A. Nicotinic acid
B. Riboflavin
C. Pantothenic acid
D. Vicasol
E. Tocopherol

11. Which vitamin is the component of coenzyme, which participates in reactions of transamination and decarboxylation of amino acids?

A. Ubiquinone
B. Vitamin B_6
C. Vitamin PP
D. Vitamin B_2
E. Vitamin P

12. Patient complains of general muscular weakness, pains in heart. Objectively: inflammations of the tongue and lips, keratitis, vascularization of the cornea of the eye. Which vitamin hypo- or hypervitaminosis is the cause of this state?

A. Hypervitaminosis of vitamin A
B. Hypovitaminosis of vitamin A
C. Hypervitaminosis of vitamin B_2
D. Hypovitaminosis of vitamin B_2
E. Hypovitaminosis of vitamin C

13. Chemical nature of intrinsic factor of vitamin B_{12}:

A. Lipid

B. Mucoprotein

C. Simple protein

D. Polypeptide

E. Nucleoprotein

14. The decrease of acidity of gastric juice and disordered activity of nervous system were found in examination of patient. Macrocytic anemia, presence of big erythrocytes (megalocytes) were observed in blood. Which vitamin hypo- or hypervitaminosis may be believed?

A. Hypovitaminosis of vitamin PP

B. Hypervitaminosis of vitamin PP

C. Hypervitaminosis of vitamin B_6

D. Hypovitaminosis of vitamin B_6

E. Hypovitaminosis of vitamin B_{12}

Chapter 7 THE PRINCIPLES OF BIOENERGETICS. BIOLOGICAL OXIDATION. THE CITRIC ACID CYCLE

Bioenergetics, or biochemical thermodynamics is the study of the energy changes accompanying biochemical reactions.

Biological system confirm to the general laws of thermodynamics.

The first law of thermodynamics states that the total energy of a system, including the surrounding, remains constant. This is the law of energy conservation.

The second law of thermodynamics states that the total entropy of a system must increase if a process is to occur spontaneously. Entropy (S) is a measure of the system disorder. Entropy becomes maximal in a system as it approaches true equilibrium.

Under condition of constant temperature and pressure, the relationship between the free energy change (ΔG) of a reacting system and the change in entropy (ΔS) is given by the following formula, which combines the two laws of thermodynamics:

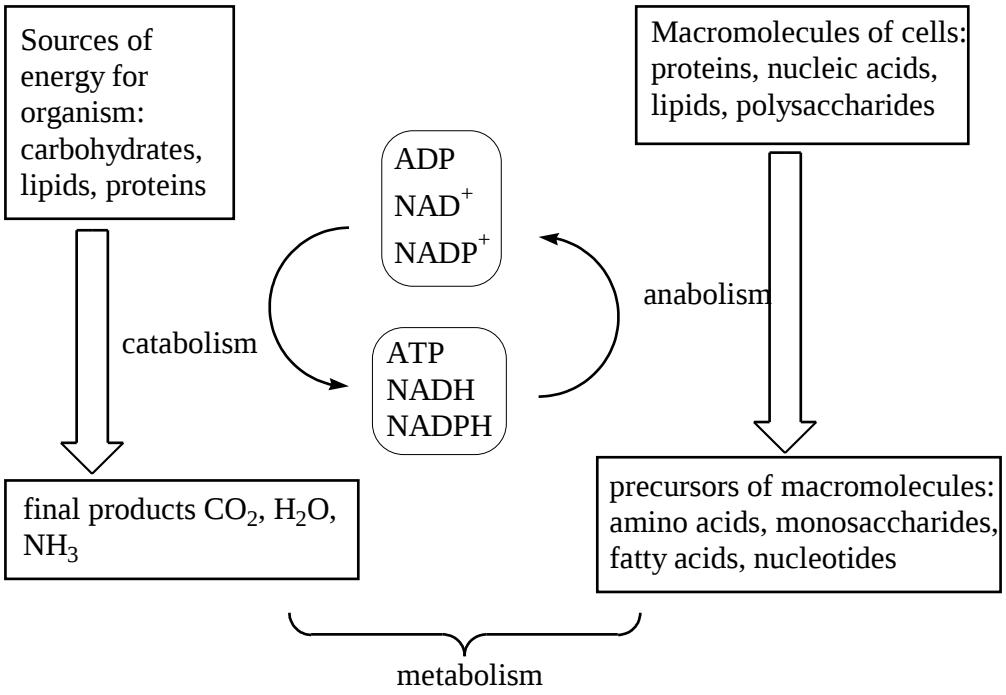
ΔG = ΔH – TΔS,

where ΔH is the change of enthalpy (enthalpy – heat content); ΔG – free energy change. The free energy is a portion of the system's energy that can be used to perform work at constant temperature and pressure. T is absolute temperature.

If ΔG is negative in sign, the reaction proceeds spontaneously, with loss of free energy; i e, it is exergonic.

If ΔG is positive, the reaction proceeds only if free energy can be gained; it is endergonic.

Exergonic reactions (reactions of break down and oxidation) are called catabolic ones. Reactions of synthesis are called anabolic. Sum total of catabolic and anabolic reactions is called metabolism.



The vital processes (e.g.: synthetic reactions, muscular contraction, nerve impulse induction, active transport) obtain energy from oxidative reactions.

Basic components of food – carbohydrates, lipids, proteins – serve sources of the energy .

The sum total of oxidative reactions in organism is called biological oxidation.

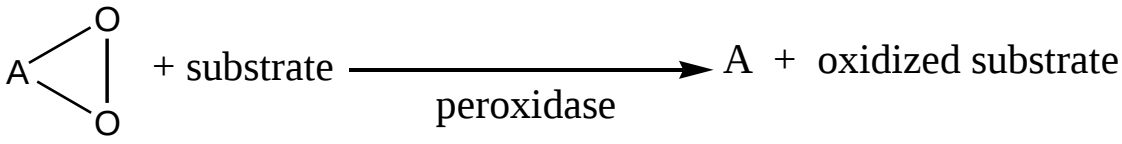
7.1 The Development of Conceptions about Biological Oxidation.

The pioneering studies of oxidation processes in the organism were initiated in the 18th century by A. Lavoizier. He believed that combustion and slow oxidation of nutrients in the organism were essentially identical processes. But biological oxidation proceeded at low temperature (body temperature), with no flame visible, and, finally, in the presence of water content of which in tissues was as high as 65% of the total mass.

That’s why the following theories of the biological oxidation have been linked with consideration of specific oxygen “activation” in the organism cells.

One of the earlier theories of biological oxidation, based on the oxygen activation, was that developed by A.N.Bach. He believed that the activation of molecular oxygen was effected by the rupture of one of its two bonds and subsequent addition of oxygen to oxygenase (A), capable to autooxidation.

The high-molecular peroxides donate the activated oxygen to the substrate.



The hypothesis was named the peroxide hypothesis.

Positive moment of this hypothesis This hypothesis demonstrates that the process of biological oxidation is polyenzymatic one. *Negative moment*. This hypothesis has not explained the process of oxidation in anaerobic organisms.

The next theory of biological oxidation is the theory of V.Palladin. V.Palladin demonstrated the process of biological oxidation on the glucose example.

Oxidation consists of 2 stages:

anaerobic: $C_6H_{12}O_6 + 6H_2O + 12R \rightarrow 6CO_2 + 12RH_2$

and aerobic: $12RH_2 + 6O_2 \rightarrow 2R + 12H_2O$.

R are intermediate hydrogen carriers. The initial stage of tissue respiration leads to formation of carbon dioxide (CO_2) and requires no participation of oxygen.

The oxygen of CO_2 is the oxygen of water or of substrate.

Specific enzymes (dehydrogenases) catalyze the cleavage of hydrogen from the substrate to be oxidized. Heat-resistant agents of dehydrogenases (named chromogens by Palladin) take up hydrogen ions from substrate and are converted into reduced forms. According to modern conceptions, acceptors of hydrogen atoms are coenzymes NAD, NADP, FMN, FAD.

The atmospheric oxygen plays a role of final hydrogen acceptor.

This theory could explain the process of oxidation both in aerobic and in anaerobic organisms.

Under anaerobic conditions another metabolites act as final acceptors of hydrogen.

7.2 Modern Conceptions about Biological Oxidation

Biological oxidation is effected gradually via numerous intermediary enzymatic stages, with a multiple transfer of protons and electrons (or electrons only) from one compound (donor) onto another (acceptor).

Chemically, oxidation is defined as the removal of electrons and reduction of the gain of electrons.

Electrons may be removed:

- by dehydrogenation;
- by electron transfer;
- by incorporation of oxygen.

The ability of reactants to donate or accept electrons is characterized by oxidation-reductive potential (or redox potential) – E_o.

It is usual to compare the redox potential of a system (E_o) against the potential of hydrogen electrode which at pH 0 is designated as 0,0 volts. However, for biological systems, it is normal to express the redox potential (E_o`) at pH 7,0, at which potential of the hydrogen electrode is -0,42 volts.

System	E_o volts	
H^+/H_2	-0,42	
$NAD^+/NADH$	-0,32	Δ 0,27
$FAD^+/FADH_2$	-0,05	
$CoQ/CoQH_2$	+0,04	Δ 0,21
cyt b Fe^{3+}/Fe^{2+}	+0,07	
cyt c_1 Fe^{3+}/Fe^{2+}	+0,23	
cyt c Fe^{3+}/Fe^{2+}	+0,25	
a	+0,29	Δ 0,53
a_3	+0,55	
O_2/H_2O	+0,82	

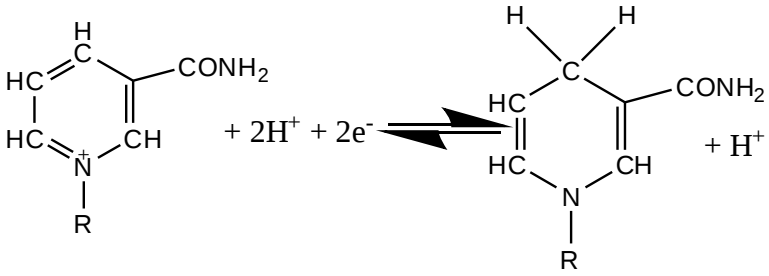
$\Delta E_{ATP} = 0,15 – 0,22$

Enzymes involved in oxidation and reduction are designated oxidoreductases. They are classified into:

1) *Oxidases* catalyze the removal of hydrogen from a substrate using oxygen as a hydrogen acceptor. They usually form hydrogen peroxide as a reaction product. Oxidases are flavoproteins. Examples: amino acid oxidases, xanthine oxidase, aldehyde dehydrogenase.

2) *Dehydrogenases* catalyze the removal of hydrogen from a substrate and cannot use oxygen as a hydrogen acceptor. They are divided into:

a) Pyridine-dependent dehydrogenases. They contain NAD or NADP as coenzymes. The capacity of NAD^+ and $NADP^+$ to play the role of an intermediate hydrogen carrier is associated with the occurrence of nicotinamide moiety in their structures.

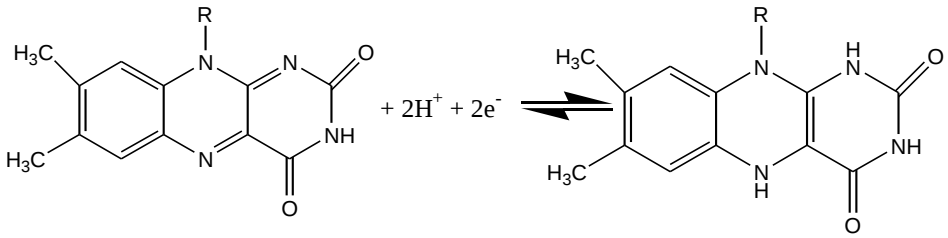


One of two reduction equivalents is inserted into the reduced coenzyme as hydrogen, and the other one, as an electron. The free ion H^+ remains in the medium.

In the cells, the NAD-dependent dehydrogenases are mostly involved in the transport of electrons and protons from organic substrates to other intermediate carriers and finally to oxygen.

In turn, the NADP-dependent dehydrogenases play a decisive role in biosynthetic reactions (for example, in the biosynthesis of higher fatty acids, sterols and other compounds).

b) Flavin-dependent dehydrogenases. FMN or FAD are coenzymes of these enzymes.



They are divided into:

- *Primary dehydrogenases*. They transfer reducing equivalents directly from the substrate to respiratory chain. Examples: succinate dehydrogenase, acyl-CoA dehydrogenase, mitochondrial glycerol-3-phosphate dehydrogenase.
- *Secondary dehydrogenases,* for example NADH-dehydrogenase, which is carrier of electrons and protons between NADH and CoQ.

3) *Cytochromes* participate in the transfer only electrons. They are iron-containing hemoproteins in which the iron atom oscillates between Fe^{3+} and Fe^{2+} during oxidation and reduction.

$$Cyt(Fe^{2+}) \underset{+\bar{e}}{\overset{-\bar{e}}{\rightleftarrows}} Cyt(Fe^{3+})$$

Cytochromes occur in the mitochondrial respiratory chain, e.g., cytochromes b, c_1, c, aa_3. Besides respiratory chain cytochromes are found in other location, e.g., the endoplasmic reticulum (cytochromes P450 and b_5).

4) *Hydroperoxidases* use hydrogen peroxide or organic peroxides as substrate. Two types of enzymes belong to this category: peroxidases and catalase. Hydroperoxidases protect the body against harmful peroxides.

a) Peroxidases. In the reactions catalyzed by peroxidases hydrogen peroxide is reduced at the expense of several substrates that will act as electron donors such as ascorbate, quinones, glutathione etc.

Overall reaction is as follows:

$$H_2O_2 + AH_2 \xrightarrow{\text{peroxidase}} 2H_2O + A$$

Example: glutathione peroxidase.

b) Catalase. Catalase is hemoprotein. It uses one molecule of H_2O_2 as electron donor and another one as electron acceptor.

$$2H_2O_2 \xrightarrow{\text{catalase}} 2H_2O + O_2$$

5) *Oxygenases*. Enzymes of this group catalyze the incorporation of oxygen into a substrate molecule. Oxygenases may be divided into 2 subgroups:

a) Dioxygenases incorporate both atoms of molecular oxygen into the substrate. The basic reaction is shown below:

$$A + O_2 \rightarrow AO_2$$

Examples:

- homogentisate dioxygenase
- 3-hydroxyantranilate dioxygenase
- L-tryptophan dioxygenase.

b) Monooxygenases (hydroxylases) incorporate only one atom of molecular oxygen into the substrate. The other oxygen atom is reduced to water, and additional electron donor or cosubstrate is necessary for this purpose.

$A\text{-}H + O_2 + ZH_2 \rightarrow AOH + H_2O + Z$

substrate cosubstrate

Example: microsomal cytochrome P450 monooxygenase.

Types of biological oxidation: mitochondrial, microsomal, free radical oxidation.

	Mitochondrial oxidation	**Microsomal oxidation**
Location	In mitochondria	In endoplasmic reticulum
Oxidation of substrate	is performed by dehydrogenation	is performed by incorporation of oxygen into substrate molecules
Role of O_2	Oxygen acts as final electron acceptor	Activated oxygen is directly incorporated into the substrate
Enzymes	Dehydrogenases, cyt. b, c_1, c, aa_3	Oxygenases, cyt. b_5, P450
Functions- -	Energy function - Thermoregulation	Plastic function – the synthesis of substrates needed for organism - Detoxification – oxidation of xenobiotics, endogenous toxins.
	80% of O_2	20% of O_2

In mitochondrial oxidation part of energy is accumulated as high-energy phosphates. Macroergic (energy-rich) bonds are those which, when are subjected to hydrolysis under standard conditions, produce within the system a change in free energy greater than 21kJ/mol (or 5kcal/mol).

Examples of substances, which have energy-rich bonds:

Phosphoenolpyruvate - 61,7 kJ

1,3-bisphosphoglycerate - 49,2 kJ

Creatine phosphate - 42,5 kJ

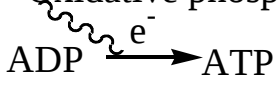
ATP → ADP + P_i – 30,4 kJ

Pyrophosphate – 28,3 kJ etc.

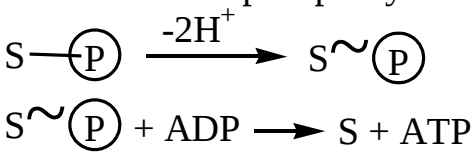
ATP plays major role in energy metabolism:

1. ATP is very thermodynamically instable molecule. It is used for about 1 minute after synthesis. Therefore it serves as universal energy carrier in cells.
2. In the energy scale ATP takes a midway position, and therefore ATP may be formed both oxidative and substrate level phosphorylation.

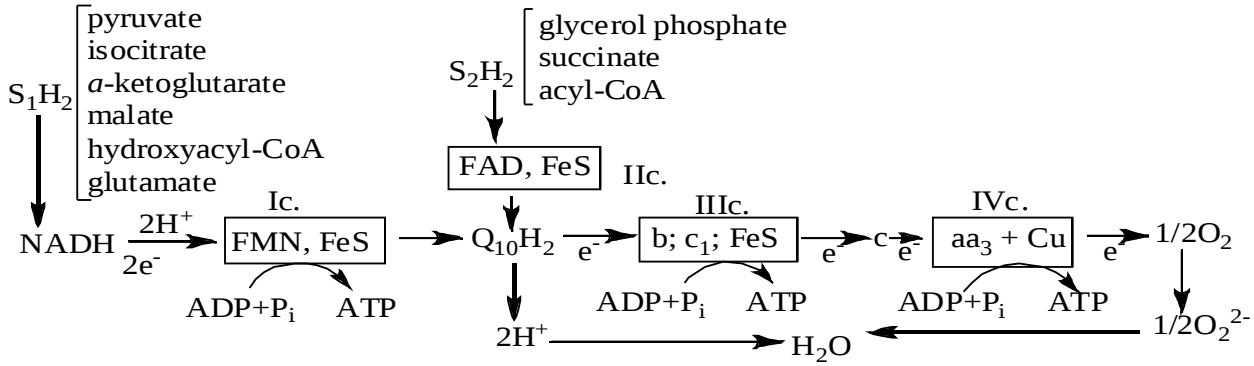
Qxidative phosphorylation



Substrate level phosphorylation



Mitochondrial Respiratory Chain



I complex: NADH, CoQ-oxidoreductase consists of NADH-dehydrogenase (coenzyme FMN) and FeS-containing proteins.

II complex: depending on substrate: { succinate dehydrogenase; acyl-CoA dehydrogenase; glycerol phosphate dehydrogenase

III complex: CoQ, cytochrome c-oxidoreductase.

IV complex: cytochrome oxidase.

The components of respiratory chain are present in the *inner mitochondrial membrane* as four protein-lipid respiratory chain complexes that cross the membrane. Cytochrome c is the only soluble cytochrome and, together with Q,

seems to be a more mobile component of the respiratory chain connecting the fixed complexes.

Components of respiratory chain are distributed depending on increasing redox potential.

The transfer of electrons from one component of respiratory chain to another is accompanied by diminishing free energy:

$NADH + H^+ + 1/2O_2 \rightarrow NAD^+ + H_2O + 220$ kJ (52,7 kcal)

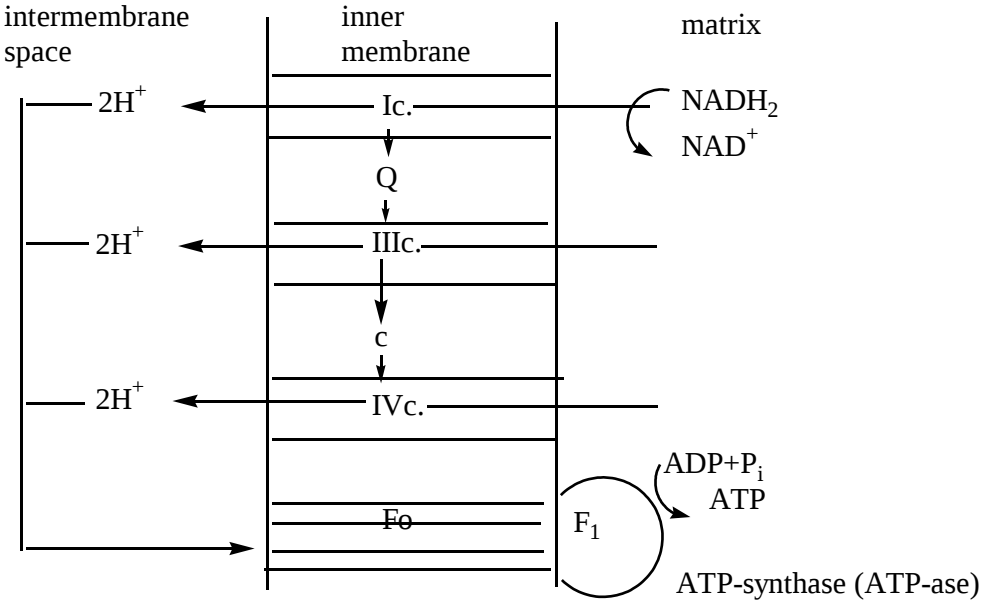
Part of this energy (~40%) is accumulated as macroergic bonds of ATP. Respiratory chain has three points of coupling oxidation and phosphorylation.

Mechanism of Oxidative Phosphorylation

The chemiosmotic theory was proposed by Mitchell in 1961 – 1966. Mitchel's chemiosmotic theory postulates that the energy from oxidation of components in the respiratory chain generates the hydrogen potential.

The electrochemical potential difference resulting from the asymmetric distribution of the hydrogen ions is used to drive the mechanism responsible for the formation of ATP.

Each of the respiratory chain complexes I, II and IV act as proton pumps.



The inner membrane is impermeable to ions in general and particularly to protons which are accumulated outside the membrane, creating an electrochemical potential difference across the membrane ($\Delta\mu H^+$).

This consists of a chemical potential (difference of pH) and an electrical potential.

The electrochemical potential difference is used to drive a membrane-located ATP-synthase which in the presence of P_i + ADP forms ATP. Ratio between formation of ATP and consumption of $1/2O_2$ is called coefficient of phosphorylation:

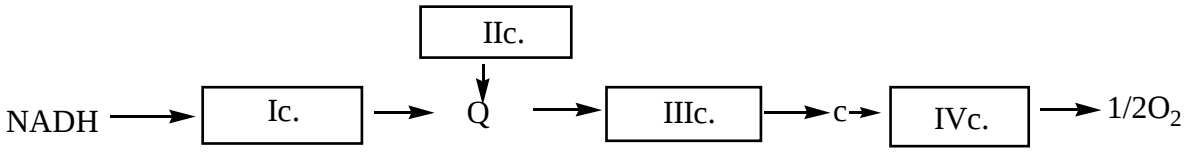
P/O = 3 (from NADH + H^+ to O_2)

P/O = 2 (from $FADH_2$ to O_2)

The rate of respiration in mitochondria is controlled by the concentration of ADP.

Uncouplers (e.g. dinitrophenol, T_3, T_4, dicoumarol) are amphipathic and increase the permeability of mitochondria to protons. In this way, oxidation can proceed without phosphorylation.

Inhibitors of Electron Transport



Complex I – rotenone, amobarbital (FMN-dehydrogenase → CoQ).

Complex II – carboxin.

Complex III – antimycin A (cyt.b → cyt.c_1).

Comlex IV – cyanides (CN^-), CO.

Inhibitor of oxidative phosphorylation (ATP-ase) – oligomycin.

Brown adipose tissue

Brown adipose tissue promotes thermogenesis. It is found in hibernating of animals, in newborns.

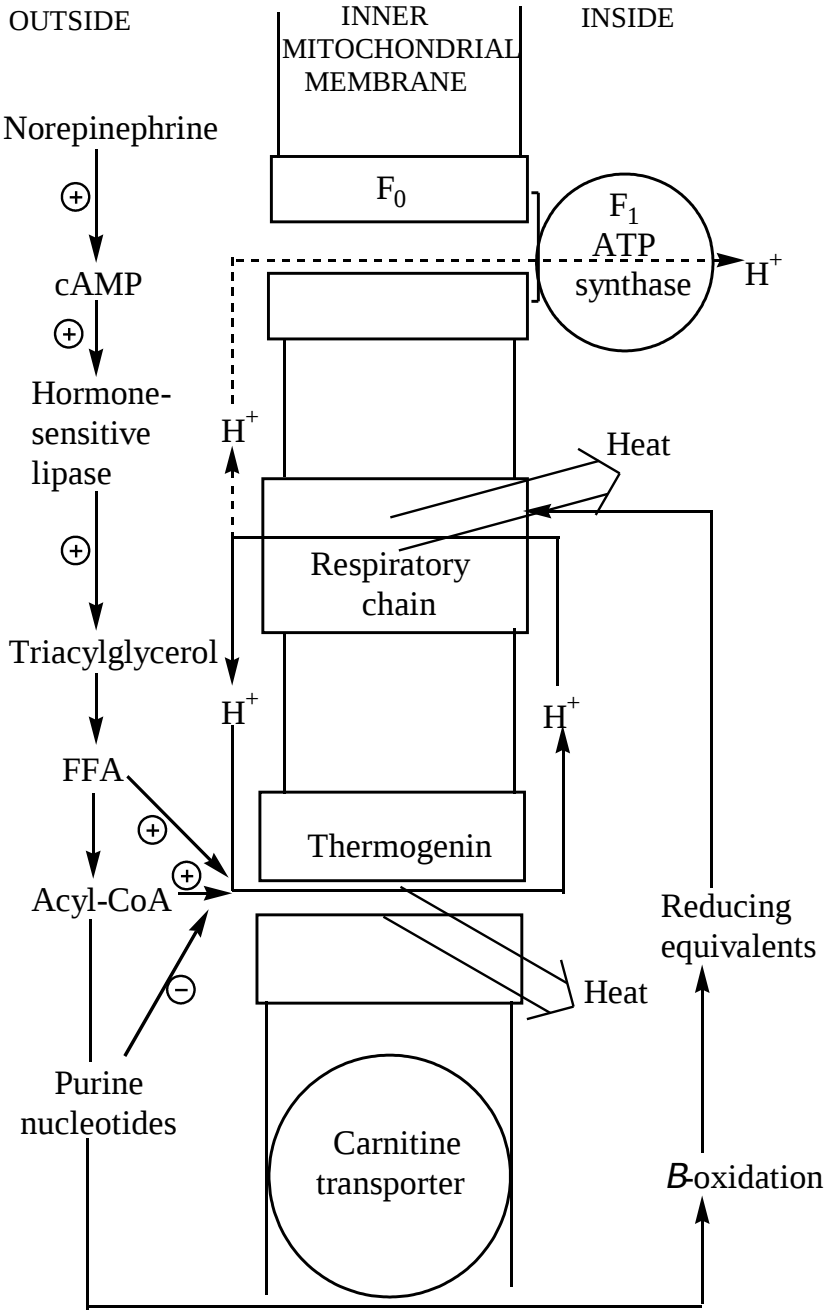


Figure 7.1 Tthermogenesis in brown adipose tissue

Brown adipose tissue is characterized by a well-developed blood supply and a high content of mitochondria and cytochromes but low activity of ATP synthase. Oxidation and phosphorylation are not coupled of this tissue. Proton gradient is continually dissipated in brown adipose tissue by a thermogenic uncoupling protein *thermogenin,* which acts as a proton conductance pathway.

Free Radical Processes

Oxidative processes under aerobic conditions are accompanied by forming reactive oxygen species (ROS).

Reactive oxygen species:

$OH^{\cdot}$ - hydroxyl free radical

$`O_2$ – singlet oxygen (electrons in the O_2 molecule are at a higher energy level)

$O_2^{\cdot-}$ - superoxide free radical

H_2O_2 – hydrogen peroxide

ClO^- - hypochlorite anion.

Sources of reactive oxygen species:

1. Microsomal oxidative system:

Monooxygenases, dioxygenases.

MOS is the main source of ROS. Detoxification of xenobiotics is usually accompanied by activation of free radical processes.

2. Oxidases (oxidases of D- and L- amino acids, xantine oxidase) → $H_2O_2, O_2^{\cdot-}$

3. Respiratory chain.

Ubiquinone is reduced through semiquinone form $QH^{\cdot}$. This radical can immediately interact with oxygen, forming superoxide free radi*ca*l $O_2^{\cdot-}$, which may be converted into other reactive oxygen species.

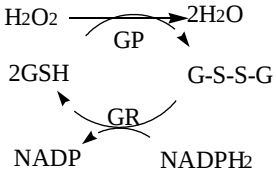
From the first, $O_2^{\cdot-}$ and H_2O_2 are formed conversion of which leads to forming $`O_2$ and $OH^{\cdot}$

Elimination of $O_2^{\cdot-}$

by means of SOD (superoxide dismutase)	spontaneous dismutation with forming $H_2O_2 + `O_2$
$O_2^{\cdot-} + O_2^{\cdot-} + 2H^+ \rightarrow H_2O_2 + O_2$	(it is very slow at physiological value of pH)

Detoxification of H_2O_2

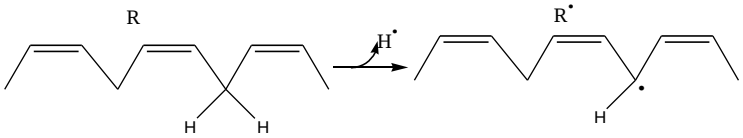
by means of catalase	by means of glutathione	Fenton reaction
$2H_2O_2 \rightarrow 2H_2O + O_2$	peroxidase (GP)	$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + OH^{\bullet}$



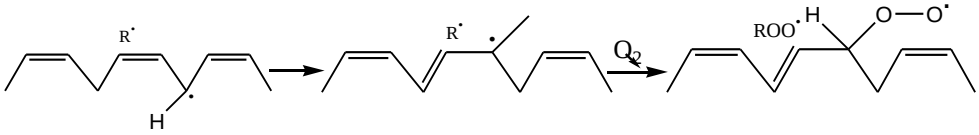
Reactive oxygen species are initiators of free radical processes. They are able to modify proteins, nucleic acids, to induce lipid peroxidation.

Lipid peroxidation:

1. *Initiation.* Reaction is frequently initiated by $OH^{\bullet}$, which removes H^+ from CH_2^- group of polyunsaturated fatty acid

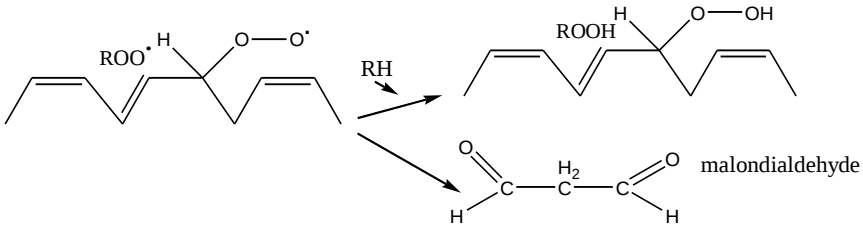


2. *Propagation* is performed by adding O_2, which leads to formation peroxyl free radical



$R^{\bullet} + O_2 \rightarrow ROO^{\bullet}$

3. *Destruction* of lipid structure. Final products of polyunsaturated fatty acids peroxidation are malondialdehyde and hydroperoxide of fatty acid (lipid peroxide).



4. *Termination* - interaction of radicals.

$ROO^{\bullet} + ROO^{\bullet} \rightarrow ROOR + O_2$

$ROO^{\bullet} + R^{\bullet} \rightarrow ROOR$

$R^{\bullet} + vit.E \rightarrow RH + vit.E^{\bullet}$

$Vit.E^{\bullet} + R^{\bullet} \rightarrow RH + vit.E_{ox}$

Free radical processes occur under normal conditions, but in limited range. They:

- induce apoptosis (programmed cell destruction);
- activate phospholipase A_2 → synthesis of eicosanoids;
- determine the rate of cell division;
- determine the state of oxidative phosphorylation;
- $O_2^{\cdot-}$ activates NO-synthase;
- they regulate membrane permeability;
- they regulate the function of many enzymes;
- participate in formation of cell immunity.

In granulocytes:

$NADPH + 2O_2 \xrightarrow{\text{NADPH-oxidase}} NADP^+ + 2O_2^{\cdot-} + H^+$

$NADH + H^+ + O_2^{\cdot-} \xrightarrow{\text{NADH-oxidase}} NAD^+ + H_2O_2$

$H_2O_2 + Cl^- \xrightarrow{\text{myeloperoxidase}} H_2O + HOCl \rightarrow ClO^-$ - hypochlorite anion

Reactive oxygen species provide bactericidal action.

But intensification of free radical processes and lipid peroxidation leads to disturbances of structure and functions of membranes, proteins, enzymes and nucleic acids.

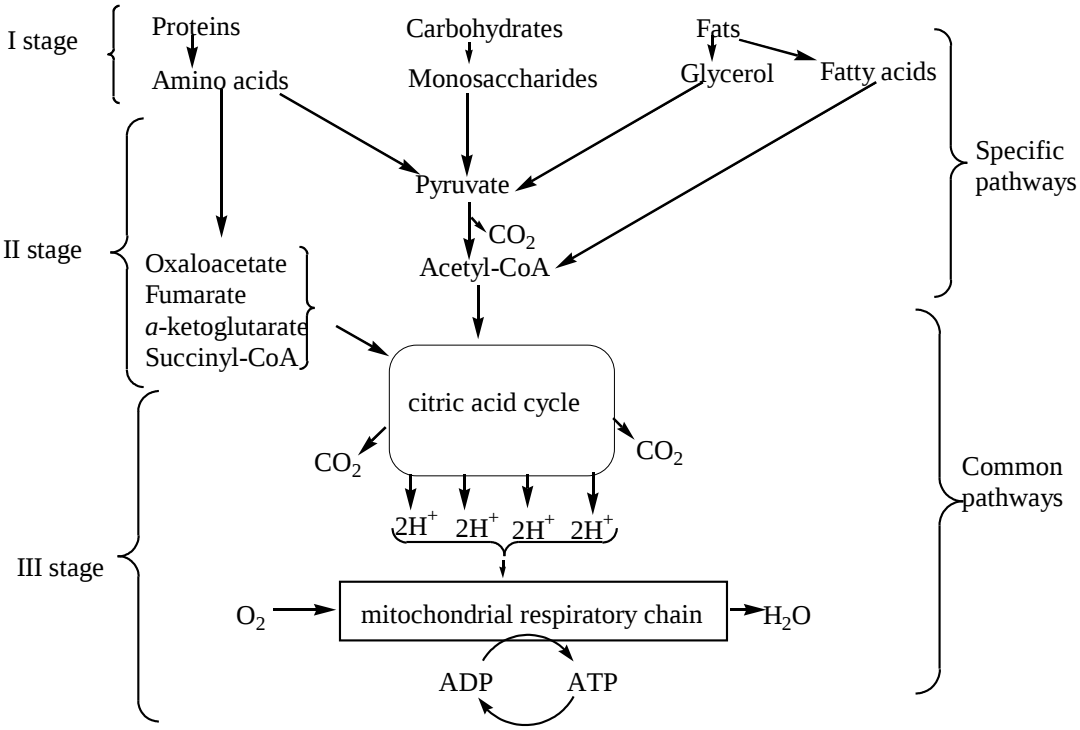
Therefore the potent antioxidant system (AOS) exists in every organism.

AOS

Enzymatic	Nonenzymatic
1. Superoxide dismutase	1. Tocopherol: $O_2^{\cdot-}$, $`O_2$, peroxyl free radicals (at high O_2 partial pressure)
2. Catalase	2. Vitamin A : $O_2^{\cdot-}$, $`O_2$, peroxyl free radicals (at low O_2 partial pressure)
3. Glutathione peroxidase	

3. Vitamin C
4. Glutathione
5. Taurine (ClO^-)
6. Carnosine ($OH^•$)

Stages of energy liberation:



7.3 Oxidative Decarboxylation of Pyruvate

Before pyruvate can enter the citric acid cycle, it must be transported into the mitochondrion via a special pyruvate transporter that aids its passage across the inner mitochondrial membrane. Within the mitochondrion, pyruvate is oxidatively decarboxylated to acetyl-CoA.

This reaction is catalyzed by multienzyme complex which is designated as ***pyruvate dehydrogenase complex.***

This complex is associated with the inner mitochondrial membrane.

It is analogous to the α-ketoglutarate dehydrogenase complex of the citric acid cycle.

Pyruvate dehydrogenase complex:

1) Pyruvate dehydrogenase (coenzyme – thiamin diphosphate or thiamin pyrophosphate – TDP or TPP) E_1-TDP.
2) Dihydrolipoyl transacetylase (lipoamide-E_2).
3) Dihydrolipoyl dehydrogenase (FAD-E_3).

5 coenzymes:

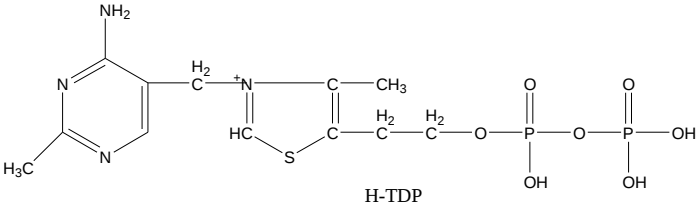
TDP – B_1;

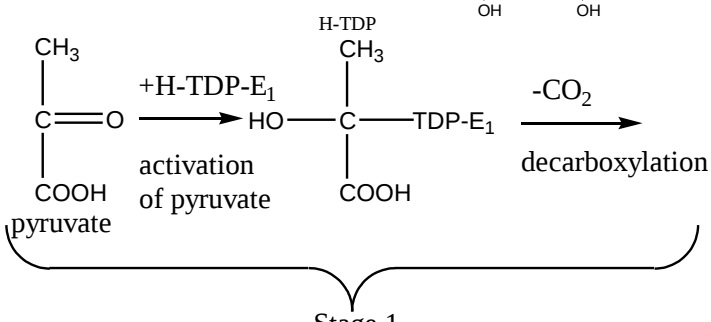
HSCoA – pantothenic acid;

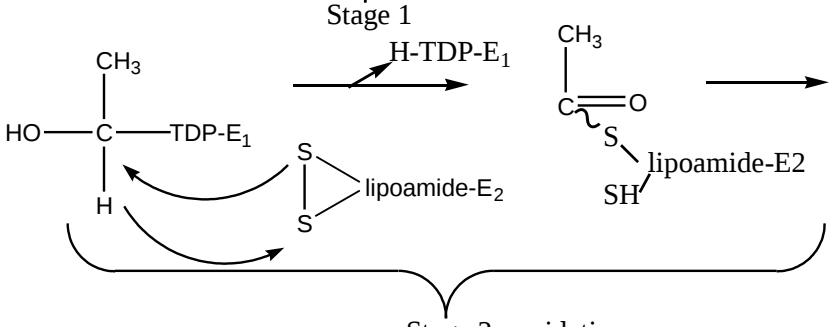
lipoamide;

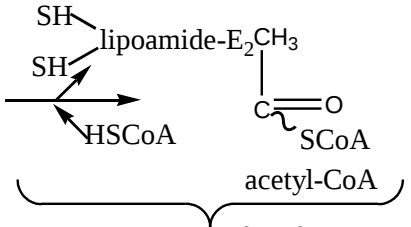
FAD – B_2;

NAD – PP.

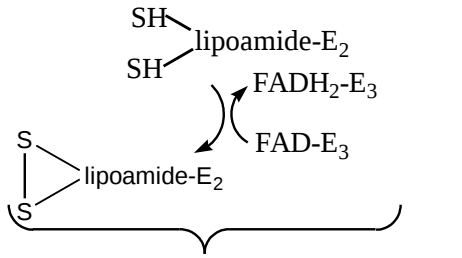








Stage 3 - the transfer of acetyl group to CoA



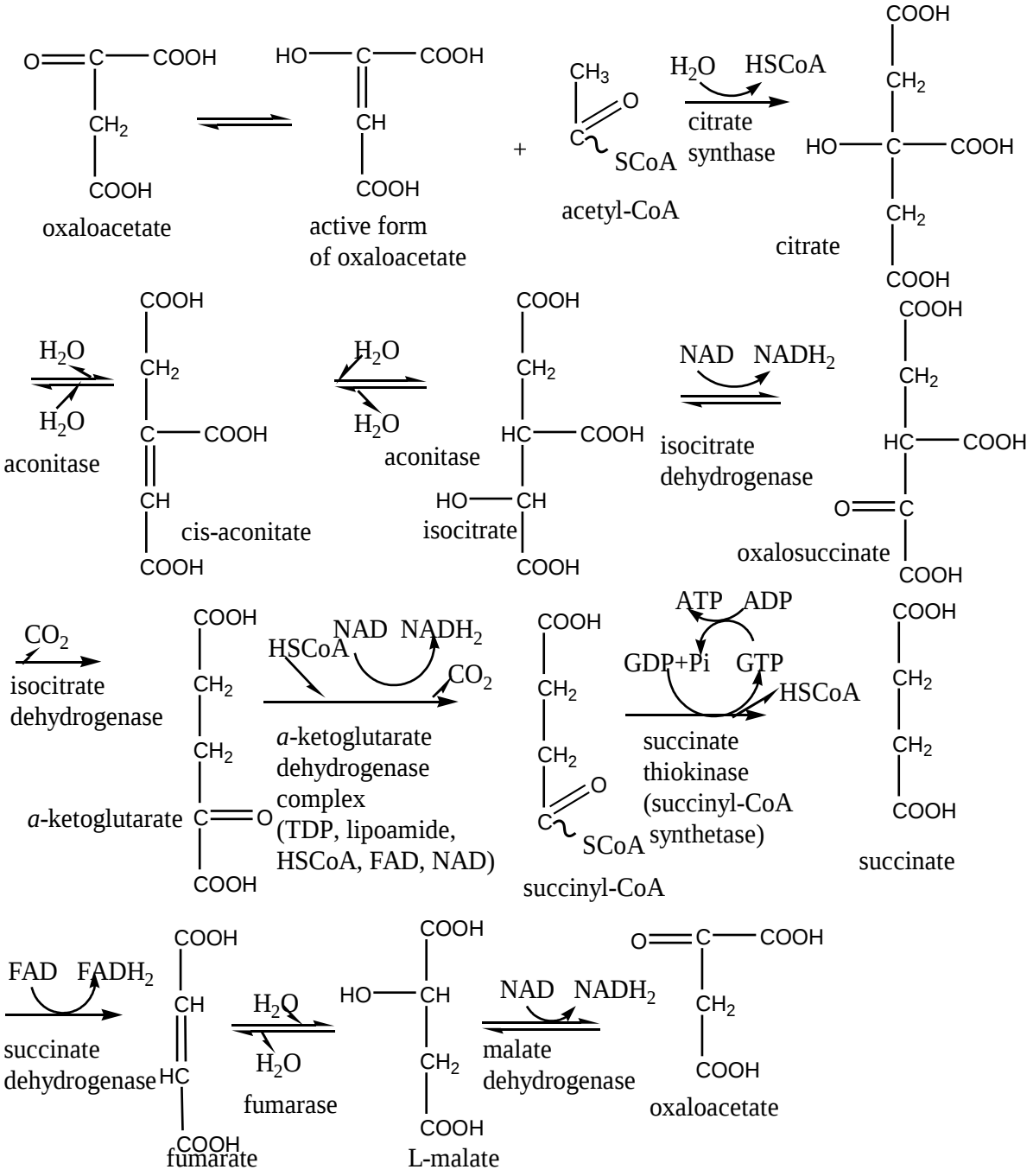
Stage 4 - oxidation of dihydrolipoamide

Stage 5 - oxidation of $FADH_2$

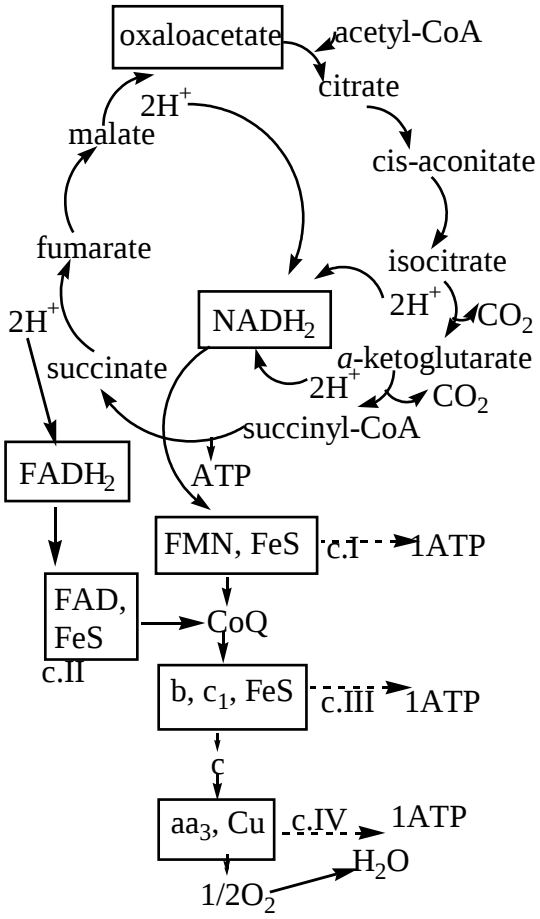
7.4 The Citric Acid Cycle

Full oxidation of acetyl residues is performed in citric acid cycle. This cycle was discovered by G. Krebs in 1937.

The enzymes of the citric acid cycle are located in the mitochondrial matrix. Succinate dehydrogenase is attached to the inner membrane of mitochondrion.



Interrelation of citric acid cycle and tissue respiration



3 $NADH_2$ → 3 x 3 → 9 ATP

1 $FADH_2$ → 2 ATP

1 ATP → substrate level phosphorylation

Σ 12 molecules ATP

Functions:

1) Catabolic (final common pathway of amino acids, carbohydrates, lipids oxidation).
2) Anabolic (metabolites of cycle are used in reactions of other components synthesis):

Succinyl-CoA → heme.

Oxaloacetate → aspartate, glucose.

The participation of citric acid cycle in the both catabolic and anabolic processes is called *amphibolic* function.

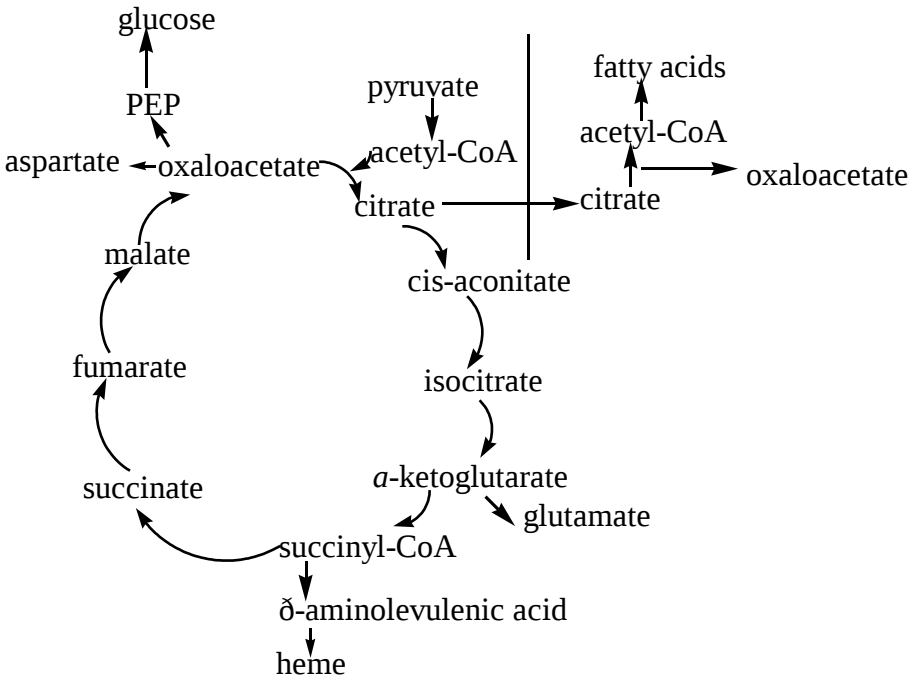
3) Integrative.

4) Hydrogen generating.

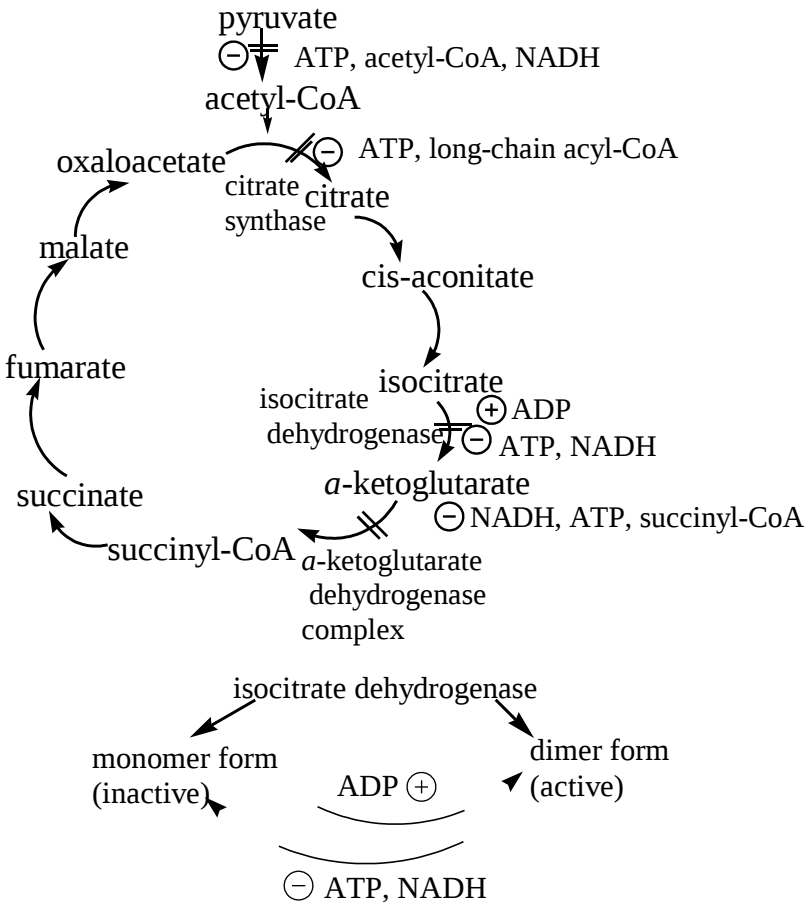
5) Energy.

6) Generation of CO_2, which is used for synthetic processes.

Amphibolic function of citric acid cycle



Regulation of the citric acid cycle



Tests for Self-control

1. Cytochromes were found to be distributed in respiratory chain between CoQ and oxygen. What predetermines the sequence of their including in respiratory chain?

A. Reductive-oxidative potential
B. Molecular mass
C. The presence of different metal ions in structure
D. Amount of peptide chains
E. Difference of heme structure

2. Electrochemical potential of inner membrane is created by means of:

A. Functioning ATP-synthase
B. Anaerobic oxidation of substrates
C. Oxidative phosphorylation
D. Substrate level phosphorylation
E. Functioning respiratory chain

3. The substrates of microsomal oxidation are:

A. Pyruvate and acetyl-CoA
B. Succinate and malate
C. Steroids hormones and cholesterol
D. Isocitrate and α-ketoglutarate

4. Show the point of coupling oxidation and phosphorylation in respiratory chain, which is blocked by barbiturate:

A. $FMNH_2DH \rightarrow CoQ$
B. $CoQH_2 \rightarrow 2b(Fe^{3+})$
C. $2b(Fe^{2+}) \rightarrow 2c_1\ (Fe^{3+})$
D. Cytochrome oxidase $\rightarrow 1/2O_2$
E. $NADH \rightarrow FMNDH$

5. Show the point of coupling oxidation and phosphorylation in respiratory chain, which is blocked by antibiotic antimycin A:

A. $FMNH_2DH \rightarrow CoQ$
B. $CoQH_2 \rightarrow 2b(Fe^{3+})$
C. $2b(Fe^{2+}) \rightarrow 2c_1\ (Fe^{3+})$
D. Cytochrome oxidase $\rightarrow 1/2O_2$
E. $NADH \rightarrow FMNDH$

6. Show the point of coupling oxidation and phosphorylation in respiratory chain, which is blocked by carbon monooxide:

A. $FMNH_2DH \rightarrow CoQ$
B. $CoQH_2 \rightarrow 2b(Fe^{3+})$
C. $2b(Fe^{2+}) \rightarrow 2c_1\ (Fe^{3+})$
D. Cytochrome oxidase $\rightarrow 1/2O_2$
E. $NADH \rightarrow FMNDH$

7. Energy effect of oxidation of 1 mole of isocitrate to α-ketoglutarate is equal to 3 ATP. Show the change of this value with appearance of rotenone in cell:

A. No change

B. The decrease
C. The increase
D. Zero
E. Negative value

8. Experimental animals were treated by substance, which removes the pH gradient on the inner mitochondrial membrane, to uncouple the tissue respiration and oxidative phosphorylation. Which substance has been injected?

A. Dinitrophenol
B. Cholesterol
C. Ketone bodies
D. Urea
E. Somatotropin

9. After the treatment with Phenobarbital, which is the inducer of cytochrome P_{450} synthesis, patient has the intensified:

A. Microsomal oxidation
B. Peroxide oxidation of lipids
C. Biological oxidation
D. Oxidative phosphorylation
E. Substrate level phosphorylation

10. The purpose of brown fat tissue in newborns is:

A. To serve as the plastic material
B. To serve as the thermoinsulator
C. To serve as source of heat by means of uncoupling oxidation and phosphorylation
D. To perform the mechanic protection of tissues and organs
E. The source of the formation of ketone bodies

Chapter 8. HORMONES

A remarcable feature exhibited by the living organisms is their capacity to maintain the homeostatic constancy by means of self-regulated mechanisms.

In higher animals, the coordinated course of all the biological processes both in the whole organism and in microspace of a single cell and its organelles is defined by the neurohumoral mechanisms.

By means of these mechanisms the organism responds to a variety of environmental and internal factors and exerts a precise control over the intensity of metabolic processes.

Hormones mediate between the functions of nervous system and the action of enzymes immediately involved in the metabolism rate control.

The term “hormone” was first applied by Ernest Starling in 1905 for secretin produced by intestinal mucosa.

Hormones are biologically active substances, products of specific metabolism of specialised cells of endocrine glands or single neuroendocrine cells, which exert regulatory effects on metabolism and physiological functions.

8.1 Features of Biological Action of Hormones

- Hormones show their biological action at very low concentrations (in the range of $10^{-11} – 10^{-6}$ M).
- Hormones realize their effects by means of interaction with specific receptors.

Receptors

Intracellular receptors:
- cytosolic
- nucleus

Receptors of plasmatic membrane

Receptors have a protein nature. All receptors have at least 2 functional domains:

- a recognition domain binds the hormone;
- second region generates a signal that couples hormone recognition to some intracellular function.

- Many hormones require the systems of second messengers for realisation of their effects.
- They have a high specifity of biological action which is provided:

- by presence of specific receptors in target organs;
- by presence of different systems of chemical signal transduction;
- by features of metabolism in target organs.

- Hormones realize their action or by changing rate of enzyme synthesis or by influence on the enzyme efficiency.
- Production and secretion of hormones are controled by central nervous system.
- Endocrine glands and produced by them hormones constitute integral system components of which are tihgtly linked by means of direct and feed-back mechanisms.

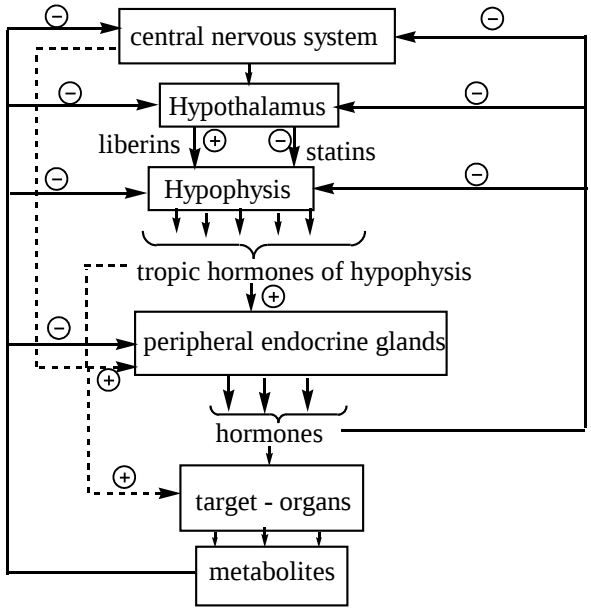


Figure 8.1. Feed-back mechanism of hormonal action

- Distant character of action (telecrine effect) is typical for most of hormones. But some hormones act on adjacent cells (paracrine effect) and even on the cells producing them (autocrine effect).
- Many hormones of protein-peptide nature are synthesized as precursors. Their activation is performed by means of limited proteolysis.

8.2 Classification of Hormones

There are different classifications of hormones

Anatomic classification or classification depending on the localization of hormone synthesis.

1. ***Hormones of hypothalamus***:

- Growth hormone-releasing hormone
- Growth hormone release-inhibiting hormone (somatostatin)
- Thyrotropin- releasing hormone
- Prolactin release-inhibiting hormone
- Gonadotropin- releasing hormone
- Corticotropin- releasing hormone

2. ***Pituitary hormones (hormones of hypophysis)***

The anterior pituitary

- Growth hormone (GH, STH)
- Thyroid-stimulating hormone (TSH)
- Luteinizing hormone (LH)
- Follicle-stimulating hormone (FSH)
- Prolactin (PRL, lactogenic hormone, mammotropin)
- ACTH
- Lipotropin

Intermediate lobe

- MSH

Posterior pituitary

- Antidiuretic hormone (vasopressin, ADH)
- Oxytocin

3. ***Hormones of epiphysis (pineal gland)***

- melatonin
- adrenoglomerulotropin

4. ***Hormones of thymus gland***

- Thymopoietin, thymosin

5. ***Hormones of thyroid gland***

- Calcitonin
- Thyroxin (T_4), triiodothyronine (T_3)

6. ***Hormones of parathyroid gland***

- Parathyroid hormone

7. ***Pancreatic hormones***

- Insulin
- Glucagon
- Somatostatin

8. ***Hormones of adrenal glands***

a) hormones of adrenal cortex:

- glucocorticoids
- mineralocorticoids

b*) hormones of adrenal medulla*

- adrenaline (epinephrine)
- noradrenaline (norepinephrine)

9. ***Hormones of sex glands***

- female sex hormones
- male sex hormones

Classification depending on the chemical structure

1. Conjugated proteins (glycoproteins)

- Thyroid-stimulating hormone (TSH)

- Luteinizing hormone (LH)

- Follicle-stimulating hormone (FSH)

- Chorionic gonadotropin

2. Simple proteins

- Prolactin

- Somatotropic hormone

- Parathyroid hormone
- Insulin
- Chorionic somatomammotropin

3. Peptides

- Hormones of hypothalamus
- ACTH
- MSH
- Vasopressin (ADH)
- Oxytocin
- Glucagon

4. Amino acids derivatives

- Derivatives of tyrosine: T_3, T_4, catecholamines (adrenaline and noradrenaline)
- Derivatives of tryptophan: melatonin

5. Steroids

- Sex hormones
- Corticosteroids (glucocorticoids, mineralocorticoids)

6. Derivatives of polyunsaturated fatty acids (eicosanoids)

- Prostaglandins
- Thromboxanes
- Prostacyclins
- Leukotrienes

Classification depending on the mechanism of action

Group I. Hormones which are able to diffuse through the plasmatic membrane, have intracellular receptors and affect gene expression (lipophilic hormones belong to this

Group II Hormones have membrane receptors and use intracellular messengers (hydrophilic hormones belong to this group: proteins, peptides,

group: steroids, T_3, T_4) adrenaline, noradrenaline)

Cytosolic mechanism **Membrane-intracellular mechanism**

Cytosolic mechanism of action

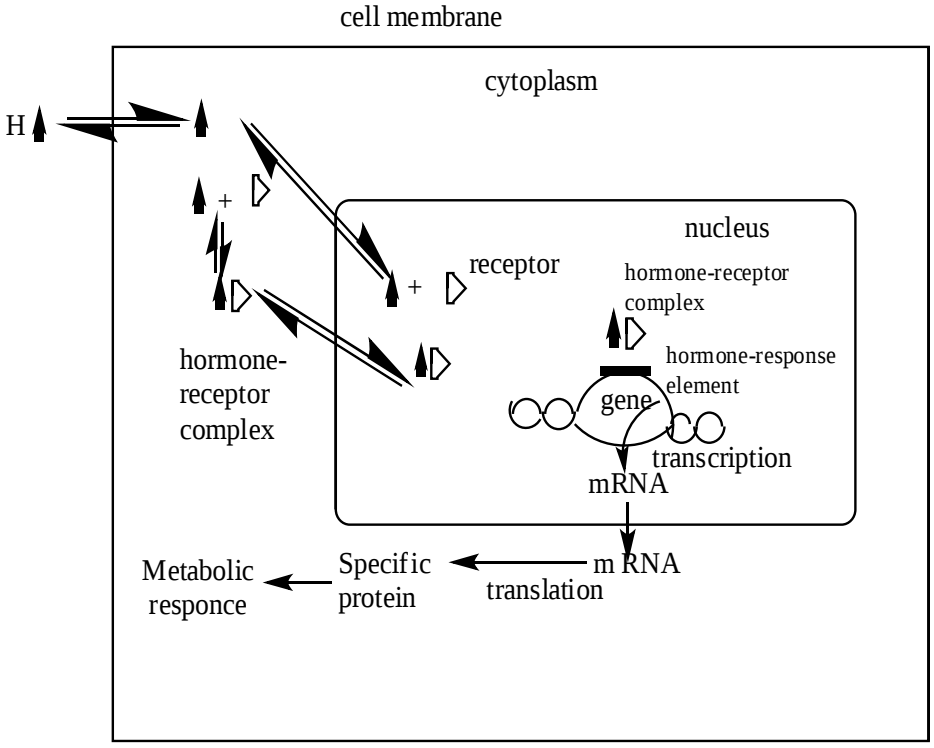


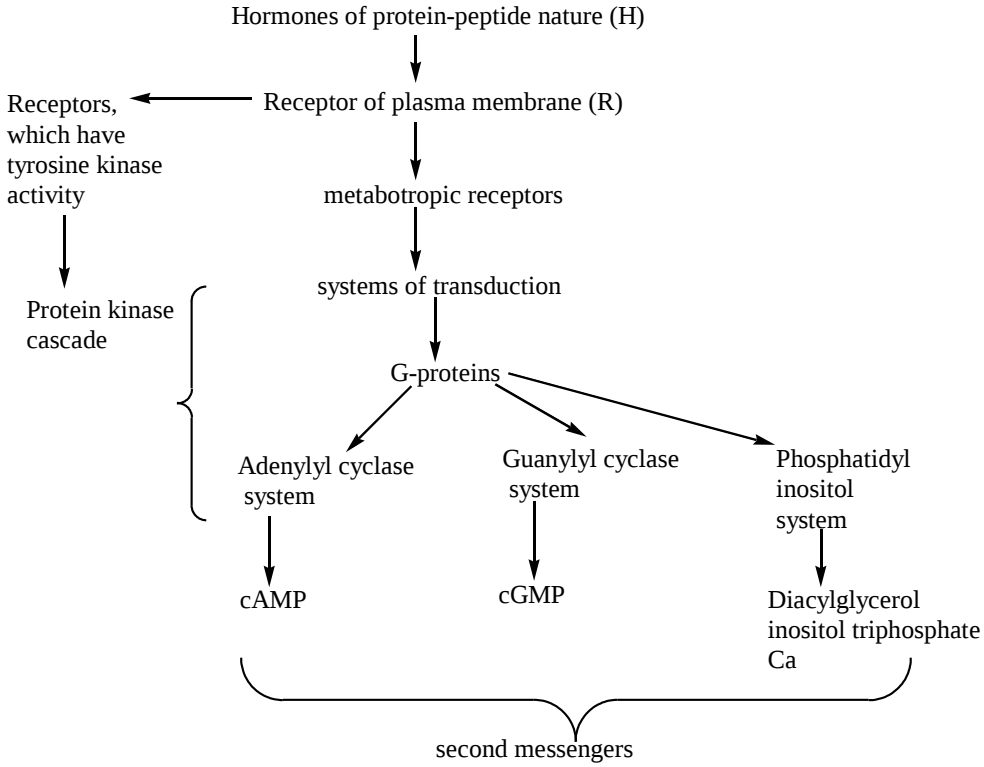
Figure 8.2. Cytosolic mechanism of action.

- Lipophilic hormones diffuse through the plasmatic membrane.
- They interact with cytoplasm or nucleus receptor with formation of hormone-receptor complex.
- Hormone-receptor complex is then activated. This activation leads to size, conformation and surface charge changes.
- Activated hormone-receptor complex penetrates to nucleus.
- It binds to chromatin (to a specific region of DNA – hormone-response element).
- This leads to activation or inactivation of specific genes.
- Activation of specific genes leads to mRNA synthesis.
- mRNA penetrates into cytosol.

- Translation of specific enzymes is activated.
- This leads to metabolic effects.

Membrane-intracellular mechanism of action

Hydrophilic hormones can not pass through plasma membrane. Their receptors are located on the plasma membrane. Their effects are provided by formation of the second messengers.



I Adenylyl cyclase messenger system.

Second messenger is cAMP

cAMP is the most investigated and spread second messenger.

Examples of hormones acting through adenylyl cyclase system:

- catecholamines

β_i and β_2-receptors. → G_s → ↑[cAMP]

α_2-receptors → G_i → ↓[cAMP]

- ACTH, ADH (in renal channels), calcitonin, LPH, MSH etc.

● Receptor binds with hormone.

● G-proteins have such name because they are bound with guanine nucleotides. In the absence of hormone G-protein is ibound with GDP. Binding of hormone to receptor leads to conformational change of the receptor and to exchange of GDP and GTP on the α-subunit of G-protein. GTP-α-subunit complex dissociates from G-protein and activates adenylyl cyclase.

● Adenylyl cyclase catalyzes the reaction of conversion of ATP into cAMP.

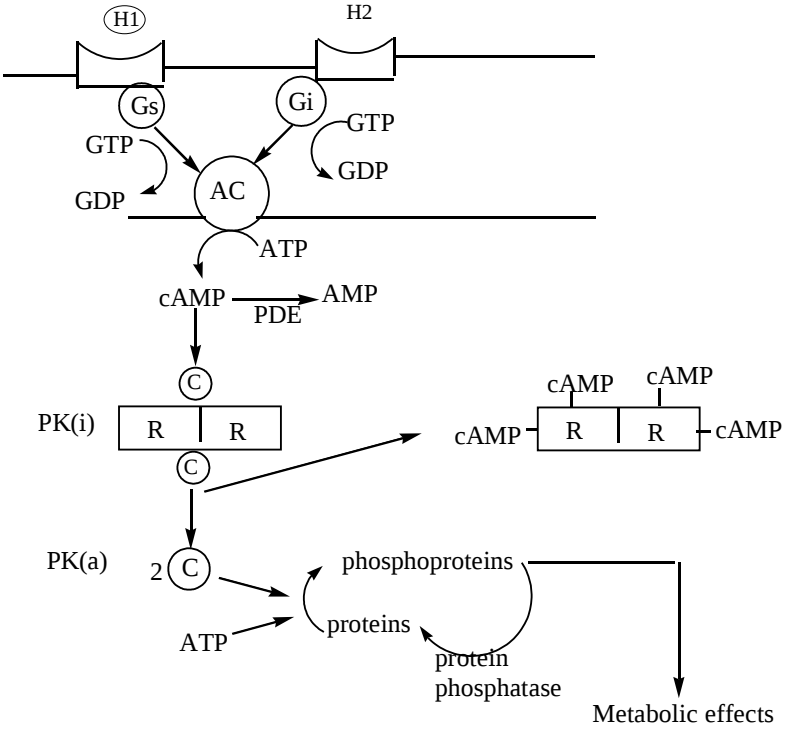


Figure 8.3. Adenylyl cyclase messenger system.

● cAMP is a second messenger which realizes the effect of hormone in cells.

● cAMP activates protein kinase which consists of two regulatory and two catalyst subunits.

● Active protein kinase phosphorylates different enzymes.

● This leads to changing enzymatic activity and to metabolic effects.

● Elimination of cAMP action is provided by phosphodiesterase (PDE). cAMP is destroyed by this enzyme.

Protein-peptide hormones can also influence the protein synthesis by means phosphorylation of regulatory proteins (factors of translation, transduction, transcription).

II Guanylyl cyclase messenger system.

The second messenger is cGMP. Atrial natriuretic factor (ANF) and nitric oxide (NO) act through guanylyl cyclase messenger system.

Cyclic GMP is made from GTP by the enzyme guanylyl cyclase, which exists in soluble and membrane-bound forms.

Atrial natriuretic factor binds to and activates the membrane-bound form of guanylyl cyclase.

Nitric oxide activates the soluble form of guanylyl cyclase.

III Phoshatidyl inositol system (second messengers – diacylglycerol, inositol triphosphate, Ca^{2+}).

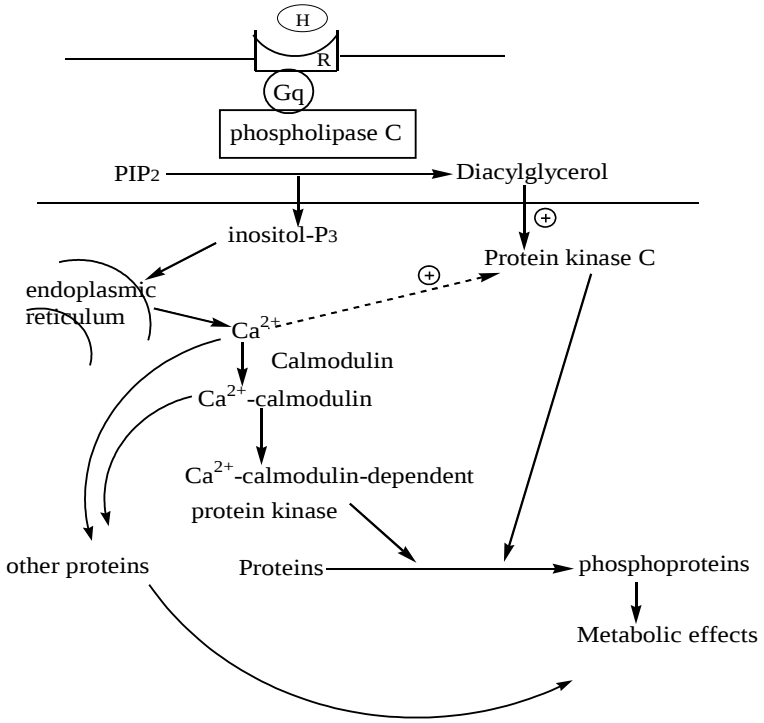
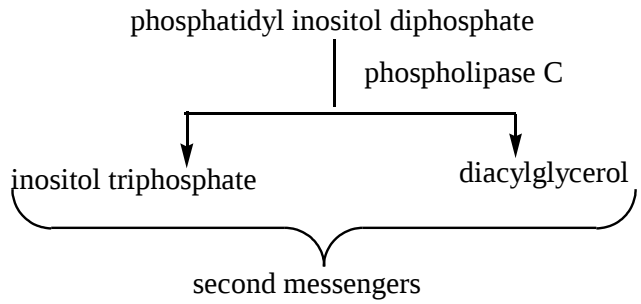


Figure 8.4. Phoshatidyl inositol system.

The effect of many hormones (angiotensin II, cholecystokinin, gastrin, gonadotropin-releasing hormone, vasopressin – vasocontractive action, catecholamines – through receptor α_1) are realized by phosphatidyl-inositol system.

- Receptor binds with hormone.
- Hormone-receptor complex leads to activation of phospholipase C through G-protein.
- Activated phospholipase C cleaves membrane-bound phosphatidyl inositol-diphosphate.



● Inositol-triphosphate causes a rapid liberation of Ca^{2+} from intracellular stores. The increase of intracellular [Ca^{2+}] permits the formation of Ca^{2+}-calmodulin complex that mediates a wide range of effects.

● Diacylglycerol activates a membrane bound protein kinase C.

● Protein kinase C phosphorylates proteins.

IV Protein kinase cascade

Some hormones act through a protein kinase cascade (insulin, insulin-like growth factors – IGF-I, IGF-II). Receptors to these hormones have intrinsic tyrosine kinase activity.

Tyrosine kinase activation initiates phosphorylation and dephosphorylation cascade that involves the action of several other protein kinases and the counterbalancing action of phosphatases.

Example: insulin action.

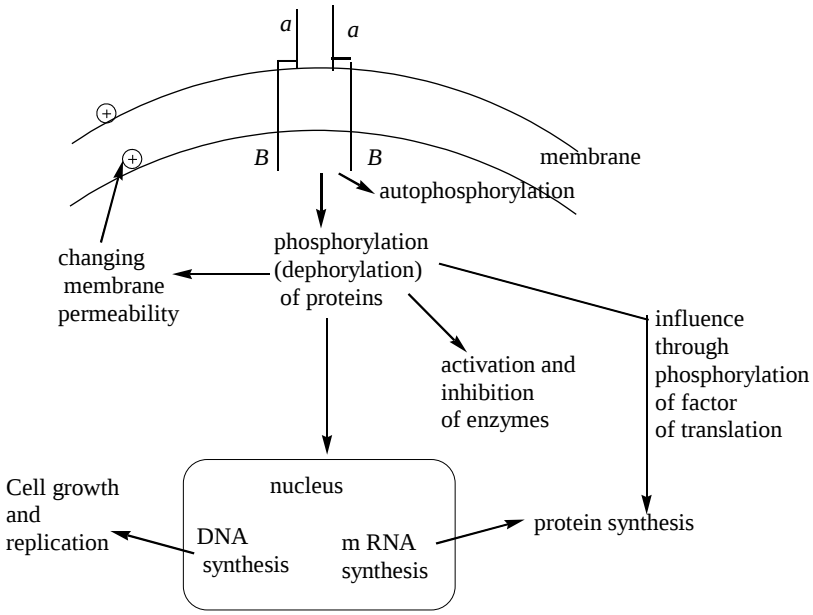


Figure 8.5. Protein kinase cascade.

Physiological classification of hormones.

1. **Hormones determing a reproductive function of organism:**
   a) Hypothalamic hormones:
      - gonadotropin-releasing hormone;
      - prolactin release-inhibiting factor
   b) Pituitary hormones:
      - follicle stimulating hormone;
      - luteinizing hormone;
      - prolactin.
   c) Sex hormones:
      - female – estrogens, progestins;
      - male – androgens.
2. **Hormones influencing on a growth and a differentiation of tissues and forming an immunology status:**
      - growth hormone;
      - T_3, T_4;
      - thymus hormones (thymopoetin, thymosin).
3. **Hormones reacting on the food supply:**
      - insulin;
      - glucagon.
4. **Stress hormones:**
      - catecholamines (hormones of acute stress);
      - glucocorticoids (hormones of chronic or prolonged stress).
5. **Hormones regulating water-salt metabolism:**
      - ADH;
      - mineralocorticoids.
6. **Hormones providing a calcium homeostasis:**
      - parathyroid hormone;
      - calcitonin;

- GH, sex hormones, insulin, glucocorticoids etc.

8.3 Hypothalamic and Pituitary Hormones

Hypothalamic Hormones

- Hypothalamus is the site for immediate interaction between the CNS and endocrine system.

- Hormonal agents produced by the hypothalamic nerve cells are carried via portal capillary system to the pituitary gland where their function is to regulate the secretion (and, probably, synthesis) of hypophysal hormones.

- They are divided into releasing factors (or liberins) and inhibiting factors (or statins).

- They realize their effects through cAMP, Ca^{2+} or phosphatidyl-inositol messenger systems.

Gonadotropin-releasing hormone realizes its effects through phosphatidyl-inositol messenger system, Ca^{2+}. It stimulates the release of FSH, LH, prolactin.

Prolactin release-inhibiting hormone inhibits the release of prolactin. Stimulates the secretion of gonadotropic hormone.

Growth hormone-releasing hormone (somatoliberin) increases the biosynthesis and secretion of growth hormone.

Growth hormone release-inhibiting hormone (somatostatin) acts through adenylyl cyclase messenger system. It decreases the secretion of GH, TSH, FSH.

Thyrotropin-releasing hormone realizes its effects through phosphatidyl-inositol messenger system. It stimulates the secretion of TSH and prolactin.

Corticotrophin-releasing hormone realizes its effects through adenylyl cyclase messenger system. It increases the secretion of ACTH, MSH, LPH, endorphins.

Pituitary Hormones

Anterior Pituitary Hormones

Growth hormone (somatotropic hormone)

- It is synthesized in somatotrophes, a subclass of the pituitary acidophilic cells.

- Its secretion is impulsive. It is secreted every 20-30 minutes. A marked rise in growth hormone secretion is within the first hours of deep sleep.

- GH is simple protein whish consists of 191 amino acids and has two disulfide bridges.

- It realizes its effects through proteinkinase cascade and through somatomedines (IGF-I, II), which are synthesized in liver.

Physiological and biochemical effects:

1. Main function is the stimulation of postnatal growth.
2. It influences protein and NA metabolism.

- It increases the transport of amino acids into cells.
- It increases the protein, DNA and RNA synthesis.

3. Influence on carbohydrate metabolism. It generally antagonizes the effects of insulin.

- It decreases the peripheral utilization of glucose.
- It stimulates gluconeogenesis.
- Growth hormone increases liver glycogen.

4. The influence on lipid metabolism.

- It stimulates the fat mobilization in adipose tissue.

5. It has prolactin-like effects.

A lot of the biological effects of GH are mediated by IGF-I and IGF-II (somatomedins). IGH-I correlates most directly with GH effects.

Disturbances

Deficiency of GH leads to dwarfism. Hypophysal dwarfism is characterized by a proportional under-development of the whole body, including the skeleton, without any significant psychic abnormalities. Several types of dwarfism illustrate the

GH excess, usually from acidophilic tumor, causes gigantism if it occurs before the epiphysial plates close, since there is accelerated growth of the long bones.

Acromegaly results from excessive release of GH that begins after

importance of somatomedin in realization of GH effects.

For example, Laron type dwarfs have excessive amount of GH but they lack functional hepatic GH receptors and therefore low level of IGF-I.

epiphysal closure. Acromegaly (from the Greek akron – extremities + megal - great) is characterized by facial changes (protruding jaw, enlarged nose) and enlargement of the hands, feet and skull. Other findings include a variety of metabolic problems, including diabetes mellitus.

Prolactin (lactogenic hormone)

- It is produced by acidophilic cells of anterior pituitary.
- It contains 199 amino acids with the S-S linkages. Dopamine suppresses the prolactin secretiom.

Functions: It stimulates the mammary gland development (mammotropic action), the secretion of milk (lactogenic action), the growth of internal organs, the secretory function of corpus luteum (yellow body).

Pathophysiology

- Tumors of prolactin-secreting cells cause ***amenorrhea*** (cessation of menses); ***galactorrhea*** (milk discharge) in women.

- Excessive PRL has been associated with ***impotence and gynecomastia*** (breast enlargement) in men.

Chorionic somatomammotropin (CS, placental lactogen) has no definite function in humans. In bioassays, CS has lactogenic and luteotropic activity and metabolic effects that are qualitatively similar to those of growth hormone.

Group of glycoprotein hormones (thyroid-stimulating hormone, luteinizing hormone, follicle-stimulating hormone and chorionic gonadotropin). They have remarkable structural similarities, they act through adenylyl cyclase messenger system.

Each of these hormones consists of two subunits α and β, joint by noncovalent bonds. The α-subunits are identical for all of these hormones within a species. The specific biologic activity is determined by β-subunits.

Thyroid-stimulating hormone (TSH)

- It is synthesized by basophilic cells of anterior pituitary.
- It is glycoprotein composed of two subunits (α and β)
- Target organ of TSH is thyroid gland.

Functions of TSH:

- It stimulates the synthesis of thyroid hormones at all the stages.
- It enhances the release of stored thyroid hormones.
- It increases synthesis of DNA, RNA, protein, cell size.
- It increases lipolysis.

Human chorionic gonadotropin is a glycoprotein synthesized in syncytiotrophoblast cells of the placenta. It increases in blood and urine shortly after implantation hence its detection is the basis of many pregnancy tests.

Gonadotropic hormones (gonadotropins)

- They are synthesized in the anterior pituitary (basophilic cells).
- They are glycoproteins consist of α and β subunits.
- They act through cAMP.

FSH

In females	In males
- Stimulates the follicular maturation of the ovary. - Stimulates the secretion of estrogens	- Supports the spermatogenesis. - Increases the sensitivity of Leydig cells to LH

Luteinizing hormone (LH)

In females	In males
- It causes the final maturation of Graffian follicle.	- It stimulates the development and functional activity of

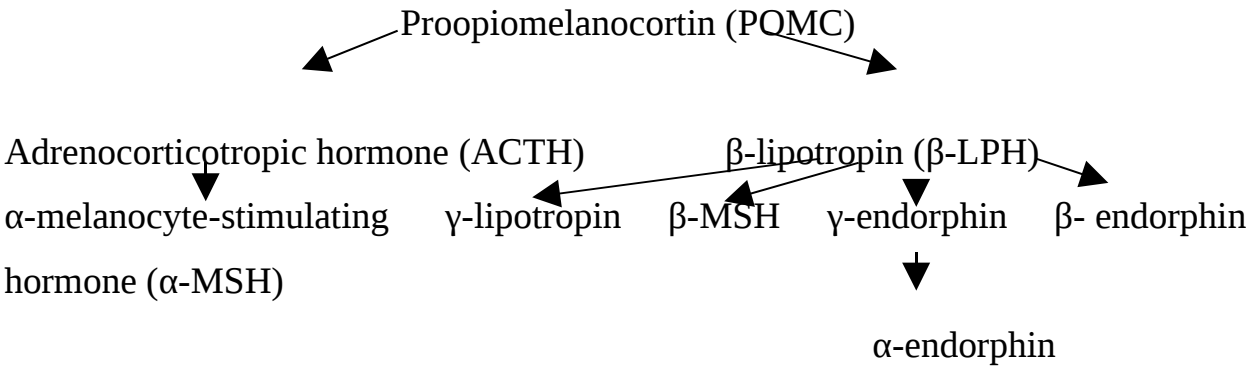
- Stimulates ovulation.
- Stimulates secretion of progesterone.
- Helps in the formation and development of corpus luteum.

Leydig cells (interstitial).

- Stimulates the secretion of testosterone.

Group of proopiomelanocortin consists of peptides that act as hormones (ACTH, LPH, MSH) and others that may serve as neurotransmitters or neuromodulators (endorphins). Proopiomelanocortin is synthesized in basophyl cells of hypophysis.

Processing proopiomelanocortin mainly occurs in anterior and intermediate lobes of pituitary. Middle lobe is active only in embryonal period and in pregnant women.



ACTH

- It is synthesized by basophilic cells of anterior pituitary.
- It is peptide (consists of 39 amino acids).

Effects:

- It acts through cAMP.
- Major function is the stimulation of glucocorticoids synthesis and secretion.
- It exhibits fat mobilizing activity.
- Melanocyte stimulating activity.

Lipotropin (91 amino acids)

- It causes lipolysis and fatty acid mobilization but its physiological role is minimal.

- It probably serves only as the precursor for endorphins.

Melanocyte-stimulating hormone increases the activity of melanocytes.

The Posterior Pituitary Hormones (Oxytocin, Vasopessin).

● ADH is synthesized in the supraoptic nucleus and oxytocin in the paraventricular nucleus of hypothalamus.

● They are transported through axons in association with specific carriers proteins called neurophysins.

● Both hormones are nonapeptides.

Oxytocin:

- acts through adenylyl cyclase messenger system;
- stimulates the contraction of smooth uterine muscles in labour;
- stimulates the contraction of the myofibrils around the mammary alveolar ducts for milk secretion.
- Estrogen stimulates the production of oxytocin and of neurophysin-I, and progesterone inhibits the production of these compounds.

Vasopressin (antidiuretic hormone)

It has two types of receptors. V_1- receptors (smooth muscle of vessels, thrombocytes, hepatocytes). They are linked with phosphatidyl-inositol messenger system. V_2- receptors are found only on the surface of renal epithelial cells.

Effects:

- Vasoconstrictive action.
- ADH promotes the reabsorption of water from the distal renal tubules.
- One of the forms of ADH (lysyl-vasopressin) has additional function: it promotes the renovation memory, facilitates the act of reminiscence.

Regulation of secretion

- ADH secretion is stimulated by increased osmolality of plasma. This is mediated by osmoreceptors located in the hypothalamus and by baroreceptors located in the heart and other regions of the vascular system.
- Other stimuli include emotional and physical stress.

- Ethanol inhibits ADH secretion.

Pathophysiology

The atrophy of posterior pituitary leads to **diabetes insipidus** development. Diabetes insipidus is characterized by the excretion of large volumes of dilute urine.

8.4 Thymus Gland Hormones

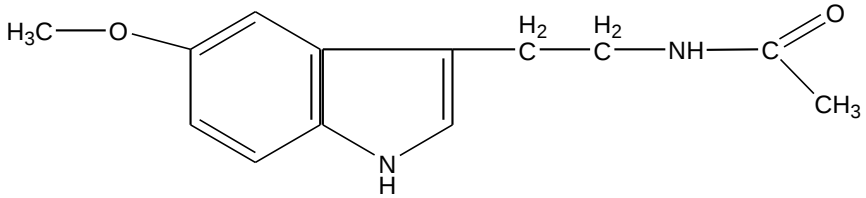
The thymus supplies, in the neonate organism, lymphoid cells to the lymph nodes and to the spleen and produces specific hormones involved in the development and maturation of lymphoid tissue cells.

Thymopoetin II is composed of 44 amino acids. It appears to be the major hormone which stimulates the production of T-lymphocytes.

Thymosin α (28 amino acids) performs a regulatory function at the late stage of T-cell differentiation.

8.5 Pineal Gland Hormones

Melatonin (N-acetyl-5-metoxytryptamine) is tryptophan derivative. It is synthesized in pinealocytes of epiphysis and in several periphery tissues: gastro-intestinal tract, eyes etc. Melatonin production has a cycle character; it increases at darkness and is inhibited by bright light.



melatonin

Functions:

- Regulation of day rhythms (acceleration of asleeping).
- Regulation of season rhythms.
- Inhibition of pituitary hormones, STH, thyroid hormones, corticosteroid secretion.
- Stimulation of several immune reactions.

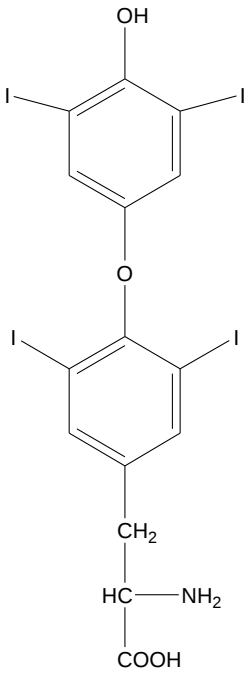
- Regulation of reproductive function: the sharp decrease of melatonin level at boys stimulates the starting puberty.
- Antioxidant properties.

8.6 Thyroid Hormones

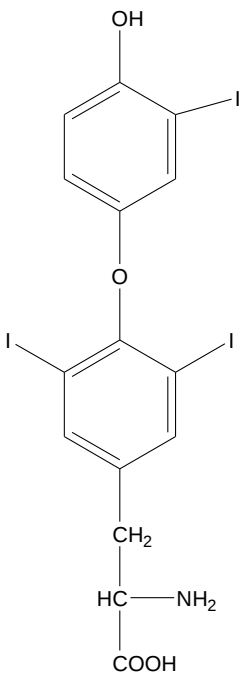
T_3 (Triiodothyronine), T_4 (Thyroxine)

● T_3 and T_4 are formed in follicular cells of thyroid gland.

● Thyroid hormones are iodinated derivatives of tyrosine.



Thyroxine (T_4)



Triiodothyronine (T_3)

Biosynthesis. Thyroid hormones are synthesized by the iodination of tyrosine residues of a large protein called thyroglobulin. Thyroglobulin is a dimeric glycoprotein which contains 115 tyrosine residues each of them is a potential site of iodination.

Steps:

1. Concentration of iodide (I^-).
2. Oxidation of iodide.
3. Iodination of tyrosine residues in thyroglobulin with formation of mono- and diiodotyrosines.
4. Coupling iodotyrosine residues with formation of triiodothyronine or tetraiodothyronine within the thyroglobulin molecules.

Thyroglobulin is a storage form of T_3 and T_4 in the colloid.

Thyroid hormones are released from thyroglobulin by hydrolysis. Hydrolysis is stimulated by TSH, but is inhibited by I^-. This is used for treatment of hyperthyroidism.

Transport. Within the plasma T_3 and T_4 are transported by:

- thyroxine-binding globulin (60%);
- thyroxine-binding prealbumin (30%);
- serum albumin (10%);

Approximately 0,05 % of the circulating thyroxine is the “free” form.

Although the circulating levels of T_3 are much lower than the corresponding T_4 levels, T_3 appears to be the major thyroid hormone metabolically, because:

- extrathyroidal deiodination converts T_4 to T_3 (80%);
- T_3 binds to the thyroid receptor in target cells with 10 times the affinity of T_4;
- T_3 is 3 to 5 times more active than T_4.

Metabolism. The thyroid hormone metabolism includes total deiodination and inactivation by deamination or decarboxylation, conjugation with glucuronic acid or sulfate.

Mechanism of action. Thyroid hormones are lipophilic. They pass through membrane. Receptors to them are located:

- On the plasma membrane. Binding with this receptor, thyroid hormones stimulate a transport of amino acids.
- On inner mitochondrial membrane. Binding with these receptors, thyroid hormones can effect the synthesis of some mitochondrial enzymes.
- Nuclear receptors. They influence the transcription.

Effects of thyroid hormones:

● In embryonal period they influence tissue differentiation.

● Thyroxine is one of the factors essential for normal growth and skeletal maturation.

● It stimulates the synthesis of GH and potentiates its effect on tissues.

● *Influence on protein metabolism*: in physiological doses thyroid hormones stimulate protein synthesis leading to N-retention. High doses of T_4 cause a protein catabolism leading to negative nitrogen balance.

● *Influence on carbohydrate metabolism*. Thyroid hormones are antagonistic to insulin.

- They increase the rate of glucose absorption from intestine.
- They decrease the glucose tolerance because they increase the degradation of insulin.
- They increase glycogenolysis in liver.
- They stimulate glycolysis and pentose phosphate pathway.
- They increase hepatic gluconeogenesis.

● *Influence on lipid metabolism*. They increase lipolysis in adipose tissue.

● Calorigenic effect. They increase consumption of oxygen and heat production. They uncouple oxydation and phosphorylation.

● They influence mineral metabolism.

●They influence cardio-vascular system.

Dysfunction of thyroid gland

The hypofunction		Endemic goiter	Hyperfunction
During infancy it leads to disease known as **cretinism.**	In adult age the insufficiency of thyroid gland leads to the development of **myxedema.**	is linked with deficiency of iodine in water and food.	leads to development of **Basedow's disease (Graves disease, hyperthyroidism, or thyrotoxicosis).**

Symptoms of cretinism:

- growth retardation;
- severe psychic disturbances;
- increased sensitivity to cold;
- dry skin and hair.

Symptoms of myxedema:

- disturbances of water-salt balance, basal and fat metabolism;
- mucous oedema;
- pathologic obesity;
- loss of hair;
- general brain disorder and psychic aberrations.

Symptoms of Basedow's disease:

- increased degradation of proteins (negative nitrogen balance);
- tachycardia (rapid heart rate);
- exophthalmos (abnormal protrusion of the eyeball);
- goiter (enlargement of thyroid gland);
- general physical wasting;
- psychic abnormalilies.

8.7 Hormones That Regulate Calcium Metabolism

Calcitonin

It is synthesized in parafollicular C-cells of thyroid glands. It is peptide hormone (32 amino acids). It acts through adenylyl cyclase messenger system.

Role of calcitonin.

- It influences Ca and P metabolism. It decreases calcium and phosphorus levels in blood.
- It inhibits osteoclast function.
- It increases the synthesis of collagen.
- It decreases the activity of collagenase.

Parathyroid Gland Hormone (PH)

It is simple protein (84 amino acids). It is synthesized as precursor.

Preparathormone (115 amino acids) → proparathormone (90 amino acids) → parathormone (84 amino acids).

It realizes its effects through adenylyl cyclase messenger system. It influences Ca and P metabolism (the increase of Ca and the decrease of P levels in blood).

Role of PH.;

In bones:

● It increases the bones resorption.

● It inhibits the collagen synthesis and enhances the resorption of bone tissue organic matrix.

In kidney:

● It lowers the phosphorus reabsorption and slightly elevates the calcium reabsorption.

In intestine:

● It enhances the calcium absorption in intestine (indirectly through stimulation of 1,25-dihydroxycholecalciferol synthesis).

8.8 Hormones of Pancreas and Gastro-intestinal Tract

Pancreatic hormones:

α-(or A)-cells produce glucagon;

β-(or B)-cells produce insulin;

D-cells produce somatostatin;

F-cells produce the little studied pancreatic polypeptide.

Insulin is synthesized as preproinsulin (107 amino acids) → proinsulin (84 amino acids) → insulin (51 amino acids).

The insulin molecule, which contains 51 amino acids, is composed of two polypeptide chains linked through disulfide bonds.

Insulin influences the carbohydrate, protein, lipid and, indirectly, on the water-salt metabolism. The glucose concentration in the blood plays a dominant role in the physiological control of insulin secretion. Elevated content of glucose in the blood stimulates the increased insulin secretion in the pancreatic islets.

Receptor to insulin consists of two α- and two β- subunits. The α-subunits are extracellular and they bind insulin. The β-subunits perform the signal transduction, as the cytoplasmic portion of the β-subunits has tyrosine kinase activity and they are able to autophosphorylation.

The phosphorylated insulin receptor phosphorylates insulin receptor substrate. This leads to cascade of phosphorylation and finally to activation of protein phosphatases.

The influence on the carbohydrate metabolism:

- Stimulation of glucose transport from extracellular space through membrane to cells. This effect is observed in muscles, adipose tissue, lymphocytes. Insulin does not influence the membrane transport of glucose in hepatocytes, brain and kidney cells.
- It stimulates glycolysis.
- It inhibits gluconeogenesis.
- It activates pentose-phosphate pathway of glucose oxidation.
- It stimulates the glycogen synthesis and inhibits its degradation.

The influence on the lipid metabolism:

- It inhibits lipolysis.
- It increases the synthesis of fatty acids.
- It stimulates lipogenesis.

The influence on protein metabolism:

- It increases the membrane permeability to amino acids.
- It stimulates the protein synthesis.

It indirectly influences the metabolism of K^+ and P. It influences the processes of embryogenesis, growth and differentiation of tissues.

Deficiency or resistance to the action of insulin leads to development of diabetes mellitus.

Symptoms: hyperglycemia, glucosuria, ketonemia, ketonuria, polydipsia, polyuria, polyphagia, loss of weight, dry skin, itch of skin.

About 90% of persons with diabetes mellitus have non-insulin-dependent type (type II) of diabetes mellitus. Such patients are usually obese, have elevated plasma insulin levels and have down-regulated insulin receptors. Ketonemia and ketonuria are not observed in these patients.

Glucagon is synthesized by α-cells of pancreas as preproglucagon.

preproglucagon → proglucagon → glucagon (29 amino acids).

The secretion of glucagon is inhibited by glucose.

Target organs: liver, myocardium, adipose tissue, **but not skeletal muscles**.

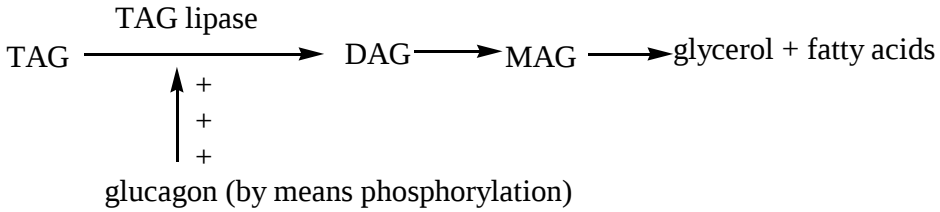
Glucagon is antagonist of insulin.

Mechanism of action: membrane-intracellular, second messenger is cAMP.

The influence on carbohydrate metabolism:

- It stimulates glycogen mobilization.
- It inhibits glycolysis and stimulates gluconeogenesis.
- It inhibits glycogen synthesis.

In adipose tissue it stimulates lipolysis (mobilization of fats).



Gastrointestinal hormones

Hormone	Location	Major action
Gastrin	Gastric antrum, duodenum	Hydrochloric acid and pepsin secretion
Secretin	Duodenum, jejunum	Pancreatic bicarbonate secretion
Cholecystokinin	Duodenum, jejunum	Stimulation of gallbladder contraction, stimulation of pancreatic enzymes secretion.
Gastric inhibitory protein	Small intestine	Enhances glucose-mediated insulin release; inhibits gastric acid secretion
Vasoactive intestinal polypeptide	Pancreas	Smooth muscle relaxation, stimulates pancreatic bicarbonate secretion
Motilin	Small intestine	Initiates interdigestive intestinal motility
Somatostatin	Stomach,	Numerous inhibition effects

	duodenum, pancreas	
Pancreatic polypeptide	Pancreas	Inhibits pancreatic bicarbonate and protein secretion
Enkephalins	Stomach, duodenum, gallbladder	Opiate-like actions
Substance P	Entire gastro-intestinal tract	Physiologic action uncertain
Bombesin	Stomach, duodenum	Stimulates release of gastrin and cholecystokinin
Neurotensin	Ileum	Physiologic action is unknown
Enteroglucagon	Pancreas, small intestine	Physiologic action is unknown

8.9 Hormones of Adrenal Medulla

Catecholamines – epinephrine (adrenaline) and norepinephrine (noradrenaline) are synthesized in chromaffin cells of adrenal medulla, sympathetic nerve ganglia and adrenergic structures of CNS from tyrosine. Epinephrine is mainly hormone and norepinephrine – neurotransmitter. They act mainly through adenylyl cyclase system.

They have, at least, four types of receptors: β_1- and β_2- adrenoreceptors are linked with adenylyl cyclase through G_s protein, α_2-adrenoreceptors are coupled with adenylyl cyclase through G_i-protein; α_1- adrenoreceptors are linked with phosphatidyl inositol messenger system.

Epinephrine binds to and activates both α- and β-receptors, so that its action in a tissue having both depends on the relative affinity of these receptors for the hormone. ***Norepinephrine*** at physiologic concentrations primarily binds to α-receptors.

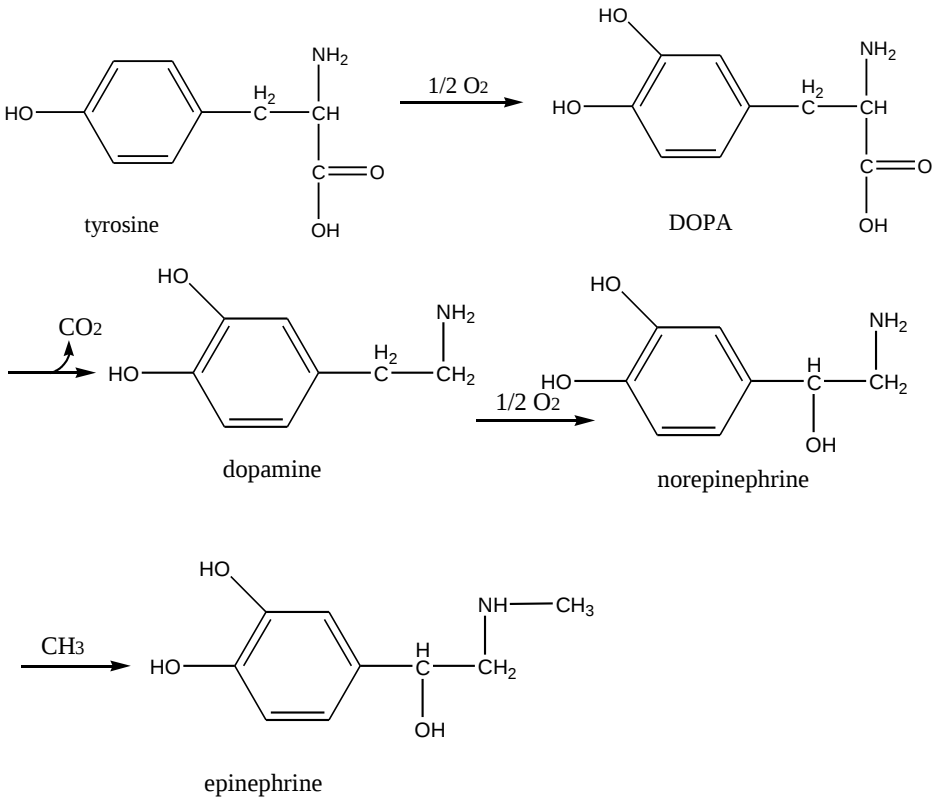


Figure 8.6 Catecholamine synthesis.

Physiological functions of catecholamines:

- Epinephrine increases the rate and strength of cardiac muscle contraction and cardiac output (β_1 effect).
- Norepinephrine exerts an over-all vasoconstrictor effect (α_1 effect).
- Epinephrine relaxes the smooth muscles of gastro-intestinal tract ($\alpha 2$ and β effects) and bronchioles, ciliary muscles of eye, urinary bladder ($\beta 2$ effect).

Metabolic effects of epinephrine:

- The influence on carbohydrate metabolism by glycogen phosphorylase activation (glycogenolytic action) in muscles and liver, producing hyperglycemia.
- The influence on lipid metabolism is characterized by lipolytic effect through TAG lipase stimulation in adipocytes.

Catecholamine breakdown is catalyzed by mitochondrial monoamine oxidase and catechol-O-methyltransferase. End products of catecholamine breakdown are 3-metoxy-4-mandelic acid and oxoadrenochrome.

Pheochromocytomas are tumors of adrenal medulla. These tumors are usually not detected unless they produce and secrete enough epinephrine or norepinephrine

to cause a severe hypertension syndrome. The ratio of norepinephrine to epinephrine is often increased in pheochromocytoma. This may account for differences in clinical presentation, since norepinephrine is thought to be primarily responsible for hypertension and epinephrine for hypermetabolism.

8.10 Hormones of Adrenal Cortex

Glucocorticoids

Glucocorticoids are synthesized in zona fasciculata cells. Synthesis is regulated by hypophysal ACTH. In its turn the synthesis of ACTH in the pituitary gland is controlled by hypothalamus, which in response to stress secretes corticoliberin.

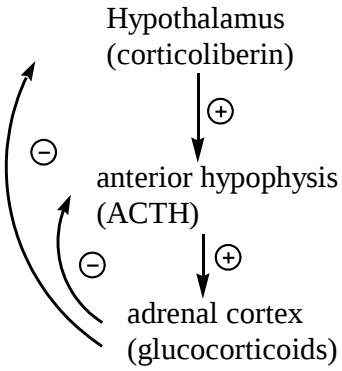


Figure 8.7. Regulation of glucocorticoids secretion

ACTH shows a fast (short-term) and slow (chronic) actions on the adrenal glands.

Short-term effect is linked with the increase of corticosteroid synthesis. Chronic action of ACTH involves the growth and reproduction of adrenal cells. The ACTH action is mediated by adenylyl cyclase system.

Synthesis of glucocorticoids

Esters of cholesterol

↓

cholesterol

↓

pregnenolone → 17-α- hydroxypregnenolone

↓ ↓

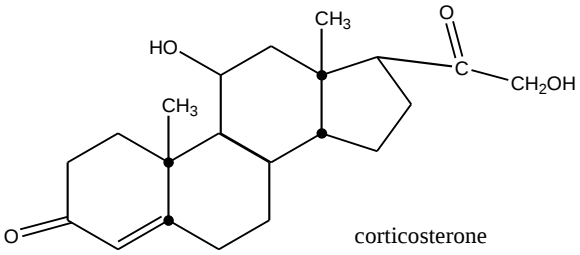
progesterone → 17-α- hydroxyprogesterone

↓ ↓

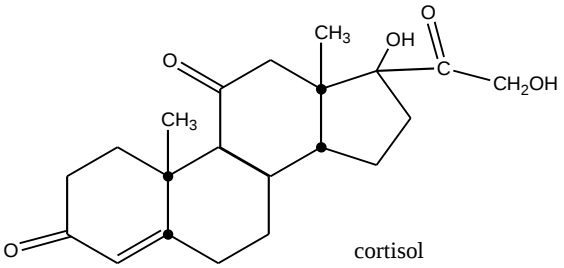
11-deoxycorticosterone 11-deoxycortisol

↓ ↓

corticosterone cortisol (hydrocortisone)



corticosterone



cortisol

Cortisol is main glucocrticoid of human.

Transport:

- 60% of cortisol is bound to a specific cortisol-binding protein (transcortin);
- 30% is bound to albumin;
- 8% - free cortisol – biologically active form.

Metabolism:

- Inactivation by reduction of ring and 3-keto group. Reduced metabolites conjugate with glucuronic acid and are excreted with urine.
- The other way of inactivation is oxidation leading to the cleavage of a side chain at carbon atom 17 with formation of 17-ketosteroids.

They have cytosolic mechanism of action.

Target tissues: liver, muscles, adipose tissue, lymphatic tissue, skin, bone, fibroblasts etc.

They perform catabolic action in muscular, lymphatic, connective and adipose tissue and anabolic action in liver.

Influence on carbohydrate metabolism (they have hyperglycemic effect):

- They decrease the permeability of cell membrane to glucose in muscles, adipose tissue.
- They inhibit glycolysis in peripheral tissues.
- They increase gluconeogenesis in liver because:
    a) they induce the sysnthesis of key enzymes of gluconeogenesis;
    b) they increase accessibility of substrates of gluconeogenesis by means of protein catabolism in extrahepatic tissues.
- They increase the synthesis of glycogen in liver.

Influence on lipid metabolism.

They stimulate lipolysis in adipose tissue.

Influence on protein metabolism:

- In peripheral tissues they stimulate the protein breakdown.
- In liver they induce the synthesis of proteins.

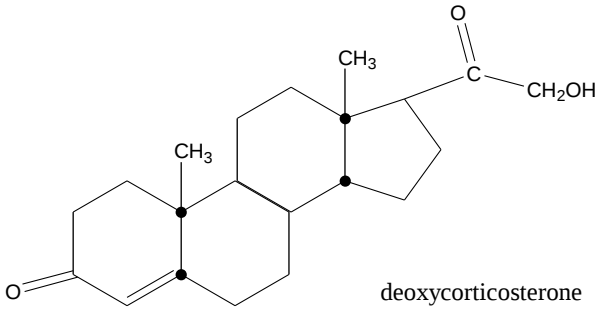
Therapeutic doses of glucocrticoids exert an anti-inflammatory effect:

- They stabilize lysosomal membrane.
- They prevent the formation of bradikinin.
- They decrease the permeability of capillary walls.
- They decrease the formation of prostaglandins and leukotrienes by inhibiting phospholipase A_2.
- They prevent the release of histamine from mast cells.
- They decrease the number of circulating lymphocytes.

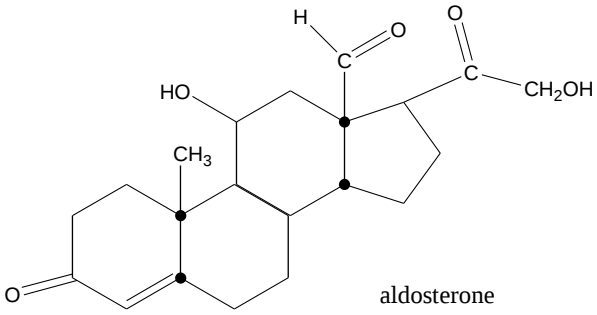
Therefore they are used as anti-inflammatory drugs in treatment of rheumatoid arthritis, acute glomerular nephritis.

They have antiallergic and antiimmune activities. Therefore they are used in organ transplantation and for treatment of bronchial astma.

Mineralocorticoids (Deoxycorticosterone and Aldosterone)



deoxycorticosterone



aldosterone

They are synthesized in zona glomerulosa cells.

They are involved in the regulation of sodium, potassium, chloride metabolism. They favour the retention of sodium and chloride ions in the organism and facilitate the urinary excretion of potassium ions.

The primary regulators of aldosterone production are the rennin – angiotensin system and potassium. Sodium, ACTH and neural mechanisms are also involved.

Disfunction of adrenal cortex.

Hypoproduction	Hyperproduction
1) Primary deficiency of adrenal cortex (Addison's disease): - the decrease of stability to stress; - hypoglycemia; - decrease of blood pressure; - hyponatriemia; - hyperpigmentation of skin (patients develop abnormal	1) Cushing's disease (hyperproduction of ACTH), Cushing's syndrome (malignant tumors of the cortex): - hyperglycemia; - osteoporosis; - increasing adiposity of the face, neck and trunk (so called "moon" face and Buffalo-fat distribution); - hypertension;

bronze pigmentation of skin and mucous membranes) from the increased production of ACTH.

2) Secondary deficiency of adrenal cortex – deficiency of ACTH (signs the same except the last)

- impotence in males and sterility in female.

2) Hyperaldosteronism

- Primary aldosteronism (Conn's syndrome)

- hypernatriemia, hypokaliemia, alkalosis.

- Secondary aldosteronism. Renal arteriole stenosis can lead to hyperplasia and hyperfunction of juxtaglomerular cells and cause elevated levels of rennin and angiotensin II.

Secondary aldosteronism resembles the primary form, except the elevated rennin and angiotensin II levels.

8.11 Sex Hormones

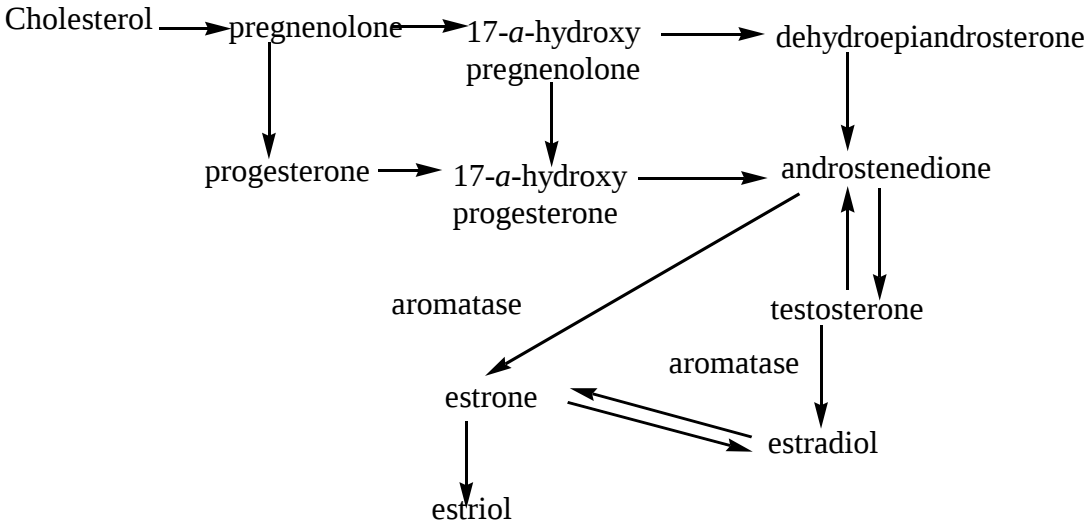


Figure 8.8 General scheme of sex hormones synthesis

Catabolism of sex hormones:

Testosterone → 17-ketosteroids → conjugation with glucuronic or sulphuric acid in liver

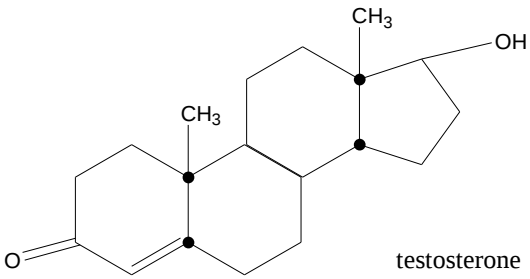
Estradiol, estrone → estriol → conjugation with glucuronic or sulphuric acid.

Progesterone → pregnanediol → conjugation with glucuronic acid

Androgens

Biosynthesis: mainly in the testes and particularly in the ovaries and adrenal glands.

Main androgen : testosterone.



testosterone

- Androgens exert already in the embryonic period a significant influence on the differentiation of male sex glands as well as on differentiation of other tissues, for example CNS, determining the secretory activity of gonadotrotpic hormones.
- Some functions of testosterone in CNS (especially functions, which belong to behaviour and functional differentiation of gonadostate) are not performed by testosterone itself, but are performed by estradiol, which is formed from testosterone in specific neurons.
- In target cells testosterone acts mainly as its reduced derivative – **dehydrotestosterone,** which is formed from circulating testosterone directly in sensitive cells.

Functions of androgens. They are involved in:

- sexual differentiation;
- spermatogenesis;
- development of secondary sexual organs;
- anabolic effects:

- they stimulates a protein anabolism;
- stimulate the growth of bones before the closure of epiphyseal cartilage;
- androgens cause a rather selective increase in size and weight of the kidneys (“renotropic” action);

- male-pattern behaviour.

Testosterone and its synthetic analogues have found application in medicine practice as drugs for treatment of breast cancer.

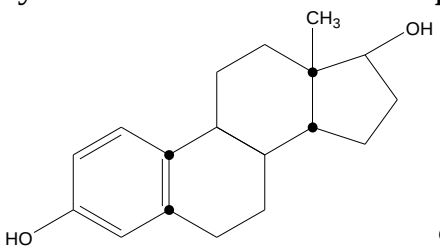
Female Sex Hormones

The major sites of the synthesis are ovaries and corpus luteum; they are also formed in the adrenal glands, testes and placenta.

Velocity of female sex hormones secretion significantly varies depending on the phase of the sex cycle.

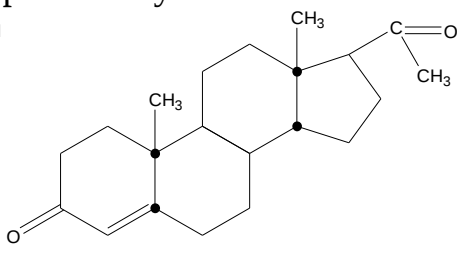
Female sex hormones

Estrogens (C_{18}) are predominantly synthesized on the follicular phase

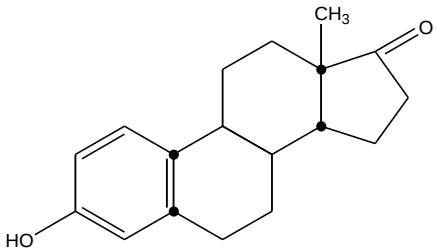


estradiol

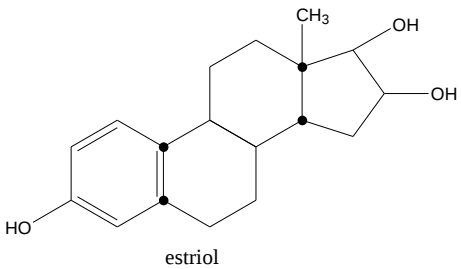
Progestins (C_{21}) are mainly synthesized on the luteal phase



progesterone



estrone



estriol

Main functions of estrogenes:

- Formation of second sex signs.
- Formation of sexual instinct.

- They create optimal conditions for the eventual fertilization of ovum, for pregnancy, delivery (childbirth), lactation.
- Anabolic effects.

Progesterone functions:

- It prepares the endometrium (mucous membrane of the uterus) for effective implantation of the fertilized ovum.
- It provides the normal course of pregnancy.
- It stimulates the growth of mammary gland tissue.
- It inhibits ovulation.
- It inhibits the contraction of the uterus.

Natural hormones and synthetic preparations with estrogenic activity have found wide applications in medical practice for example as contraceptives.

Stilbestrol, stilben have also found application in oncologic practice as inhibitors of tumoral growth of the prostate.

In the female organism in gestation another endocrine organ, the placenta, functions to produce estrogens and progesterone. However, as has been established, the placenta alone is not capable of synthesizing the steroid hormones and the functionally effective endocrine organ is a complex of fetus and placenta, the so-called fetoplacental unit. A specific feature of fetoplacental estrogen synthesis is that the parent material, cholesterol, is supplied by maternal organism; in the placenta, cholesterol is stepwise converted to pregnenolone and progesterone. The subsequent synthesis occurs only in the fetal tissues.

8.12 Eicosanoids

The ***eicosanoids*** are group of signaling substances that arise from the C-20 fatty acid *arachidonic acid* and therefore usually contain 20 C atoms (Greec eicosa = 20).

Biosynthesis. Almost all of the body’s cells form eicosanoids. Membrane phospholipids that contain the polyunsaturated fatty acid arachidonic acid provide the starting material.

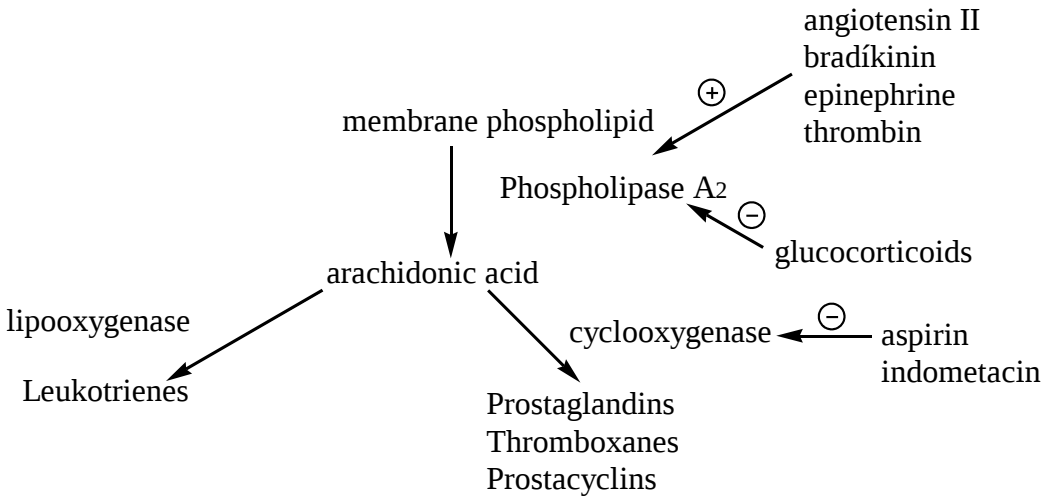


Figure 8.10 Regulation of eicosanoids formation

Initially ***phospholipase A₂*** releases the arachidonic acid from these phospholipids. The activity of phospholipase A_2 is strictly regulated. It is activated by hormones and other signals via G proteins. The arachidonate released is a signaling substance itself. However, its metabolites are even more important.

Two different pathways lead from arachidonic acid to ***prostaglandins***, ***prostacyclins*** and ***thromboxanes***, on the one hand, or ***leukotrienes*** on the other. The key enzyme for the first pathway is ***prostaglandin exoperoxide synthase,*** which posesses two separate enzyme activities, ***cyclooxygenase*** and ***peroxidase***. Using up O_2, it catalyzes in a two-step reaction the cyclization of arachidonic acid to prostaglandin H_2, the parent substance for prostaglandins, prostacyclins and thromboxanes. ***Lipooxygenase*** start the synthesis of leukotrienes.

Effects of eicosanoids

Eicosanoids act via membrane receptors in the immediate vicinity of their site of synthesis, both on the synthesizing cell itself (autocrine action) and on neighbouring cells (paracrine action). Many of their effects are mediated by second messengers cAMP and cGMP.

They influence a large number of physiological processes.

As they can stimulate or inhibit smooth muscle contraction depending on the substrate concerned, they affect blood pressure, respiration, and intestine and uterine activity, among other properties. In the stomach prostaglandins inhibit HCl secretion via G_i proteins. At the same time, they promote mucus secretion, which

protect the gastric mucosa against the acid. Prostaglandins are also involved in bone metabolism and in the activity of the sympathetic nervous system. In the immune system, prostaglandins are important in the inflammatory reaction. Among other things, they attract leukocytes to the site of infection. They are also involved in the development of pain and fever.

Prostaglandins:

- They act on the smooth muscles of gastrointestinal tract, reproductive and respiratory tissues and blood vessels. These effects depend on the types of prostaglandin: PGE_2 has vasodilatory effect, decreases arterial pressure; $PGF_{2\alpha}$ causes contraction of vessels, increases arterial pressure.
- They modulate the activity of other hormones.
- They perform the autonomous control of nervous excitation.
- They regulate inflammatory processes.
- They regulate renal blood flow rate.

Thromboxanes are synthesized in platelets and cause vasoconstriction and platelet aggregation.

Prostacyclins are produced by blood vessel walls and are potent inhibitors of platelet aggregation. They have vasodilatory effect.

Leukotrienes. They participate in inflammatory processes, allergic and immune reactions. They increase the supply of leukocytes to inflammation site, they cause activation of leukocytes.

Metabolism. Eicosanoids are inactivated within a period of seconds to minutes. This takes place by enzymatic reduction of double bond and dehydrogenation of hydroxyl groups.

Eicosanoid metabolism is an important ***drugs target***. Acetylsalicylic acid and related non-steroid anti-inflammatory drugs (NSAIDs) selectively inhibit the ***cyclooxygenase*** activity of prostaglandin synthase and consequently the synthesis of most eicosanoids. This explains their analgesic, antipyretics and antirheumatic effects. Frequent side effect of NSAIDs also results of eicosanoids synthesis inhibition. They impair hemostasis because the synthesis of thromboxanes by

thrombocytes is inhibited. In the stomach NSAIDs increase HCl secretion and inhibit the formation of protective mucus. Long-term NSAIDs use can therefore damage the gastric mucosa.

8.13 Cytokines

Cytokines are hormone-like peptides and proteins, which are synthesized and released by cells of immune system and other cell types. The cytokines differ from hormones only in certain respects: they are released by many different cells and they regulate a wider variety of target cells than hormones.

As peptides or proteins, cytokines are hydrophilic signaling substances that act by binding to receptor on the cell surface. Binding of a cytokine to its receptor leads via several intermediate steps to the activation of specific genes transcription.

There is an extremely large number of cytokines. Cytokines include ***interleukins (IL), lymphokines, monokines, chemokines, interferons (IFN) and colony-stimulating factors (CSF).***

Numerous biological functions of cytokines operate in these areas:

- they regulate the development and homeostasis of the immune system;
- they control the hematopoietic system;
- they are involved in non-specific defense, influencing inflammatory processes, blood coagulation and blood pressure.

In general, cytokines regulate the growth, differentiation and survival of cells. They are also involved in regulating apoptosis.

Tests for Self-control

1. Which hormone stimulates the activity of adenylyl cyclase?
A. Adrenaline
B. Aldosterone
C. Testosterone
D. Progesterone
E. Calcitriol

2. Some hormones are the products of protein hydrolysis and modification. Which protein is the precursor of lipotropin, ACTH and MSH?
A.Proopiomelanocortin
B.Neuroalbumin
C.Neurostromin
D. Neuroglobulin
E. Thyroglobulin

3. Calcium ions can function as second messengers. They are activators of many processes if they react with:
A. Calcitonin
B. Calmodulin
C. Calciferol
D. Myosin
E. Phosphorylase

4. Patient, age 67, suffers from diabetes insipidus after hemorrhage of brain with damage of hypothalamic nuclei. Diabetes insipidus is accompanied by polyuria in result of:
A. Hypoglycemia
B. Decreased potassium reabsorbtion
C. Enhance of kidney filtration
D. Hyperglycemia
E. Decreased water reabsorption

5. Which of the below mentioned hormones is hydrophilic and doesn’t require the specific transport protein?
A. Dihydrotestosterone
B. Progesterone
C. Parathyroid hormone
D. Aldosterone
E. Estradiol

6. Which of the below mentioned hormones diffuses to cell and interacts with cytosolic receptors?
A. Estradiol
B. Oxytocin
C. Parathyroid hormone
D. Adrenaline
E. Growth hormone

7. The formation of cAMP from ATP is provided by activation of the enzyme:
A. ATP-ase
B. Adenylyl cyclase
C. Phosphatase
D. Phosphodiesterase
8. Which hormones act on the genetic apparatus of cell?
A. Protein nature
B. Peptide nature
C. Steroid nature
D. Derivatives of amino acids
E. Polypeptide nature
9. Which hormone stimulates the biosynthesis of glycogen and increases anabolic pocesses?
A. Adrenaline
B. Noradrenaline
C. Cholecystokinin
D. Insulin
E. Thyroxine
10. Tachycardia, loss of weight, increased temperature of body, over excitation are observed in the patient. This state is caused by increased level of hormone:
A. Thyroxine
B. Vasopressin
C. Growth hormone
D. Insulin
E. Adrenocorticotropic hormone
11. Disturbance of the hormone synthesis results in development of different pathologic states. Which hormone synthesis deficiency leads to inhibition of growth without disturbance of mental activity?
A. Thyroxine
B. Prolactin
C. Growth hormone
D. Adrenaline
E. Gonadotropin
12. A 50-year-old man was in the state of severe stress. Adrenaline and noradrenaline levels in blood are increased. Which enzymes catalyze the inactivation of these hormones?
A. Glucosidases
B. Monoaminoxidases
C. Pepidases
D. Carboxylases
E. Tyrosinases
13. A patient is observed with losing their weight, over excitation, light increase in body temperature, exophthalmus, hyperglycemia, azotemia. Which hormone overproduction results in the appearance of these symptoms?

A.Adrenaline
B.Thyroxine
C. Calcitonin
D.Aldosterone
E.Vasopressin

14. Patient is in hypoglycemic coma. Which hormone overdose can result in this situation?

A. Insulin
B. Cortisol
C. Somatotropin
D. Progesterone
E. Corticotpopin

15**.** Hyperglycemia is observed in Cushing's disease. Which process is stimulated in this state?

A. Glycolysis
B. Phosphorolysis of glycogen
C. Krebs cycle
D.Pentose phosphate pathway of glucose oxidation
E. Gluconeogenesis

16. Examination of a patient revealed hypokaliemia, hypernatremia and hypervolemia. What is the possible cause of that state?

A. Hyperaldosteronism
B. Hypoaldosteronism
C. Addison's disease
D. Basedow's disease
E. Diabetus mellitus

17. Aspirin shows anti-inflammatory action because it inhibits the activity of cyclooxygenase. Which biologically active substances level will decrease?

A. Catecholamines
B. Prostaglandins
C. Melatonin
D. Iodothyronines
E. Mineralocorticoids

Chapter 9. CARBOHYDRATE METABOLISM

9.1 Carbohydrate Digestion

More than a half of carbohydrates ingested by humans is starch. Starch digestion begins in the mouth and continues in the duodenum. Starch and glycogen digestion begins in the *oral cavity* by means of salivary amylase. It is α-amylase, its optimal pH is 6,8-7,0. It hydrolysis internal α-1.4-linkages. It has an obligatory requirement for chloride ions. Saliva amylase produces dextrins and small amount of maltose and maltotriose.

Stomach contains no enzymes of carbohydrate digestion. In the stomach, the activity of salivary amylase ceases, since the stomach contents exhibit a strongly acid reaction (pH = 1,5-2,5). However in the interior of the ingested food globus the salivary amylase retains its activity.

The crucial stage of starch and glycogen degradation occurs in the *duodenum* under the action of pancreatic α-amylase. Here the pH medium changes to about neutral value, which corresponds to optimal pH of pancreatic amylase. It hydrolyzes starch, glycogen and dextrins to α-limit dextrins (mixture of branched oligosaccharides), maltose, maltotriose.

The intestinal juice contains enzymes which complete digestion of carbohydrates:

- *Sucrase – isomaltase complex*, which is found as the proenzyme on one polypeptide chain but as active enzymes on separate polypeptides. *Sucrase* hydrolyzes *sucrose* to glucose and fructose. *Isomaltase* catalyzes the hydrolysis of α-1,6-glycosidic bond in α-limit dextrins liberating glucose.
- *α-Glucosidase (maltase)* removes single glucose residues from α (1→4)-linked oligosaccharides and disaccharides (maltose).
- *β-Glycosidase (lactase)* removes galactose from lactose and attacks cellobiose and other β- glycosides. In addition it has a second catalytic site that splits glycosylceramides.
- *Trehalase* hydrolyzes trehalose.

Many of these hydrolases are located on the outer surface of epithelial cells lining the small intestine. These cells have many fingerlike folds called microvilli that markedly increase their surface area for digestion and absorption of nutrients.

9.2 Absorption of Monosaccharides

The monosaccharides released in digestion then pass with the help of various *sugar-specific transporters* into the cells of intestinal epithelium. Absorption of monosaccharides is carried out by means of facilitated diffusion and the secondary active transport. *Secondary active transport* serves for the uptake of *glucose* and *galactose,* which are transported against of concentration gradient in cotransport with Na^+.

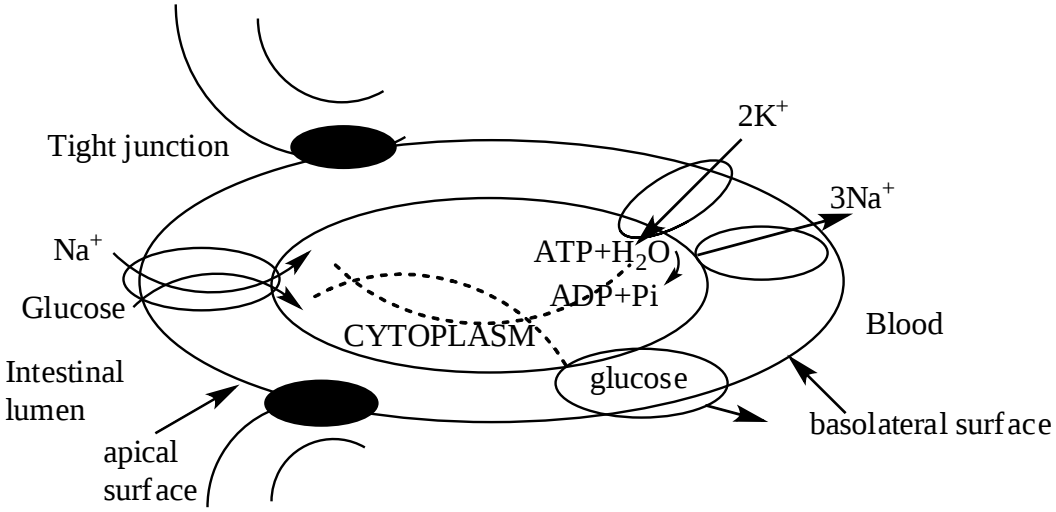


Figure 9.1 Secondary active transport

A specific transporter removes glucose from the lumen, against a concentration gradient. The transporter will also carry galactose and xylose, but glucose competitively inhibits the carriage of both these sugars. Active uptake of glucose occurs because its transport takes place simultaneously together with uptake of Na^+ which enters the cells from its higher to the lower concentration according to the gradient. The energy source for the process is ATP which is used by the Na^+, K^+ - ATPase (in the basolateral surface) to produce an electrochemical gradient of Na. On the basolateral surface, glucose carrier is a single uniporter that allows to facilitate the glucose diffusion from the cells into the blood, according to the concentration gradient.

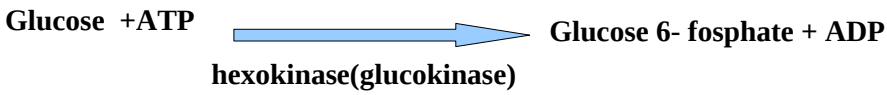
Fructose is taken up by a specific type of transporter using facilitated diffusion.

9.3 Glycogen Metabolism and Its Regulation

Liver has an important role in supporting constant glucose concentration in blood. One of mechanisms which regulates the blood glucose level is the synthesis and degradation of glycogen. Two major sites of glycogen storage are liver and skeletal muscles. The glycogen concentration is higher in liver (about 5%) than in skeletal muscle (about 1%), but more guantity of glycogen is stored in skeletal muscles because of their much greater mass.

Glycogen Synthesis

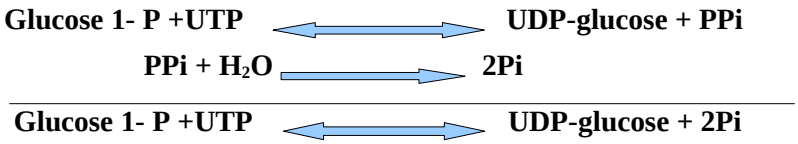
Glycogen synthesis begins from glucose phosphorylation. The reaction is catalyzed by hexokinase. The reaction is essentially irreversible:



In different tissues there are several enzymic forms. Most hexokinases have a low Km for glucose, so they are saturated with this substrate, and an increased intracellular glucose concentration does not increase hexokinases activity.

However hexokinase IV (also called glucokinase), which is the major isoenzyme in liver, is induced by insulin and has Km about 10 mM glucose. It is not inhibited by physiological concentrations of glucose-6-phosphate. Therefore the rate of phosphorylation by glucokinase increases with the raise the intracellular glucose concentration, so that the liver can respond to an elevated plasma glucose concentration by increasing the rate of glucose-6-phosphate formation.

Glucose 6-phosphate is converted into glucose-1-phosphate by phosphoglucomutase. The glucose donor in the biosynthesis of glycogen is an activated form of glucose, that is, UDP - glucose. UDP - glucose is synthesized from glucose 1- phosphate and uridine triphosphate (UTP) during the reaction catalyzed by glucose 1-phosphate uridyltransferase (UDP-glucose-pyrophosphorylase). The above reaction is readily reversible.



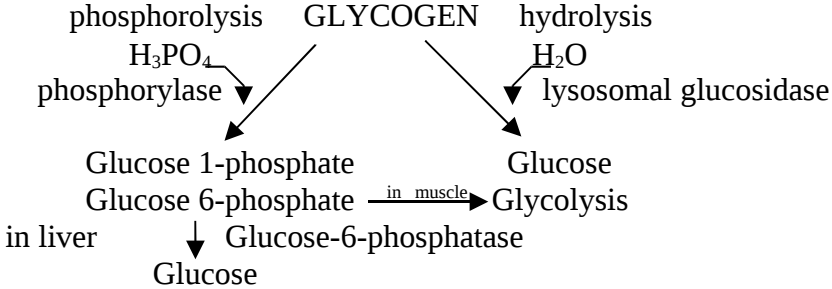
However pyrophosphate is rapidly hydrolyzed in vivo to orthophosphate by an inorganic pyrophosphatase. Practically the irreversible hydrolysis of pyrophosphate drives the synthesis of UDP-glucose (many biosynthetic reactions are driven by the hydrolysis of pyrophosphate).

New glucosyl units are added to the nonreducing terminal residues of glycogen. The activated glucosyl unit of UDP-glucose is transferred to hydroxyl group at a C-4 terminus of glycogen to form an α-1,4 -glycosidic linkage. This reaction is catalyzed by glycogen synthase, which can add glucosyl residues only if the polysaccharide chain already contains more than four residues.Thus glycogen synthesis requires a primer, which in this case consist of some glucosyl units. This priming function is carried out by a protein containing an oligosaccharide of α-1,4-glucose units attached to the phenolic oxygen atom of a tyrosine residue.

Glycogen synthase catalyzes only the synthesis of α-1,4-linkages. Another enzyme namely branching enzyme or amylo – (1,4→1,6)-glucosyltransferase, is needed to form the α-1,6-linkages that make glycogen as branched polymer. *Branching* is important because it increases the solubility of glycogen. Furthermore, branching creates a large number of terminal residues, which are the sites of action of glycogen phosphorylase and synthase. Thus, branching increases the rate of glycogen synthesis and degradation.

Glycogen Breakdown

Glycogen cleavage is performed by two pathways: hydrolysis and phosphorolysis.



Hydrolysis is carried out by hydrolytic amylases (which are digestion enzymes) and by acid glucosidase which is located in lysosomes.The deficiency of the latter enzyme causes glycogen accumulation in many tissues and is fatal. This disease is called type II glycogen storage disease or Pompe's disease.

Phosphorolysis is performed by glycogen phosphorylase which catalyzes the sequential removal of glucosyl residues from the nonreducing end of the glycogen molecule. The bond between the C-1 carbon atom of the terminal residue and C-4 of the adjacent one is cleaved by orthophosphate.

Glycogen + Pi ⟺ glucose-1-phosphate + glycogen
(n residues) (n-1 residues)

The reaction catalyzed by phosphorylase is readily reversible in vitro. However, phosphorolysis proceeds far in the direction of glycogen breakdown in vivo because the ratio of inorganic P to glucose 1-phosphate is usually greater than 100.

The phosphorolytic cleavage of glycogen is energetically advantageous because the released sugar is phosphorylated. In contrast, a hydrolytic cleavage would yield glucose, which would have to be phosphorylated at the expense of an ATP to enter the glycolytic pathway. An additional advantage of phosphorolytic cleavage for muscle cells is that glucose 1-phosphate, ionized under physiological conditions, cannot diffuse out of the cell, whereas glucose can do. Pyridoxal phosphate participates in the phosphorolytic cleavage of glycogen. In muscles 50-80 % of pyridoxal phosphate is linked with phosphorylase.

Glycogen is degraded to a limited extent by phosphorylase alone. The α-1,6-glycosidic bonds at the branch points are not susceptible to cleavage by phosphorylase. Phosphorylase stops cleaving α-1,4-linkages when it reaches the fourhth terminal residue from a branch point.

Debranching is brought about by two reactions. The first: the transfer of a part of α-1,4 chain from one side chain to another, leaving a single glucose residue linked α-1,6.

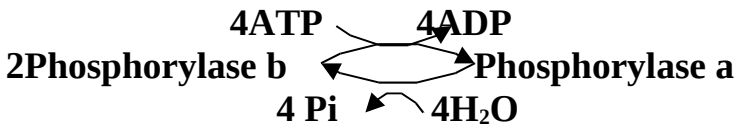
The second: hydrolysis of the α-1,6 linked “stump” to give a molecule of free glucose.The two reactions are both catalyzed by the same protein (debranching enzyme). Debranching enzyme shows both transferase and amylo α-1,6-glucosidase activity. Now phophorylase can attack the remaining α-1,4-linked chain. The isomerization of the product, that is, glucose 1-phosphate, to glucose-6-phosphate is catalyzed by phosphoglucomutase. Phosphorylated glucose, in contrast to glucose, cannot readily diffuse out of cells. The liver contains a hydrolytic enzyme, glucose-6-phosphatase, that enables glucose to leave that organ. Glucose 6-phosphatase is also present in kidneys and intestine, but it is absent in muscles and brain.

Liver stores and releases glucose primarily for the benefit of other tissues.

Regulation of Glycogen Metabolism

The synthesis and degradation of glycogen are under hormonal control, acting through phosphorylation of glycogen synthase and glycogen phosphorylase.

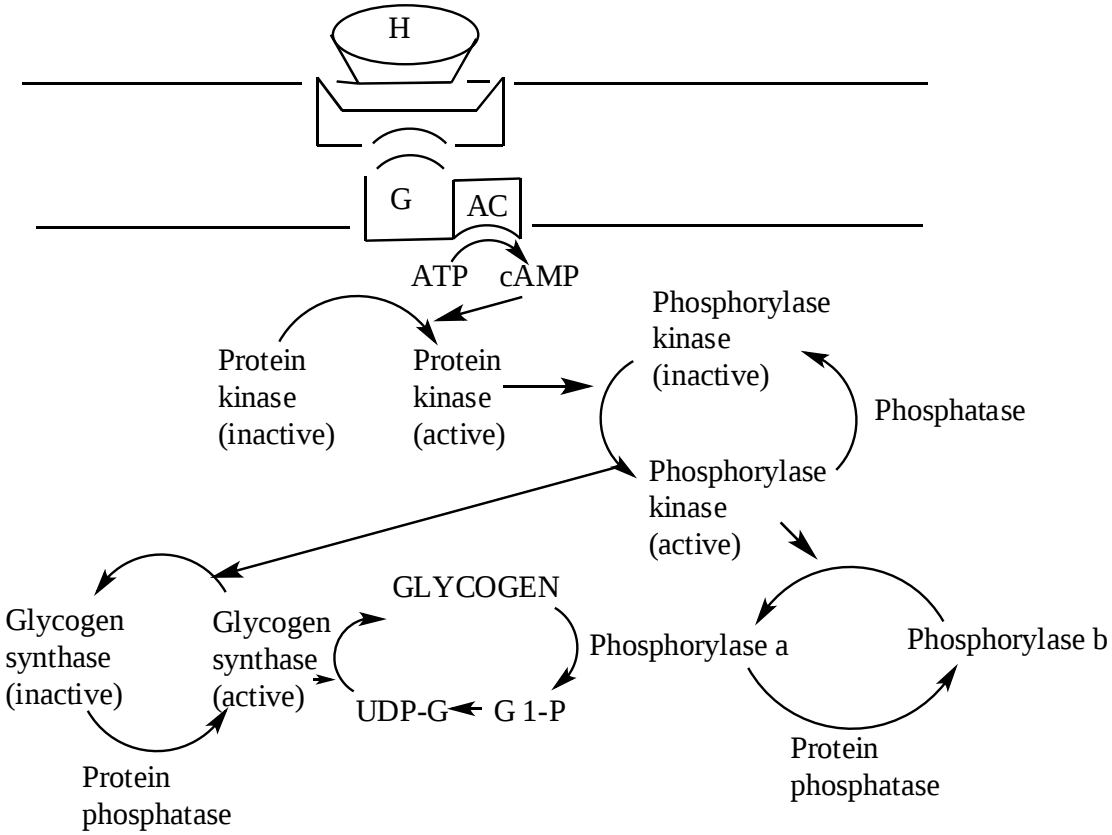
Cyclic AMP, the concentration of which is increased by glucagon and adrenaline, activates cAMP-dependent protein kinase. The latter, that is, protein kinase, phosphorylates a specific protein kinase, called phosphorylase kinase. Glycogen phosphorylase exists in two forms, called a and b. Phosphorylase b is a dimer of identical subunits each of which can be phosphorylated on a single serine. During phosphorylation, the dimers associate to form tetrameric phophorylase a, which is much more active than the b form.



Both cAMP-dependent protein kinase and phosphorylase kinase can activate phosphorylase by this way.

The glycogen synthase activity is also hormonally controlled. The interconversion of active (dephosphorylated) and inactive (phosphorylated) forms is catalysed by cAMP-dependent protein kinase. Insulin increases protein

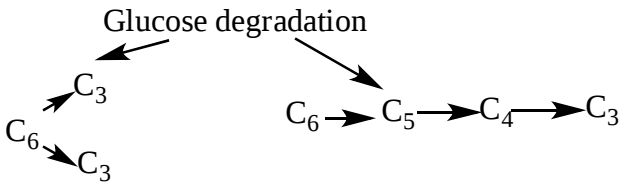
phosphatase activity. The consequent activation of glycogen synthase and deactivation of phosphorylase leads to a build-up of the glycogen store.



- Hormones such as epinephrine and glucagon bind with the receptors in plasma membrane and trigger the activation of adenylyl cyclase.
- Adenylyl cyclase in the plasma membrane catalyzes the formation of cAMP from ATP.
- The increased intracellular level of cyclic AMP activates protein kinase.
- The protein kinase phosphorylates both phosphorylase kinase and glycogen synthase. The phosphorylation of both enzymes is the basis of the coordinated regulation of glycogen synthesis and breakdown. Several other kinases also act on glycogen synthase.

9.4 Aerobic and Anaerobic Oxidation of Glucose

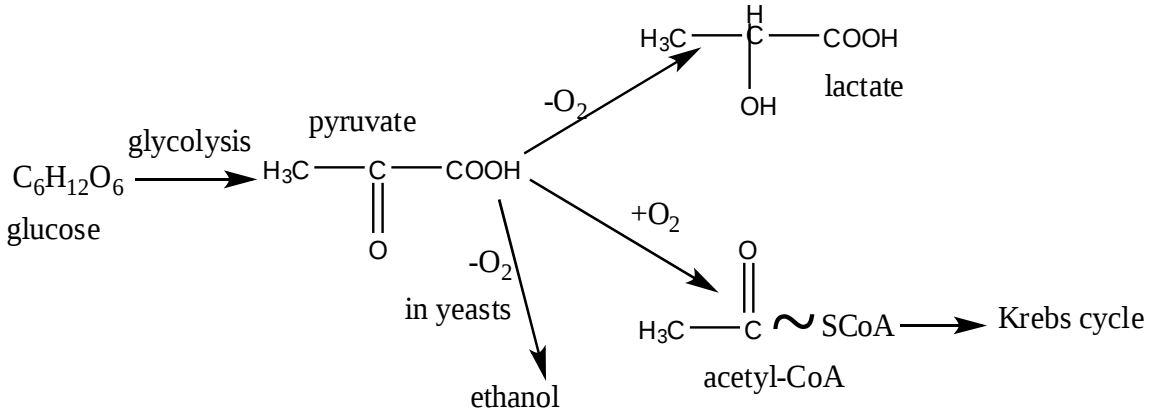
Glucose plays an important role for living organisms. In organism glucose may be degraded by 2 pathways: dichotomic and apotomic ones.



dichotomic pathway
(or glycolytic,
Embden-Meyerhoff
indirect oxidation)

apothomic pathway
(or pentose phosphate
pathway, direct oxidation)

Glycolysis is the sequence of reactions that convert glucose into pyruvate with the concomitant production of ATP. In aerobic organisms glycolysis is the prelude to the citric acid cycle and the electron-transport chain. Citric acid cycle and electron-transport chain together harvest most of the energy contained in glucose. Under aerobic conditions pyruvate enters mitochondria, where it is completely oxidized to CO_2 and H_2O. If the oxygen supply is insufficient, as it takes place in actively contracting muscles, pyruvate is converted into lactate. In some anaerobic organisms, such as yeast, pyruvate is transformed into ethanol.

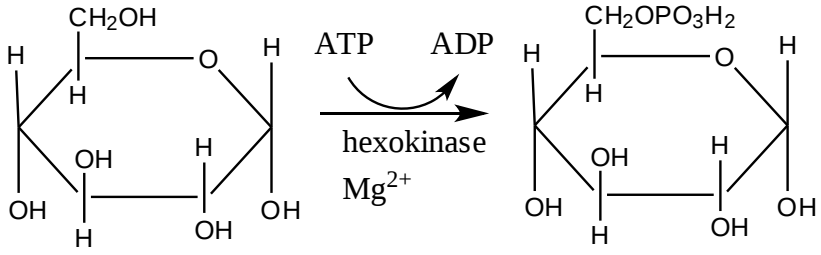


In the cell cytosol glycolysis is performed in two stages.

The preceding stage

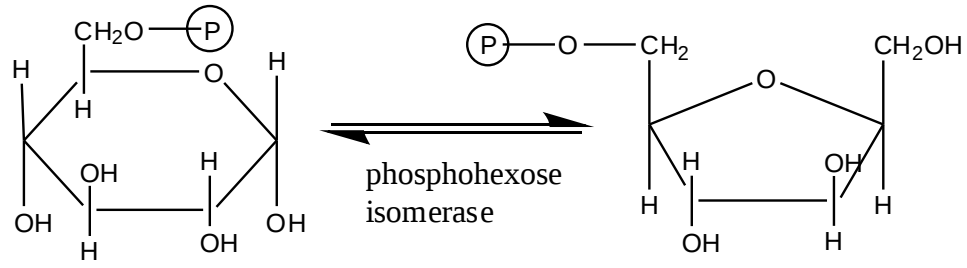
The strategy of this stage is to form compound that can be readily cleft into phosphorylated three-carbon units.

Glucose enters most cells by a specific transport protein and has one principal fate: it is phosphorylated by ATP to form glucose-6-phosphate. Phosphoryl group transfer from ATP to the hydroxyl group on C-6 of glucose is catalysed by hexokinase.



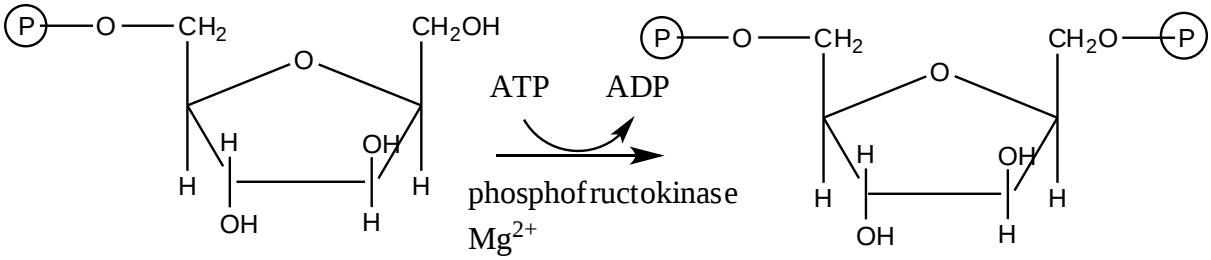
glucose glucose 6-phosphate

Phosphoryl transfer is a basic reaction in biochemitry. An enzyme that catalyzes the transfer of a phosphoryl group from ATP to an acceptor is called a kinase. Hexokinase, like all other kinases, reguires Mg (or another divalent metal ion such as Mn) for activity. The divalent metal ion forms a complex with ATP. The next glycolysis step is the isomerization of glucose-6-phosphate to fructose-6-phosphate.



glucose 6-phosphate fructose 6-phosphate

The second phosphorylation reaction follows the isomerization step. Fructose 6-phosphate is phosphorylated by ATP to fructose 1,6-bisphosphate. This compound was formely known as fructose 1,6-diphosphate. Bisphosphate means two separated phosphate groups, whereas diphosphate means two joined phosphate groups. Hence, the name fructose 1,6-bisphosphate is preferable.

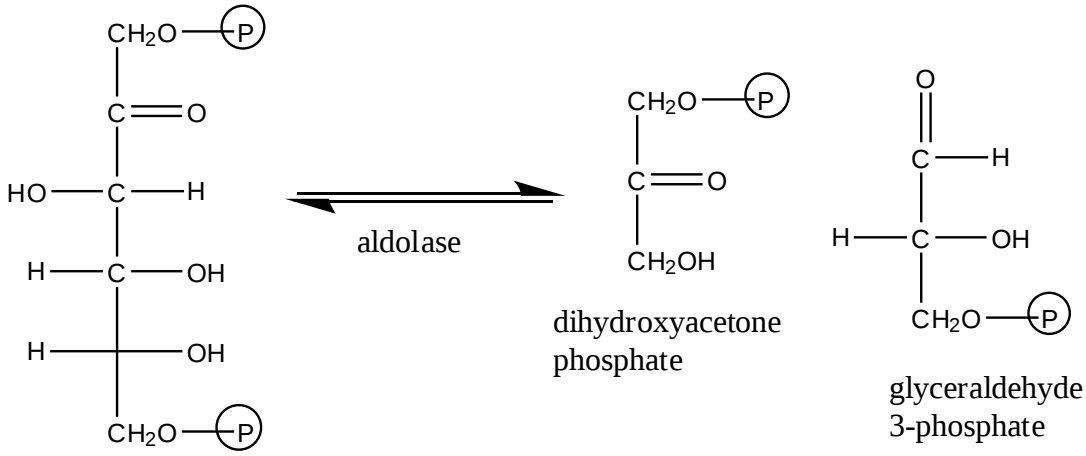


fructose 6-phosphate fructose 1,6-bisphosphate

This reaction is catalyzed by phosphofructokinase, an allosteric enzyme. The process of glycolysis is critically dependent on the level of activity of

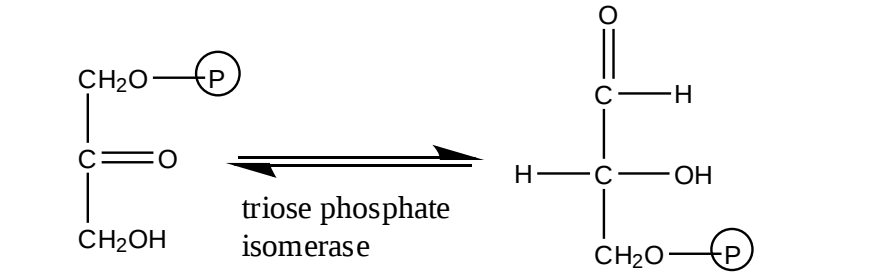
phosphofructokinase, which is allosterically controlled by ATP and several other metabolites.

Next reaction is the splitting fructose 1,6-bisphosphate into glyceraldehyde 3-phosphate and dihydroxyacetone phosphate.



fructose 1,6-bisphosphate

This reaction is catalyzed by aldolase. Glyceraldehyde 3-phosphate enters the second stage of glycolysis, while dihydroxyacetone phosphate does not, but it can be readily converted into glyceraldehyde 3-phosphate.The isomerization of these three-carbon phosphorylated sugars is catalyzed by triose phosphate isomerase.



dihydroxyacetone phosphate (*a ketose*)

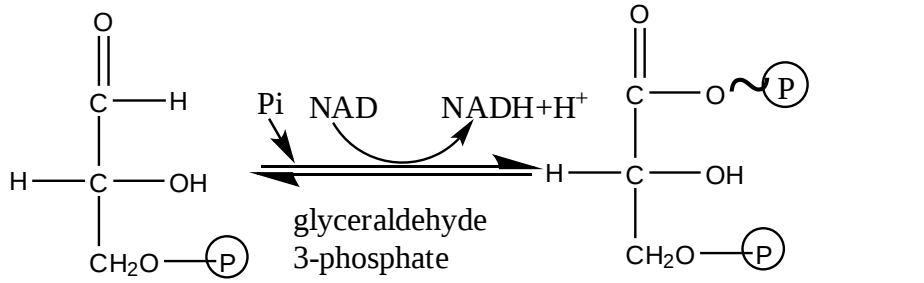
glyceraldehyde 3-phosphate (*an aldose*)

This reaction is rapid and reversible. At equilibrium, 96% of the triose phosphate is dihydroxyacetone phosphate. However, the reaction proceeds readily from dihydroxyacetone phosphate to glyceraldehyde 3-phosphate because of efficient removal of this product.

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

The second stage. The oxidation of glyceraldehyde 3-phosphate and energy conservation.

The preceding steps in glycolysis transform one molecule of glucose into two molecules of glyceraldehyde 3-phosphate and no energy is extracted. On the contrary, two molecules of ATP are formed in the second stage. The initial reaction in this sequence is the conversion of glyceraldehyde 3-phosphate into 1,3-bisphosphoglycerate (1,3-BPG). A reaction is catalyzed by glyceraldehyde 3-phosphate dehydrogenase.

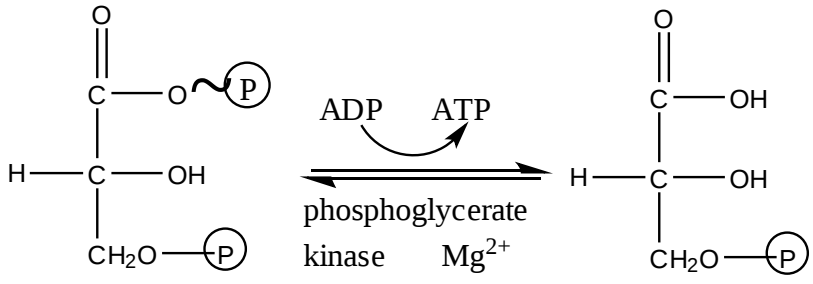


glyceraldehyde 3-phosphate

1,3-bisphosphoglycerate

A high-energy phosphate compound is generated in this oxidation-reduction reaction.

During the next step, the high phosphoryl transfer potential of 1,3-BPG is used to generate ATP. Indeed, this is the first ATP-generating reaction in glycolysis. Phosphoglycerate kinase catalyzes the transfer of the phosphoryl group from the acyl phosphate of 1,3-BPG to ADP. This is an example of substrate-level phosphorylation.



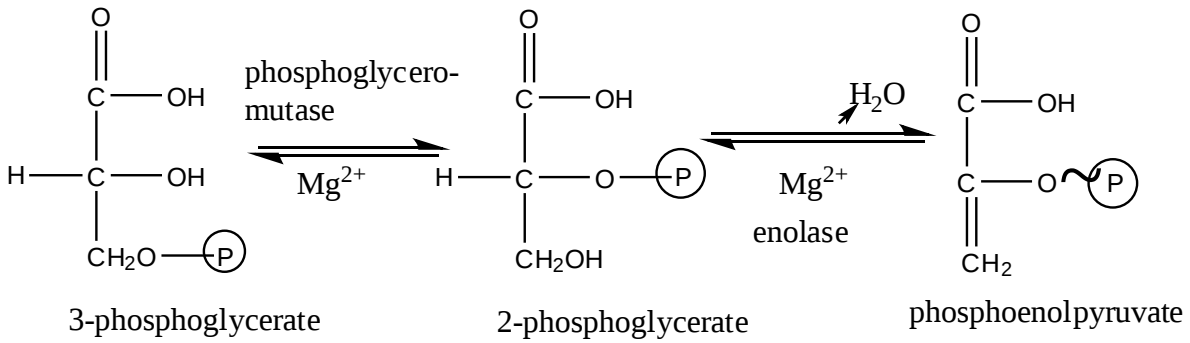
1,3-bisphosphoglycerate

3-phosphoglycerate

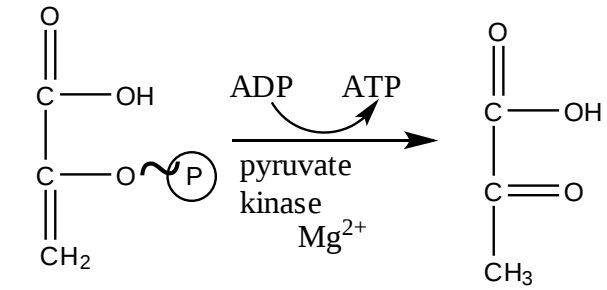
3-Phosphoglycerate is converted into 2-phosphoglycerate by phosphoglyceromutase. In general, a mutase is enzyme that catalyzes the intramolecular shift of a chemical group, such as phosphoryl one.

During the next reaction, an enol is formed by the dehydration of 2-phosphoglycerate. Enolase catalyzes the formation of phosphoenolpyruvate. An enol phosphate has a high phosphoryl-transfer potential.

In the last reaction, pyruvate is formed, and ATP is generated concomitantly. The virtually irreversible transfer of a phosphoryl group from phosphoenolpyruvate to ADP is catalyzed by pyruvate kinase.This is another example of substrate-level phosphorylation.

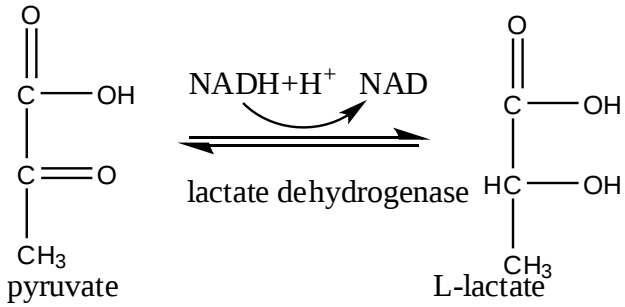


3-phosphoglycerate 2-phosphoglycerate phosphoenolpyruvate



phosphoenolpyruvate pyruvate

Under anaerobic conditions pyruvate is converted into lactate. The reduction of pyruvate by NADH to form lactate is catalyzed by lactate dehydrogenase.

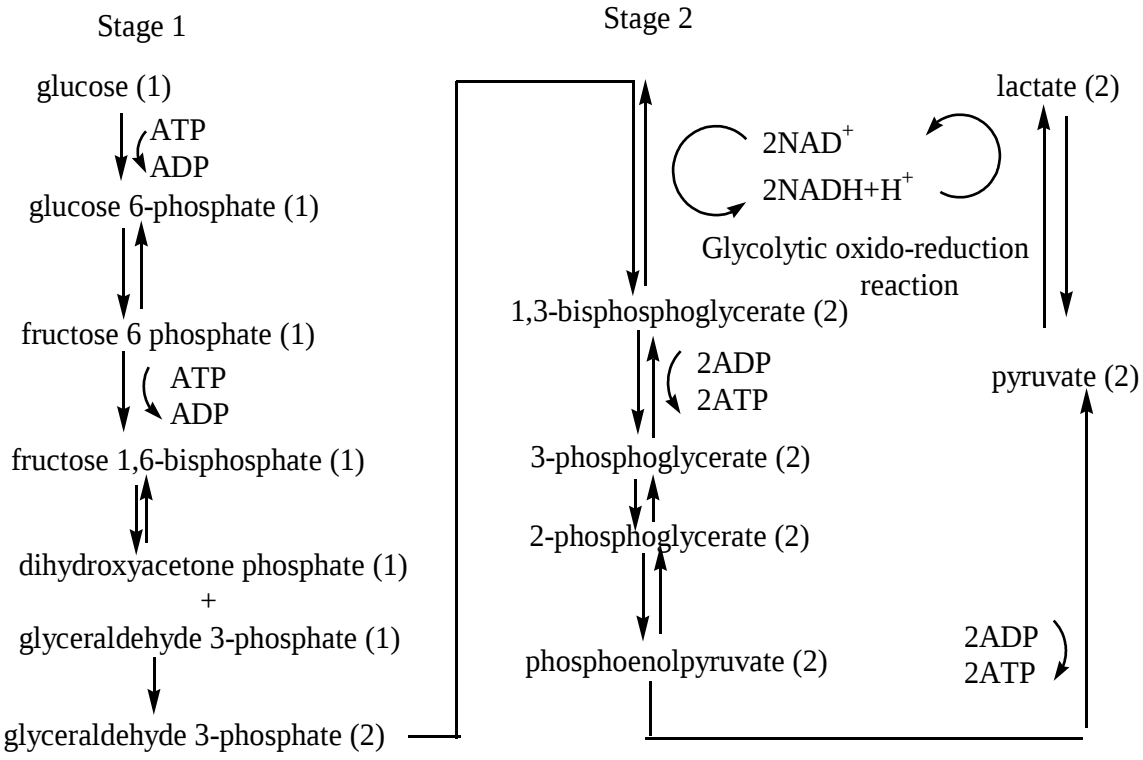


pyruvate L-lactate

Under aerobic conditions pyruvate is converted into acetyl-CoA. This reaction, which is catalyzed by the pyruvate dehydrogenase complex, takes place inside mitochondria.

And then acetyl-CoA is oxidized in citric acid cycle.

Glycolytic oxidation-reduction reaction consists in generation of oxidized NAD for glycolysis.



Under aerobic conditions electrons and protons from NADH are carried through the mitochondrial membrane into the respiratory chain by shuttles.

Shuttle mechanisms of transport of electrons and protons from cytosolic NADH to mitochondrial respiratory chain

Glycerol phosphate shuttle mechanism. One carrier is glycerol 3-phosphate, which readily traverses the outer mitochondrial membrane. The first step in this shuttle is the electrons transfer from NADH to dihydroxyacetone phosphate to form glycerol 3-phosphate. This reaction, catalyzed by glycerol 3-phosphate dehydrogenase, occurs in the cytosol. Glycerol 3-phosphate is reoxidized to dihydroxyacetone phosphate on the outer surface of the inner mitochondrial membrane, that is, an electron pair from glycerol 3-phosphate is transferred to the FAD prosthetic group of the mitochondrial glycerol phosphate dehydrogenase. Then the dihydroxyacetone phosphate diffuses back into the cytosol to complete the shuttle.

The reduced flavin inside mitochondria transfers its electrons to the electron carrier Q, which then enters the respiratory chain as QH_2. Consequently, two ATP

are formed when cytoplasmic NADH transported by the glycerol phosphate shuttle is oxidized by the respiratory chain.

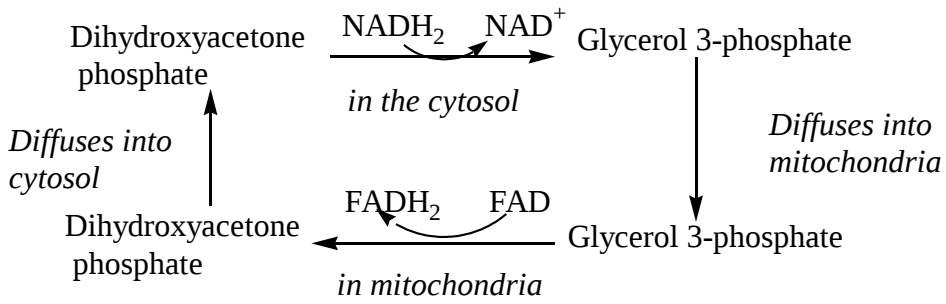


Figure 9.2 Glycerol phosphate shuttle mechanism.

The malate-aspartate shuttle mechanism. At first electrons are transferred from NADH in the cytoplasm to oxaloacetate, forming malate, which traverses the inner mitochondrial membrane and then is reoxidized by NAD in the matrix to form NADH. The resulting oxaloacetate does not readily cross the inner mitochondrial membrane, and so the transamination reaction is needed to form aspartate, which can be ransported to the cytosolic side.

Three ATP are synthesized per NADH transferred.

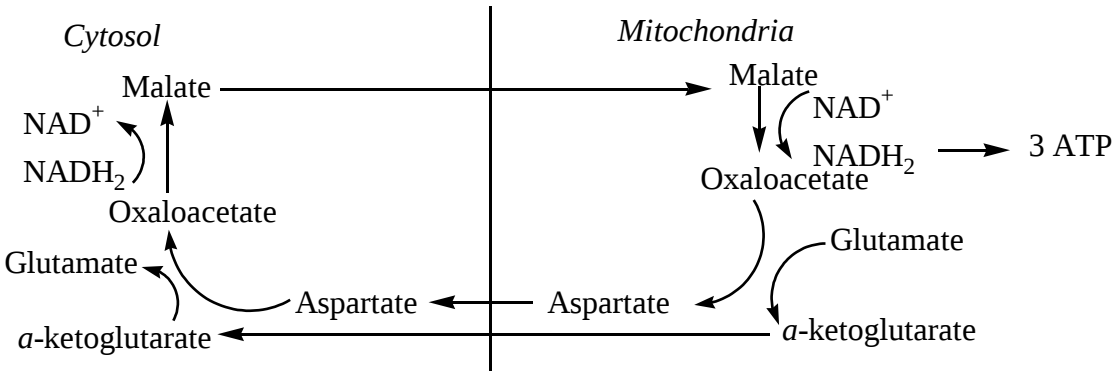


Figure 9.3 Malate-aspartate shuttle mechanism

Energy balance of aerobic and anaerobic glucose degradation

Reaction sequence	**in aerobic conditions**	**in anaerobic conditions**
Glucose phosphorylation	**-1 ATP**	**-1 ATP**
Fructose 6-phosphate phosphorylation	**-1 ATP**	**-1 ATP**
Dephosphorylation of 2 mol of 1,3-BPG	**+2 ATP**	**+2 ATP**
Dephosphorylation of 2 mol of PEP	**+2 ATP**	**+2 ATP**
Oxidation of 2 mol of GAP (2 NADH)	**+4(6)ATP**	**-**

Conversion of 2 molecules of pyruvate into acetyl-CoA (2 NADH)	**+6 ATP**	**-**
Acetyl-CoA (2) TAC	**12x2=24 ATP**	**-**
	Σ=36(38)ATP	**Σ=2 ATP**

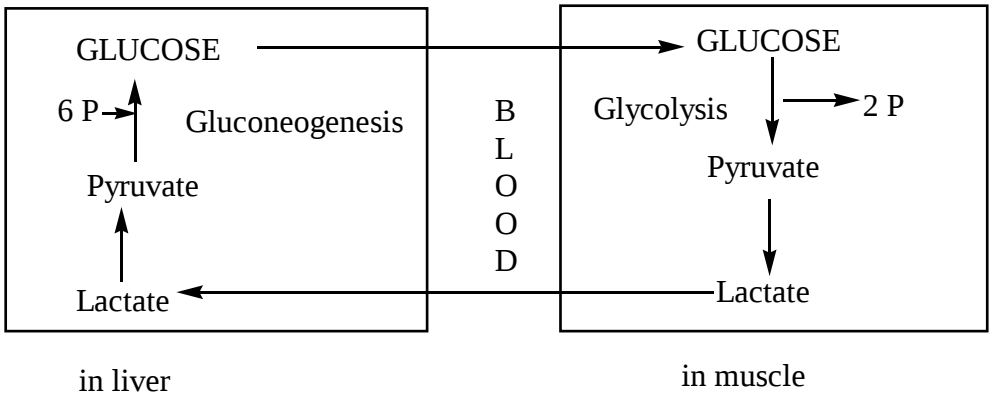
O_2 ⟹ 38 ATP

without O_2 ⟹ 2 ATP 19 mol of glucose without O_2 ⟹ 38 ATP

Anaerobic glucose oxidation is the only energy source under anaerobic conditions. The above mentioned oxidation plays an important role during intensive muscle work, under the conditions of oxygen lack (for example, in cardio-vasculary diseases, while tourniqueting). Moreover, anaerobic pathway of glucose oxidation is the only energy source in mature erythrocytes, which don't have mitochondria. French scientist Louis Pasteur is known to study the problem of fermentation by yeast and to discover the inhibition of glycolysis under aerobic conditions. This effect was called Pasteur's effect. Under aerobic condition the consumption of glucose decreases and accumulation of lactate stops. The inhibition of phosphofructokinase by citrate and ATP occurs during the Pasteur effect.

9.5 Gluconeogenesis. Regulation of Gluconeogenesis and Glycolysis

The lactate concentration in blood is normally about 1 mM. Almost all the rapid changes in blood lactate levels are due to glucose catabolism in muscles. The lactate that enters the liver is oxidized to pyruvate, a reaction which is favoured by the low NADH/NAD ratio in the liver cells cytosol. Then pyruvate is converted into glucose by the gluconeogenic pathway in liver. After that glucose enters the blood and is taken up by skeletal muscles. Thus, liver supplies glucose to contracting skeletal muscle, which derives ATP from glucose glycolytic conversion into lactate, and then glucose is synthesized from lactate by the liver. These reactions constitute the **Cori cycle**.



The interconversions of pyruvate and lactate are facilitated by differences in the catalytic properties of lactate dehydrogenase enzymes in different tissues. Lactate dehydrogenase is a tetramer of two kinds of subunits. The H type predominates in the heart, and the homologous M type - in skeletal muscle and the liver. These subunits associate to form five types of tetramers: H_4, H_3M, H_2M_2, HM_3, and M_4. H_4 is designed to oxidize lactate to pyruvate, which is then utilized as a fuel by the heart. In contrast, M_4 is optimized to operate in the reverse direction, to convert pyruvate to lactate under anaerobic conditions.

Glucose synthesis from noncarbohydrate precursors is **called gluconeogenesis.**

The major noncarbohydrate precursors are **lactate, amino acids** and **glycerol.**

Gluconeogenesis is activated

during long intensive working	**during starvation**
Precursors:	**Precursors:**
1) lactate (from skeletal muscles)	**1) glycerol (from adipocytes)**
2) glycerol (from adipocytes).	**2) amino acids (from break-down of proteins in skeletal muscles)**

The gluconeogenesis from lactate

The major site of gluconeogenesis is liver. Gluconeogenesis also occurs in kidney cortex, but the total amount of glucose formed there is about one-tenth of

that formed in liver. All the glycolysis reactions except reactions catalyzed by hexokinase, phosphofructokinase and pyruvate kinase, are reversible.

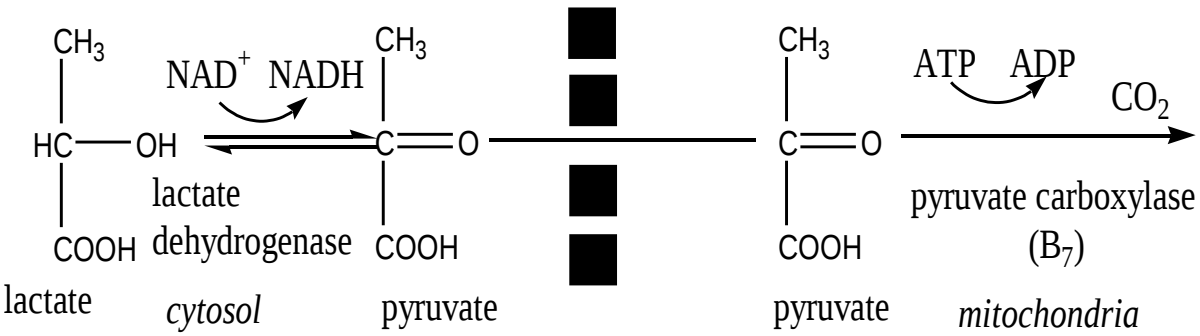
These reactions, which virtually irreversible, are bypassed in gluconeogenesis by the following steps:

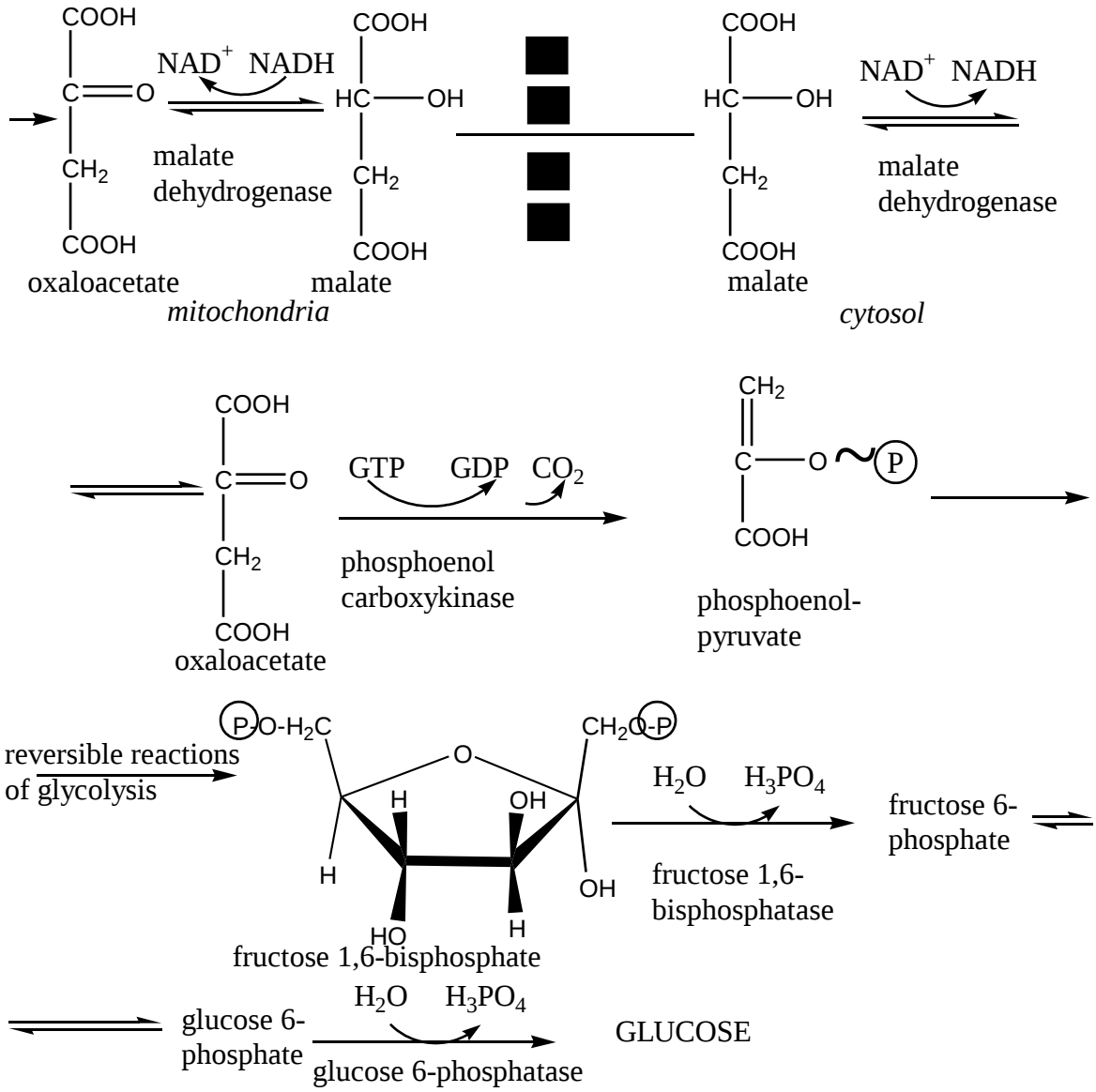
1. Phosphoenolpyruvate is formed from pyruvate by oxaloacetate way. First, pyruvate is carboxylated to oxaloacetate at the expense of ATP. Then, oxaloacetate is decarboxylated and phosphorylated to yield phosphoenol pyruvate at the expense of the second high-energy phosphate bond. The first reaction is catalyzed by pyruvate carboxylase, and the second one by phosphoenol-pyruvate carboxykinase.

2. Fructose 6-phosphate is formed from fructose 1,6-bisphosphate by hydrolysis of the phosphate ester at C-1. Fructose 1,6-bisphosphatase catalyzes this hydrolysis.

3. Glucose is formed by hydrolysis of glucose 6-phosphate in a reaction catalyzed by glucose 6-phosphatase.

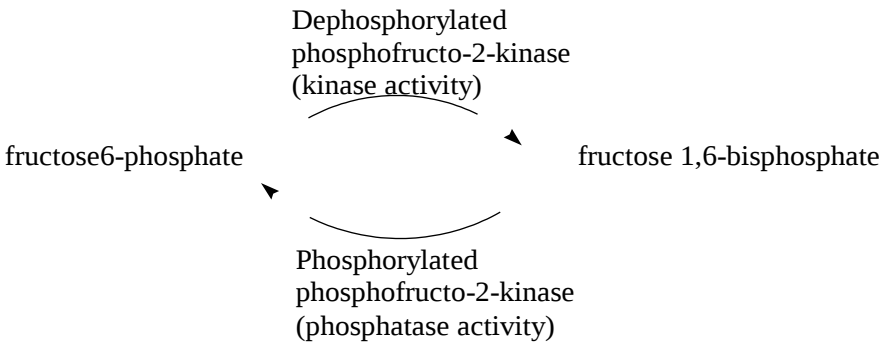
Oxaloacetate utilized in the cytosol for gluconeogenesis is formed in the mitochondrial matrix. Oxaloacetate leaves mitochondria in the form of malate, which is reoxidized to oxaloacetate in the cytosol.



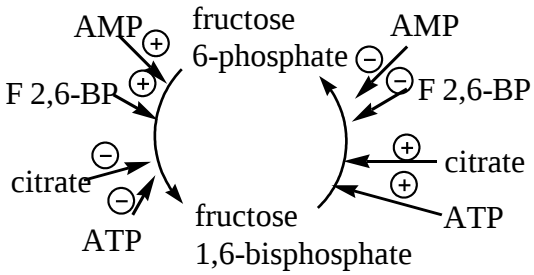


Glycolysis and Guconeogenesis Regulation

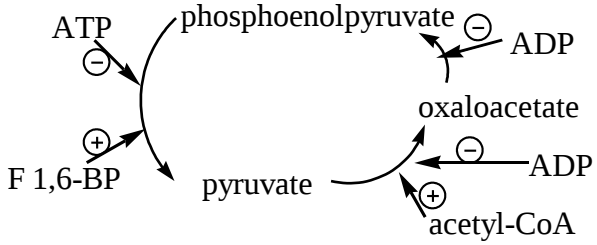
Gluconeogenesis and glycolysis are reciprocally regulated. The intercorversion of fructose 6-phosphate and fructose 1,6-bisphosphate is a key point of gluconeogenesis and glycolysis control. AMP stimulates phosphofructokinase, whereas AMP inhibits fructose 1,6-bipshosphatase. Citrate has the opposite effect. Fructokinase and fructose-1,6-biphosphatase are controlled by fructose 2,6-bisphosphate, a signal molecule derived from fructose 6-phosphate.



Fructose 2,6-bisphosphate (F 2,6-BP) stimulates phosphofructokinase and inhibits fructose 1,6-bisphosphatase. Thus, fructose 2,6-bisphosphate plays an important role in determining whether glucose has to be degraded or synthesized.



Pyruvate kinase and pyruvate carboxylase are also reciprocally regulated. Fructose 1,6-bisphosphate stimulates but ATP inhibits pyruvate kinase, whereas acetyl CoA stimulates but ADP inhibits pyruvate carboxylase. Phosphoenolpyruvate carboxykinase is also nhibited by ADP.



The principal control of gluconeogenesis is hormonal one. Glucocorticoids induce the synthesis of several gluconeogenic enzymes (glucose 6-phosphatase, fructose 1,6-bisphosphatase and phosphoenol-pyruvate carboxykinase).

Glucagon is the most important gluconeogenic hormone, which increases the cAMP concentration in liver and the mentioned increase has two direct stimulatory effects on gluconeogenesis:

1. Protein kinase activation leads to phosphorylation of phosphofructo-2-kinase, reducing its kinase and increasing its phosphatase activity. That leads to a

fall in the concentration of fructose 2,6-bisphosphate, favouring the conversion of fructose 1,6-bisphosphate to fructose 6-phosphate.

2. Pyruvate kinase is also phosphorylated and thereby is inactivated. This directs PEP toward gluconeogenesis, rather than production of acetyl CoA.

Recent studies show, that the muscle phosphofructo-2-kinase catalyzing the synthesis of F-2,6-BP is stimulated, but not inhibited, by cAMP-induced phosphorylation. Thus, adrenaline stimulates glycolysis in muscles but inhibits glycolysis in liver because of the key difference between the isoenzymes. The increased breakdown of liver glycogen induced by adrenaline serves to supply glucose to muscles which rapidly consume it to generate ATP for contractive activity. Insulin increases glycolysis but inhibits gluconeogenesis, because:

- it stimulates all the key glycolysis enzymes (glucokinase, phosphofructokinase and pyruvate kinase);
- insulin stimulates pyruvate carboxylase, but inhibits phosphoenolpyruvate carboxykinase.

9.6 Pentose Phosphate Pathway

The source of readily available reducing power in cells is NADPH. There is a fundamental distinction between NADPH and NADH in most biochemical reactions. NADH is oxidized by the respiratory chain to generate ATP, whereas NADPH serves as electron donor in reductive biosyntheses.

NADPH is generated in the pentose phosphate pathway.

The pentose phosphate pathway is sometimes called the pentose shunt, the hexose monophosphate pathway, or the phosphogluconate oxidative pathway. All the reactions of pentose phosphate pathway occur in the cytosol.

Functions of pentose phosphate bathway:

- One of them is the synthesis of ribose 5-phosphate, which is essential for the synthesis of nucleotides. Nucleotides are used for nucleotide coenzymes formation such as NAD, FAD and HSCoA. In addition, nucleotides are required for the synthesis of RNA and DNA.
- The other function of pentose phosphate pathway is to be a source of NADPH.

- NADPH is utilized for cholesterol and fatty acid syntheses, for detoxification processes.

- NADPH is necessary for stabilization of erythrocyte membranes.

- NADPH is utilized by NADPH oxidase in production of reactive oxygen species needed for phagocytosis.

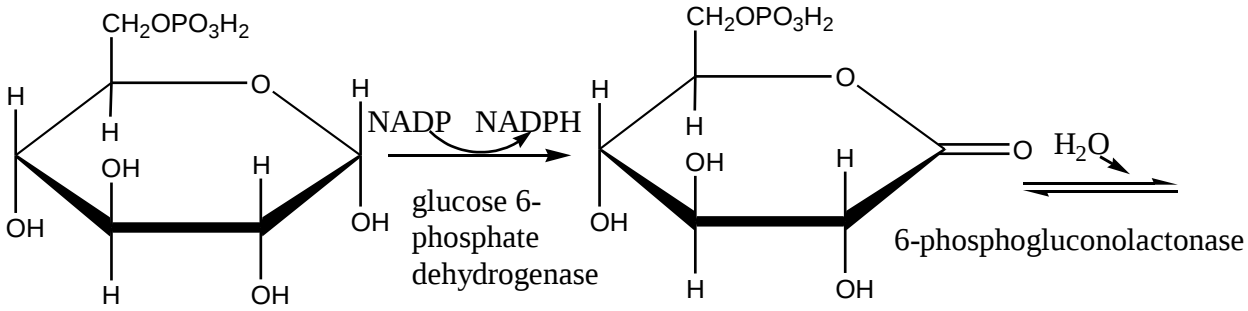
- Pentose phosphate pathway generates CO_2 for synthetic processes.

In mammals, the pentose phosphate pathway is relatively high in liver, adrenal glands, embryonal and adipose tissues, mammary glands in lactation, but it is practically absent in skeletal muscles.

Pentose phosphate pathway consists of two stages: oxidative and nonoxidative phases. Nonoxidative phase includes conversion of pentose phosphates to generate the initial glucose 6-phosphate.

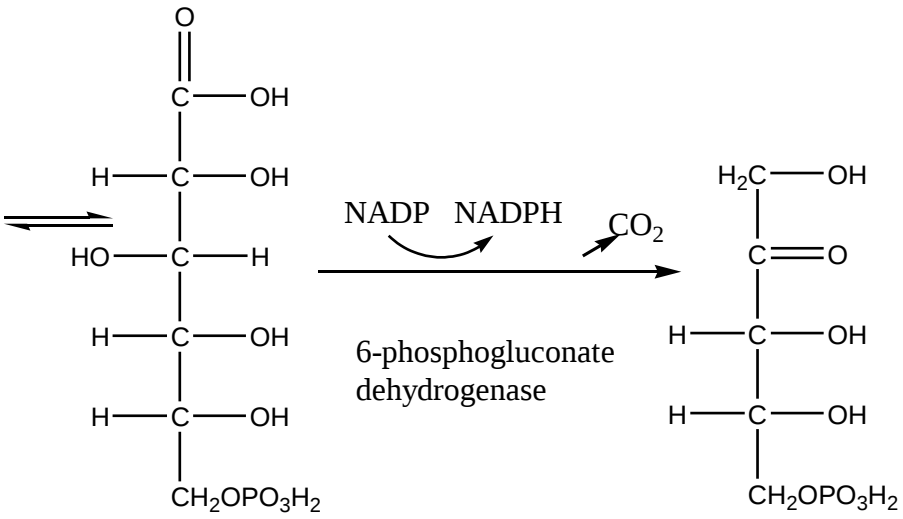
Oxidative phase

The pentose phosphate pathway starts with dehydrogenation of glucose 6-phosphate at C-1. The reaction is catalyzed by glucose 6-phosphate dehydrogenase (coenzyme NADP). The next step of the pathway is the hydrolysis of 6-phosphogluconolactone by a specific lactonase to form 6-phosphogluconate. This six-carbon sugar is then oxidatively decarboxylated by 6-phosphogluconate dehydrogenase to yield ribulose 5-phosphate. And NADP becomes the electrons acceptor again.



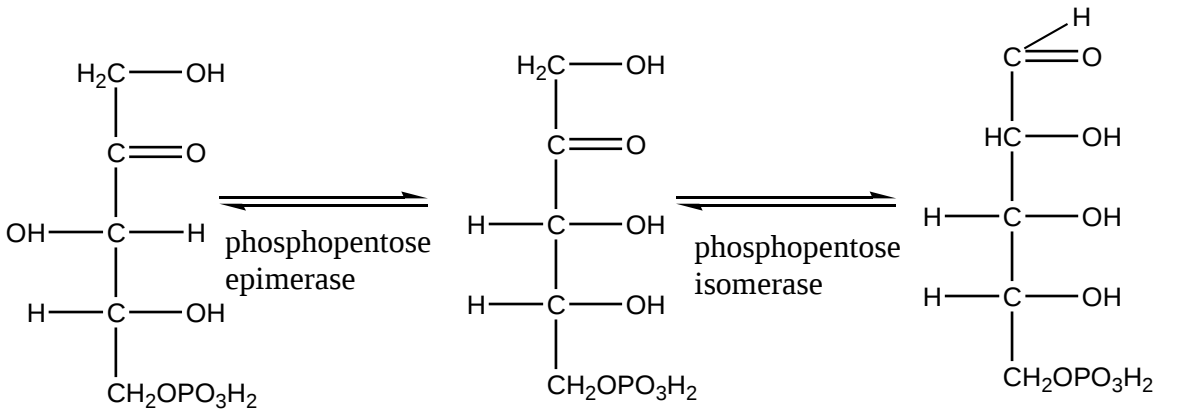
glucose 6-phosphate

6-phosphogluconolactone



6-phosphogluconate ribulose 5-phosphate

This pentose is converted into ribose 5-phosphate by phosphopentose isomerase or into xylulose 5-phosphate by phosphopentose epimerase.



xylulose 5-phosphate ribulose 5-phosphate ribose 5-phosphate

The preceding reactions yield two NADPH and one ribose 5-phosphate for each glucose 6-phosphate oxidized. However, many cells need NADPH for reductive biosynthesis much more than ribose 5-phosphate for incorporation into nucleotides and nucleic acids. In this case, pentoses are involved into the second stage of the pentose phosphate pathway and are converted into glyceraldehyde 3-phosphate and fructose 6-phosphate by transketolase and transaldolase.

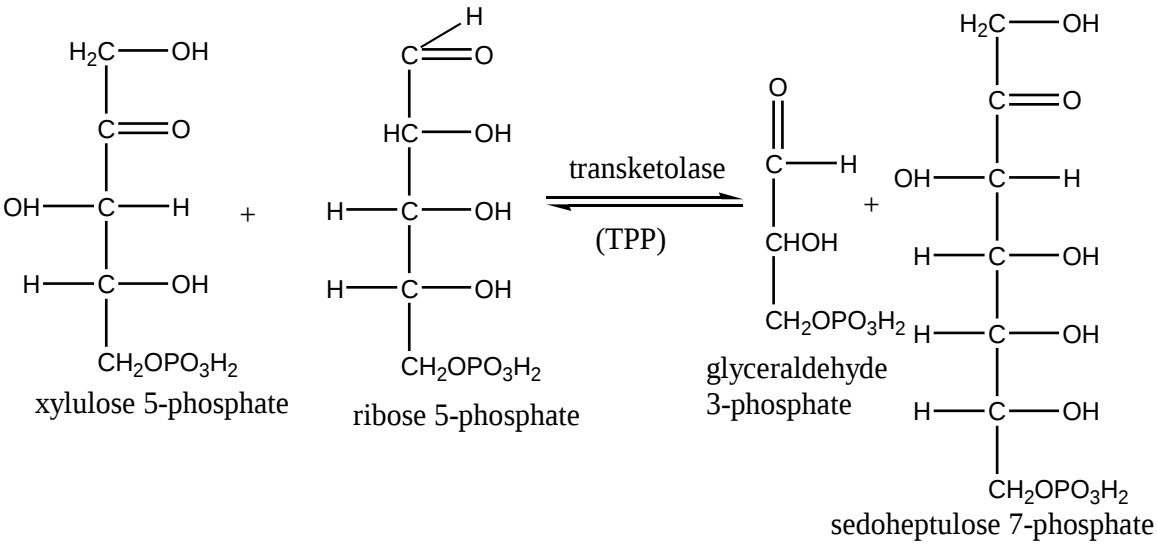
These above mentioned enzymes create a reversible link between the pentose phosphate pathway and glycolysis by catalysing the following three reactions:

C_5 + C_5 transketolase C_7 + C_3

C_3 + C_7 transaldolase C_6 + C_4

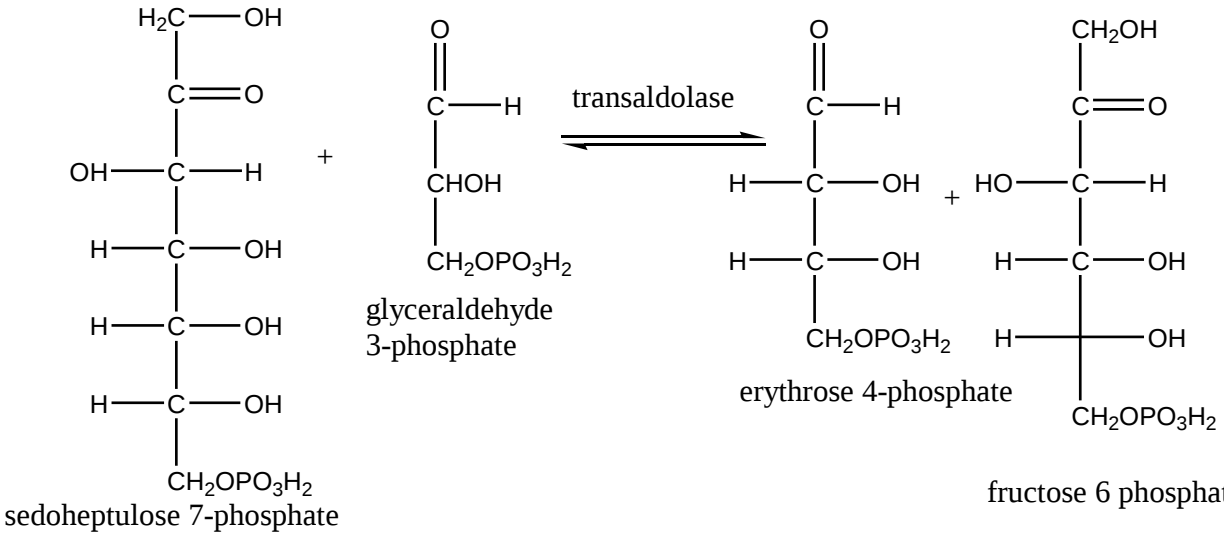
C_5 + C_4 transketolase C_3 + C_6

The net result of these reactions is the formation of two hexoses and one triose from three pentoses. The first of the three reactions is the formation of glyceraldehyde 3-phosphate and sedoheptulose 7-phosphate from two pentoses.

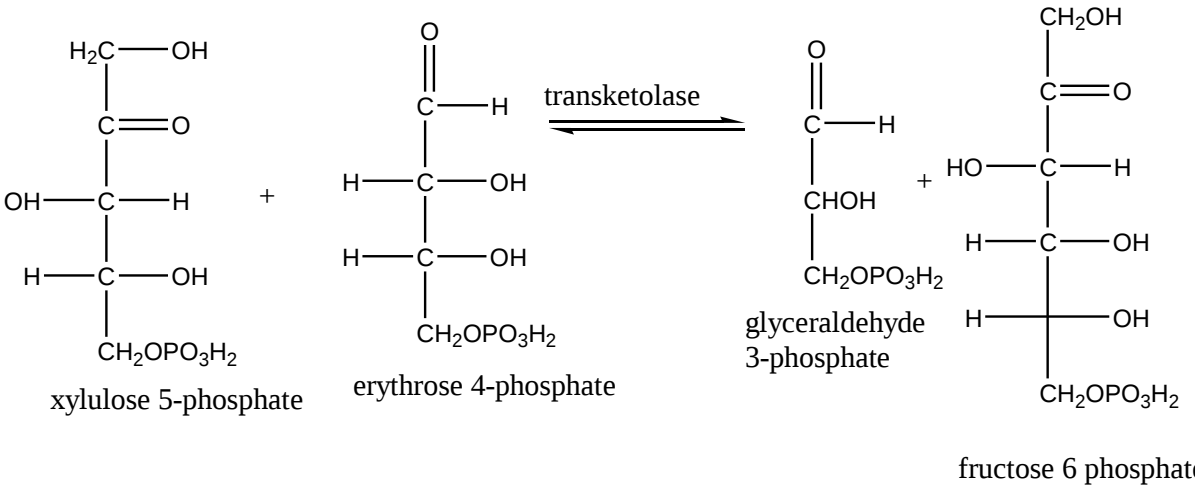


Transketolase contains a tightly bound thiamine pyrophosphate (TPP) as its prosthetic group. The donor of the twocarbon unit in the first reaction is xylulose 5-phosphate, which is an epimer of ribulose 5-phosphate.

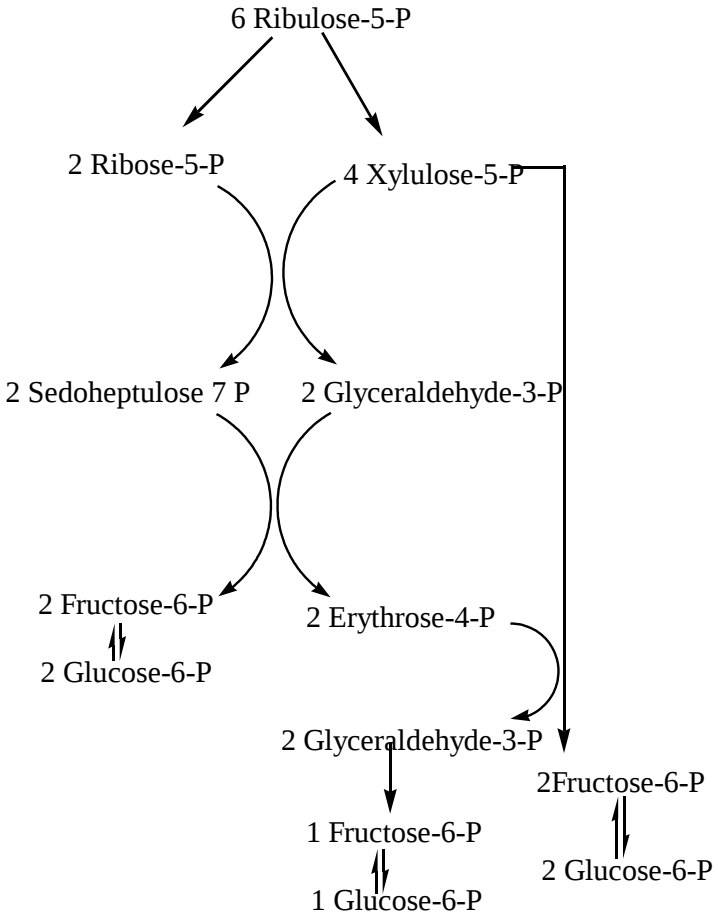
During the second reaction glyceraldehyde 3-phosphate and sedoheptulose 7-phosphate react to form fructose 6-phosphate and erythrose 4-phosphate. This reaction is catalyzed by transaldolase.



In the third reaction, transketolase catalyzes the synthesis of fructose 6-phosphate and glyceraldehyde 3-phosphate from erythrose 4-phosphate and xylulose 5-phosphate.



Thus six pentoses are converted into four hexoses and two trioses. Two latters form hexose.



Pentose phosphate pathway regulation

The first reaction in the oxidative branch of the pentose phosphate pathway, namely the dehydrogenation of glucose 6-phosphate, is essentially irreversible. In fact, this reaction is rate-limiting under physiological conditions and serves as the control site. The most important regulatory factor is the level of NADP, which is

the electron acceptor in the oxidation of glucose 6-phosphate to 6-phosphogluconolactone.

The ratio of NADP to NADPH in the cytosol of liver cells from a well fed rat is about 0,014, that is, several orders of magnitude lower than the ratio of NAD to NADH, which is 700 under the same conditions.

Insulin is known to stimulate the pentose phosphate pathway by activating glucose 6-phosphate dehydrogenase.

Pentose phosphate pathway disturbances

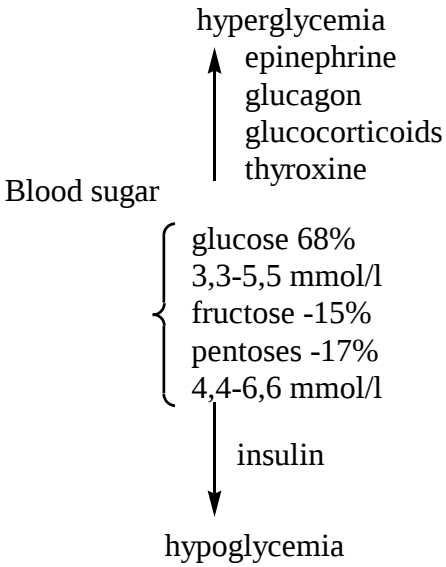
There are two pentose phosphate pathway disturbances:

1. The first, so called ***Wernicke-Korsakoff syndrome***, a striking neuropsychiatric disorder, is caused by a lack of thiamine in the diet of susceptible persons. Transketolase from patients with Wernicke-Korsakoff syndrome binds thiamine pyrophosphate tenfold less avidly than does the enzyme from normal persons. The disease is characterized by paralysis of eye movements, abnormal postage and gait, and markedly diminished mental function.

2. ***Drug-induced hemolytic anemia***. The basis of this disease is the deficiency of glucose 6-phosphate dehydrogenase in red cells. The pentose phosphate pathway is the only source of NADPH in red cells because of lack of mitochondria, and so the production of NADPH by them is markedly diminished by this deficiency. The major role of NADPH is to reduce the disulfide form of glutathione to the sulfhydryl form. Reduced glutathione is essential for maintaining the normal structure of red cells and for keeping hemoglobin in the ferrous state. Cells with a lowered level of reduced glutathione are more susceptible to hemolysis.

9.7 Regulation of Blood Sugar

The concentration of true glucose in the blood normally ranges from about 3,3 to 5,5 mmol/L. The term “blood sugar” includes glucose and other monosaccharides levels. Normal blood sugar is 4,4-6,6mmol/L.



Supporting glucose constant level in blood is important for organism, because the increase and decrease of glucose lead to negative consequences. The increased glucose level leads to atherosclerosis, obesity and diabetes mellitus. The decreased level of glucose results in fainting.

Glucose takes an active part in its level regulation itself. The rise in the plasma glucose concentration is the major stimulus for releasing insulin. Insulin is stored in the β -cells of the pancreatic islets of Langerhans, and is released by exocytosis. Glucose concentration up to 5 mM has little stimulatory effect. But the insulin secretion stimulation is half maximally in glucose concentration of 8 mM. Insulin stimulates the uptake and utilization of glucose. Namely:

- In skeletal muscle and adipose tissue insulin stimulates the rate of glucose uptake.
- Insulin stimulates glycogen deposition by activation of glycogen synthase and inhibition of glycogen phosphorylase.
- It stimulates glycolysis by induction of glucokinase, phosphofructokinase and pyruvate kinase.
- Insulin inhibits gluconeogenesis by decreasing activity of phosphoenolpyruvate carboxykinase.
- Glucose conversion to fatty acids is stimulated in all tissues through effect on pyruvate dehydrogenase and acetyl CoA carboxylase.

The decreased glucose level leads to glucagon releasing. Glucagon shows hyperglycemic effect as it increases glycogen degradation by stimulation of glycogen phosphorylase and decreases glycogen synthesis by inhibition of glycogen synthase. Glucagon also stimulates gluconeogenesis and inhibits glycolysis by decreasing fructose 2,6-bisphosphate concentration.

Blood glucose level is influenced by adrenaline which shows hyperglycemic effect, because it elevates the glycogen degradation by cAMP-dependent mechanism. Glucocorticoids increase glucose level because they stimulate gluconeogenesis enzyme synthesis.

9.8 Disturbances of Carbohydrate Metabolism

Carbohydrate metabolism disturbances

- The diseases caused by deficiency of enzymes
- The disturbances of the hormonal regulation of carbohydrate metabolism

Diseases caused by enzyme deficiencies:

I. Lactose intolerance is due to the lack of *lactase*.

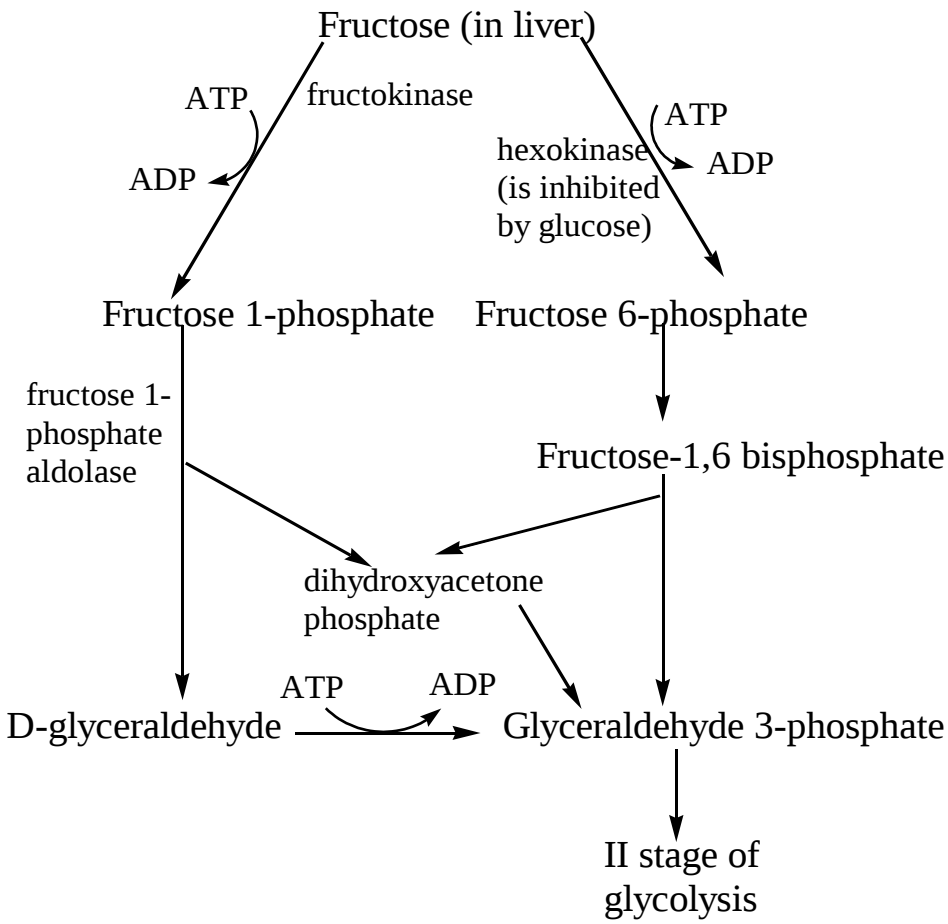
Symptoms: abdominal cramps, diarrhea, and flatulence.

II. Sucrase Deficiency: There is an inherited deficiency of sucrase and isomaltase. These two deficiencies coexist, because sucrase and isomaltase occur together as a complex enzyme. Symptoms occur in early childhood and are the same as those described in lactase deficiency.

III. Hereditary disturbances of fructose metabolism

- **Essential fructosuria**
- **Hereditary fructose intolerance**

The main way of fructose involvement into glycolysis



- ***Fructosuria*** is caused by the lack of *fructokinase*

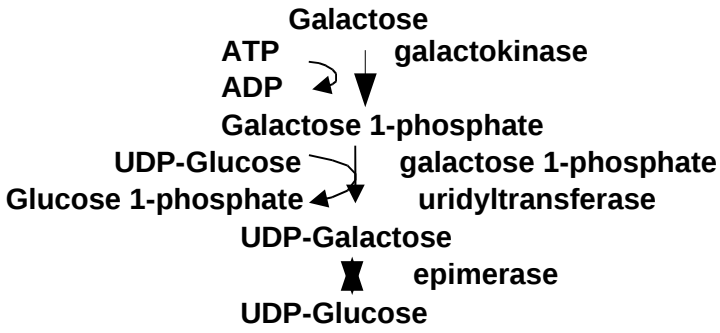
- Clinical symptoms are absent
- Fructosemia
- Fructosuria

} are observed

- ***Hereditary fructose intolerance*** is due to absence of hepatic *fructose-1-phosphate aldolase. Clinical manifestations:*
  - Severe failure of liver
  - Convulsions
  - Vomiting
  - Faintness

IV. Galactosemia is linked with the deficiency of *galactose-1-phosphate uridyl transferase.*

Galactose is involved in glycolysis by two following reactions sequence:



The symptoms of galactosemia:

- *Liver enlargement (often accompanied by jaundice)*
- *Cataract*
- *Mental retardation*

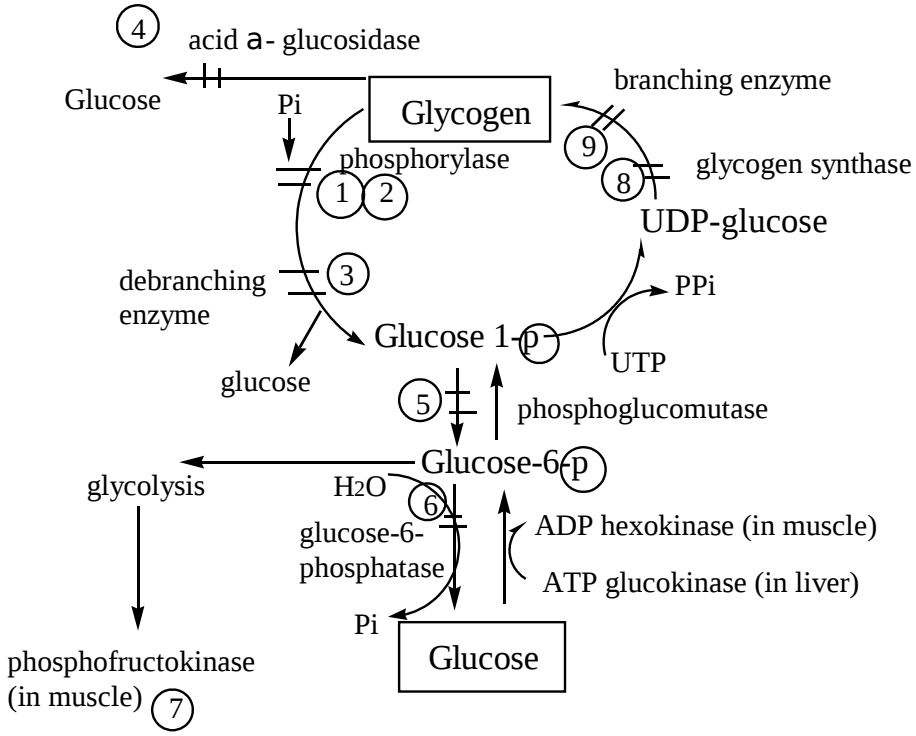
The course of galactosemia treatment consists of the ***exclusion of galactose from diet.***

Since the epimerase reaction is freely reversible glucose can be converted to galactose, so that preformed galactose is not essential in the diet. Galactose is required in the body not only in the formation of lactose but also as a constituent of glycolipids (cerebrosides), proteoglycans and glycoproteins.

V. Drug-induced hemolytic anemia. The basis of this disease is the deficiency of *glucose-6-phosphate dehydrogenase in erythrocytes.*

VI. Glycogen storage diseases. The common symptoms of most of glycogen storage disease are: hypoglycemia, acidosis, lipidemia.

Scheme of synthesis and degradation of glycogen



1. Type VI - Her's disease *is due to a deficiency of liver phosphorylase*

- Accumulation of glycogen of normal structure.
- Hepatomegaly
- Mild hypoglycemia
- Lipidemia
- Mild acidosis
- Adrenaline and glucagon tests are negative (after administration of adrenaline or glucagon the rise of glucose in blood doesn't occur)

2. Type V Mc Ardle's disease is caused by deficiency of *muscle phosphorylase.*

- Accumulation in muscles of glycogen of normal structure
- Muscle cramps on exercise
- Pain
- Weakness
- Adrenaline test is positive

3. Type III. Cori's (or Forbe's) disease *(limit dextrinosis) is caused by* deficiency of *debranching enzyme*

- Glycogen of *abnormal* structure (short outer chains) is accumulated in liver, muscle and heart; leukocytes
- Hepatomegaly
- Moderate hypoglycemia
- Acidosis
- Progressive myophathy
- Adrenaline and glucagon tests are negative

4. Type II. Pompe's disease is due to a deficiency of *acid α-glucosidase (lysosomal enzyme)*

- Glycogen of normal structure is accumulated in different tissues
- Hypoglycemia, lipidemia and ketonuria *are absent*
- Adrenaline and glucagon test are positive
- Enlargement of heart
- Muscle hypotonia

Death usually occurs before 9 months.

5. Thompson's disease is caused by a deficiency of *phosphoglucomutase (in liver and / or muscle)*

- Accumulation of glycogen of normal structure

6. Type I. Von Gierke's disease is caused by a deficiency of *glucose-6-phosphatase*

- Accumulation of glycogen of normal structure in liver, intestine and kidney.
- Hepatomegaly
- Severe hypoglycemia
- Increased level of cholesterol
- Metabolic acidosis
- Symptoms of gout
- Adrenaline and glucagon tests are negative

7. Type VII. Tarui's disease is due to a deficiency of *phosphofructokinase of muscle*

- Glycogen of normal structure is accumulated in skeletal muscles
- Clinical manifestations are similar to type V (Mc Ardle's disease)

8. ***Type 0*** *– deficiency of glycogen synthase*

- Liver glycogen amount is very low
- Severe hypoglycemia is on an empty stomach
- Hepatomegaly

9. Type IV. Andersen's disease *(amylopectinosis)* is caused by deficiency of branching *enzyme* (in liver, muscle, heart)

- Glycogen of abnormal structure (linear glycogen)
- Spleenomegaly
- Hepatomegaly
- Hypoglycemia
- Cirrhosis of liver
- Muscle hypotonia

Prognosis is usually fatal.

VII. Deficiencies of enzymes that degrade glycosaminoglycans result in mucopolysaccharidoses.

Clinical symptoms: cloudy corneas, mental retardation, short stature, nonproportional growth, deformation of vertebrate, stiff joints.

Fibroblasts, leukocytes, tissues, amniotic cells or serum can be used for the assay of many of the above enzymes.

Disturbances of hormonal regulation lead to hyperglycemia (it means the increased glucose concentration) or hypoglycemia which shows the decreased glucose concentration.

Diabetes mellitus is developed in insulin deficiency. Insulin plays an important role in carbohydrate metabolism regulation: it increases the permeability of membranes of insulin-sensitive cells for glucose; stimulates glycolysis and inhibits gluconeogenesis; increases glycogen synthesis and decreases its degradation. In diabetes mellitus the blood glucose concentration is increased

(which is called hyperglycemia), glucose is found in urine (glucosuria), and at the same time ketone bodies accumulation is observed (see chapter 8).

Hyperglycemia can arise not only because of the lack of insulin, but it can also be due to disordered function of the other endocrine glands, as an example, it can be hyperglycemia which occurs in hypophyseal diseases, adrenal cortex tumors and in hyperactivity of thyroid gland.

Occasionally, hyperglycemia is observed in pregnancy. Finally, hyperglycemia may develop in organic lesions of CNS, cerebral circulation disturbances, in inflammatory or generative liver diseases.

Hypoglycemia is observed in hypophyseal cachexia, Addison’s disease and hypothyroidism.

Tests for Self-control

1. Vomiting, diarrhea, general dystrophy, hepato- and spleenomegaly were observed in a newborn. These symptoms decrease after exclusion of milk from diet. Which main hereditary defect results in the pathology?

A. Disturbance of galactose metabolism
B. Disturbance of phenylalanine metabolism
C. Hypersecretion of endocrine glands
D. Disturbance of glucose metabolism
E. Deficiency of glucose-6-phosphate dehydrogenase

2. Fructose is mainly supplied to organism in composition of:

A. Maltose
B. Sucrose
C. Starch
D. Lactose
E. Glycogen

3. Newborn has good feeling after breast-feeding. Vomiting, abdominal ache, diarrhea, hypoglycemia appear after adding fruits and juices to food. Which is the cause?

A. Hyperglycemia
B. Ketosis
C. Gierke's disease
D. Glucosuria
E. Hereditary fructose intolerance

4. Postsynthetic covalent modification plays the important role in enzyme activity regulation. Glycogen phosphorylase and glycogen synthase activities are regulated by:

A. Methylation
B. Adenylation
C. Limited proteolysis
D. ADP-ribosylation
E. Phosphorylation-dephosphorylation

5. Fatty liver, galactosuria and aminoaciduria are observed in newborn. Which substance must be excluded from diet?

A. Milk sugar
B. Fatty acids
C. Phenylalanine
D. Cholesterol
E. Sucrose

6. Glucose-6-phosphatase absence, hypoglycemia and hepatomegaly were found in a child with point mutation of genes. What pathology is characterized by these signs?

A. Gierke's disease
B. Cori's disease
C. Addison's disease

D. Parkinson’s disease
E. Mc-Ardle’s disease

7. Which substance is included in reaction of substrate phosphorylation in glycolysis?
A. Glucose-6-phosphate
B. Phosphoenolpyruvate
C. Fructose-1,6-bisphosphate
D. Glyceraldehyde-3-phosphate
E. 2-Phosphoglycerate

8. Choose the substance, which is the substrate of gluconeogenesis:
A. Glycogen
B. Glucose
C. Pyruvate
D. Fructose
E. Galactose

9. Gluconeogenesis in liver is activated in sportsman after intensive training. Point the main substrate of this process:
A. Serine
B. Lactate
C. α-Ketoglutarate
D. Aspartate
E. Glutamate

10. Point end products of anaerobic glycolysis:
A. CO_2 and H_2O
B. Oxaloacetate
C. Malate
D. Pyruvate
E. Lactate

11. Deficiency of pyruvate kinase in erythrocytes is found in a child with symptoms of anemia. Which process in erythrocytes is disturbed?
A. Oxidative phosphorylation
B. Tissue respiration
C. Anaerobic glycolysis
D. Elimination of peroxides
E. Deamination of amino acids

12. Choose the main regulatory enzyme of glycolysis:
A. Phosphofructokinase
B. Phosphorylase
C. Lactate dehydrogenase
D. Succinate dehydrogenase
E. Pyruvate kinase

13. Point the enzyme which catalyzes the conversion of pyruvate under aerobic conditions:
A. Pyruvate dehydrogenase
B. Lactate dehydrogenase

C. Aldolase
D. Hexokinase
E. Triosophosphate isomerase

14. Point the end products of aerobic conversion of glucose in human's tissues:

A. Lactate
B. Pyruvate
C. CO_2 and H_2O
D. Malate
E. Acetone

15. The increased hemolysis of erythrocytes is observed in a 3-year-old child with increased temperature after intake of aspirin. Which enzyme congenital deficiency could cause hemolytic anemia?

A. Glycerol phosphate dehydrogenase
B. Glucose 6-phosphatase
C. Glycogen phosphorylase
D. Glucose 6-phospate dehydrogenase
E. γ-Glutaminyltransferse

16. In old woman a cataract was developed as a consequence of a diabetes mellitus background. Which process stimulation is the cause of cloudy crystalline lens:

A. Protein glycosylation
B. Proteolysis of proteins
C. Lipogenesis
D. Lipolysis
E. Gluconeogenesis

Chapter 10. LIPID METABOLISM

Daily requirement:

1 – 1,5g / kg of body weight (80 – 100g)

40% (20 – 30g) of them – oils

4 – 8 g of polyunsaturated fatty acids

5 - 6 g of phospholipids

0,3 – 0,6 g of cholesterol

10.1 Digestion and Absorption of Lipids

About 90% of lipids of food are TAG.

Necessary conditions for lipids digestion: presence of **emulsified** fats; presence of **active lipase.**

In adults:

Oral cavity: fats undergo no change because the human saliva contains no fat-splitting enzymes.

Stomach:

- Lipase, contained in a small amount in gastric juice of adult humans, is not active because its optimal pH is **3-6** (the gastric juice pH in adults is about **1,5**).
- The conditions for fats emulsification are not provided in stomach.
- In adult human the lipoprotein complexes unedrgo a partial degradation

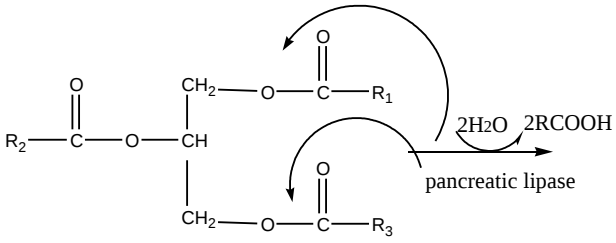
Duodenum:

- In duodenum the hydrochloric acid of gastric juice is neutralized by bicarbonates contained in the pancreatic juice.
- Fat emulsification occurs. The most potent emulsifiers are **bile acid salts.**
- Pancreatic lipase is activated by participation of colipase in presence of bile acid salts. Colipase anchors the lipase to its triacylglycerol substrate.

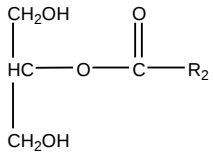
Pancreatic lipase (inactive) + colipase $\xrightarrow[\text{salts}]{\text{bile acid}}$ Pancreatic lipase (active)

Pancreatic lipase is the main enzyme of fat digestion in adults.

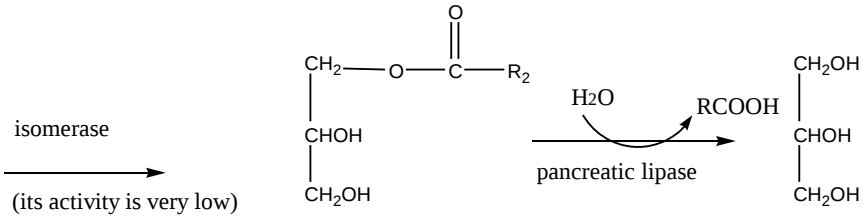
Pancreatic lipase hydrolyzes 1- and 3- acyl groups of TAG. The products are free fatty acids and 2-acylglycerols. 2-monoacylglycerols are isomerizated into 1-monoacylglycerols, which are hydrolyzed by pancreatic lipase.



TAG

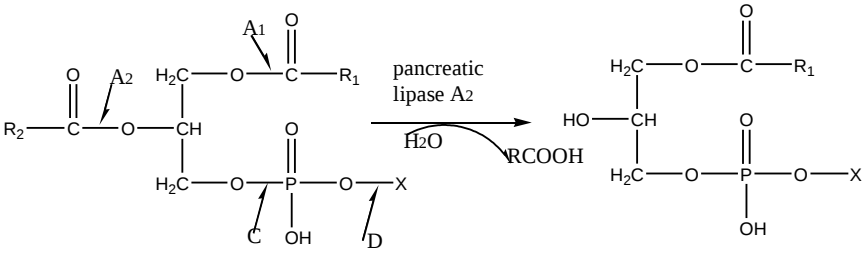


2-MAG



Digestion of Phospholipids

In duodenum



Lysophospholipids

In small intestine:

Lysophospholipids $\xrightarrow[\text{Pi, nitrogen component}]{\text{phospholipases}}$ glycerol + fatty acids

Digestion of Cholesterol Esters

Cholesterol esters $\xrightarrow[\text{cholesterol esterase}]{\text{pancreatic}}$ cholesterol + fatty acids

Digestion of Lipids in Infants

The main source of lipids for babies is emulsified milk fats.

Preduodenal lipases play the important role in these lipids digestion in infants:

- *A lingual lipase* is secreted by the dorsal surface of the tongue
- *Gastric lipase*

Both lipases have optimal pH 3 – 6,0. The gastric juice pH in babies is about 5,0 which favours the digestion of emulsified milk fats by these lipases.

Bile Acids

Bile acids are formed in liver from cholesterol. They are hydroxylated forms of cholanic acid.

Cholic and chenodeoxycholic acids are formed in liver. They are called “the primary bile acids”.

Cholesterol (C_{27}) → bile acids (C_{24})

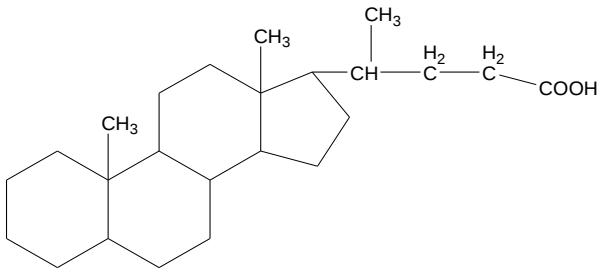


Figure 10.1. Cholanic acid

Bile acids:

3,7,12-trihydroxycholanic (cholic)
3,12-dihydroxycholanic (deoxycholic)
3, 7-dihydroxycholanic (chenodeoxycholic)

+ glycine NH_2-CH_2-COOH
+ NH_2-CH_2-CH_2-SO_3H taurine

Bile acids are conjugated with glycine or taurine. If the diet is rich in carbohydrates the relative content of glycine conjugates is seen to increase. In contrast, the taurine conjugates are predominant in the digestion of protein-rich diet. In large intestine the primary bile acids are converted into the secondary bile acids (deoxycholic and litocholic) by means of microflora enzymes. Deoxycholic acid is absorbed therefore it supplies to liver.

Bile acids are emulsifiers because they have both hydrophobic and hydrophilic parts, they are amphypatic substances.

Function of Bile Acid Salts

- ***Emulsification of fats.*** Bile acids diminish a surface tension at the fat/water interface. They not only facilitate the emulsification but also stabilize the formed emulsion.

Only the combination: bile acid salt + unsaturated fatty acid + monoacylglycerole is capable of providing for the needed degree of fat emulsification

- They ***activate pancreatic lipase.***
- They ***form mixed micelles*** and therefore play the important role in absorption of lipid digestion products.
- They ***stimulate intestinal peristalsis***.
- They ***stimulate bile formation in liver***.
- They ***support cholesterol***, which is contained in bile, in dissolved state.
- They ***regulate the cholesterol synthesis***.

Absorption of Fats in the Intestine

- The uptake of fats occurs in the proximal portion of the small intestine.
- Part of the emulsified fats (the size of emulsified fat droplets is not larger than 0,5μm) can be absorbed through the intestinal wall **without hydrolysis** (about 10%) by pinocytosis.
- The short-chain fatty acids (with the number of carbon atoms less than 10) and glycerol are readily absorbed in the intestine and supply to the portal vein blood and then to liver.
- The uptake of long chain fatty acids and monoacylglycerols is performed by means of **mixed micelles**.

Mixed micelles consist of:

- hydrophobic core (fatty acids, MAG, cholesterol, fat-soluble vitamins);
- hydrophilic shell (bile acids salts and phospholipids).

Micelles penetrate by pinocytosis into the epithelial cells and undergo to breakdown. Liberated MAG and fatty acids are used for the **resynthesis of fats in**

intestinal wall. Bile acids supply to liver via the portal vein and then they return back to the bile.

Continuous circulation of bile acids between the liver and the intestine takes place. This process is called **hepatoenteric circulation**.

Only 10 – 15% of bile acids are newly synthesized by the liver, most of them (85 – 90%) are reabsorbed and resecreted as bile components. In humans the total pool of bile acids is about **2,8 – 3,5g**, they circulate at a rate **5 - 6** cycles per day.

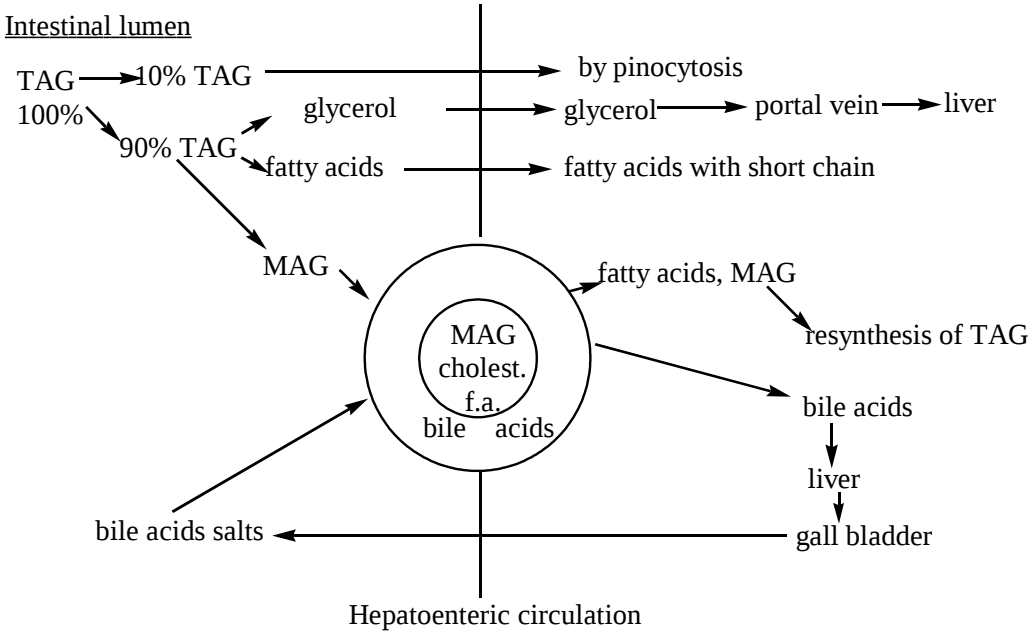


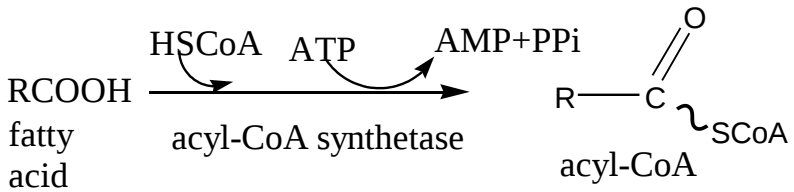
Figure 10.2. Scheme of fat digestion product absorption.

10.2 Synthesis of Fats

Main sites of fat synthesis:

- Small intestine.
- Liver.
- Adipose tissue.

Activation of fatty acids (in all tissues):



Resynthesis of fats in intestinal wall

Monoacylglyceride (main) pathway Through phosphatidic acid

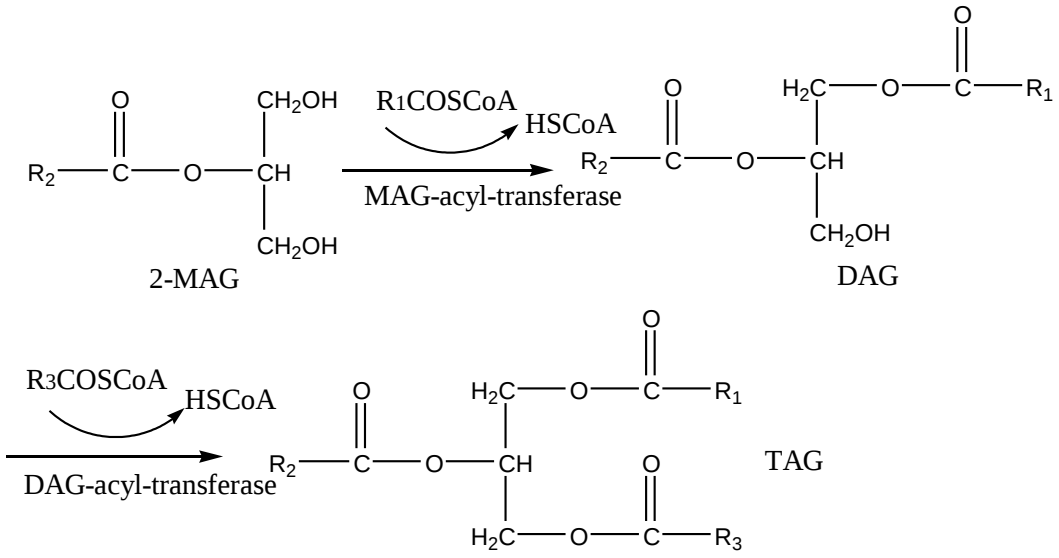


Figure 10.3 Monoacylglyceride pathway

Synthesis of fats in the liver and adipose tissue is performed through phosphatidic acid (figure 10.4).

In liver glycerol-3-phoshate, which is needed for the synthesis of phosphatidic acid, may be synthesized by 2 pathways: from glycerol and from dihydroxyacetone phosphate. In adipose tissue the activity of glycerol kinase is very low therefore the production of glycerol-3-phosphate is associated with reduction of dihydroxyacetone phosphate (intermediate of glycolysis).

Only in 2 organs (small intestine and in liver) TAG are synthesized for "export". Therefore transport forms of lipids (lipoproteins) are formed in these organs.

10.3 Transport Forms of Lipids

Four major groups of plasma lipoproteins have been identified:

1. **Chylomicrons**
2. Very low density lipoproteins (**VLDL or pre-β-lipoproteins**)
3. Low density lipoproteins (**LDL or β-lipoproteins**)
4. High density lipoproteins (**HDL or α-lipoproteins**)

All lipoproteins consist of the same components, but differ from one another by ratio of the components.

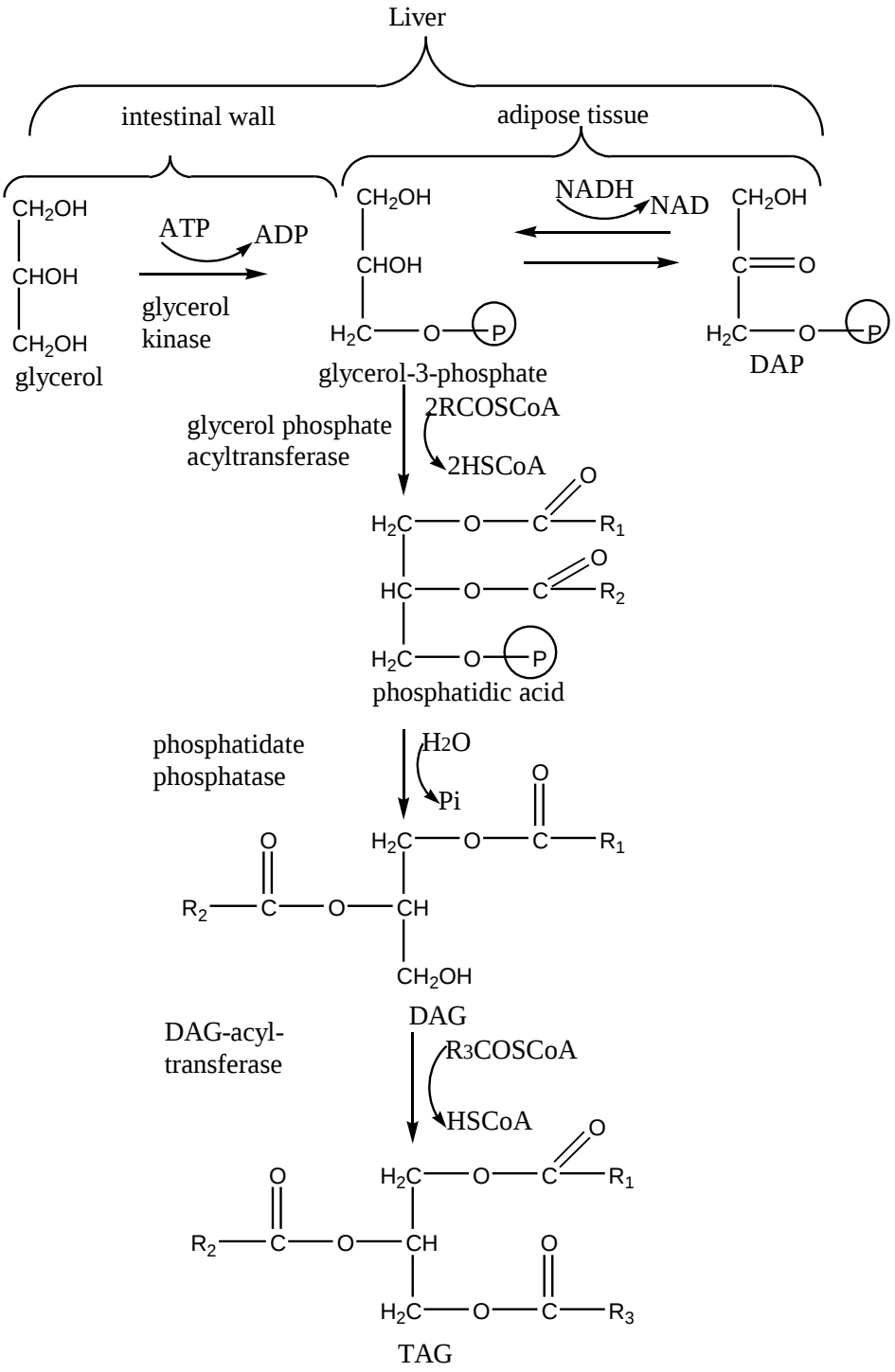
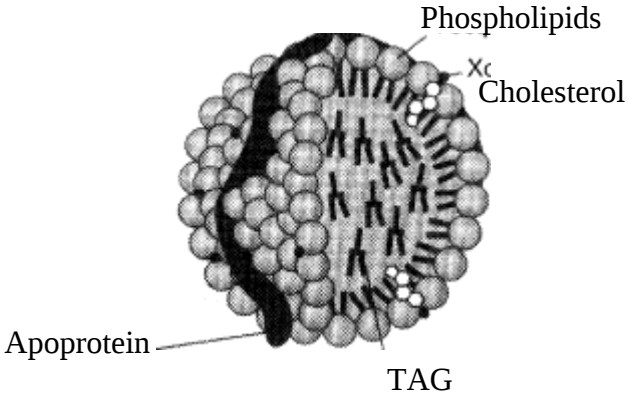


Figure 10.4. Scheme of TAG synthesis through phosphatidic acid.

They include:

- **The hydrophobic core** which consists of mainly nonpolar lipids: TAG, cholesterol esters.
- **Hydrophilic shell** which consists of cholesterol, phospholipids, integral and peripheric apoproteins.

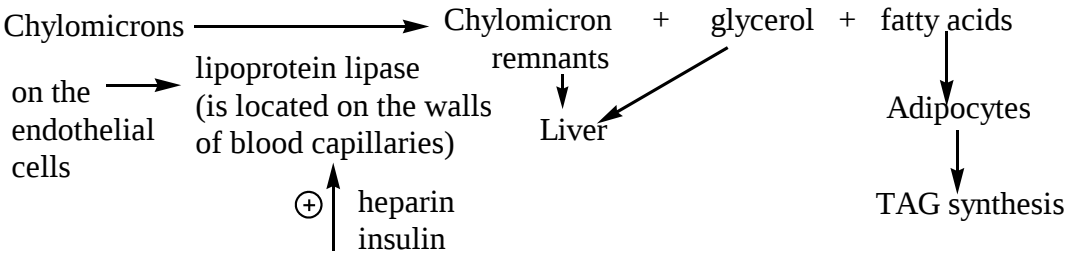


Pure fat is less dense than water; it follows that if the proportion of lipid to protein in a lipoprotein increases, the density decreases (table 10.1)

1. **Chylomicrons** are synthesized in enterocytes and serve as transport form of exogenous TAGs. Their triacylglycerols are derived from dietary lipids, and their major apoprotein is apo B-48. Chylomicrons pass into the spaces between the intestinal cells and then into the lymphatic system draining the intestine and finally pass to the systemic blood via the thoracal duct.

Chylomicrons (enterocytes) → Lymph → Thoracal duct → Blood → Different tissues, mainly adipose tissue

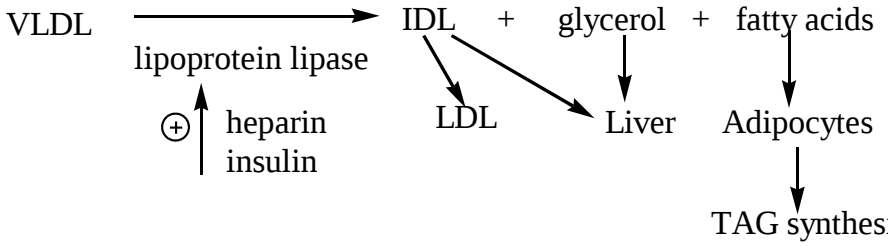
Apo C, Apo C-II and Apo E are transferred to nascent chylomicrons from HDL, and mature chymicrons are formed. In peripheral tissues, particulary adipose and muscle, the TAGs of chylomicrons are digested by lipoprotein lipase. Apo C-II is the activator of lipoprotein lipase.



Some of the released free fatty acids return to the circulation attached to albumins, but the **bulk** is transported into the adipose tissue. The chylomicron remnants interact with receptors on liver cells and are taken by endocytosis.

2. VLDL are mainly synthesized in liver, but some part of VLDL may be synthesized in intestine. They transport the endogenous TAGs. In peripheral

tissues VLDL TAGs are digested by lipoprotein lipase, and VLDLs are converted to IDLs.



Part of IDLs returns to the liver, is taken up by endocytosis and is degraded by lysosomal enzymes. In humans a much larger proportion of IDLs forms LDLs.

3. **LDL** Most LDLs appears to be formed from VLDL in bloodstream, but there is evidence for some production directly by liver. The role of LDLs is the transport of cholesterol to peripheral tissues and they regulate de novo cholesterol synthesis at these sites.

Different cells take up LDL. A lot of tissues have receptors to LDL. LDL are destroyed in lysosomes and receptors return to the plasmatic membranes.

Cholesterol, which is liberated, regulates its own synthesis in cell by inhibition of β-hydroxymethylglutaryl-CoA-reductase. In its turn the rate of LDL receptors synthesis is regulated by feedback mechanism.

4. **HDLs** are mainly formed in liver, but particularly in intestine. Functions: transport of phospholipids to tissues and cholesterol from tissues to liver. **HDLs take up cholesterol from lipoproteins and tissues by means of lecithin cholesterol acyltransferase.** HDLs have the important function of preventing cholesterol accumulation in tissues. They are involved in other lipoproteins metabolism. The major protein of HDL is apo A.

Table 10.1. Composition of the Blood Lipoproteins (%)

Fraction	Total		Percentage of total lipids				Apopro-teins
	Protein	Lipids	TAG	Phospho-lipids	Choleste-rol (free)	Choleste-rol ester	
Chylomicrons	1 - 2	98-99	88	8	1	3	B-48, C-II, E
VLDL	7 - 10	90-93	57	20	8	15	B-100, C-II, E

LDL	21	79	14	28	10	48	B-100
HDL	45	55	18	44	8	30	A-I, C-II, E

10.4 Lipid Metabolism in Adipose Tissue

Adipose tissue is the main store of triacylglycerols in the body.

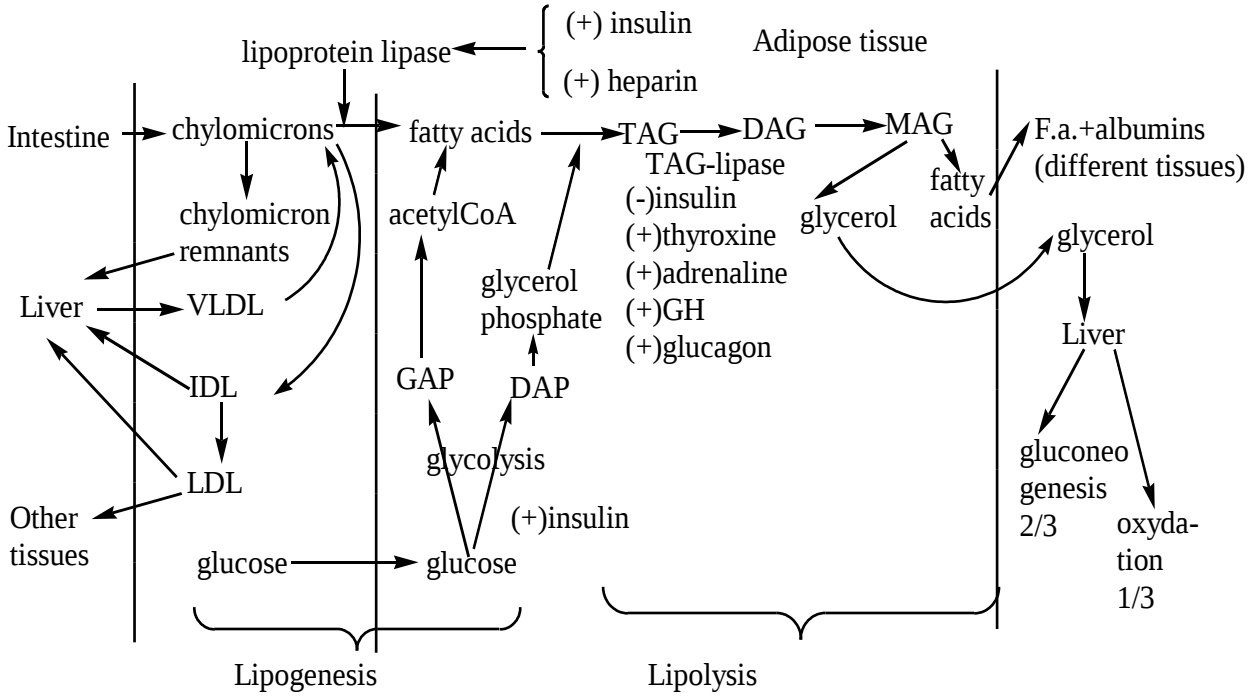


Figure 10.5 Lipogenesis and lipolysis in adipose tissue

Lipogenesis is stimulated by excess of fats and carbohydrates in diet. Insulin increases lipogenesis: increases activity of lipoprotein lipase; increases the permeability of membranes to glucose by causing the translocation of glucose transporters from the Golgi apparatus to the plasma membrane; increases activity of pyruvate dehydrogenase, acetyl-CoA carboxylase and glycerol phosphate acyltransferase. The triacylglycerol stores in adipose tissue are continually undergoing lypolysis and reesterification.

Triacylglycerol lipase is hormone sensitive. It is activated by means phosphorylation. Such hormones as adrenaline, noradrenaline, glucagon, growth hormone, thyroid hormones, glucocorticoids promote lipolysis (figure 10.6). Insulin inhibits the activity of hormone-sensitive lipase. Adipose tissue is much more sensitive to insulin than many other tissues.

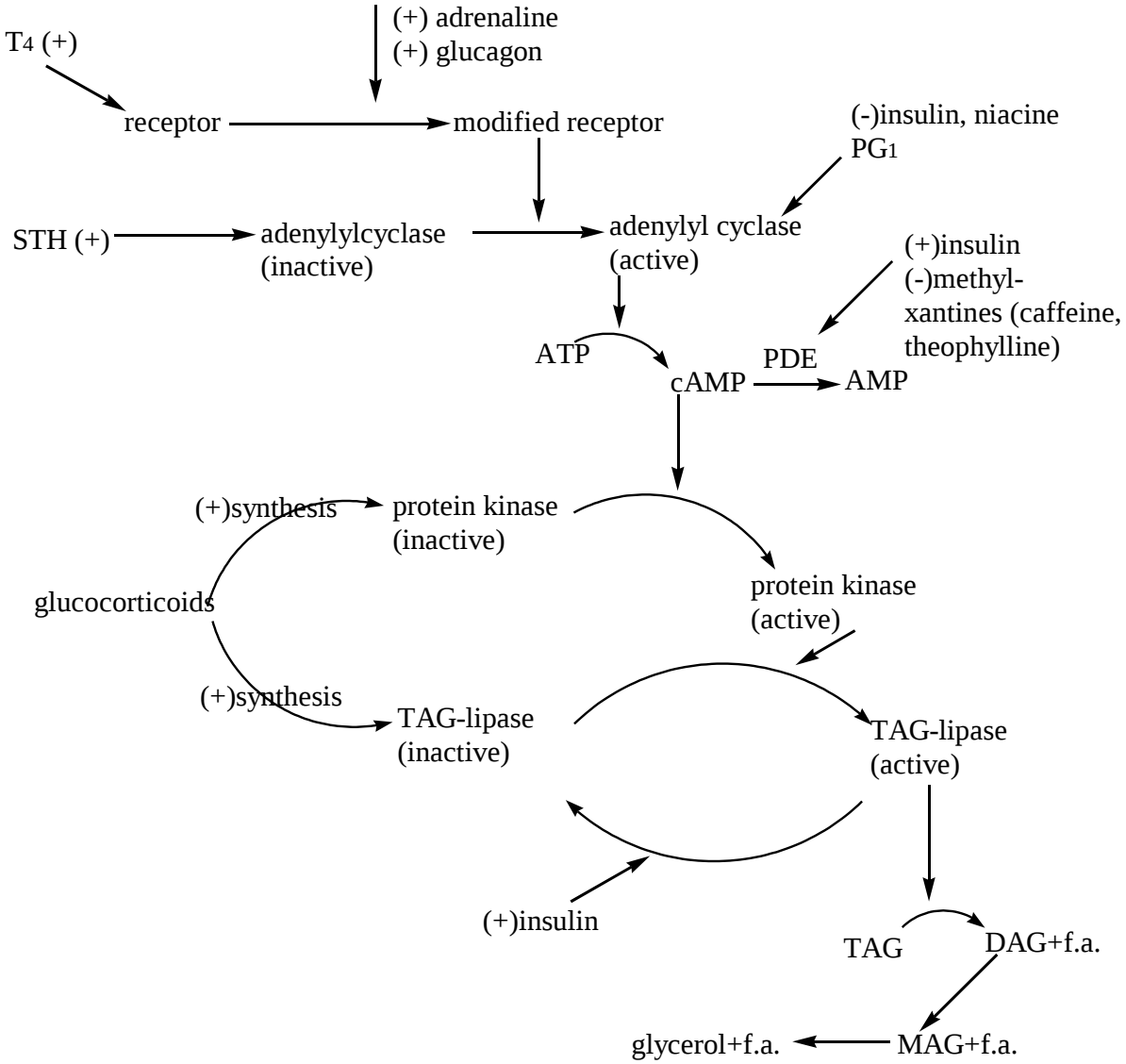


Figure 10.6.Regulation on lipolysis in adipose tissue

Methylxantines (such as caffeine and theophylline) inhibit the activity phophodiesterase (enzyme of cAMP degradation). It is significant that the drinking of coffee, contained caffeine, causes elevation of plasma FFA in humans.

The free fatty acids formed by lipolysis can be reconverted in the tissue to acyl-CoA by acyl-CoA synthetase and reesterified with flycerol-3-phosphate to form triacylglycerol. Thus, there is a continuous cycle of lipolysis and reesterification within the tissue. However, when the rate of reesterification is not sufficient to match the rate of lipolysis, free fatty acids accumulate and diffuse into the plasma, where they bind to albumin and raise the concentration of plasma free fatty acids. These are a most important source of fuel for many tissues. Because the

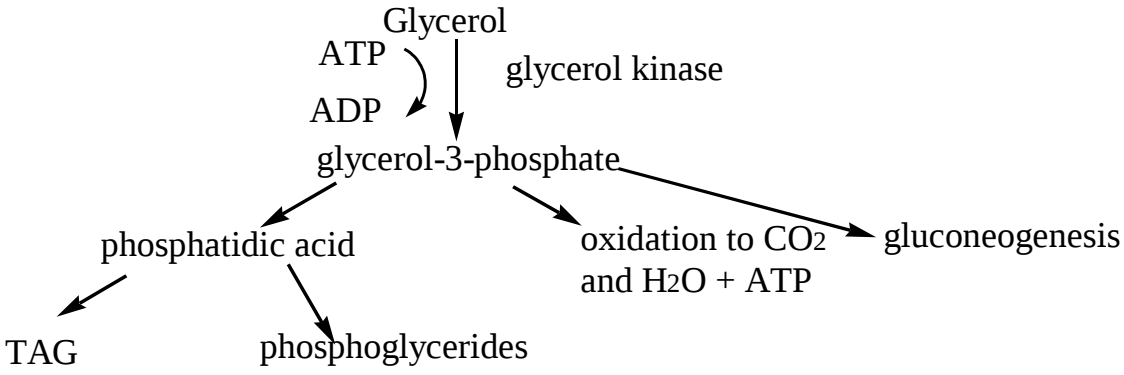
enzyme glycerol kinase is low in activity in adipose tissue glycerol cannot be utilized.

10.5 Conversions of Glycerol

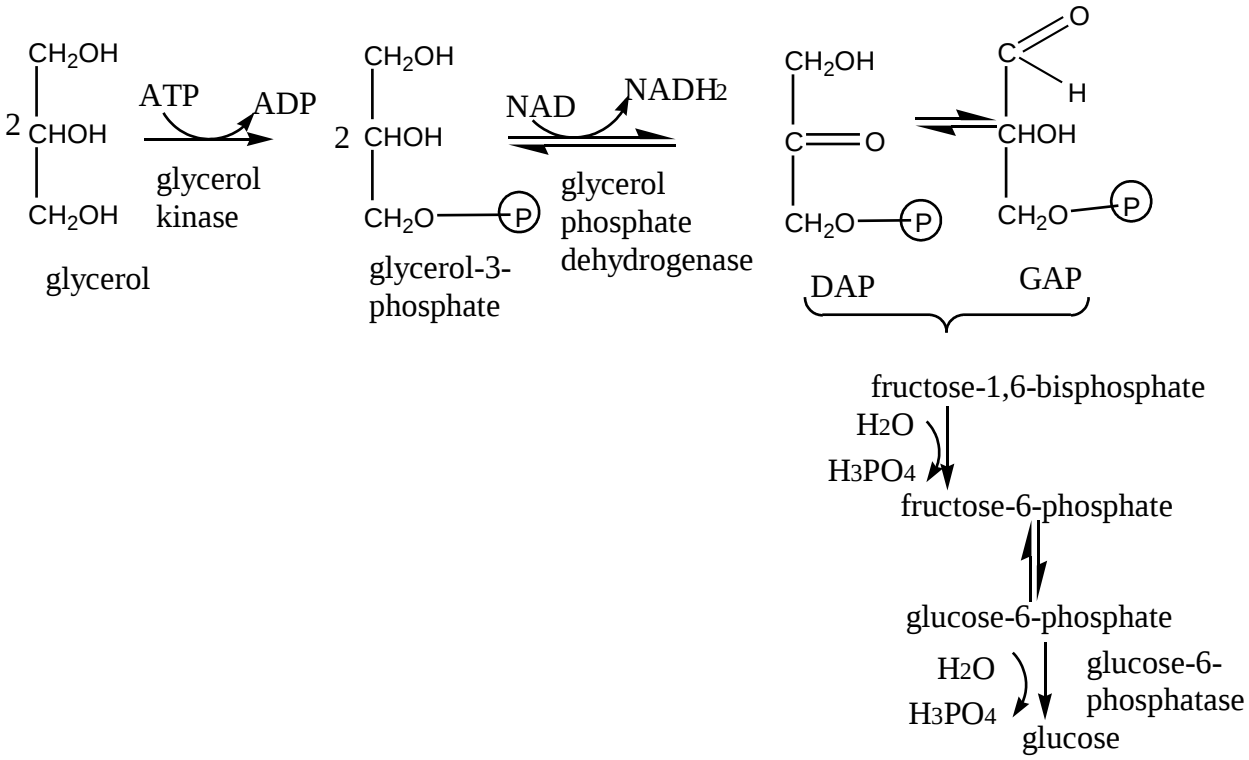
Glycerol may be used by tissues which have glycerol kinase.

Considerable amount of glycerol kinase is found in: liver; kidney; intestine; brown adipose tissue; mammary glands in lactation.

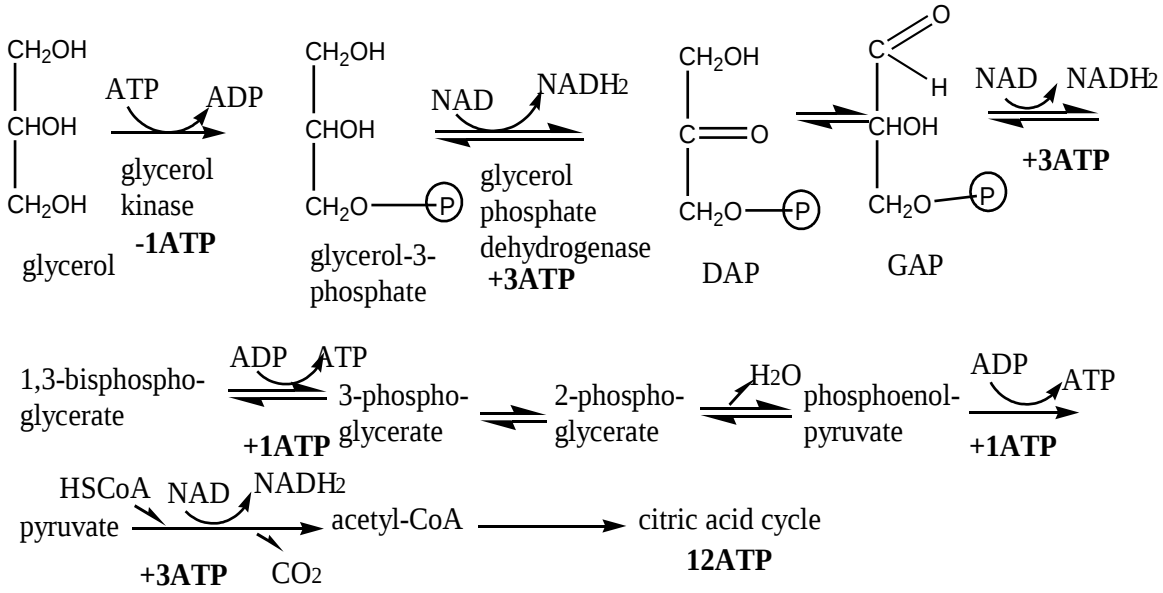
The highest amount of glycerol is metabolized in **liver**.



- ***Gluconeogenesis***



- ***Oxidation of glycerol***



Σ 22ATP

10.6 Oxidation of Fatty Acids

Fatty acids which are mainly formed by means of lipolysis in adipose tissue are combined with albumin and are transported to different tissues with exception of brain. They are taken up by tissues and are metabolized. Half life of fatty acids in blood is 2 – 4 minutes.

Fatty acids may be oxidized by α-, β- and ω-oxidation.

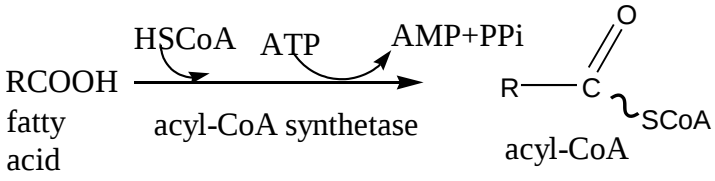
ω β α

CH_3-…CH_2-CH_2-COOH

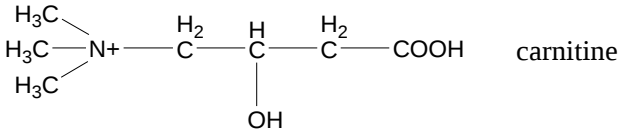
ω-oxidation	**β- oxidation**	**α- oxidation**
It is normally a very minor pathway and it occurs in the endoplasmic reticulum of liver and leads to dicarboxylic acid formation.	It is the most important pathway of fatty acids oxidation. It occurs in mitochondria of all the tissues and cells and plays the energy function.	It has been detected in endoplasmic reticulum of brain. It leads to α-hydroxyacid formation and it is important for sphyngolipid synthesis.

β-Oxidation includes some stages:

I. Fatty acids activation



II. Fatty acid transport from cytosol to mitochondria is performed by means of ***carnitine***



carnitine

β-hydroxy-γ-trimethylaminobutyrate is synthesized from lysine and methionine in liver and kidney.

Carnitine acyl tranferase I which is present in the outer mitochondrial membrane converts long-chain acyl-CoA to acylcarnitine.

Carnitine acylcarnitine translocase acts as inner membrane carnitine exchange transporter. The acylcarnitine then reacts with HSCoA, catalyzed by **carnitine acyl tranferase II**, located on the inside of the inner membrane. Acyl-CoA is reformed in the mitochondrial matrix and carnitine is liberated.

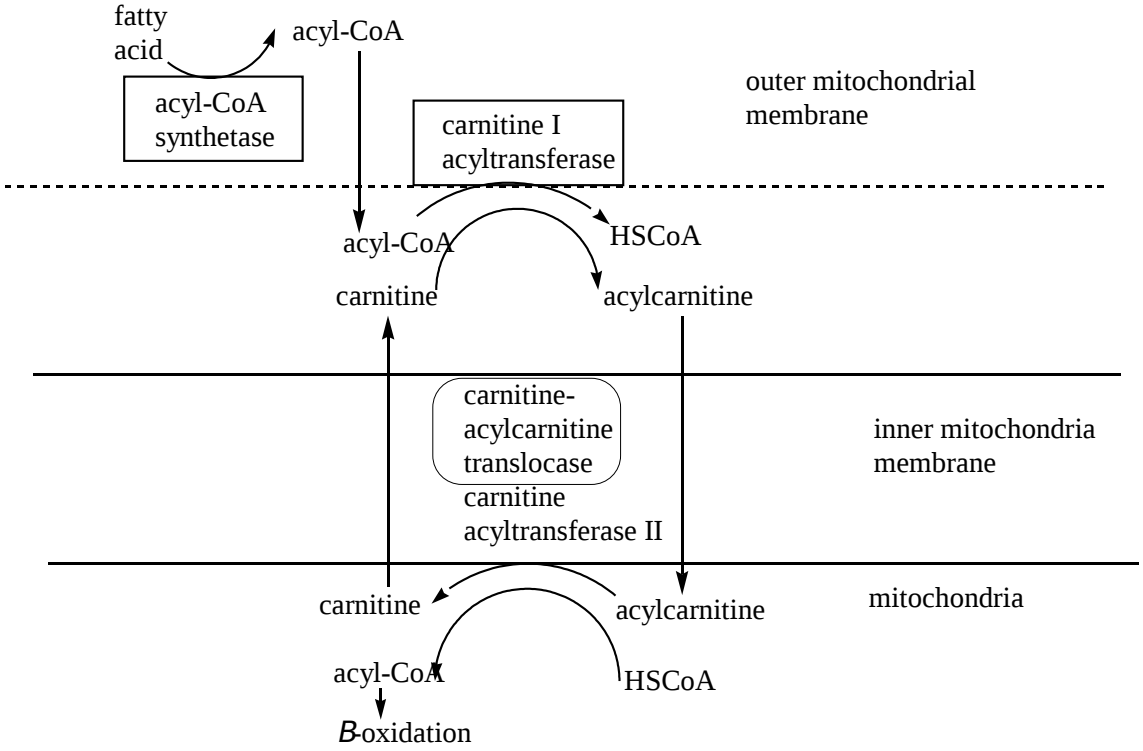


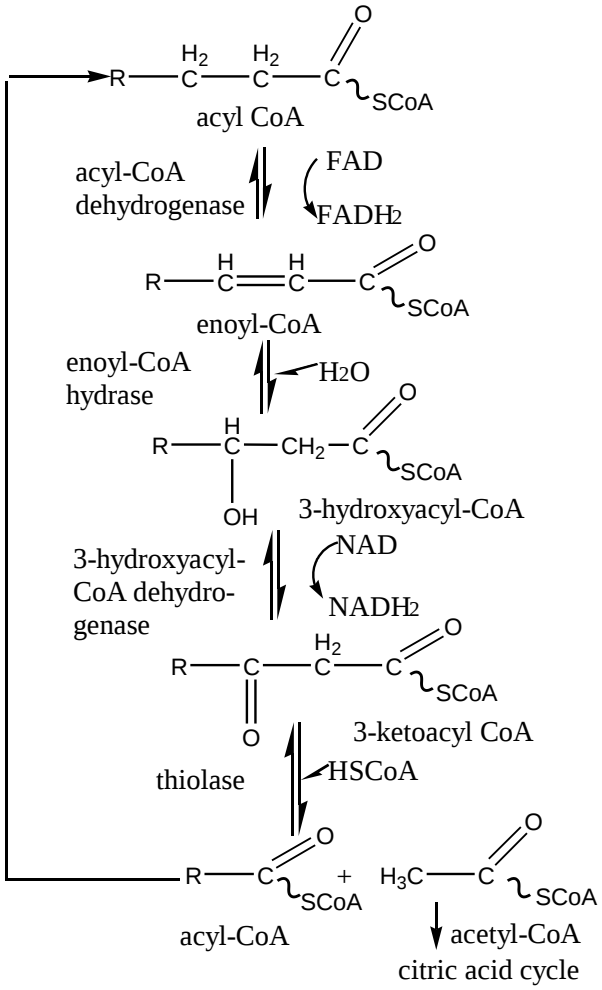
Figure 10.7 Fatty acids transport from cytosol to mitochondria

III. Oxidation

The enzymes of β-oxidation are found in the mitochondrial matrix. They catalyze the oxidation of acyl-CoA to acetyl-CoA.

The products of these reactions are acetyl-CoA and acyl-CoA derivative containing two carbons less than the original acyl-CoA molecule.

The acyl-CoA formed in the cleavage reaction reenters the oxidative pathway. In this way, a long chain fatty acid is finally degraded to acetyl-CoA.



IV. Oxidation of acetyl-CoA in citric acid cycle

Energy balance

The oxidation of fatty acid with n carbon atoms includes **(n/2 - 1)** cycles of β-oxidation and leads to formation of **n/2** molecules of acetyl CoA.

Each β-oxidation cycle is accompanied by formation of **1 molecule of $FADH_2$ (2 ATP molecules)** and **1 molecule of $NADH+H^+$ (3 ATP molecules) 2+3=5 ATP.**

The oxidation of one molecule of acetyl-CoA in Krebs cycle leads to accumulation of 12 ATP molecules.

Therefore: **[(n/2-1)·5+n/2·12-1] ATP**

For example: palmitic acid C_{16}

(16/2-1)·5+16/2·12-1=130ATP.

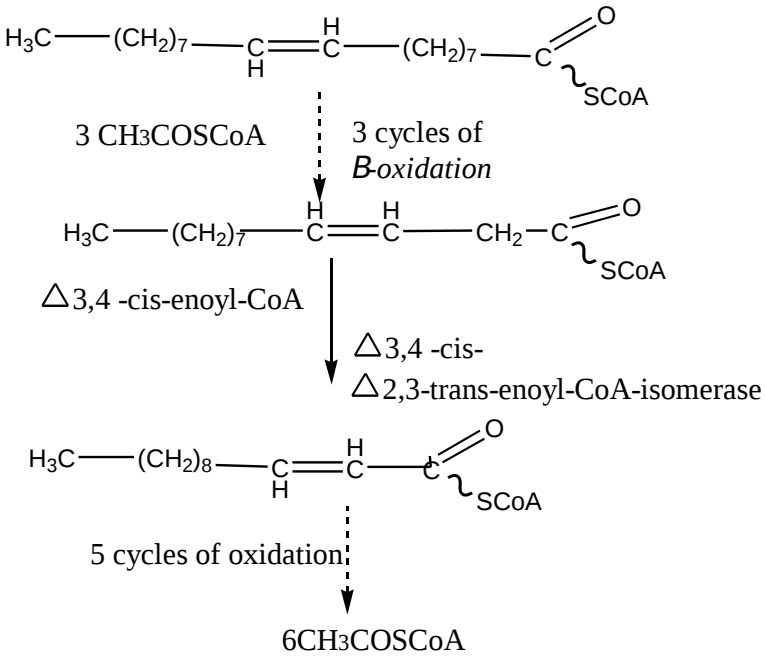
Oxidation of unsaturated fatty acids

The oxidation of unsaturated fatty acids is similar to oxidation of saturated fatty acids with exception of two processes.

The double bonds of natural unsaturated fatty acids possess a cis-configuration.

The step with removal of two-carbon fragments on oxidation of the unsaturated fatty acid chain to the first double bond gives $\Delta^{3,4}$-cis-enoyl-CoA instead of $\Delta^{2,3}$-trans-enoyl-CoA, which is an intermediate product in the β-oxidation of saturated fatty acids.

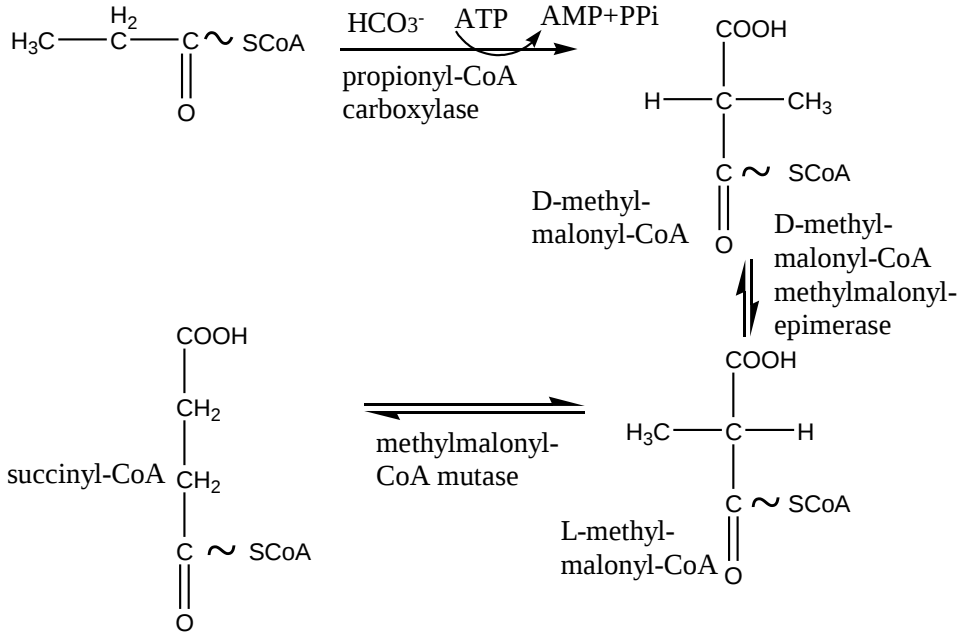
Enzyme $\Delta^{3,4}$-cis- $\Delta^{2,3}$-trans-enoyl-CoA-isomerase translocates the double bond from the position 3-4 to position 2-3 and alters the double bond configuration form cis to trans.



Oxidation of odd-numbered fatty acids

β-Oxidation of long-chained odd-numbered fatty acid result in acetyl-CoA and propionyl-CoA as end products.

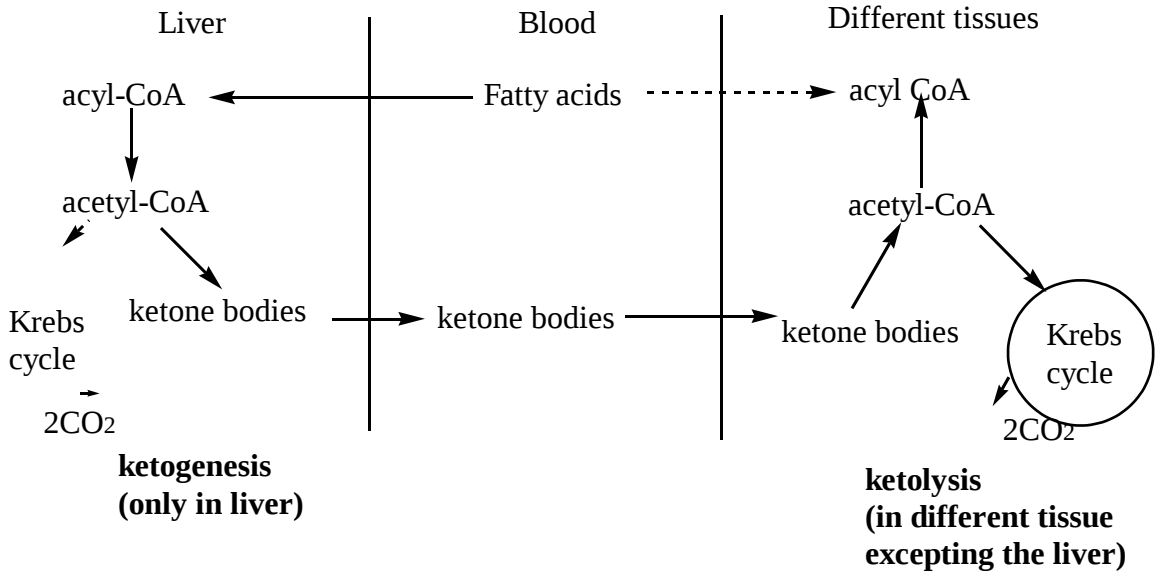
Propionyl-CoA is converted to succinyl-CoA, which is a metabolite in the Krebs cycle.



10.7 Ketogenesis and Ketolysis

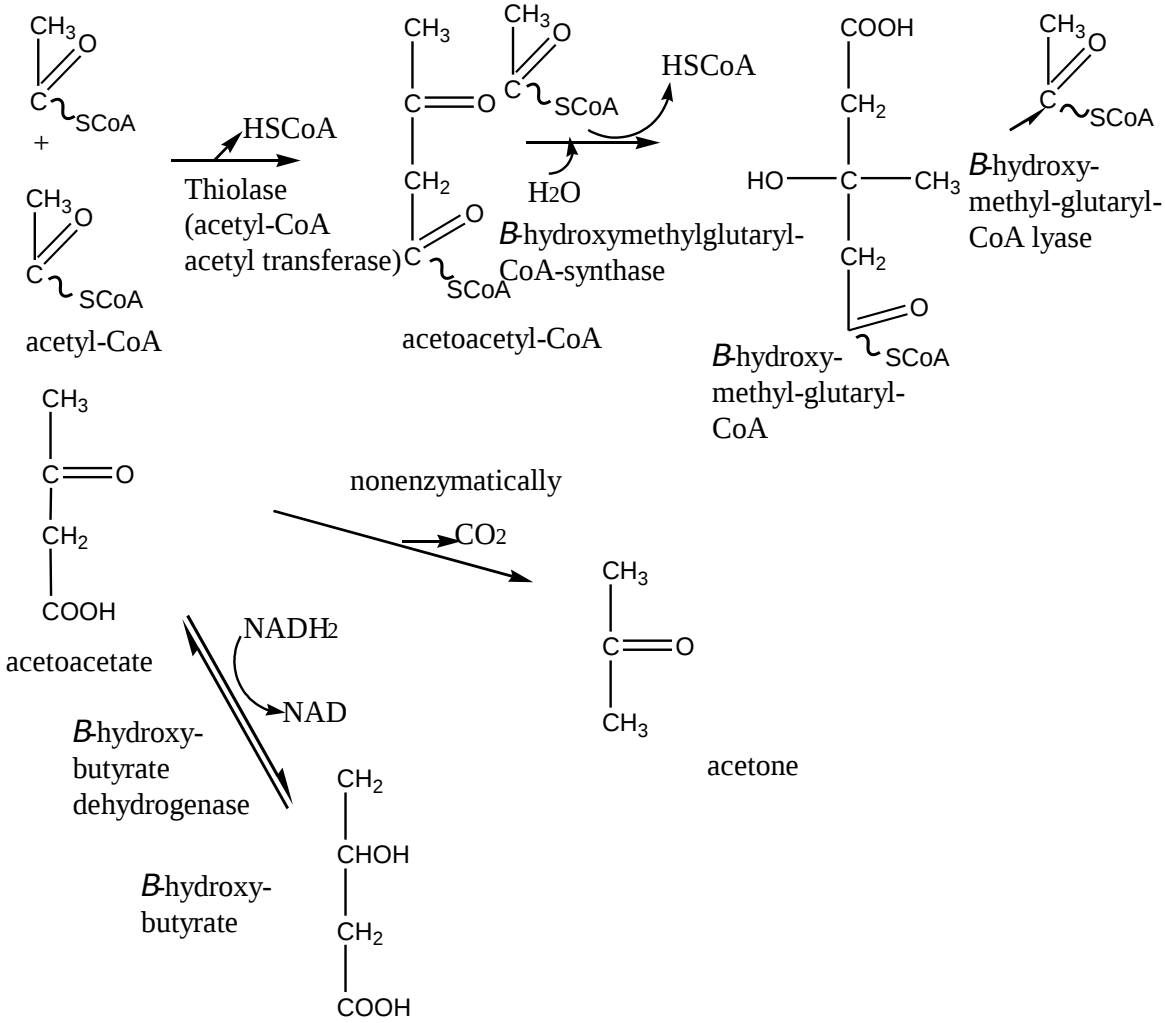
The main organ of fatty acids oxidation is liver Excessive production of acetyl-CoA in liver is used for formation of alternative metabolic fuel molecules – ketone bodies.

Ketone bodies: acetoacetate; β-hydroxybutyrate; acetone.

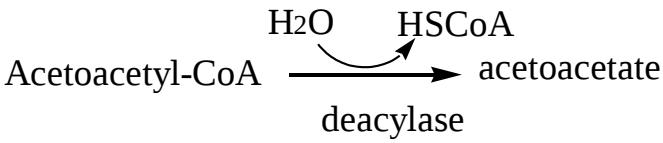


Ketogenesis

I (main) pathway occurs in the liver. Enzymes responsible for ketone body formation are associated mainly with the mitochondria.



II pathway



Normal contents of ketone bodies

Blood – 0,1 – 0,6 mmol/L

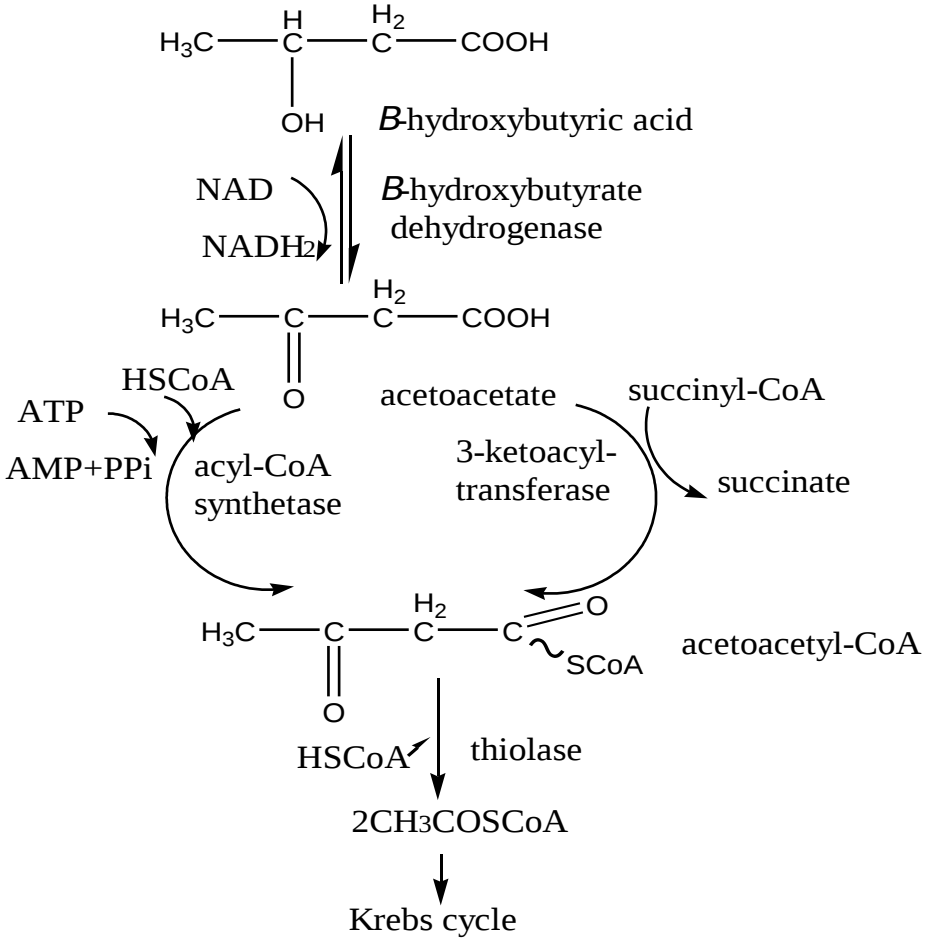
Urine – 20 – 30 mg/day.

Functions of Ketone Bodies

- ***Energy function.*** Extrahepatic tissues utilize them as energy substrates. They are important energy source for heart, skeletal muscles, kidney cortex.

- ***Transport function.*** They are carriers of acetyl-CoA to extrahepatic tissues.
- ***Biosynthetic function*** (from acetyl-CoA)
- ***Regulatory function.*** They prevent the extraordinary mobilization of fatty acids from the fat depots.

Ketolysis (in different tissues excepting liver)



Accumulation of ketone bodies occurs in:

1. Diabetes mellitus.

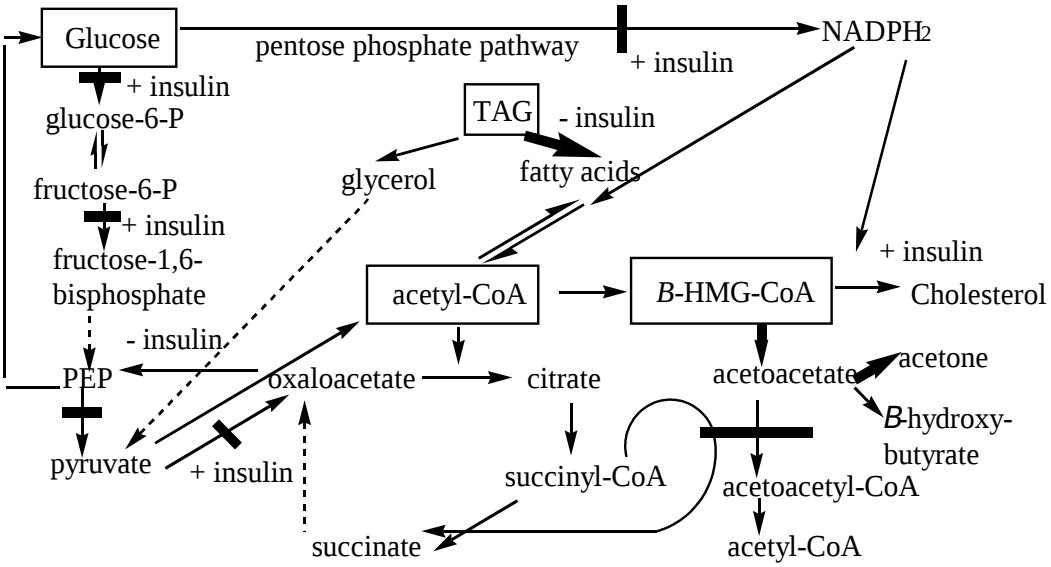
2. Starvation.

3. Thyrotoxicosis.

4. Infectious diseases (Ketonemia is often attended to infectious diseases: scarlatina, influenza, tuberculosis and meningitis. In these diseases ketonemia is a secondary effect and has little diagnostic value).

5. Long hard work.

Interrelation between carbohydrate and lipid metabolism and its disturbances



10.8 Synthesis of Fatty Acids.

The most active synthesis of fatty acids occurs in: adipose tissue; liver; mammary gland in lactation.

Intensive synthesis of fatty acids occurs in: intestine; brain; kidney cortex; lungs.

The synthesis of fatty acids to palmitic acid occurs ***in the cytoplasm***.

Elongation of fatty acid chain and *insertion* of new double bond occurs in ***mitochondria and microsomes (endoplasmic reticulum).***

It should be noted that monounsaturated fatty acids may be synthesized in our organism.

The formation of double bonds is performed in the microsomes of liver cells and adipose cells with the participation of a specific oxygenase and molecular oxygen.

The tissues of humans and certain animals are incapable to synthesize *linoleic* and *linolenic* acids.

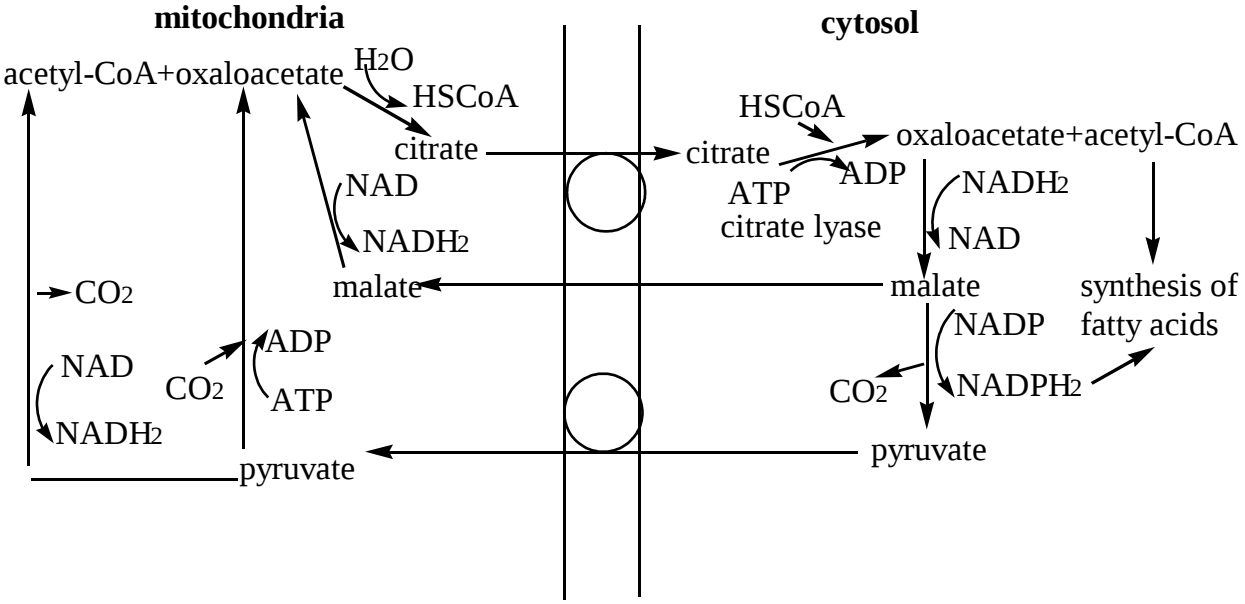
All other polyunsaturated fatty acids, found in mammals, are formed from 4 precursors (palmitooleic, oleic, linolenic and linoleic acids).

Synthesis of fatty acids from acetyl-CoA to palmitate:

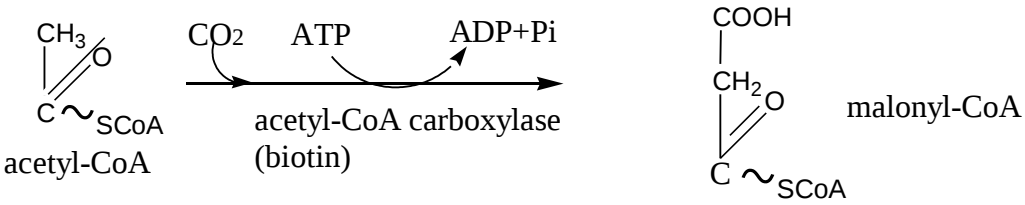
I. Transport of acetyl-CoA from mitochondria to cytoplasm

Acetyl-CoA serves as a building material for the synthesis of fatty acids in the cytoplasm. Acetyl-CoA is chiefly formed in mitochondria by oxidative decarboxylation of pyruvate and β-oxidation of fatty acids. Acetyl-CoA can not penetrate into the cytoplasm by diffusion. Therefore, initially the mitochondrial acetyl-CoA interacts with oxaloacetate to yield citrate. This reaction is catalyzed by citrate synthase. The citrate is transported into cytosol by ***tricarboxylate transport system.*** In the cytosol citrate is converted into acetyl-CoA and oxaloacetate by means of ATP-dependent citrate lyase.

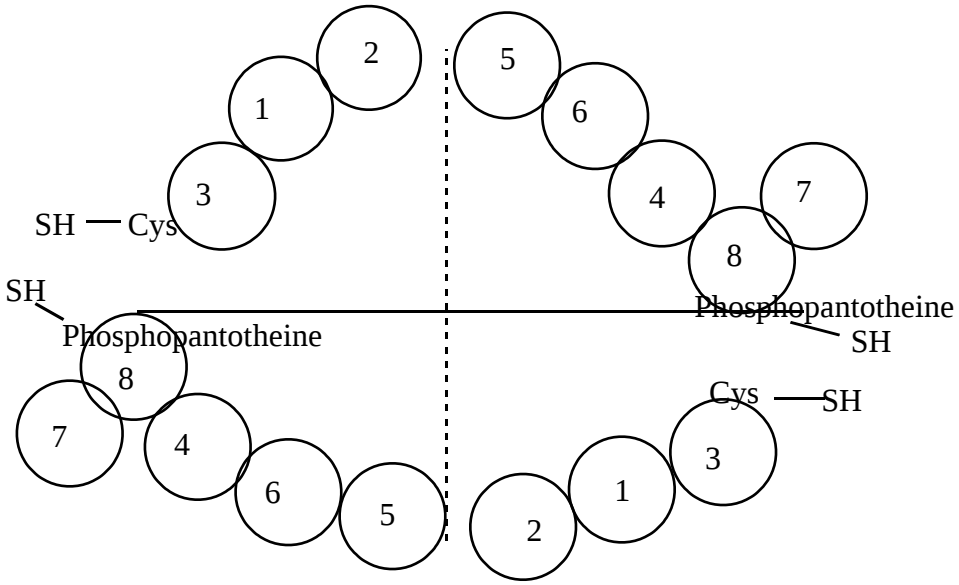
Oxaloacetate can form malate via NADH-linked malate dehydrogenase. Then malate is converted to pyruvate by malic enzyme. This process is linked with generation of NADPH which is used in fatty acid synthesis. Alternatively, malate can be transported into the mitochondria where it is able to reform oxaloacetate.



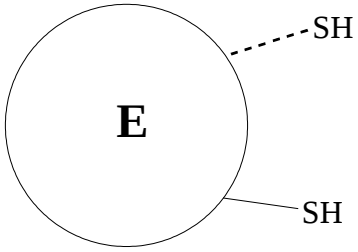
II. Activation of Acetyl-CoA



III Synthesis of fatty acids in mammals consists of 2 identical polypeptide chains. Every chain has 8 domains and 7 enzymatic activities:



1) acetyltransferase;
2) malonyltransferase;
3) β-ketoacylsynthetase (condensing enzyme);
4) β-ketoacylreductase;
5) β-hydroxyacyldehydrase;
6) enoylreductase;
7) deacylase (thioesterase).
8) acyl carrier protein (ACP)



---SH-group of cysteine of condensing enzyme

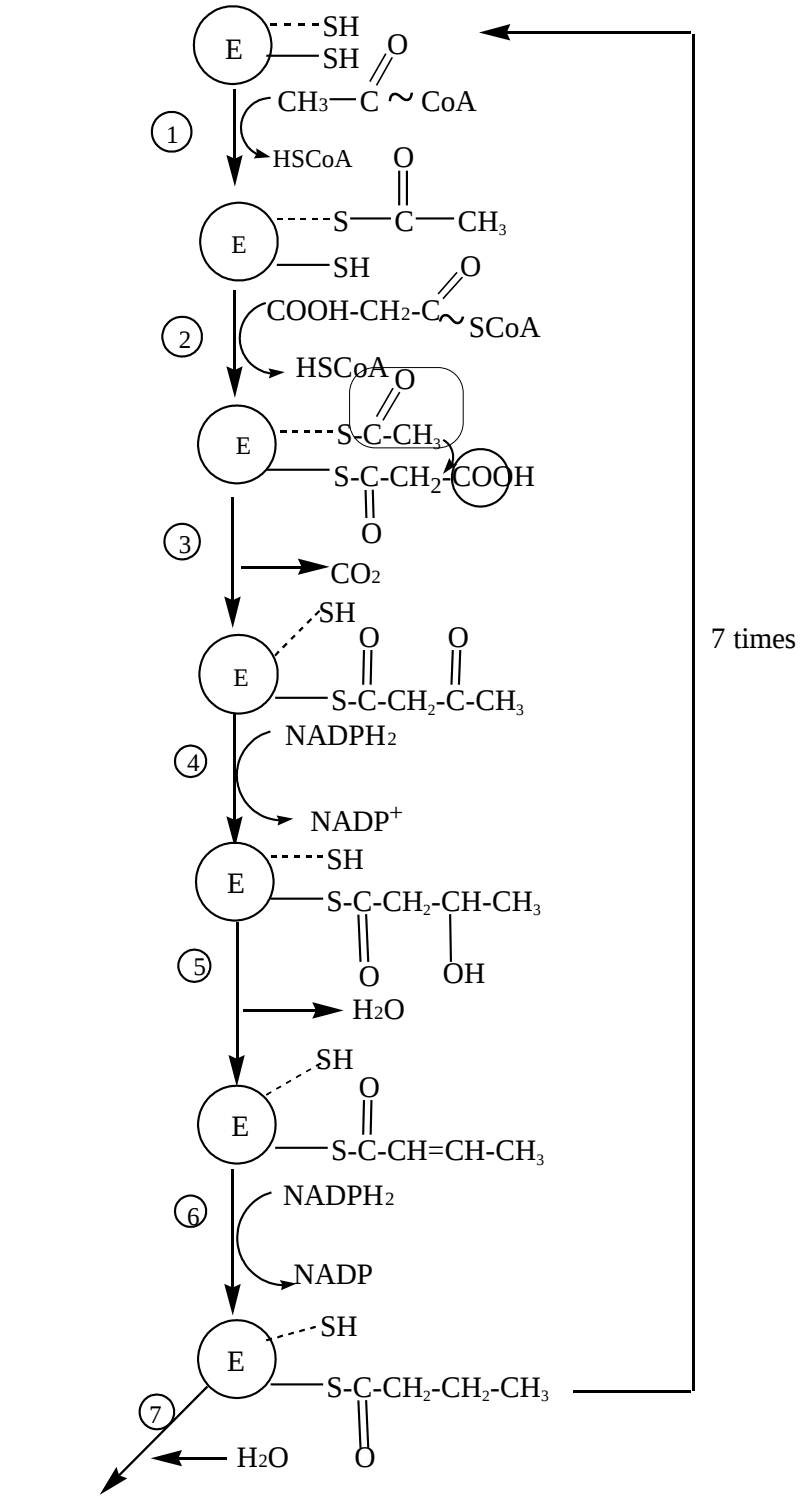
— SH-group of 4-phosphopantotheine

Total reaction

$CH_3COSCoA + 7HOOC\text{-}CH_2\text{-}COSCoA + 14NADPH_2 \rightarrow CH_3\text{-}(CH_2)_{14}\text{-}COOH + 7CO_2 + 6H_2O + 8HSCoA + 14NADP^+$

Sources of $NADPH_2$:

1. Pentose phosphate pathway of glucose oxidation.
2. Reaction malate → pyruvate.



Palmitic acid $C_{15}H_{31}COOH$

Regulation of saturated fatty acid synthesis:

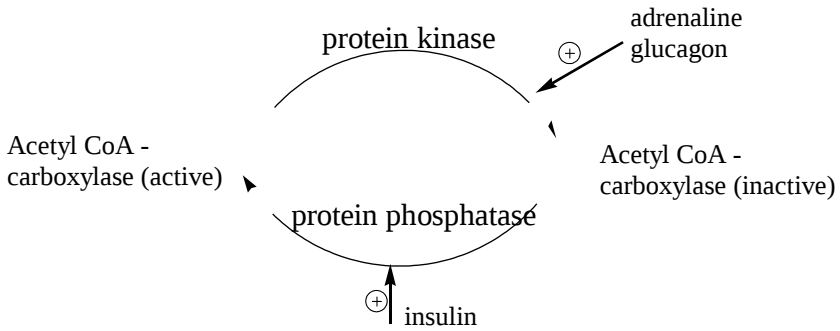
Acetyl-CoA carboxylase is the rate-limiting enzyme (key enzyme). It is regulated:

1. By means of ***allosteric regulation***:

- *citrate* increases activity of the enzyme;

- *acyl-CoA, palmitate* inhibit activity of this enzyme.

2. By means of ***chemical modification (phosphorylation - dephosphorylation)***.



Adrenaline, glucagon decrease the synthesis of fatty acids. ***Insulin*** increases the synthesis of fatty acids.

3. By means of ***changing enzyme synthesis rate*** (long time regulation):

- *insulin* increases the synthesis of enzyme;

- *excess of carbohydrates in food* activates the synthesis of enzyme;

- *starvation, excess of lipids in food* decrease one.

10.9 Metabolism of Cholesterol

Although, much of cholesterol is obtained from diet, the body can synthesize all the cholesterol, which is needed.

- Cholesterol of food ~ 0,5 g;

- Cholesterol, which is synthesized ~ 0,7 g.

Virtually all animal cells, with exception of mature erythrocytes, are capable to synthesize the cholesterol.

The most intensive cholesterol synthesis occurs:

1. In liver (50% - 80%).
2. In small intestine (10 – 15%).
3. Skin (5%).
4. Adrenal cortex.
5. Sex glands.

Cholesterol, which is synthesized in liver and intestine, is used not only by these organs, but is exported to other organs and tissues in VLDL, LDL and chylomicrons.

In addition, the liver is the organ, which excretes excess of cholesterol, either directly or as bile acids.

Synthesis of cholesterol

The microsomal (endoplasmic reticulum) and cytosol fractions of the cell are mainly responsible for cholesterol synthesis.

The biosynthesis of cholesterol may be divided into 5 stages:

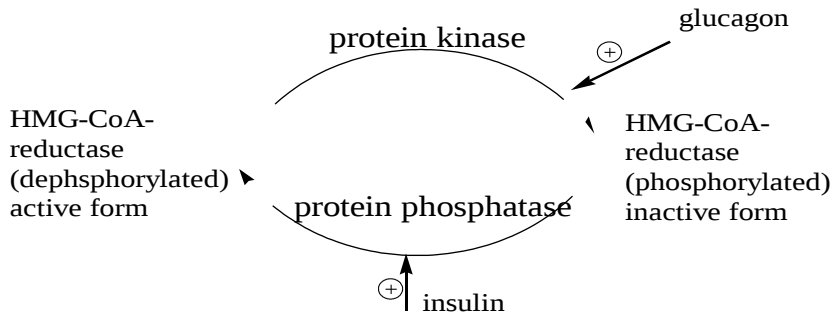
1. The conversion of acetyl-CoA into mevalonate.
2. The formation of isoprenoid units from mevalonic acid.
3. The formation of squalen by means of 6 isoprenoid units condensation.
4. Cyclization of squalen into lanosterol.
5. Conversion of lanosterol to cholesterol.

There are in fact two HMG-CoA synthetase in liver cells, one in mitochondria and one in the endoplasmic reticulum. Presumably the mitochondrial enzyme is principally for ketone body synthesis while HMG-CoA synthesized in the endoplasmic reticulum is converted into cholesterol.

Regulation of cholesterol synthesis. Cholesterol synthesis is controlled by regulation of HMG-CoA reductase. The activity of HMG-CoA reductase is regulated:

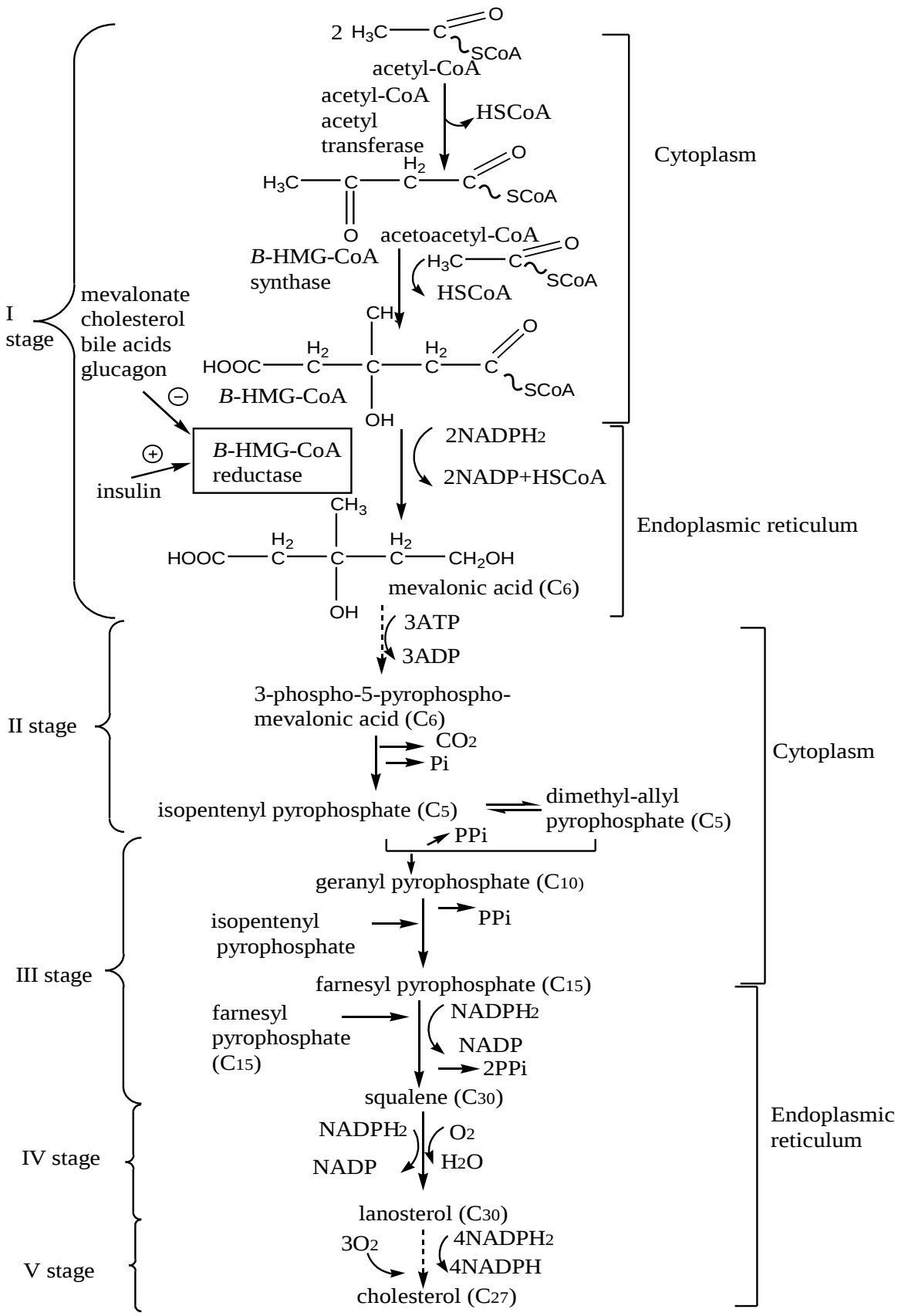
1. Through feed-back inhibition by: mevalonate, cholesterol, bile acids
2. By reversible phosphorylation. Phosphorylation inactivates HMG-CoA reductase and dephosphorylation causes reactivation.

Glucagon promotes phosphorylation of HMG-CoA reductase (it inhibits this enzyme), and insulin promotes dephosphorylation (it activates enzyme).



3. Thyroid hormones increase and glycocorticoids decrease the activity of HMG-CoA reductase.

Scheme of the cholesterol synthesis



Normal content of cholesterol in blood – 3,63 – 6,48 mmol/L (in adults – 2/3 of cholesterol is esterified).

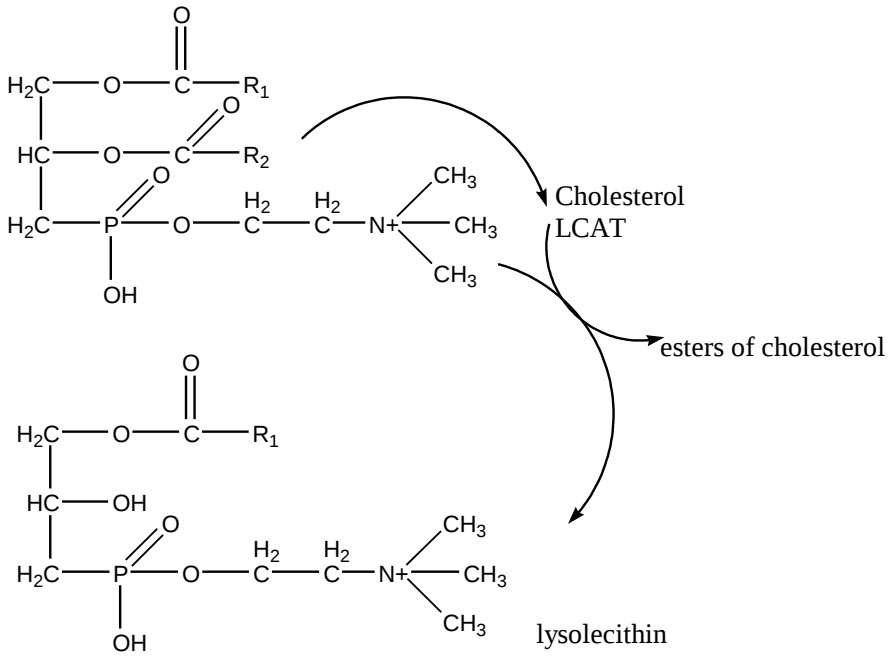
Transport of cholesterol. From intestine cholesterol is transported by chylomicrons. Chylomicrons in adipose tissue are transformed into chylomicron remnants, which are finally metabolized in liver. Therefore cholesterol of food influences the cholesterol synthesis in liver.

From liver cholesterol is initially transported by means of VLDL, part of them in bloodstream is converted into LDL. LDL transport a cholesterol to extrahepatic tissues.

The exchange by cholesterol between different forms of lipoproteins occurs in blood, but especially intensive exchange by cholesterol is observed between LDL and HDL.

In contact of lipoprotein particles cholesterol diffuses from one particle to other, but mainly to particles of HDL, because HDL are physiological substrate of lecithin: cholesterol-acyl thransferase (LCAT). LCAT is synthesized in liver and functions in plasma. It is activated by apoprotein AI, which is included into HDL.

LCAT catalyzes the transfer of acyl residue from β-position of lecithin (phosphatidyl choline) to cholesterol.



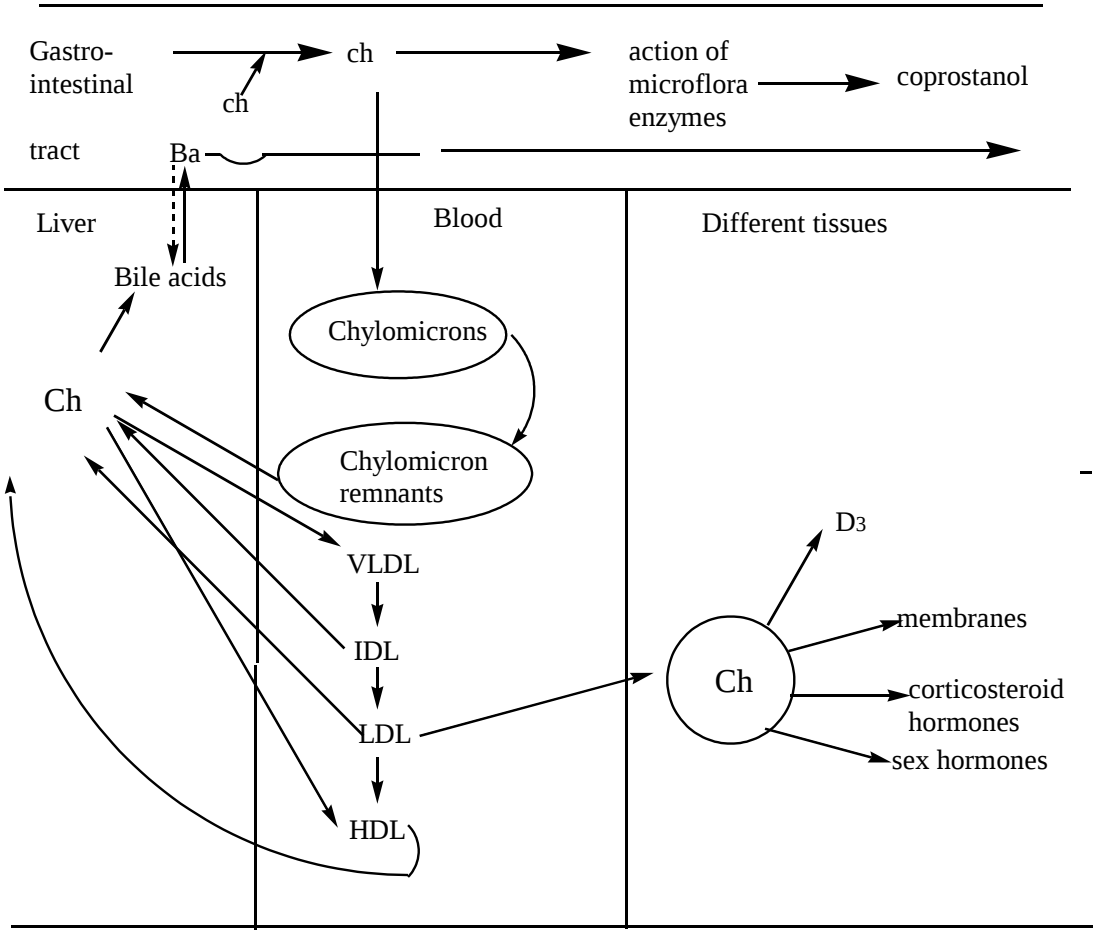
Esters of cholesterol, which are formed, diffuse into hydrophobic core of HDL particle. This leads to the diminishing concentration of free cholesterol in surface layer and to a liberation of place for new cholesterol molecules. Thus, the LCAT system is involved in the removal of excess of unesterified cholesterol from lipoproteins and from tissues.

The liver is the final site of degradation of HDL cholesterol esters.

Elimination of cholesterol. About 1g of cholesterol is eliminated from the body per day as bile acids and as cholesterol.

Cholesterol is converted into *coprostanol* by the bacteria in the large intestine. Coproctanol is the principal sterol in the feces.

Scheme of Cholesterol Metabolism



10.10 Disturbances of Lipid Metabolism

I. Atherosclerosis is linked with the disturbance of cholesterol metabolism, with accumulation of cholesterol and its esters in intima of arteries. Atherosclerotic changes of vessels result in development of hypertension, myocardial ischemia,

infarction, apoplectic stroke. Atherosclerosis correlates with increased level of LDL in blood, with a high plasma LDL/HDL cholesterol ratio.

Atherogenic cholesterol coefficient:

$$\frac{\text{LDL cholesterol + VLDL cholesterol}}{\text{HDL cholesterol}}$$

In healthy person this coefficient is < 3. The atherogenity of VLDL and LDL is considered to be manifested if they undergo peroxide oxidation.

Measures of atherosclerosis prophylaxis:

1. The decrease of fats of animal origin in diet because they contain great amount of cholesterol.
2. The increase of oils (fats of plant origin) in diet:

- β-sitosterol (plant sterol) inhibits the cholesterol absorption;
- oils contain higher level of polyunsaturated fatty acids, which diminish cholesterol level, because they stimulate the oxidation of cholesterol to bile acids, stimulate the cholesterol excretion with bile.

Drugs, which decrease the cholesterol content in blood:

1. Nicotinic acid reduces the flux of FFA (free fatty acids) by inhibition of adipose tissue lipolysis, thereby inhibiting VLDL production by the liver. But it has side effect (skin itch, tachycardia).

2. Cholestyramine resin causes a block in the reabsorption of bile acids.

3. Mevastatin, lovastatin inhibit the activity of HMG-CoA reductase.

4. Sitosterol acts by blocking the absorption of cholesterol from the gastro-intestinal tract.

II. Hyperlipoproteinemias. According to the currently accepted classification, five types of hyperlipoproteinemias are distinguished:

Hyperlipoproteinemia types

Type	Disturbance	Blood cholesterol	Plasma TAGs	Clinical manifestation
I	Excess of *chylomicrons*	increased	increased	xanthomatosis
IIa	Excess of *LDL*	increased	normal	atherosclerotic

		or normal		disturbances; myocardial ishemia
IIb	Excess of *LDL* and *VLDL*	increased	increased	atherosclerotic disturbances: myocardial ishemia
III	Excess of *chylomicron remnants* and *IDL*	increased	increased	atherosclerosis
IV	Excess of *VLDL*	increased or normal	increased	this type is associated with diabetes mellitus, obesity and myocardial ishemia
V	Excess of *VLDL* and *chylomicrons*	increased	increased	xanthomatosis

Xanthomatosis is characterized by deposition of cholesterol and (or) TAG in skin and other tissues.

Hyperlipoproteinemias may occur as primary conditions or secondary to other disorders. ***Primary hyperlipoproteinemias*** are caused by genetic abnormalities of some enzymes synthesis of lipoprotein metabolism or other proteins, for example, receptors to them or apoproteins.

Types I and V may be linked with the deficiency of lipoprotein lipase or apo C-II.

Type II may be linked with LDL receptor defect (apo B mutation).

III. Inherited diseases of sphingolipid metabolism

1. Tay-Sach's disease (GM_2 gangliosidosis) is linked with the deficiency of ***hexosaminidase A***. Accumulation of gangliosides in brain and nervous tissue takes place.

Clinical manifestation:

- mental retardation (progressive development of idiocy);
- cherry-red spots on retina (are caused by destruction of retinal ganglion cells);

- optic nerve atrophy;
- blindness.

Death occurs to 2 – 4 years.

2. **Gaushe's disease** is linked with the deficiency of ***β-glucocerebrosidase***. Accumulation of glucocerebrosides occurs in liver, spleen, nervous tissue, bones.

Clinical manifestation: hepatomegaly; splenomegaly; neuropathy, mental retardation; disturbance of bone tissue.

3. **Niemann-Pick's disease** is an inherited disorder of sphingomyelin metabolism (sphingomyelin is not degraded). It is caused by the deficiency of ***shingomyelinase***.

Manifestation: hepatomegaly; splenomegaly; mental retardation; deafness; blindness. Over 80% of infants with this disease die within 2 years.

IV. Fat liver may be linked with: excess of fats in food; disturbances of phospholipid formation; excessive supply of FFA from adipose tissue to liver.

V. Obesity may be developed in result of: excess of fats and carbohydrates in diet; genetically determined increase of lipogenesis enzymes; disturbances of hormonal control of lipogenesis and lipolysis.

VI. Ketosis (accumulation of ketone bodies) is observed: in diabetes mellitus; in starvation; thyrotoxicosis; long hard work; infectious diseases.

VII. Steatorrhea (increased amount of fat in feces). Disturbances of digestion and absorption of lipids lead to appearance of steatorrhea. It may be linked with diseases of pancreas, liver, gastro-intestinal tract.

Tests for Self-control

1. Cholesterol performs all the below mentioned functions except:

A. It is the component of cellular membranes

B. It is the substrate for the synthesis of bile acids

C. It is the substrate for the synthesis of vitamin D_3

D. It is the source of energy

E. It is the substrate for the synthesis of steroid hormones

2. Linoleic and linolenic acids are necessary for organism as precursors of eicosanoids. Basic source of these acids is:

A. Alimentary factor

B. Biosynthesis of fatty acids

C. Cholesterol degradation

D. Microsomal oxidation

E. Oxidation of fatty acids

3. Choose the second messenger, which participates in activation of hormone-sensitive triacylglycerol lipase:

A. cGMP

B. cAMP

C. Diacylglycerol

D. Ca^{2+}

E. Inositol triphosphate

4. Point the substrate which is used for the formation of glycerol-3-phosphate in process of triacylglycerol biosynthesis in adipose tissue:

A.Glyceraldehyde phosphate

B.Glycerol

C. Glycerate

D.Dihydroxyacetone phosphate

E.Pyruvate

5. Fatty liver is prevented by lipotropic substances. Which of the below mentioned substances belongs to those?

A. Methionine

B. Cholesterol

C. Bilirubin

D. Glycine

E. Glucose

6. Patient has the increased level of LDL in blood serum. Which disease may be in the patient?

A. Gastritis

B. Renal diseases

C. Acute pancreatitis

D. Atherosclerosis

E. Pneumonia

7. Which of the below mentioned substances belongs to ketone bodies?

A. Acetic acid

B. Butyric acid
C. Palmitic acid
D. Oleic acid
E. Acetoacetate

8. Point vitamin-like substance which participates in the transport of fatty acids from cytoplasm to mitochondria:
A. HS-CoA
B. Carnitine
C. Biotin
D. Pantothenic acid
E. Folic acid

9. How many carbon atoms are removed from high fatty acid by means of 1 cycle of β-oxidation?
A.3
B.4
C. 2
D.1
E. 0

10. Hyperketonemia is observed in all the below mentioned cases with exception:
A. Starvation
B. Diabetes mellitus
C. Excess of carbohydrates in diet
D. Chronic stress
E. Thyrotoxicosis

11. Choose the process which is changed in excessive supply of cholesterol with food:
A. The synthesis of endogenous cholesterol is accelerated
B. Catabolism of cholesterol to CO_2 and H_2O is activated
C. Synthesis of cholesterol in liver decreases
D. β-Hydroxymethylglutaryl-CoA reductase activity increases
E. β-Hydroxymethylglutaryl-CoA synthase activity diminishes

12. Point the regulatory enzyme of cholesterol synthesis:
A. Acetyl-CoA-acetyl transferase
B. β-Hydroxymethylglutaryl-CoA reductase
C. β-Hydroxymethylglutaryl-CoA synthase
D. Acetyl-CoA carboxylase
E. Thiolase

13. Colloid properties of bile are disturbed in inflammatory processes in gallbladder. This can result in the formation of bile stones. Which substance crystallization is the basic cause of their formation?
A. Urates
B. Chlorides
C. Cholesterol
D. Oxalates

E. Phosphates

14. The main reducing agent in the synthesis of cholesterol is:

A. $NADPH + H^+$

B. $FADH_2$

C. $FMNH_2$

D. $CoQH_2$

E. $NADH + H^+$

15. Lipolysis is the enzymatic process of fat hydrolysis to fatty acids. Fatty acids reach the bloodstream and are transported with blood in the composition of:

A. LDL

B. Globulins

C. HDL

D. Albumins

E. Chylomicrons

16. What enzyme insufficient secretion causes the impaired digestion of lipids in gastro-intestinal tract and the appearance of the large quantities of neutral fats in feces?

A. Pepsin

B. Phospholipase

C. Enterokinase

D. Amylase

E. Pancreatic lipase

Chapter 11 METABOLISM OF SIMPLE PROTEINS

Proteins are fundamental to all organisms. They are the most versatile macromolecules in the cell.

Proteins are known to be in the dynamic state. Many proteins have turnover times of less than an hour. Each day, humans turn over 1-2% of their total body protein, principally muscle proteins. Of the liberated amino acids, 75-80% are reutilized for new protein synthesis.

In spite of the above mentioned, the overall plasma amino acid concentration remains remarkably stable; although the level may rise by 30-100% for a few hours after a meal containing protein, there is no fall below normal on fasting, even if that of prolonged. Apparently there are several tissue amino acid pools, not all readily communicating with one another, and the ability of tissues to take up particular amino acids also varies widely.

The necessary amount of protein per day is 85-90 g for adults and 1,5-4 g per kg of weight for children.

But it’s important to know that not only quantity but also quality of protein are very important. Biological value of protein depends on chemical composition and assimilability. The human body requires certain amino acids to be supplied in at least minimal amounts as the organism can’t produce them itself and they are the essential amino acids. Proteins must contain all essential amino acids and the latter must be included into protein in optimal proportion.

The Food and Agriculture Organization of UNO has worked out a “protein score”, based on the essential amino acid contents of proteins.

The table 11.1 shows that tryptophan is the amino acid needed in the least amount. “Pattern” is the composition of an ideal food protein. Milk protein, of high biological value, contains 1,4 g tryptophan per 100 g protein. The ideal protein should contain per 100 g 1,4 g tryptophan, 2,8 g threonine, 4,2 g lysine and so on.

Table 11.1 Requirements for essential amino acids

Amino acid	"Pattern" (Trp=1)	Recommended intake (g/day)
Histidine	2,0	1,0
Isoleucine	3,0	1,5
Leucine	4,0	2,0
Lysine	3,5	1,8
Methionine	3,7	1,9
Phenylalanine	4,0	2,0
Threonine	2,0	1,0
Tryptophan	1,0	0,5
Valine	3,0	1,5

In general all animal proteins (except gelatine) are complete; cereal proteins are incomplete, lacking lysine. Leguminous proteins are incomplete, lacking methionine. A suitable mixture of cereals and legumes can therefore provide a full complement of essential amino acids.

A diet that is adequate in energy but limited in protein, either qualitatively or quantitatively, results in the clinical condition known as **kwasiorkor.** The condition which is caused by the deficiency of both energy and protein (protein-energy malnutrition) is called **marasmus.**

11.1 Digestion and Absorption of Proteins

Proteins are amino acid polymers. They are characterized by primary, secondary and tertiary structures and oligomer proteins have quaternary structure in addition to those above. Secondary, tertiary and quaternary structures are formed by means of non-covalent bonds, that is, by hydrogen, hydrophobic and ionic bonds. And the only covalent bond, that is, disulfide one participates in the formation of tertiary and quaternary structures. All the mentioned structures (except of primary) are destroyed by heat treatment and by exposure to the strongly acidic conditions of the stomach.

Digestion of proteins leads to breaking of peptide bonds. The purpose of protein digestion is to eliminate their type and tissue specificity and to convert them into derivatives which are able for absorption.

Proteins are degraded by the action of proteolytic enzymes (proteinases). Most proteinases show some degree of specificity for the amino acids forming the bond, particularly on the acyl side R.

The proteinases of the digestive tract are of two kinds: the endopeptidases, which attack the bond distant from the ends of peptide chains, forming peptides; and exopeptidases, which attack terminal peptide bonds, liberating amino acids.

The proteinases have no prosthetic group, but a number of exopeptidases requires a metal ion as activator.

All the proteinases are synthesized and secreted as inactive precursors, called **zymogens**, which are converted into active proteinases when they reach the digestive tract. This activation involves limited proteolysis of the zymogens.

The synthesis of proteolytic enzymes as inactive precursors is the protective mechanism from autodigestion of cells, producing these enzymes.

Endopeptidases

Pepsin is secreted in the gastric juice as an inactive precursor, **pepsinogen**. Under acidic condition, this is spontaneously converted into a pepsin-inhibitor complex and various small peptides. The reaction is very slow at pH=6.0, but almost instantaneous at pH=2.0, and is also autocatalysed by **pepsin**. Pepsin is a very acidic protein with an **isoelectric point** less than pH=1 and an optimal pH of 1.5-2.5, depending on substrate. It preferentially attacks peptide bonds formed by amine groups of aromatic amino acids and something other links. Pepsin, like **rennin**, will coagulate milk by converting the phosphoprotein casein to paracasein, which forms an insoluble complex with calcium. **Rennin (chymosin)** is important in the digestive processes of infants because it prevents the rapid passage of milk from the stomach. In the presence of calcium, rennin changes the casein of milk to paracasein. Rennin is reported to be absent from stomach of adults.

Trypsin is secreted by the pancreas as an inactive precursor, trypsinogen, which is activated by **enteropeptidase** (previously called **enterokinase**), secreted by the intestinal mucosa. Thereafter, trypsin is activated autocatalytically. A hexapeptide is cleft from the N-terminus during activation. Trypsin plays a **central**

role in activation of other proteolytic enzymes of pancreas. Trypsin has no prosthetic group. Its optimal pH is 7-9. It is quite specific, catalyzing hydrolysis only of bonds in which a strongly basic amino acid (lysine or arginine) provides the carboxyl group.

The **chymotrypsin** group of enzymes is all derived from common precursor **chymotrypsinogen** secreted by the pancreas. Its activation is initially brought about by trypsin to give an active chymotrypsin which may be converted to other chymotrypsins by autolysis. The optimal pH is 7-8, and the enzymes have no prosthetic group. They attack not only peptides, but also esters, although the chymotrypsin manifests the greatest activity to peptide bonds which are formed by carboxyl group of phenylalanine, tyrosine, tryptophan.

Elastase (pancreatopeptidase E), from pancreas, hydrolyzes peptide bonds adjacent to small neutral amino acid residues as Ala, Gly and Ser.

These endopeptidases (pepsin, trypsin, chymotrypsins and elastase) bring about the hydrolysis of large protein molecules to smaller peptide fragments. Their further hydrolysis then depends on the action of a number of exopeptidases, either secreted by the pancreas (carboxypeptidases) or to be found within the cells lining the intestinal mucosa.

Exopeptidases

A number of these enzymes, in contrast to the endopeptidases, require a metal ion as activator.

The two **carboxypeptidases** contain Zn, and are secreted as procarboxypeptidases which are activated by trypsin. **Carboxypeptidase A** hydrolyzes the peptide bonds, which are formed by carboxyterminal aromatic acids. **Carboxypeptidase B** hydrolyzes peptides with carboxyterminal lysine or arginine.

Aminopeptidases:

Alaninaminopeptidase catalyzes the hydrolysis of peptide bond formed by N-terminal alanine. **Leucinaminopeptidase** is rather unspecific, in spite of its name.

Dipeptidases, bound to the brush border of the intestine, hydrolyze dipeptides to amino acids.

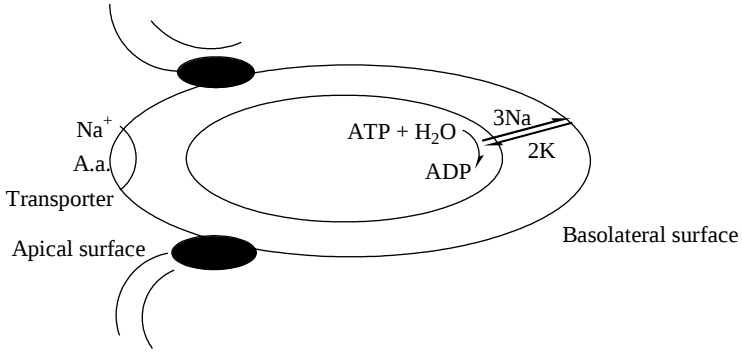
HCl plays an important role in protein digestion. It is secreted by the parietal cells of gastric mucosa. HCl secretion by parietal cells is stimulated by acetylcholine, by histamine and gastrin, a peptide hormone released into the blood by the stomach after feeding.

HCl functions:

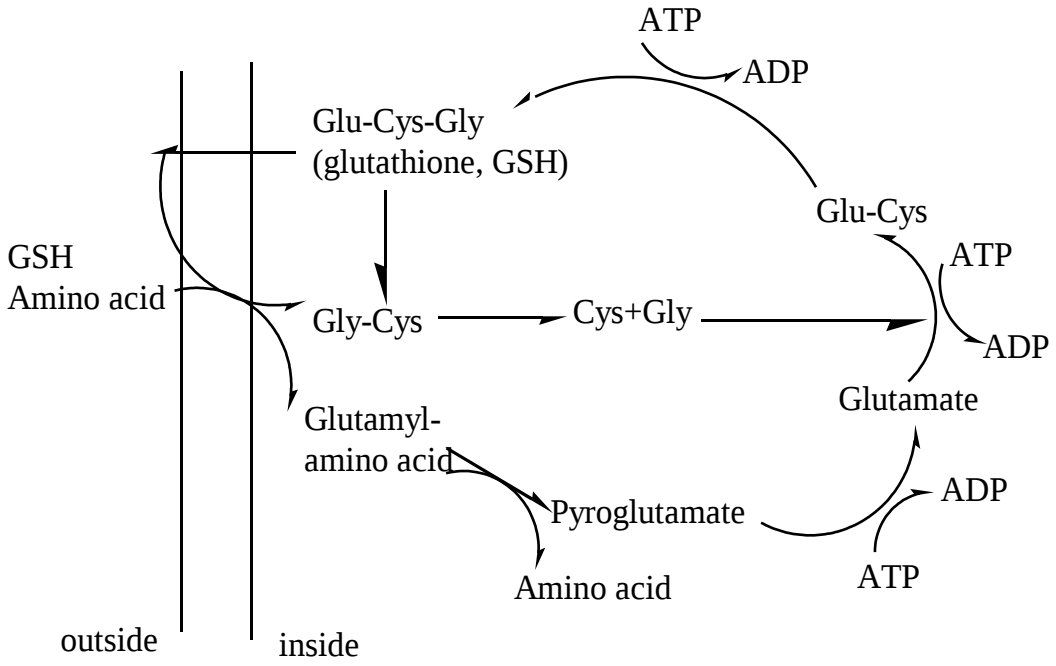
- It causes the swelling of proteins.
- It provokes the protein denaturation.
- HCl activates pepsinogen.
- HCl forms optimal pH for pepsin.
- It shows bactericidal effect.
- HCl stimulates the secretin production in intestine.
- It increases Fe absorption.
- HCl favours the formation of Castle's factor.

Absorption

The absorption of amino acids and peptides is an active process, driven by energy derived from metabolism within the mucosa cells. As in intestinal glucose uptake, amino acid transport is coupled to the uptake of Na^+, and is driven by the large difference in [Na^+] across the brush border membrane. This concentration difference is maintained by the Na^+, K^+-ATPase, so amino acid uptake is indirectly driven by ATP hydrolysis. At least seven Na^+-linked amino acid carriers of different but over lapping specificity have been found, and there are separate transporters for di- and tripeptides. This is *secondary active transport*.



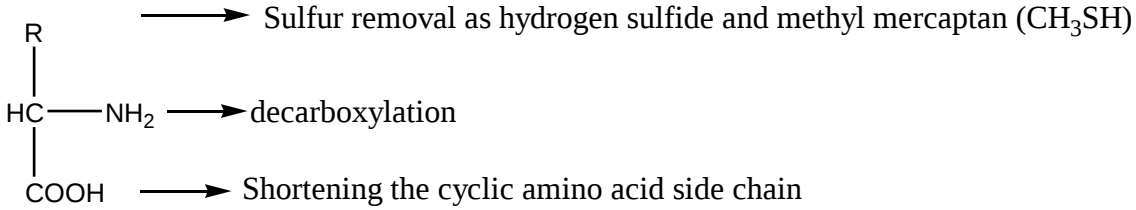
A different means of amino acid uptake is by the *γ -glutamyl cycle*. The participation of this process in intestinal absorption seems unlikely; it may be more important in the uptake of amino acids from the blood, for example by brain. The glutathione used in this process comes from the plasma; the γ -glutamyl-amino acid is translocated into the cell, where the glutamyl group is removed as pyroglutamate. The dipeptide Cys-Gly is also taken up and hydrolysed.



Third absorption mechanism is the *pinocytosis*. Food protein fragments absorption takes place in the duodenum and in the jejunum, much of them as di- and oligopeptides. In the newborn infant considerable amounts of colostrum proteins can be absorbed, particularly in the first 48 hours of life. This process is, however, very selective, greatly favoring globulins, at the expense of albumins. The absorption of the whole proteins is believed to cease after the first 2 weeks of life, and to be able to confer some valuable immunological protection. However, the increased permeability of infant intestinal mucosa can result in food allergy.

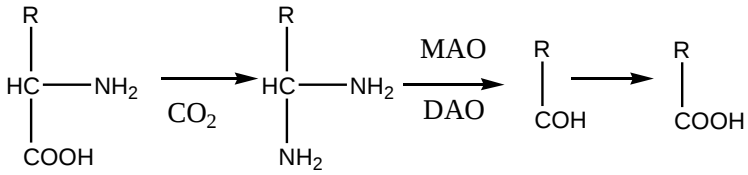
In intestine about 85% of all the amino acids are absorbed. Proteins, which aren’t hydrolysed by proteolytic enzymes, and amino acids, which are not absorbed in intestinal mucosa, are influenced by microbe enzymes in large intestine. This process is called ***protein putrefaction***.

Amino acids may undergo the following conversions by microbe enzymes:



Amino Acid Decarboxylation

Amino acid decarboxylation results in mono- and diamines formation.



Monoamines and diamines are eliminated by monoamine oxidases (MAO) and diamine oxidases (DAO) in intestinal mucosa, part of them removes from organism in inchanged state.

ornithine ⟶ putrescin

lysine ⟶ cadaverin

arginine ⟶ agmatine

Cyclic Amino Acid Conversions by Microbe Enzymes

Phenylalanine is converted into toluol and then into benzoic acid. Benzoic acid is detoxified in the liver, linking with glycine. Hippuric acid (benzoil glycocole) is formed during this reaction which is used for determination of liver detoxification function and it is called Quick-Petel's test.The test consists in the following: patients are given 4 grammes of sodium benzoate. The liver function is considered to be normal if 2 hours after 50% of sodium benzoate removes as hippuric acid and 4 hours after 70% of sodium benzoate removes as hippuric acid.

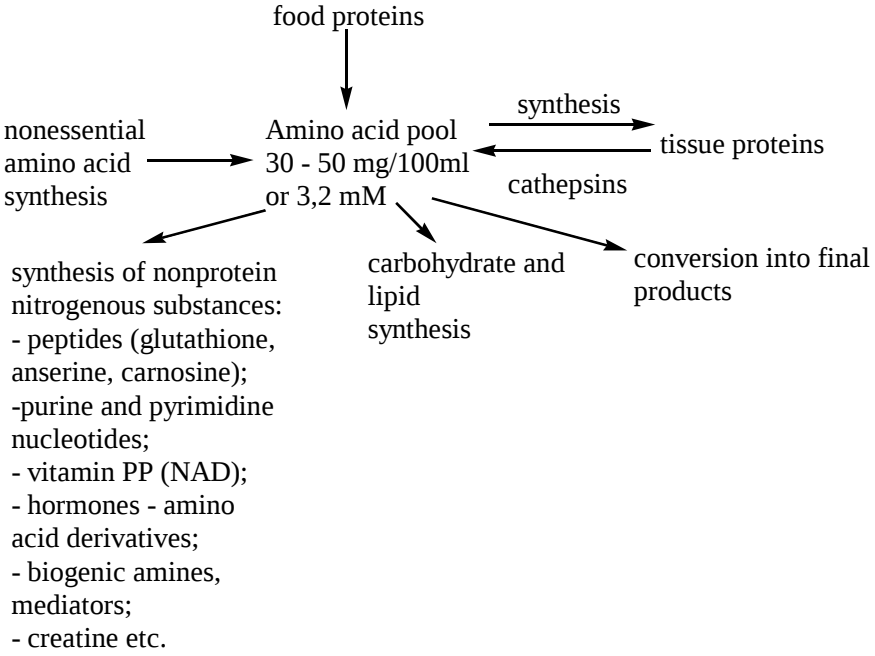
Tyrosine is converted into crezole and then into phenol, which is also detoxified in liver, linking with glucuronic acid. The latter participates in the reaction as UDP-glucuronic acid.

Tryptophan is converted into scatol and indol. In its turn in liver indol is oxidized into indoxyl, which is linked with sulfuric acid. Sulfuric acid participates

in the reaction as PAPS (3`-phospoadenosine-5`-phospho-sulfate). Indoxyl-sulfuric acid formed is converted into potassium salt of indoxyl-sulfuric acid, which is called animal indican. Urine indican amount shows the putrefaction process rate in intestine and in the whole organism. If noconjugated compounds are excreted, the liver doesn't perform the above mentioned functions.

Exogenous proteins aren't the only source of amino acids for ***amino acid pool*** formation. The other sources are tissue proteins and synthesis of nonessential amino acids. The amount of the pool is constant in normal adults. Amount of amino acids in the pool indicates the difference between that of amino acids supplied and used.

Sources of Amino Acid Pool and Pathways of Its Utilization



11.2 Tissue Proteolysis. Cathepsins

Tissue protein desintegration is performed by intracellular proteinases called cathepsins. They differ from each other by substrate specificity, optimal pH and other properties. Cathepsins are mainly located in lysosomes as many other hydrolytic enzymes. Lysosomes mostly degrade proteins ***nonselectively***. Lysosomes also have a ***selective*** pathway which is activated only after a prolonged starvation. The other intracellular proteinases are located in cytoplasm. Cytosolically based mechanism of protein degradation is ***ATP-dependent*** and ***selective***. Proteins that are selected for degradation are marked by covalently

linking to ***ubiquitin.*** Intracellular proteinases may be active in different medium. For example: lysosomal cathepsins are active in acid medium; cytosolic proteinases are active in neutral and weak basic ones. The cathepsins are divided into 2 groups like intestine proteolytic enzymes: the endopeptidases, which attack the bond distant from the ends of peptide chains; exopeptidases, which attack terminal peptide bonds, liberating amino acids.

Depending on the active site structure cathepsins are divided into:

1) serine proteinases, containing the serine in active site;

2) thiol proteinases, containing the cysteine in active site ;

3) asparagine proteinases, containing the aspartic acid in active site.

Biological meaning of cathepsins

Tissue proteolysis is necessary for:

1) renovating proteins;

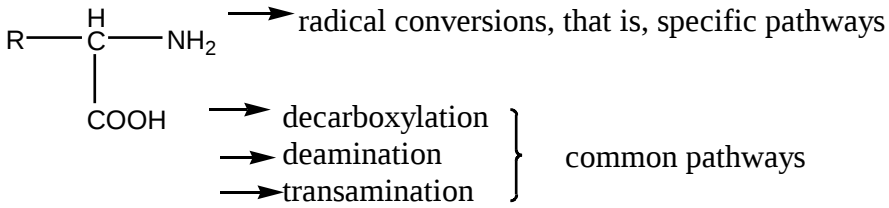
2) elimination of protein molecule defected;

3) mobilization of endogenous proteins with energy purpose;

4) regulatory function, because cathepsins are able to perform the limited proteolysis.

11.3 Common Pathways of Amino Acid Metabolism: Decarboxylation, Deamination, Transamination

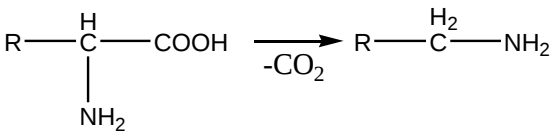
Amino acids are able to the following conversions:



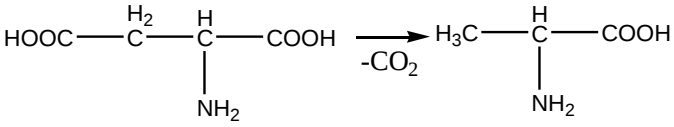
Decarboxylation

Four types of decarboxylation occur in living organisms:

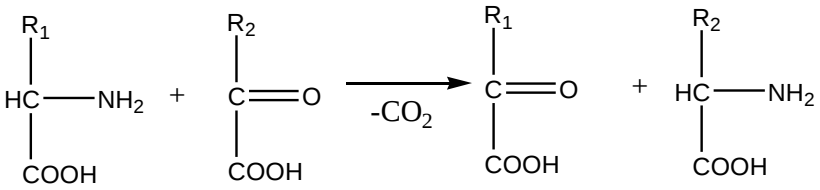
1) α -decarboxylation



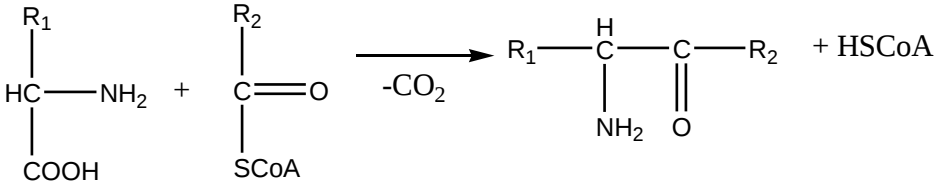
2) ω -decarboxylation (peculiar for microorganisms)



3) decarboxylation which is linked with the transamination reaction

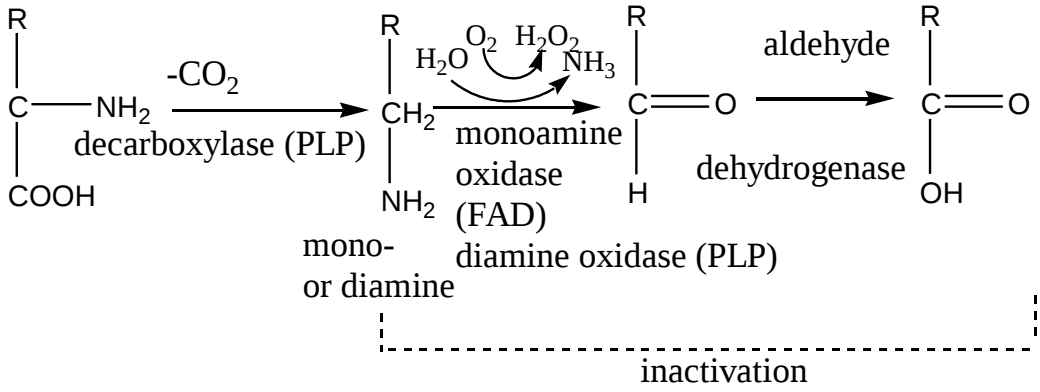


4) decarboxylation which is linked with the reaction of 2 molecules condensation



First and fourth types are typical for animal and human tissues.

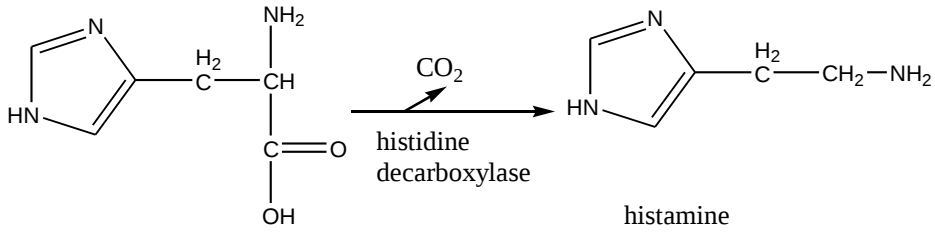
α-Decarboxylation is the most spread:



Decarboxylation reactions are catalysed by decarboxylases. Pyridoxal phosphate (PLP) is the prosthetic group of these enzymes. Biogenic amines formed during these reactions show high biological activity, thus they are rapidly inactivated by monoamine oxidases and diamine oxidases with aldehyde formation. Then aldehydes are converted into corresponding acids.

Phenylalanine derivatives, tryptophan, 5-hydroxytryptophan, cysteine, cysteinic acid, cysteine sulfinate, glutamic acid, S-adenosyl methionine, histidine, ornithine are able to decarboxylation.

Decarboxylation of histidine



histidine histamine

Histamine

H_1 receptors are coupled with phosphatidyl inositol messenger system.

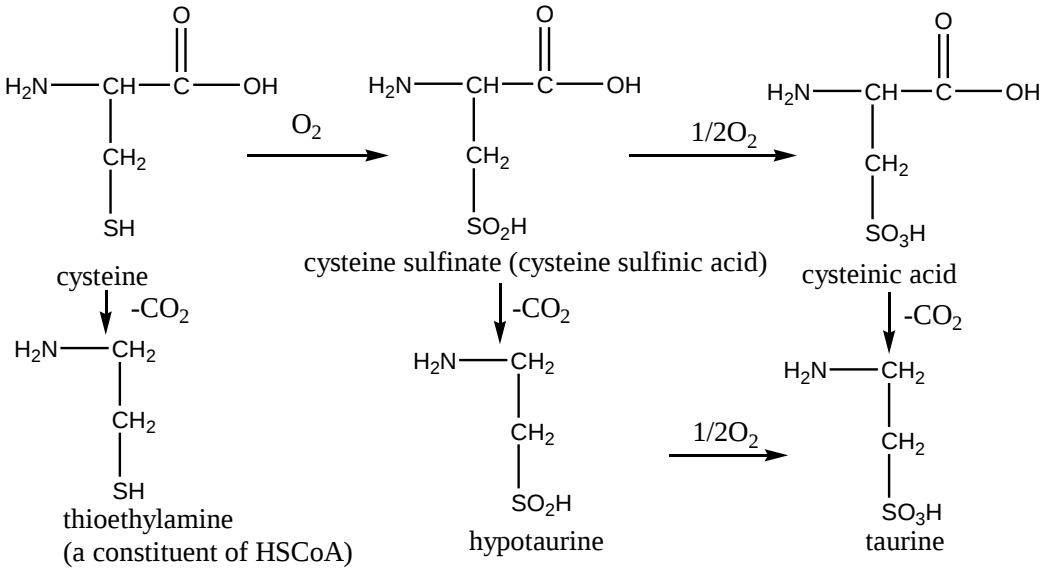
H_2 receptors are coupled with adenylyl cyclase messenger system.

Histaminergic neurones of CNS, gastric mucosa cells, basophils, mast cells are the chief source of histamine.

Functions of histamine:

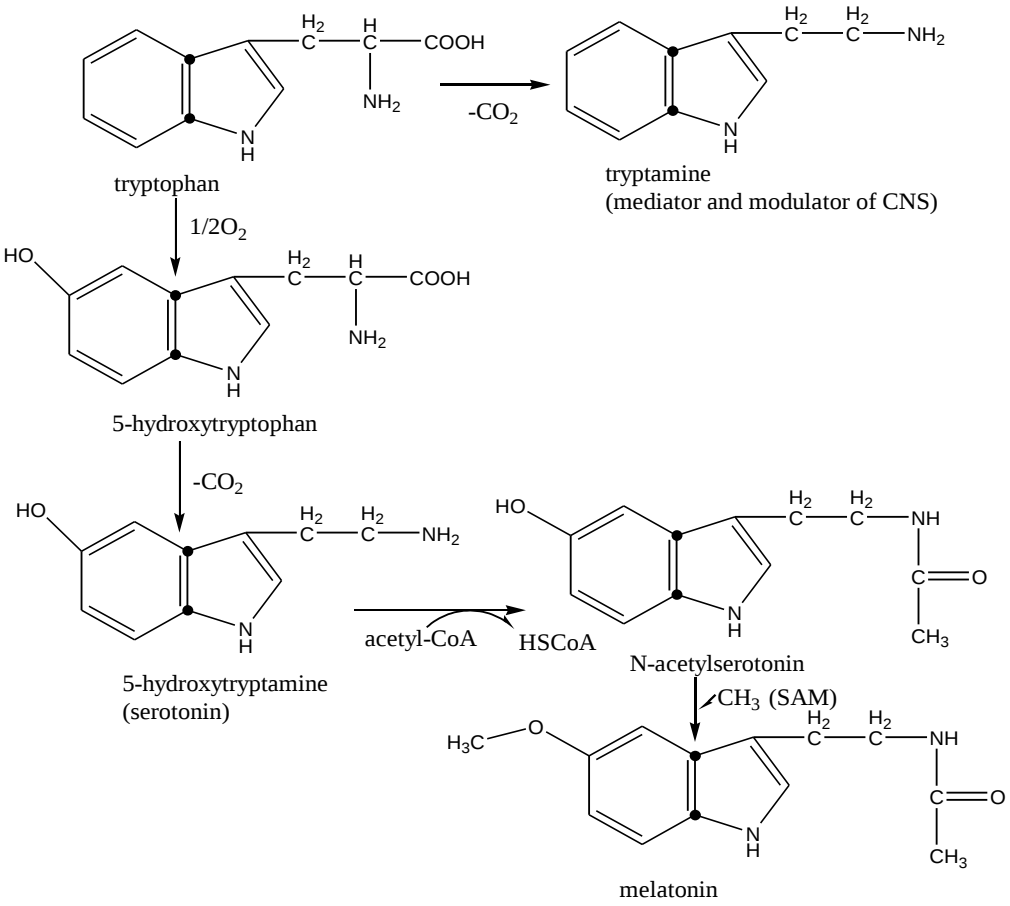
- contraction of smooth muscles of gastro-intestinal tract, bronchi;
- it increases HCL secretion in stomach;
- it shows vasodilatory effect;
- it increases vasopermeability;
- it is the inflammatory process mediator;
- it is the allergic reaction mediator;
- it is the central nervous system mediator as well.

Decarboxylation of cysteine and its derivatives



In liver taurine is conjugated with bile acids, forming bile salts, which play the important role in digestion and absorption of lipids. Taurine is also found in muscles, particulary in heart ones. It favours potassium ion delay in miocardium cells. Therefore it favours normalization of miocardium function. Taurine shows antiradiation effect. It is inhibitory mediator in central nervous system.

Decarboxylation of tryptophan and its derivatives (5-hydroxytryptophan)



Functions of serotonin:

- mediator of CNS;
- potent vasoconstrictor;
- stimulator of smooth muscle contraction (of bronchi, uterus, intestine);
- mediator of inflammation;
- participates in regulation of body temperature, breathing, renal filtration;
- modulate the process of blood clotting.

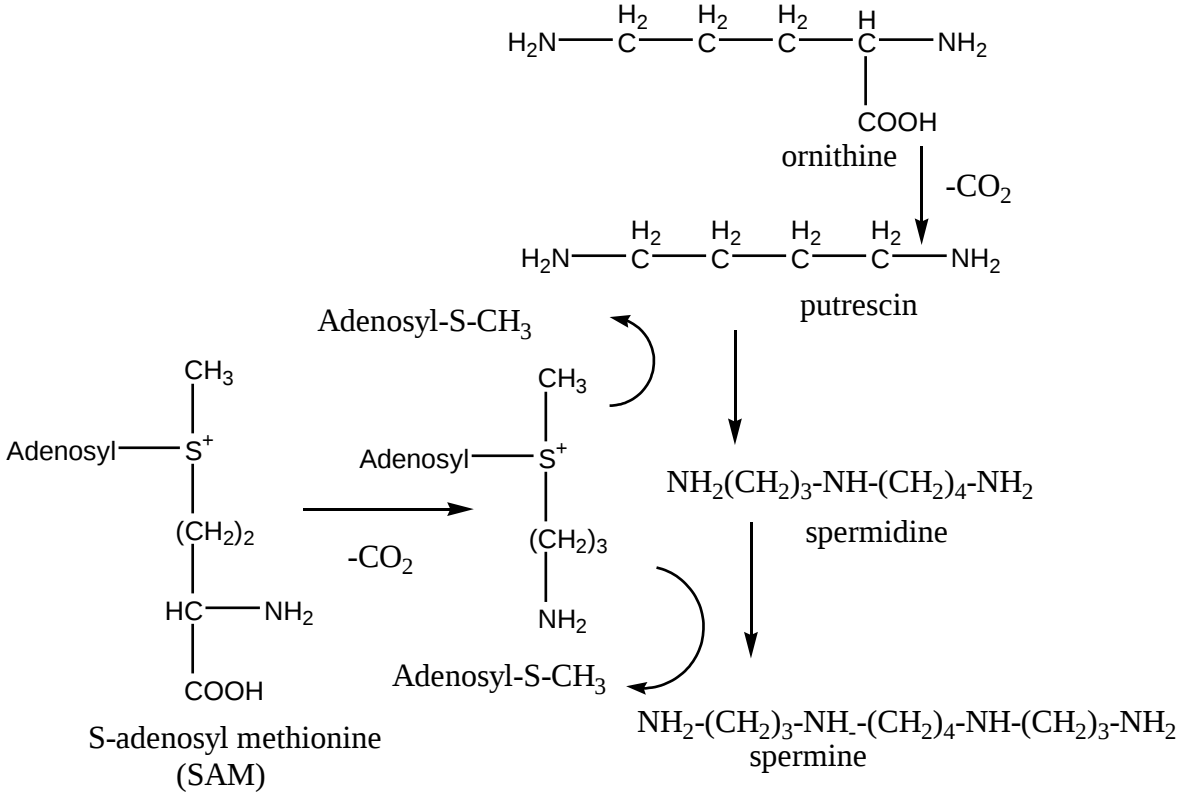
Function of melatonin (see chapter 8).

Synthesis of polyamines

Ornithine is converted into putrescin by ornithine decarboxylase, which has an extremely short half-life. Putrescin is formed in all the cells during life. It is the precursor of spermidine and of spermine (the latter is present only in eukaryotes). All cells in the body appear to be able to make at least spermidine. The mechanism involves the decarboxylation of methionine.

Copper-containing diamine oxidases oxidize putrescin, spermidine and spermine.

The polyamines spermidine and spermine have been implicated or involved in a wide variety of physiologic processes, most of which are closely related to cell proliferation. These polyamines have been implicated in the stabilization of whole cells, subcellular organelles, and membranes. Because of their highly positively charged character, they associate readily with polyanions such as RNA and DNA, and have been implicated in such fundamental processes as stabilization of DNA, stimulation of DNA and RNA synthesis, etc. The polyamines also exert diverse effects on protein synthesis and act as inhibitors of numerous enzymes including several kinases.

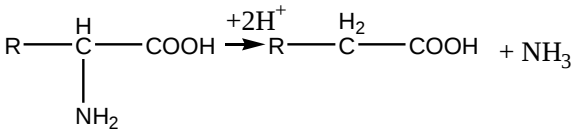


Deamination of Amino Acids

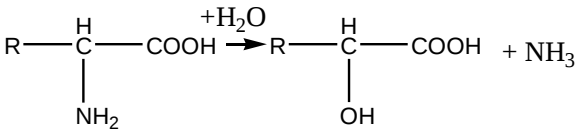
Amino acids in excess of those needed for the synthesis of proteins and other biomolecules cannot be stored in the organism in contrast to fatty acids and glucose. Rather, surplus amino acids are used as metabolic fuel. The amino group is removed and the resulting carbon skeleton is converted into major metabolic intermediates.

In living organism there are four types of deamination:

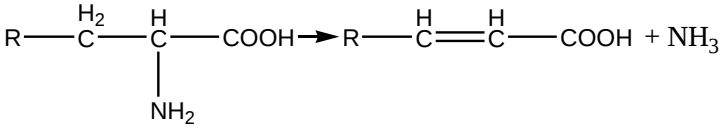
1) reductive deamination:



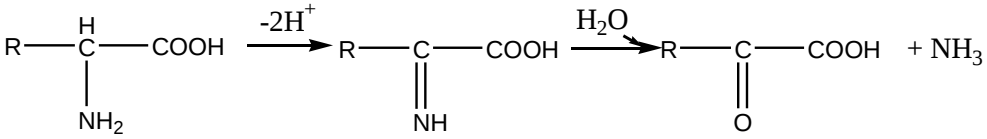
2) hydrolytic deamination:



3) intramolecular deamination:

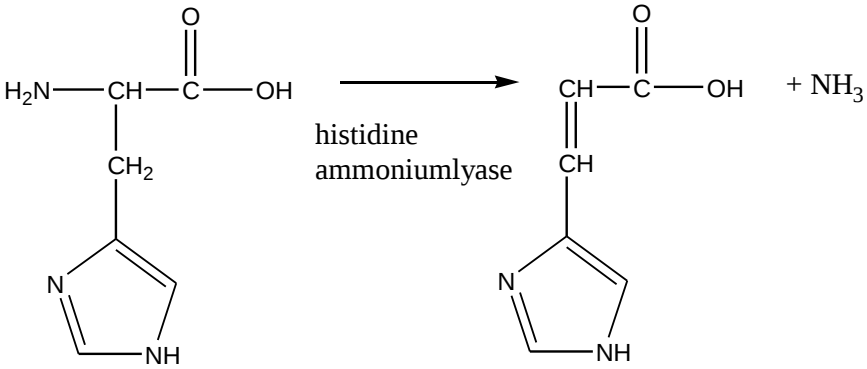


4) oxidative deamination

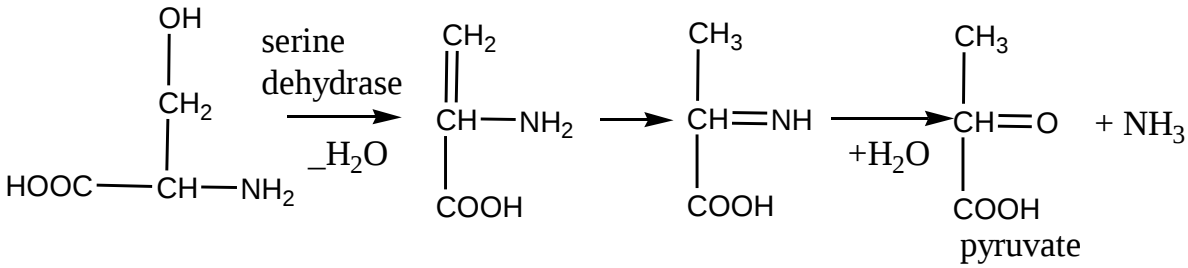


In animal's and human's tissues ***oxidative deamination prevails***.

- One amino acid namely histidine undergoes to ***intramolecular deamination***.



- Serine, threonine and cysteine α -amino group is removed by ***nonoxidative deamination***. Enzymes catalyzing these reactions are serine dehydrase, threonine dehydrase and desulfhydrase. Pyridoxal phosphate is prosthetic group of these enzymes.



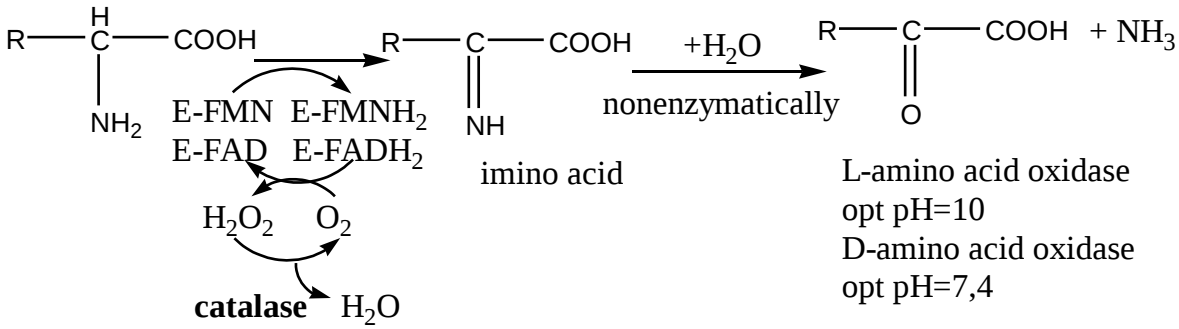
Oxidative deamination

Direct oxidative deamination Indirect oxidative deamination

- In human tissues ***direct oxidative deamination*** is performed by the following enzymes: L-amino acid oxidase, D-amino acid oxidase, glutamate dehydrogenase.

Amino acid oxidases are conjugated flavoproreins containing FMN and FAD as a coenzyme. In reaction catalyzing by amino acid oxidase the amino acid is first dehydrogenated forming an α -imino acid. This α -imino acid spontaneously adds water, then decomposes to the corresponding α -keto acid with the loss of iminonitrogen as ammonium ion. L- Amino acid oxidases can contain both FMN and FAD, while D-amino acid oxidases, only FAD as a prosthetic group.

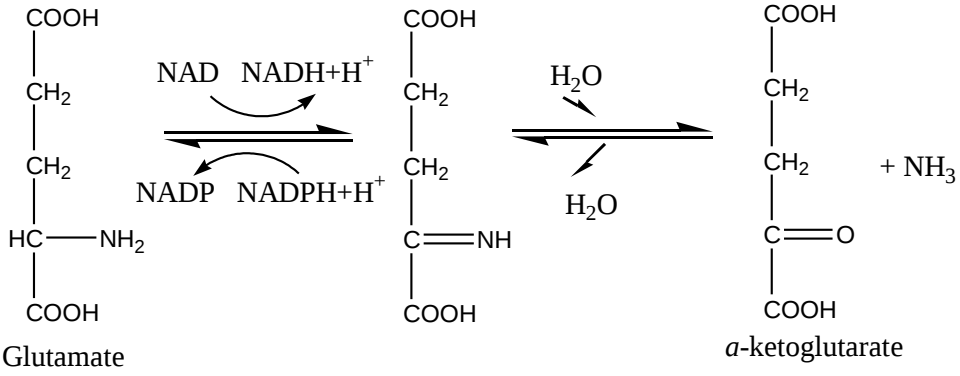
Amino acid oxidases are auto-oxidizable flavoproteins, i.e., the reduced FMN or FAD is reoxidized directly by molecular oxygen forming hydrogen peroxide (H_2O_2) without participation of cytochromes or other electron carriers. The toxic product H_2O_2 is then split to O_2 and H_2O by catalase, which occurs widely in tissues, especially in liver.



The activity of L-amino acid oxidase, however, is low even in tissues such as liver and kidney in which amino acid catabolism is rapid, because its optimal pH is 10,0. D-Amino acid oxidase is present in high concentration in kidney, in particular, and has optimal pH of 7,4. D-amino acids occur in some peptides of bacterial origin, but they are quantitatively unimportant, besides, some amino acids, such as methionine, racemize quite readily. Therefore D-amino acid oxidase seems to be a detoxifying enzyme.

Glutamate dehydrogenase reaction

Glutamate dehydrogenase requires either NAD or NADP as coenzyme. Glutamate dehydrogenase is present in high concentration in the mitochondria of some tissues. The reaction catalyzed by glutamate dehydrogenase is reversible. The reverse reaction is called the reaction of reductive amination.



The equilibrium favours glutamate synthesis, but is pulled in the direction of deamination by the continuous removal of NH_4^+. The activity of glutamate dehydrogenase is allosterically regulated. Guanosine triphosphate (GTP) and adenosine triphosphate (ATP) are allosteric inhibitors, whereas guanosine

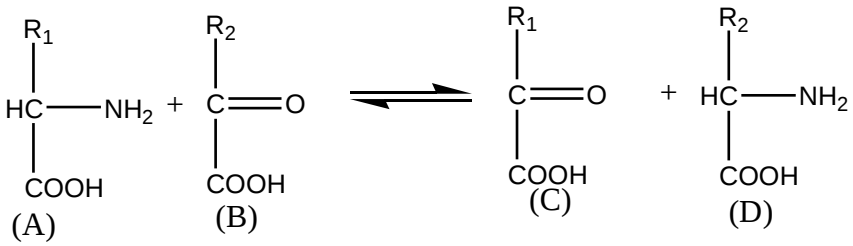
diphosphate (GDP) and adenosine diphosphate (ADP) are allosteric activators. Hence, a lowering of the energy charge accelerates the amino acids oxidation.

Direct deamination is unimportant, instead amino groups of most L-amino acids are removed by transamination.

Transamination

Transamination reaction has been discovered by Russian scientists - married couple A. Braunstein and M. Krizman.

Liver, kidneys and many other tissues contain amino transferases (also called transaminases) that catalyze the reversible transfer of amino groups from amino acid to keto acid.

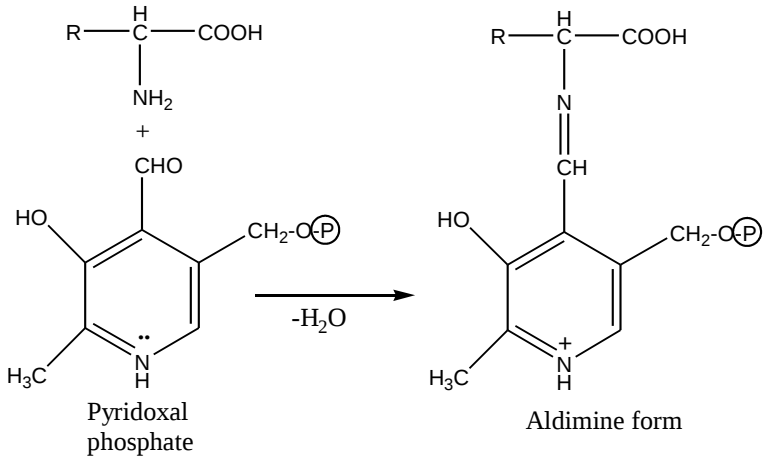


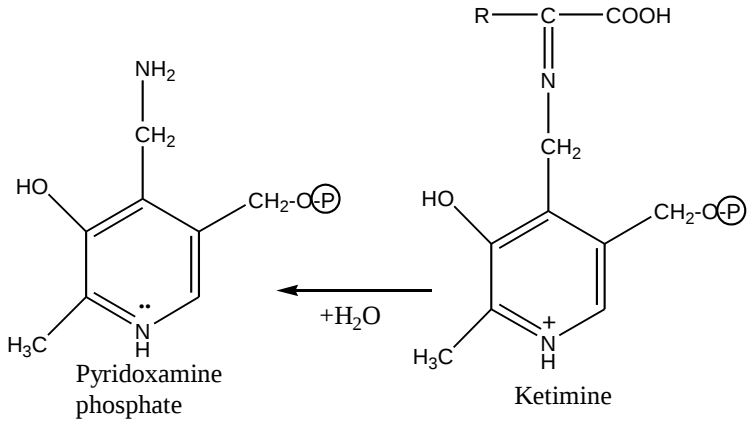
The amino donor (A) may be one of many naturally occuring amino acids (although some of them such as arginine, lysine, methionine, tryptophan and proline, are never transaminated).

The mechanism of transamination

All aminotransferases require, as prosthetic group, pyridoxal phosphate (PLP, derived from vitamin B_6). PLP was discovered by Esmond Snell in 1944. Esmond Snell and Alexander Braunstein proposed the reaction mechanism for transamination that proved to be generally valid.

Pyridoxal phosphate acts as a carrier of amino groups between amino acid and α-keto acid. During transamination, pyridoxal phosphate is transiently converted into pyridoxamine phosphate. PLP enzymes form covalent Schiff-base intermediates with their substrates. In the absence of substrate the aldehyde group of PLP is in Schiff-base linkage with the α-amino group of a specific lysine residue at the active site. The Schiff base between the amino acid substrate and PLP, termed an aldimine, converts into ketimine which contains a double bond between N an C of the substrate.





The ketimine is then hydrolyzed to an α -keto acid and pyridoxamine phosphate. The second α-keto acid reacts with the enzyme-pyridoxamine phosphate complex to yield a second amino acid and regenerate the enzyme-pyridoxal phosphate complex.

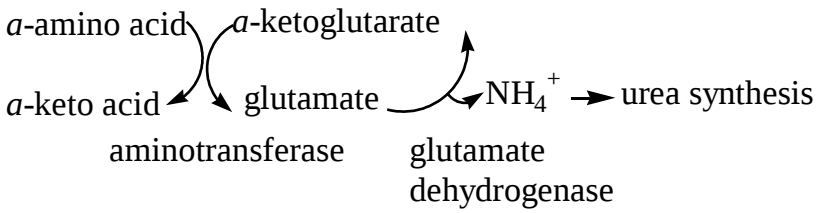
The first product leaves before the second substrate binds with the enzyme (with its prosthetic group) temporarily in an altered form. This mechanism is called a “ping-pong” one.

The most important aminotransferases are aspartate aminotransferase and alanine aminotransferase.

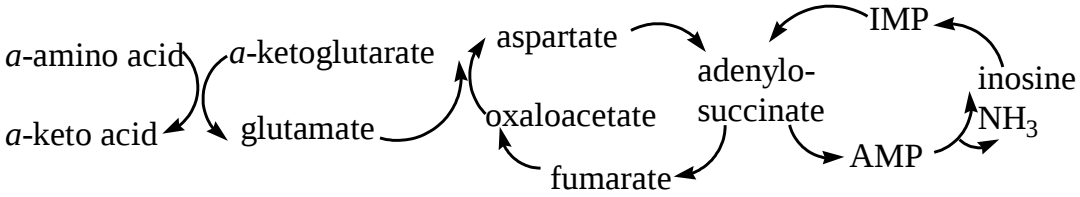
Transamination role:

1. Indirect deamination of amino acids.

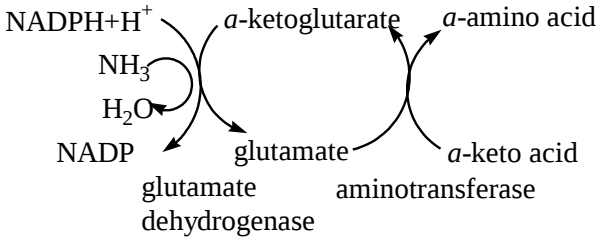
In liver:



In muscle:



2. Synthesis of non-essential amino acids.



3. Reactions of transamination are the mechanism of protection of organism from ammonia.

Glutamate dehydrogenase reaction role:

1. One of the primary mechanisms of ammonium detoxification.
2. Indirect deamination of amino acids.
3. Synthesis of nonessential amino acids.

Clinic-diagnostic significance of blood aminotransferase activity determination

Aminotransferases are tissue specific enzymes. In blood their normal activity is very low. The increasing of any aminotransferase activity in blood indicates the pathologic process localization, because these enzymes apper in blood as the result of the cell destruction. For example, elevation of aspartate aminotransferase activity shows myocardial infarction, and that of alanine aminotransferase activity indicates liver diseases.

11.4 Ammonia Detoxification.Urea Synthesis

Ammonia may be formed during amino acid, biogenic amine, dicarbonic amino acid amide, purine nucleotide deaminations and pyrimidine nucleotide decay as well. Ammonia may be also generated by enteric bacteria.

Ammonia generated by enteric bacteria is absorbed into the portal venous blood, which thus contains higher levels of ammonia than systemic blood. Since a healthy liver promptly removes this ammonia from the portal blood, peripheral

blood is virtually ammonia-free. Ammonia may rise to toxic levels in the systemic blood in impaired hepatic function or development of collateral communications between the portal and systemic veins, as may occur in cirrhosis.

The symptoms of ammonia intoxification: a peculiar flapping tremor, slurring of speech; blurring of vision.

In severe cases it leads to coma and death.

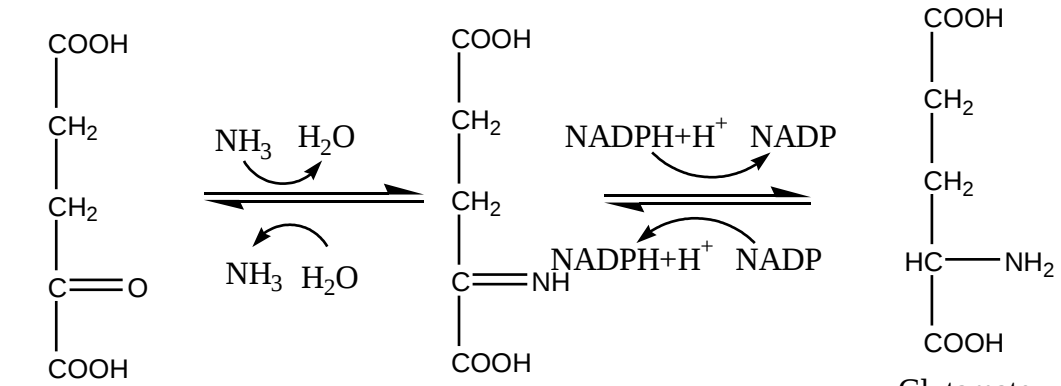
Ammonium ion is toxic for humans, especially, for the brain. A high concentration of ammonium ion shifts the equilibrium of the reaction catalyzed by glutamate dehydrogenase toward the formation of glutamate. The α –ketoglutarate concentration in the tissue drops, untill the catalytic function of the tricarboxylic acid cycle is impaired.

Therefore very effective system of ammonia detoxification exists in any organism. There are two types of ammonia detoxification mechanisms: temporary and final ones.

Temporary detoxification mechanisms are needed to transfer toxic ammonia from peripheric tissues to final detoxification organs (that is to liver and kidneys).

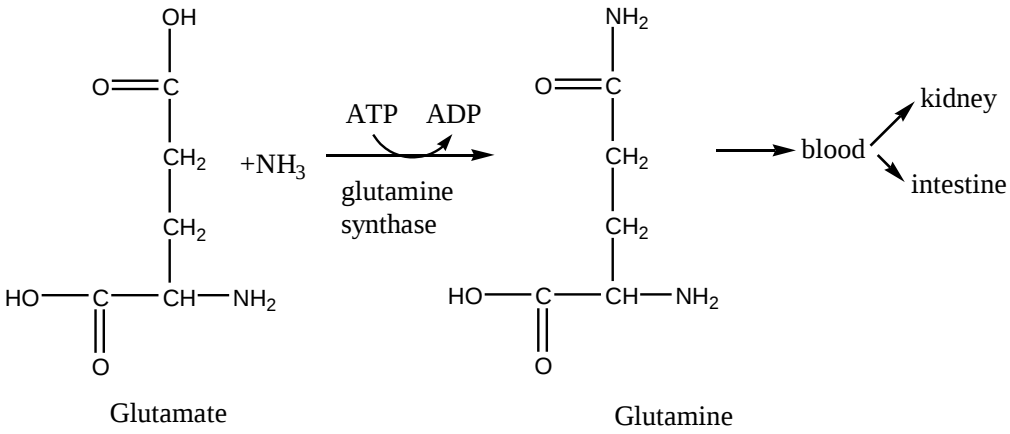
Temporary detoxification mechanisms

- Reaction of α-ketoglutarate reductive amination (catalyzed by glutamate dehydrogenase).



a-ketoglutarate Glutamate

- The formation of dicarbonic amino acid amides (Gln, Asn), mainly glutamine.



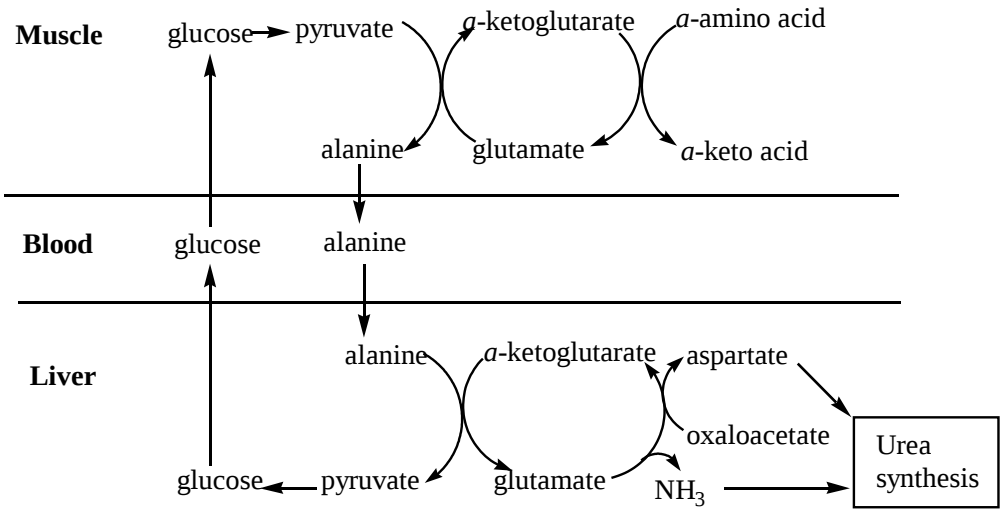
Glutamate Glutamine

Glutamine formation is the major mechanism of ammonia detoxification in brain tissue. Moreover in nervous tissue asparagine and glutamine are the products of aspartate and glutamate inactivation (aspartate and glutamate are excitatory amino acids).

Glutamine is rapidly taken up by kidney and intestine. In addition to peripheral tissues glutamine may be synthesized by liver. That glutamine acts in hepatic cells as a buffer to keep ammonia concentration low in the stage between deamination of glutamate and synthesis of citrulline. That glutamine is exported from liver to kidney or intestine.

In intestine the glutamine is the source of carbon skeletons. Glutamine is a major energy nutrient for small intestine mucosa. Of the five carbon atoms leaving the liver as glutamine, three will return as alanine, but two will be oxidized to CO_2.

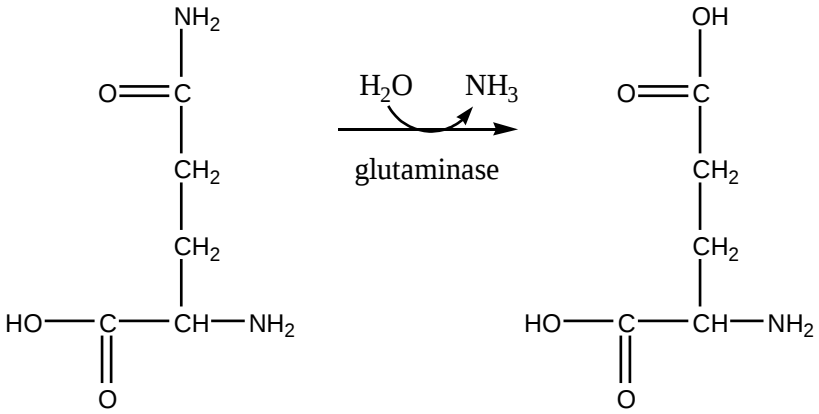
From muscle the excess of amino groups is transported to liver as alanine.



Pyruvate is formed in glycolysis. In liver alanine is deaminated by transamination with α-ketoglutarate. Pyruvate is used for synthesis of glucose.

Glutamate may be deaminated by glutamate dehydrogenase or transaminated with oxaloacetate. Free ammonia and aspartate are used for the synthesis of urea.

In kidney the glutamine is deaminated by glutaminase to provide free NH_4^+ ions for buffering urine:



Glutamine Glutamate

Activity of glutaminase depends on pH of medium. In acidosis:

- Glutaminase activity is increased.
- Ionization of ammonia is more intensive.

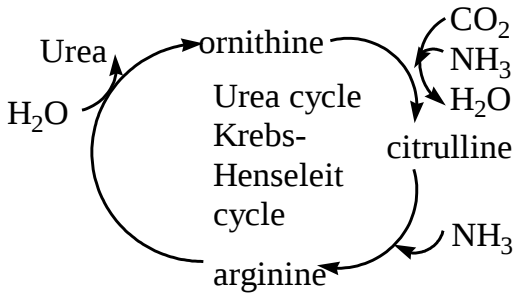
But ammonia can penetrate through cellular membrane in both directions being only in nonionic form. Therefore it distributes between urine, channel's cells and perichannel's blood depending on the hydrogen ion concentration. That's why ammonia excretion as ammonium salts rises in acidosis. It is of important value in supporting acid base balance and keeping cations. Therefore urine ammonium salts amount is a very important index of acid base balance of the organism.

Urine ammonia concentration drops mainly in vegetable diet. And its concentration elevates:

- in using proteins especially animal proteins containing great amount of sulfur and phosphorus;
- in diseases accompanied by acidosis.

Urea synthesis

Urea cycle was proposed by Hans Krebs and Kurt Henseleit (a medical student) in 1932, five years before elucidation of the citric acid cycle. In fact, the urea cycle was the first cyclic metabolic pathway to be discovered.



Urea nitrogen forms about 90% of urine total nitrogen; 20 – 35 g/day of urea are excreted with urine.

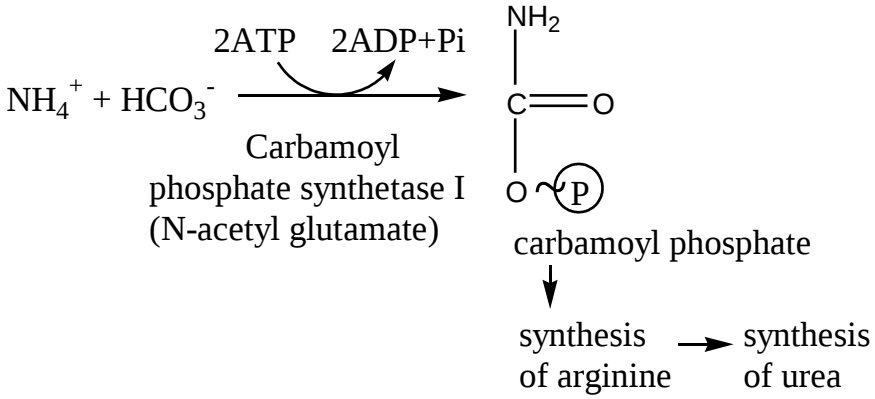
Urea synthesis occurs in the liver. The enzymes of urea synthesis are present in some other tissues but in these tissues urea cycle operates in a limited extent. For example kidney can form up to arginine but can not form urea as enzyme arginase is absent in kidney tissues. Brain can synthesize urea from citrulline, but lacks the enzyme for forming citrulline from ornithine.

Urea synthesis occurs in the liver. Urea synthesis is an energy-requiring process, and three molecules of ATP are used in the formation of each molecule of urea.

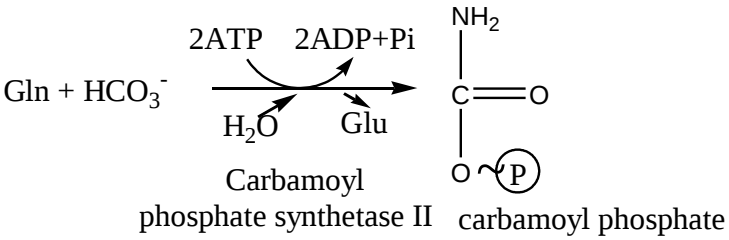
The carbon atom is derived from bicarbonate. One of the nitrogen atoms comes from ammonia, the other one from aspartate.

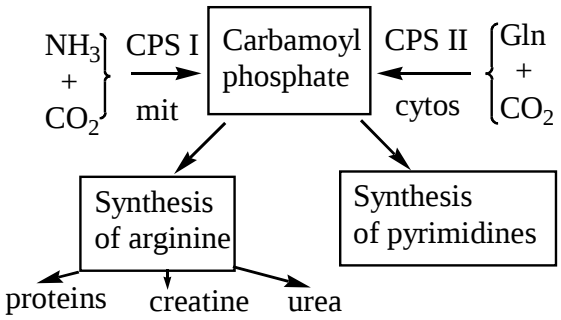
The synthesis of urea begins from synthesis of carbamoyl phosphate. In human tissues there are 2 reactions of carbamoyl phosphate synthesis.

1. One of them is the reaction, catalyzed by ammonia dependent carbamoyl phosphate synthetase. This enzyme occurs in liver mitochondria and requires N-acetyl glutamate as an activator.

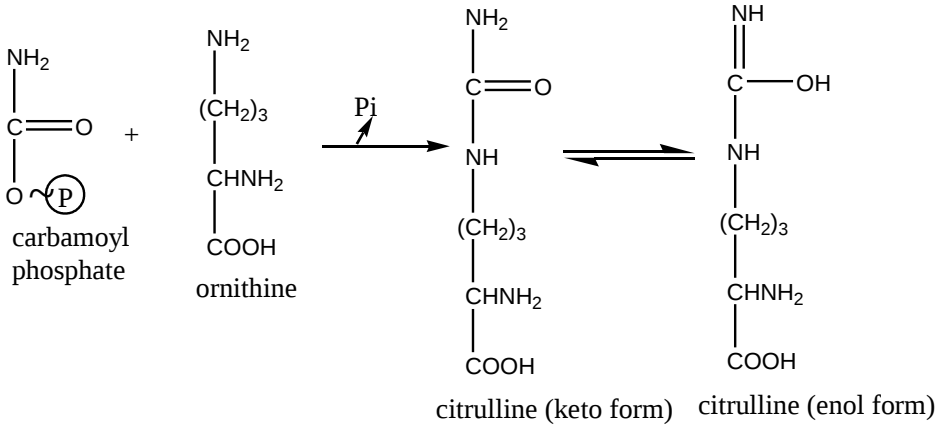


2. Another one is the reaction catalyzed by cytosolic carbamoyl phosphate synthetase II. This enzyme widely occurs in human tissues and utilizes glutamine as substrate. Its product is used for pyrimidine nucleotide synthesis.



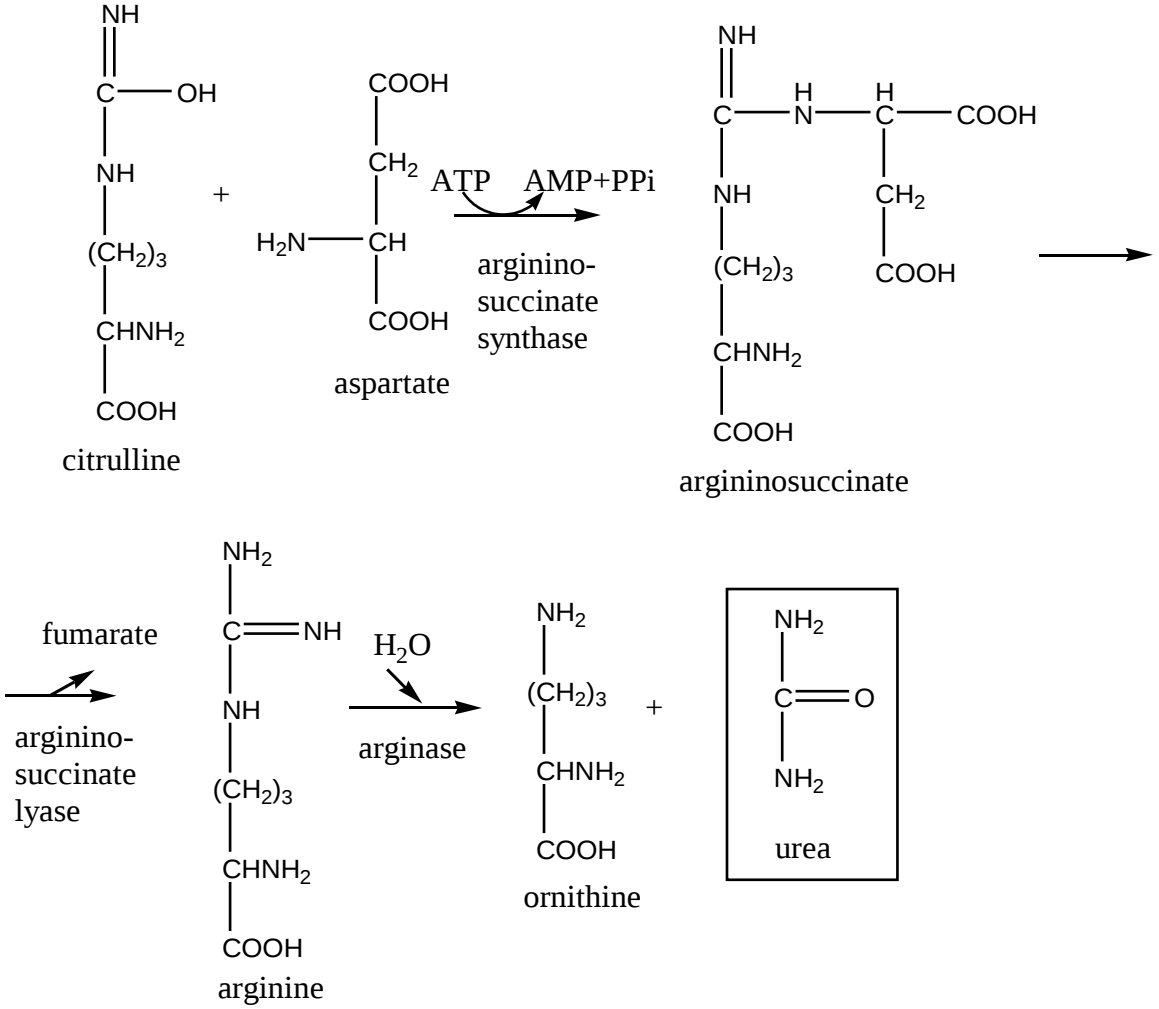


Ornithine carbamoyl transferase, which catalyzes the condensation of carbamoyl phosphate with ornithine, is also mitochondrial, while the remaining reactions of the urea cycle are cytoplasmatic ones.



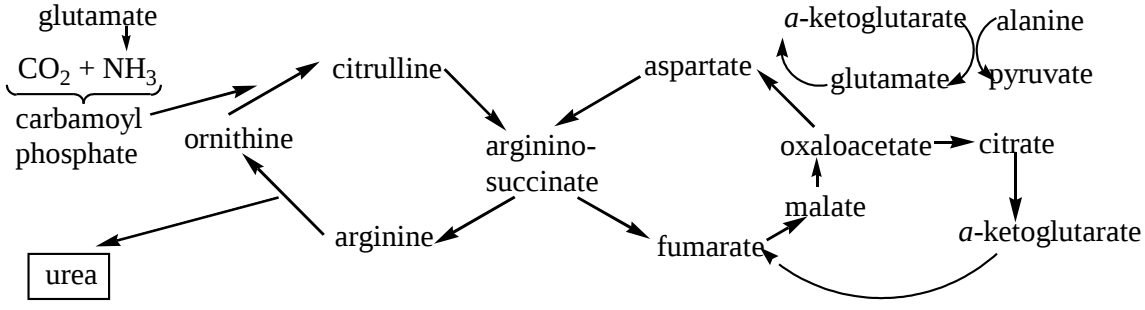
In cytosole:

Then argininosuccinate synthetase catalyzes the condensation of citrulline and aspartate. The synthesis of argininosuccinate is driven by the cleavage of ATP into AMP and pyrophosphate and by the subsequent hydrolysis of pyrophosphate.



Further argininosuccinate lyase cleaves argininosuccinate into arginine and fumarate.The immediate precursor of urea is arginine, which is hydrolyzed to urea and ornithine by arginase.

The urea cycle is linked to the citric acid cycle through fumarate.



The synthesis of fumarate by the urea cycle is important because it links the urea cycle and the citric acid one. Fumarate is hydrated to malate, which is in its turn oxidized to oxaloacetate. Oxaloacetate has several possible fates: 1) transamination to aspartate; 2) conversion into glucose by the gluconeogenic pathway; 3) condensation with acetyl CoA to form citrate; or 4) conversion into pyruvate.

Urea role:

1. This is a main product of ammonia detoxification (it is nontoxic compound).
2. Urea stabilizes protein structure rising their stability to proteolytic enzymes.
3. It participates in acid-base balance regulation.
4. Urea is an osmotically active substance.
5. It participates in maintaining nitrogenous balance.

Urea amounts: in blood - 3,3-8,32 mmol/L; in urine- 333-583 mmol/day.

In urine the urea concentration decreases: in protein deficiency in diet; in liver function disturbance (hepatitis, cirrhosis, phophorus poisoning); in acidosis, as a considerable ammonia part is used in acid neutralization; in renal diseases.

Uremia is usually a result of renal disfunction; other substances also are accumulated in the blood, more contributing to the symptomology than urea does itself.

Urea excretion increases: in meat diet; in diseases linked with enhanced destruction of protein (for instance, in malignant tumours, infectious diseases).

Congenital defects of enzymic steps in the urea cycle have been observed as well. A defect in any of these enzymes causes elevated plasma NH_4^+ (hyperammonemia). A nearly total deficiency of any of the urea cycle enzymes results in coma and death shortly after the birth. Partial deficiencies of these enzymes cause mental retardation, lethargy and episodic vomiting. A low protein diet leads to lighter course of these inherited disorders.

There are five congenital defects in urea cycle:

1) *Hyperammonemia type 1*. Hyperammonemia type 1 is linked with carbamoyl phosphate synthetase dificiency.

2) *Hyperammonemia type 2*, which is linked with the deficiency ornithine carbamoyl transferase.

3) *Citrullinemia* is linked with argininosuccinate synthase deficiency.

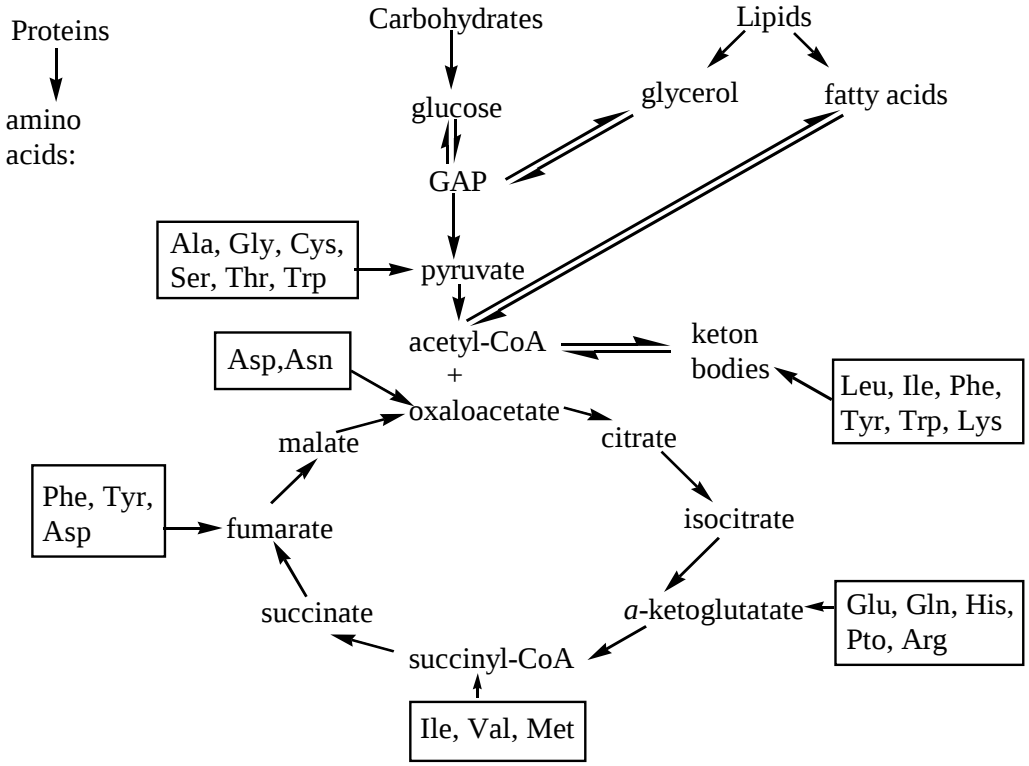
4) *Argininosuccinic aciduria* reflects the argininosuccinate lyase deficiency.

5) *Hyperargininemia* is characterized by elevated blood and cerebrospinal fluid arginine levels and arginase deficiency.

11.5 Catabolism of Carbon Skeletons of Amino Acids. Gluco- and Ketogenic Amino Acids

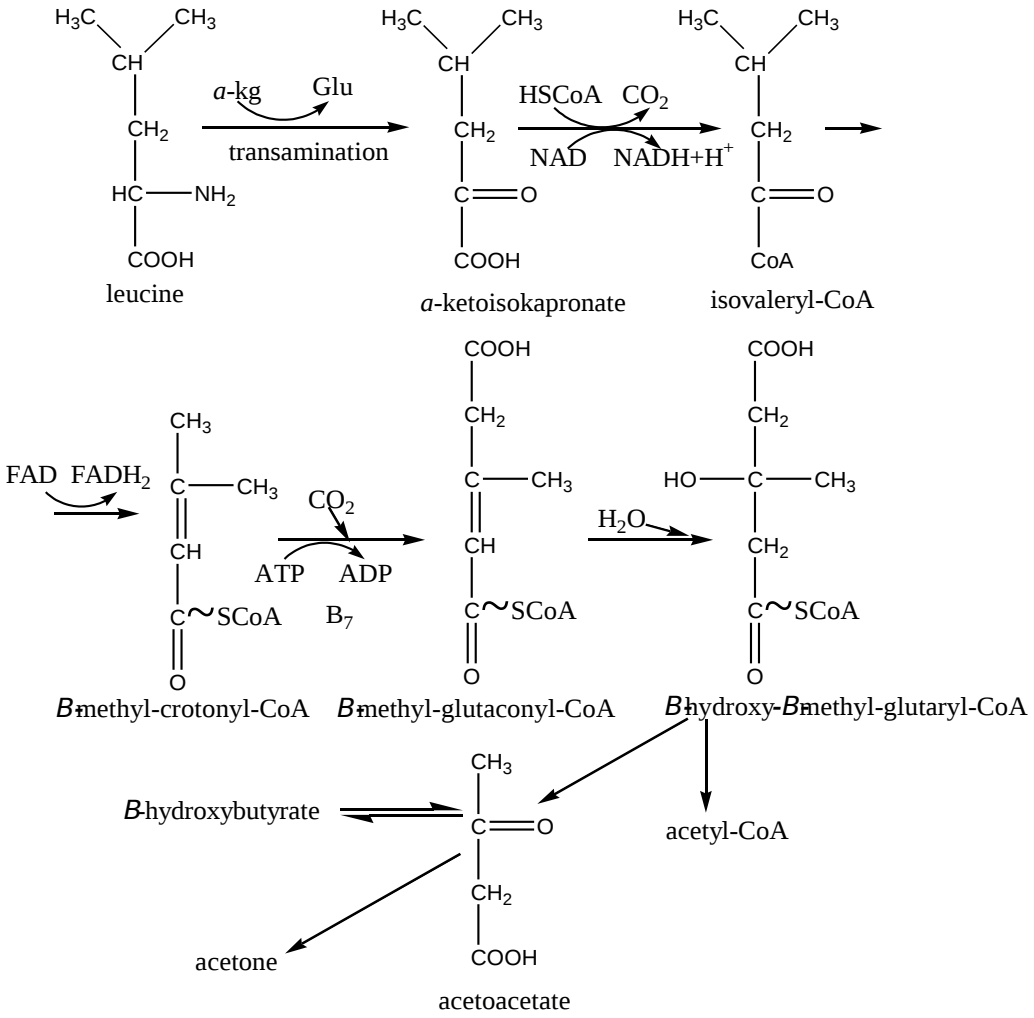
Amino acid degradation leads to formation of major metabolic intermediates that can be completely oxidized by tricarboxylic acid cycle. The carbon skeletons of the diverse set of twenty amino acids are converted into only seven molecules:

Amino acids that are degraded to acetyl-CoA or acetoacetate are termed ketogenic because they give rise to ketone bodies. In contrast, amino acids that are degraded to pyruvate, α -ketoglutarate, succinyl-CoA, fumarate, or oxaloacetate termed glucogenic. Synthesis of glucose from these amino acids is feasible because these citric acid cycle intermediates and pyruvate can be converted into phosphoenolpyruvate and then into glucose. ***Mammals haven't any pathway for glucose synthesis from acetyl CoA or acetoacetate***.



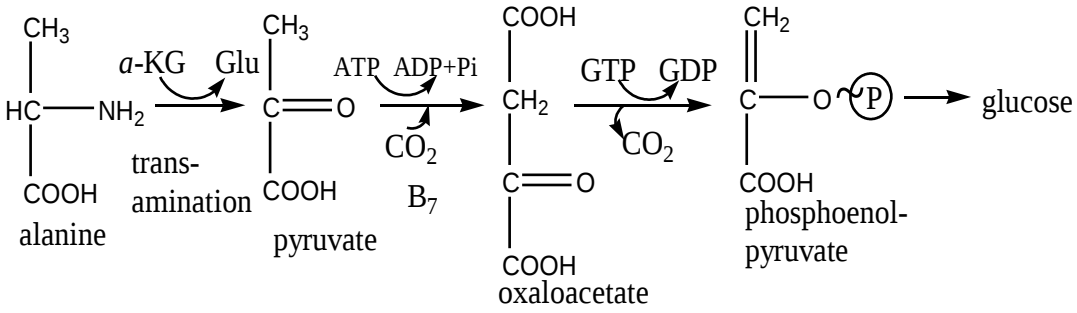
Of twenty amino acids, only leucine and lysine are purely ketogenic. Isoleucine, phenylalanine, tryptophan and tyrosine are both ketogenic and glucogenic. Some of their carbon atoms emerge in acetyl-CoA or acetoacetate, whereas the others may be used as potential purely glucogenic.

Conversion of carbon skeleton of ketogenic amino acid leucine



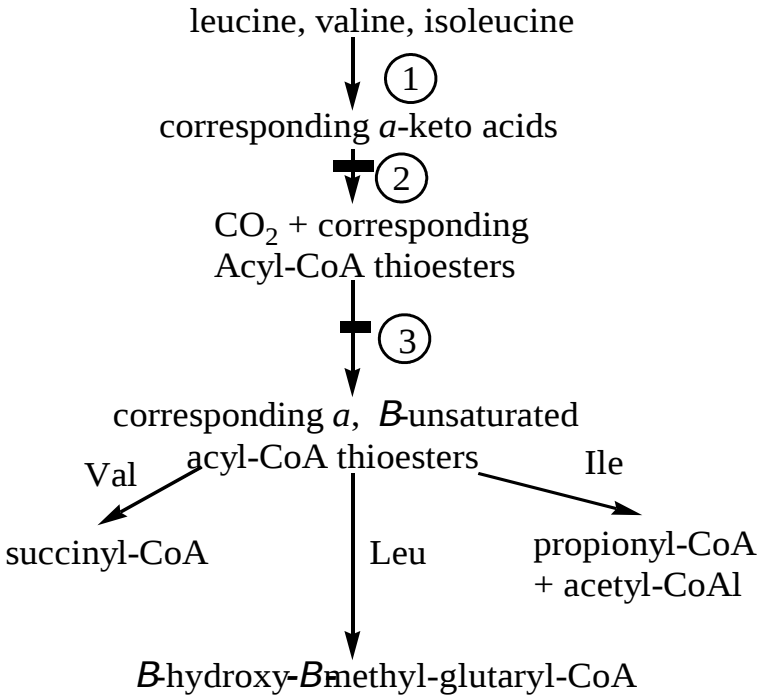
Leucine is transaminated to the corresponding α-keto acid, α-ketoisocaproate. It is oxidatively decarboxylated to isovaleryl-CoA. This reaction is analogous to the oxidative decarboxylation of pyruvate to acetyl-CoA and of α-ketoglutarate to succinyl-CoA. Isovaleryl-CoA is dehydrogenated to yield β-methylcrotonyl-CoA. β-methyl-glutaconyl-CoA is formed by carboxylation of β-methyl-crotonyl-CoA at the expense of ATP. β-Methyl-glutaconyl-CoA is then hydrated to form β-hydroxy-β-methyl-glutaryl-CoA, which is cleft to acetyl-CoA and acetoacetate.

Gluconeogenesis from alanine



Catabolism of the branched-chain amino acids

Catabolism of leucine, valine and isoleucine initially involves the same reactions. Subsequently, each amino acid skeleton follows a unique pathway to amphibolic intermediates, whose structure determines that valine is glycogenic, leucine is ketogenic and isoleucine is both.



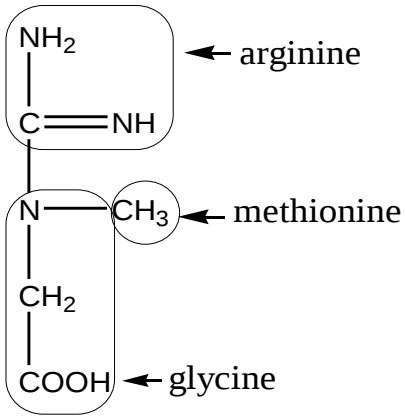
Transamination of all three branched amino acids is catalysed by a single transaminase. A mitochondrial multienzyme complex, a *branched amino acids dehydrogenase*, catalyzes the oxidative decarboxylation of α-keto acids derived from leucine, isoleucine and valine. Structure and regulation of this dehydrogenase closely resemble pyruvate dehydrogenase. Reaction 3 is analogous to dehydrogenation of of acyl-CoA thioesters in fatty acids catabolism.

Metabolic disorders of branched chain amino acid catabolism

- *Maple syrup urine disease (branched chain ketonuria)* is due to absence or greatly reduced activity of a branched chain α-ketoacid dehydrogenase. The most striking feature of this hereditary autosomal recessive disorder (incidence 1: 185000 worldwide) is the odour of the urine, which resembles that of maple syrup or burnt sugar. Plasma and urinary levels of leucine, isoleucine, valine and their α-keto acids are elevated. The disease is evident by the end of the first week of extrauterine life. The infant is difficult to feed, may vomit, and may be lethargic. Extencive brain damage occurs in surviving children. Without treatment, death usually occurs by the end of one year. Therapy involves replacing dietary protein by a mixture of amino acids that excludes leucine, isoleucine and valine. When plasma levels of these amino acids fall to normal, they are restored in the form of milk and other food in amount that never exceed metabolic demand.
- *Isovaleric acidemia.* The impaired enzyme is isovaleryl-CoA degydrogenase. Symptoms include a “cheesy” odour of the breath and body fluids, vomiting, acidosis and coma precipitated by excessive ingestion of proteins.

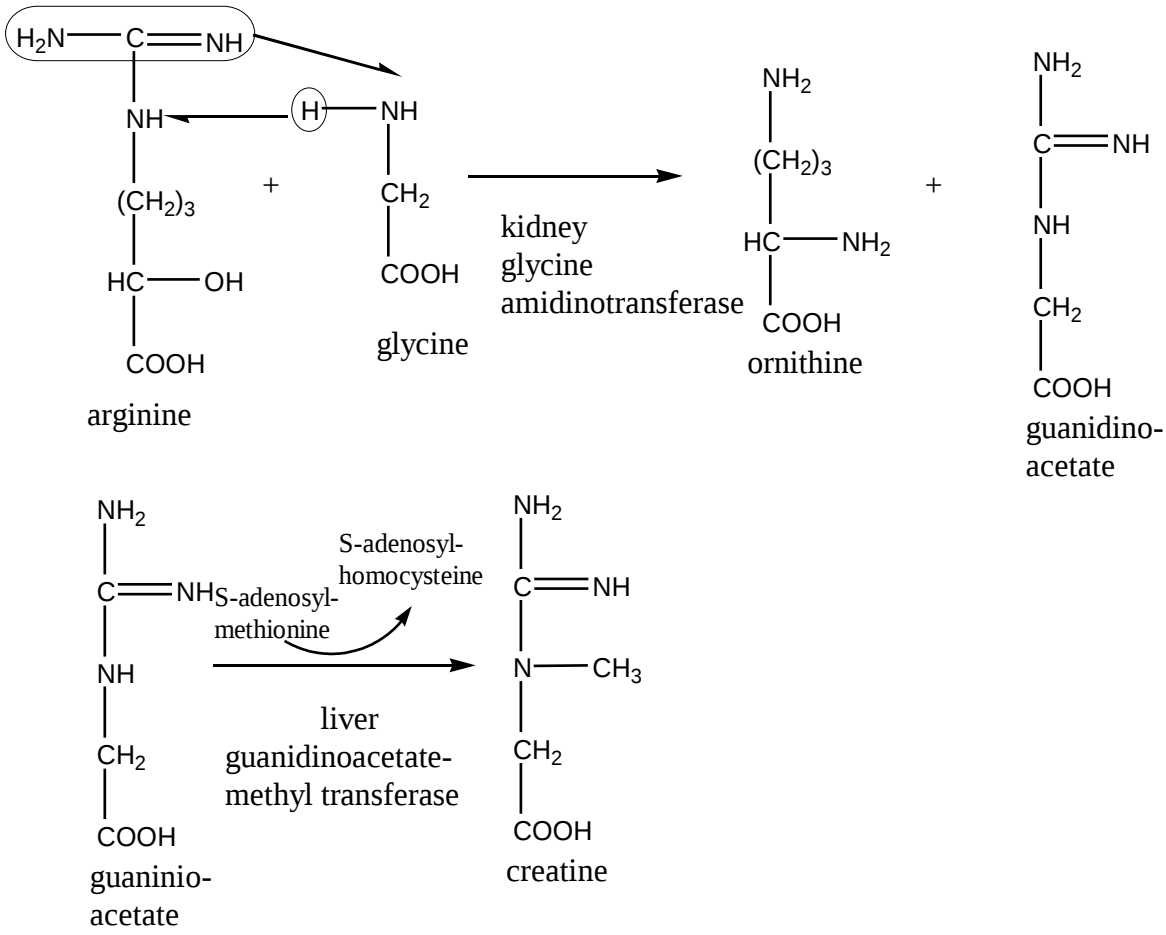
11.6 Conversion of Amino Acids to Specialised Products

Creatine metabolism



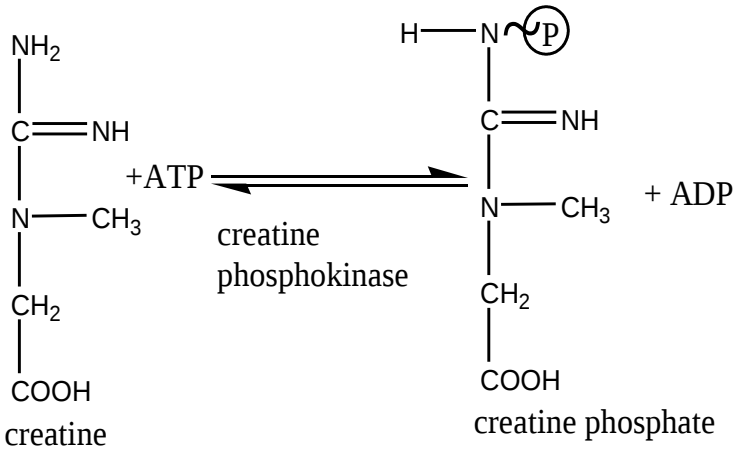
Total amount of creatine in organism is 120g. (96% - in muscles, 1,3% - in brain). Creatine occurs in high concentrations in striated muscle.

Three amino acids such as arginine, glycine and methionine participate in the creatine synthesis.

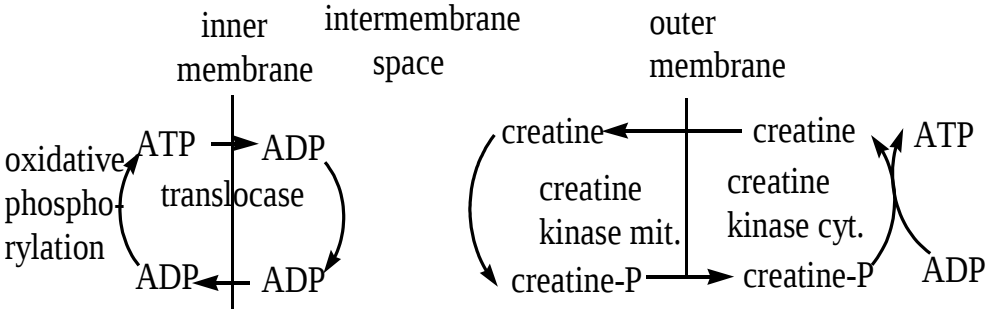


Creatine is synthesized by two steps. First, in kidney guanidinoacetate is synthesized from arginine and glycine. The reaction is catalyzed by glycine amidinotransferase. Then in liver creatine is synthesized by guanidinoacetate-methyl transferase from guanidinoacetate and methionine active form, that is, from S-adenosyl-methionine.

In 1922 Alexander Palladin suggested that creatine was linked with muscle contraction. Trained muscles contain greater creatine that untrained ones. But the energy source in muscles is known to be ATP. Creatine happened to be reversibly phosphorylated.

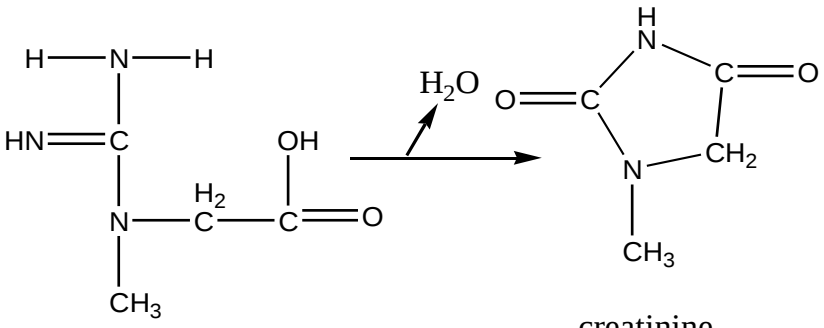


The ATP synthesis in the result of oxidative phosphorylation is know to occur in mitochondria. ATP penetrates by adenine nucleotide translocase through inner membrane. It is an antiporter which exchanges ADP to ATP. External membrane is badly permeable to ATP. It is creatine that transfers high potential phosphoryl groups from mitochondria to cytoplasm.



Furthermore creatine phosphate is a reservoir of high potential groups. Creatine phosphate maintains a high concentration of ATP during muscular exertion periods. By means of above mentioned functions creatine participates in energy metabolism, stimulates the protein synthesis, stimulates the tissue respiration and oxidative phosphorylation, regulates glycolysis.

Catabolism of creatine is linked with its dehydration. This slow nonenzymatic reaction occurs simultaneously with transphosphorylation of creatine phosphate with ADP and leads to formation of creatinine. This reaction is **irreversible**.



creatine creatinine

Creatinine is a final product of creatine metabolism. It diffuses from the muscle into the blood and is eventually excreted in the urine. The daily excretion depends on the muscle mass and is reasonably constant for individual. Creatinine clearance is the most widely used measure of renal function.

Amount of **creatinine**:

In blood – 53-106 μ mol/L;

Daily excretion: men – 1.5 – 2 g;

women – 0.8 – 1.5 g.

Creatine level in blood:

men – 15.25 – 45.75 μmol/L;

women – 45.75 – 76.25 μmol/L

Only *traces* of creatine are present in urine under normal conditions. Excretion of creatine with urine is called **creatinuria**. There are two types of creatinuria.

Creatinuria

physiological creatinuria	pathological creatinuria
occurs:	occurs:
- in children;	- in muscle diseases such as muscular dystrophy and myositis;
- in old men;	- in disturbance of carbohydrate metabolism;
- in pregnants;	- in liver damage;
- in high content of creatine in food;	- in kidney function disturbance;

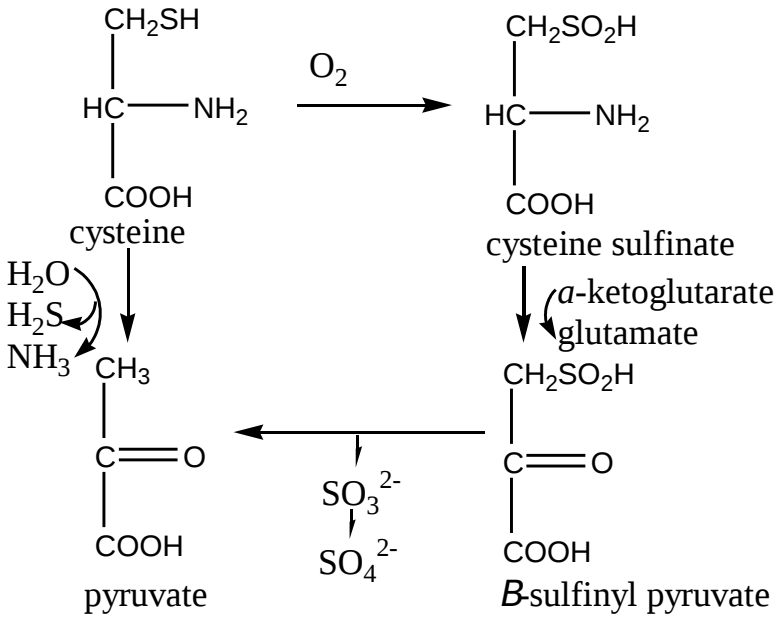
- in hormonal disbalance, for example, in diabetes mellitus and thyrotoxicosis;
- in hypovitaminoses of vit. C and E;
- in infectious diseases;
- in starvation.

Sulfur-containing Amino Acid Metabolism

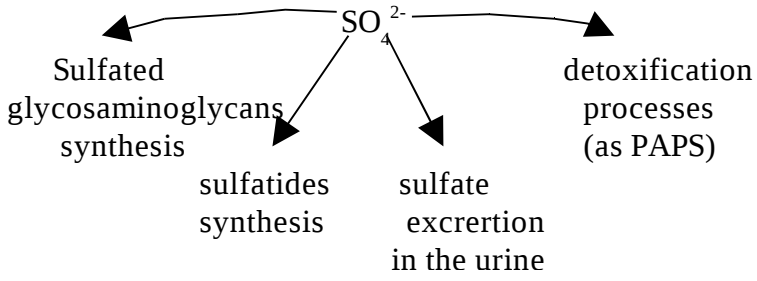
Cysteine, cystine and methionine belong to sulfur-containing amino acids.

Cysteine. If methionine is present in the diet in proper amount, cysteine is not an essential amino acid.

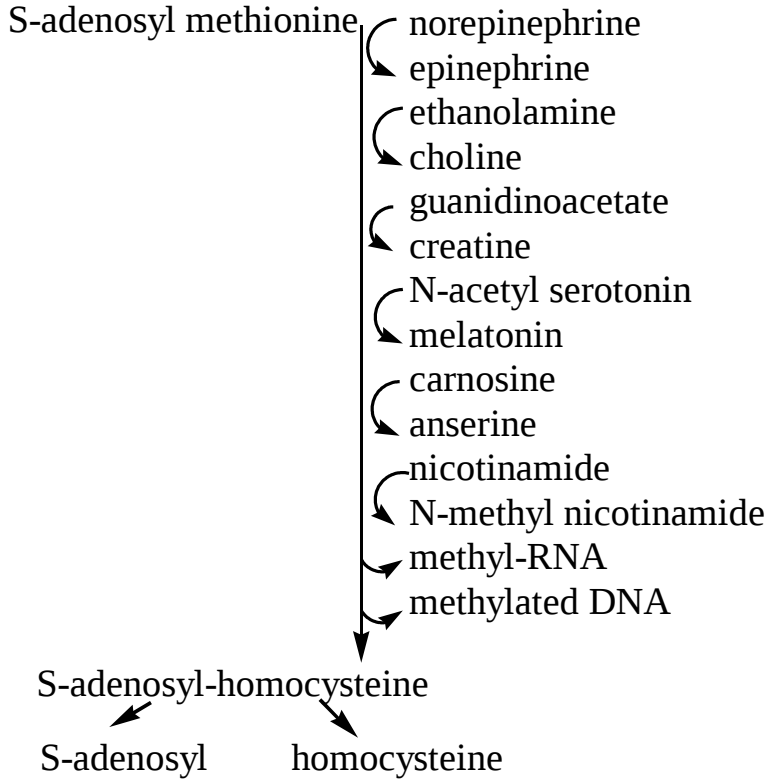
- Cysteine stabilizes protein molecule by means of disulfide bonds formation.
- Often cysteine participates in enzyme active site formation.
- Cysteine is a part of glutathione.
- Cysteine decarboxylation results in thioethylamine which is utilized for coenzyme A synthesis:
- Cysteine is also a precursor of the taurine.
- It can also be converted into pyruvate by two major ways: by nonoxidative deamination and by cysteine sulfinate transamination.



Sulfate metabolism



Generally some 20-40 mmol sulfate are excreted daily, 90% as inorganic sulfate.



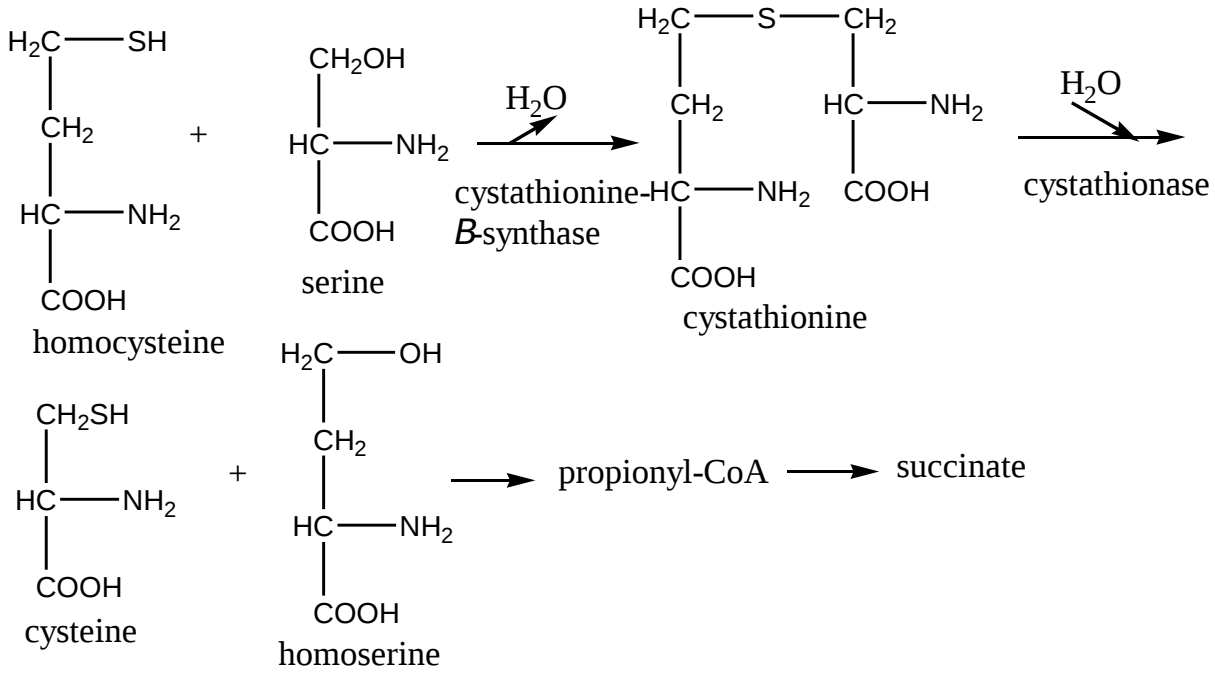
Methionine is an essential amino acid. Most of dietary methionine is probably used in methylation reactions. The methyl donor is not methionine itself, but S-adenosyl methionine (SAM).

The methyl group can be transferred to a variety of acceptors.

Quantitatively the most important use of SAM is in the synthesis of phosphatidylcholine. After methyl transfer from SAM, methionine is reformed by homocysteine methylation. The methyl group may come from methyl tetrahydrofolate, and may therefore be derived from any of sources of one-carbon

groups that are channelled through tetrahydrofolate. Betaine may also act as a methyl donor to homocysteine (choline is oxidized into betaine by a mitochondrial flaroprotein dehydrogenase).

However, the major route of its conversion is the utilization of homocysteine in the synthesis of cysteine:



The enzymes that catalyze the synthesis and breakdown of cystathionine contain pyridoxal phosphate.

SAM is also used for the synthesis of polyamines: spermine and spermidine. Methionine is the initial amino acid in protein synthesis.

Inherited disturbances of sulfur-containing amino-acid metabolism

Cystinuria (Cystine-Lysinuria). In this inherited metabolic disease, urinary excretion of **cystine** is up to 30 times normal. Excretion of lysine, arginine and ornithine is also increased, suggesting a defect in the renal reabsorptive mechanisms for these 4 amino acids. Since cystine is relatively insoluble, cystine stones are formed in the renal tubules. The mixed disulfide of L-cysteine and L-homocysteine, which is present in the urine of cystinuric patients is more soluble than cystine and hence reduces formation of cystine crystals and stones.

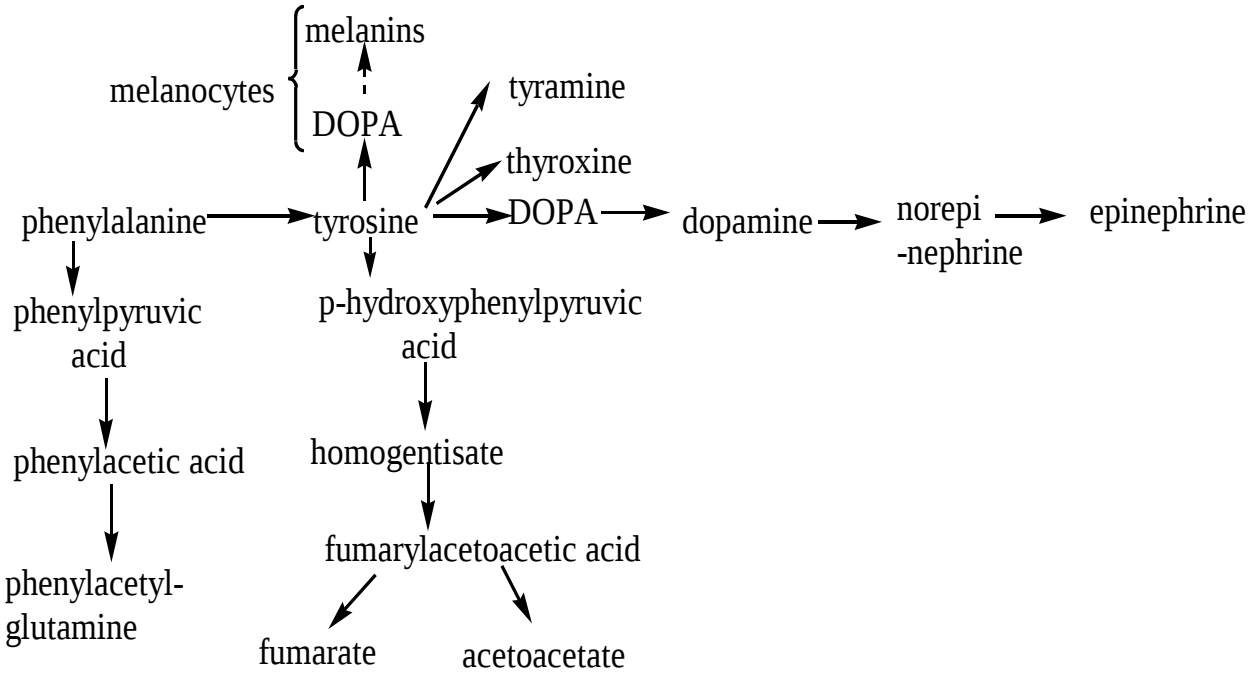
Cystinosis (cystine storage disease) is characterized by wide spread deposition of cystine in various tissues (in liver, spleen, marrow, kidney, cornea). Cystine is

accumulated with lysosomes of cells of reticulo-endothelial system. The cause is believed to be linked either with a deficiency of the enzyme **cystine reductase** or with defective carrier-mediated transport of cystine. Cystinosis is usually accompanied by a generated aminoaciduria.

Homocystinuria is linked with disturbances of methionine metabolism. Both vitamin B_6-responsive and vitamin B_6-unresponsive forms are known. At least four metabolic defects cause homocystinuria. In type I homocystinuria (defect of cystathionine β-synthase), clinical findings include thrombosis, osteoporosis and frequently mental retardation.

Phenylalanine and tyrosine

These amino acids are both glucogenic and ketogenic because the main catabolic pathway produces both fumarate and acetoacetate. Many of biologically active substances are formed from the above amino acids, in particular the catecholamines, the thyroid hormones, tyramine, melanin.



In the thyroid gland, the synthesis of the hormones such as thyroxine (T_4) and triiodothyronine (T_3) passes through the iodination of tyrosyl residues in a protein precursor, thyroglobulin. Thyroglobulin is stored in follicules, surrounded by thyroid follicular cells. Iodide is actively accumulated by the thyroid gland and is

concentrated in the lumen of the follicules. At first, iodide incorporation into the protein is catalyzed by peroxidase; then the hormones are formed by coupling two iodotyrosines in thyroglobulin, which is taken up by the thyroid cells in response to thyroid stimulating hormone. Thyroglobulin proteolytically degrades to produce T_3 and T_4.

Phenylalanine is hydroxylated to tyrosine by a monooxygenase, for which the coensyme is tetrahydrobiopterin. The catecholamines, dihydroxyphenylethylamine (dopamine), noradrenaline (norepinephrine) and adrenaline (epinephrine), occur as neurotransmitters in the central and peripheral nervous systems. Adrenaline and noradrenaline are also hormones, released into the blood stream from the adrenal medulla. They are synthesized in the brain, the adrenal medulla and nerve terminals. The starting point and rate-limiting step in their synthesis is hydroxylation of tyrosine by monooxygenase (tyrosine hydroxylase) which, like phenylalanine hydroxylase, requires tetrahydrobiopterin, as a coenzyme. Dihydroxyphenylalanine (DOPA) is the product of reaction and is decarboxylated into dopamine. In its turn dopamine is hydroxylated into noradrenaline by dopamine monooxygenase, which requires ascorbate as a coenzyme. Finally, adrenaline is produced by a SAM-dependent methylation from noradrenaline.

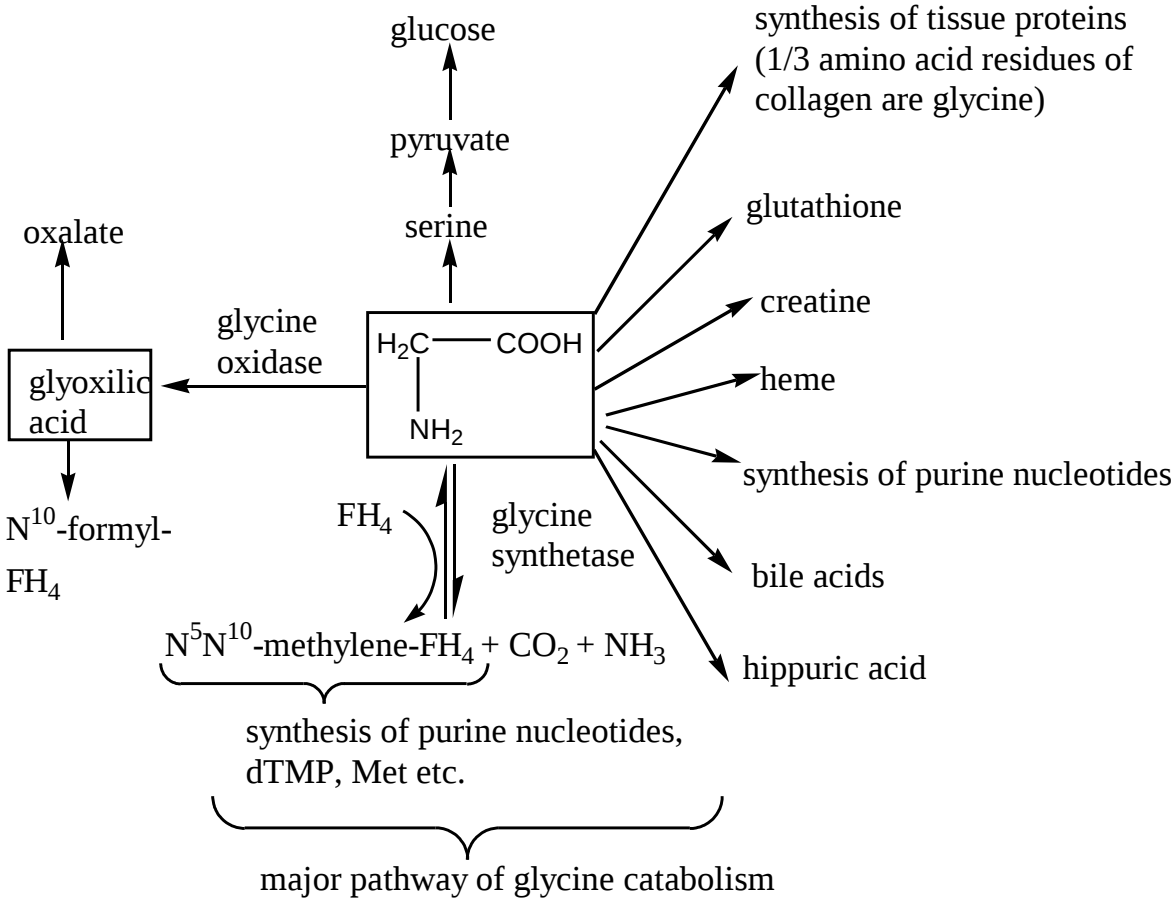
DOPA is also converted into an indole derivative, which condenses to form the high molecular weight pigment, melanin. In melanocytes, melanin is firmly bound to protein. Albinism accompanies defective melanin biosynthesis. The term ***albinism*** includes a spectrum of clinical syndromes characterized by hypomelanosis due to heritable defects of eye and skin melanocytes. Most forms of the hereditary defect albinism appear to be due to a lack of tyrosinase in melanocytes. The tyrosine hydroxylase involved in catecholamine biosynthesis is a different isoenzyme, so adrenaline production is normal in albinos.

Phenylketonuria, alkaptonuria and tyrosinosis are closely related to congenital disturbancies of metabolism in which the normal metabolism of aromatic amino acids is blocked in different points.

Phenylketonuria is caused by the absence or deficiency of phenylalanine hydroxylase or, more rarely, of tetrahydrobiopterin reductase. Phenylalanine can not be converted into tyrosine and so there is an accumulation of phenylalanine in the fluids of the whole body. Phenylpyruvate, phenyllactate, phenylacetate excrete in the urine in excessive amounts. Almost all untreated individuals with phenylketonuria are severely mentally retarded. In fact, about 1% of patients in mental institutions have phenylketonuria. Phenylketonurics have a lighter skin and hair color than their siblings. The hydroxylation of tyrosine is the first stage in the pigment melanin formation. In phenylketonurics this reaction is competitively inhibited by the high levels of phenylalanine, and that's why so less amount of melanin is formed. The therapy for phenylketonuria is a low phenylalanine diet. Early diagnosis of newborn was assayed by means of phenylalanine in blood by Gautry method (microbiological) or fluorescent examination, which is informative after 3 days from child birth. The countries with high frequency of phenylketonuria use this analysis to hold the mass screening of newborns. This procedure gives the possibility to put diagnosis before disease manifestation and to start treatment as soon as possible early and provide the normal child development. Early used method by adding $FeCl_3$ to urine, which gives an olive green color in the presence of phenylpyruvate lost the diagnostical importance. It is used for controlling diet treatment efficiency. The incidence of phenylketonuria is about 1 in 20.000 newborns in the world (in Europeans it is more: about 1 in 6000 – 7000 newborns).

Alkaptonuria is linked with the lack of homogentisate oxidase. Homogentisate is accumulated and then is excreted with urine. Homogebtisate in the urine is then oxidized by O_2 in air to a brownish-black pigment. Most striking clinical manifestation of alkaptonuria is darkening of urine that stands in air. In older patients darkening of the subcutaneous cartilage (ochronosis), degeneration of the intervertebral discs and the articular surfaces of the large joints occur.

Metabolism of glycine



Inherited disorders:

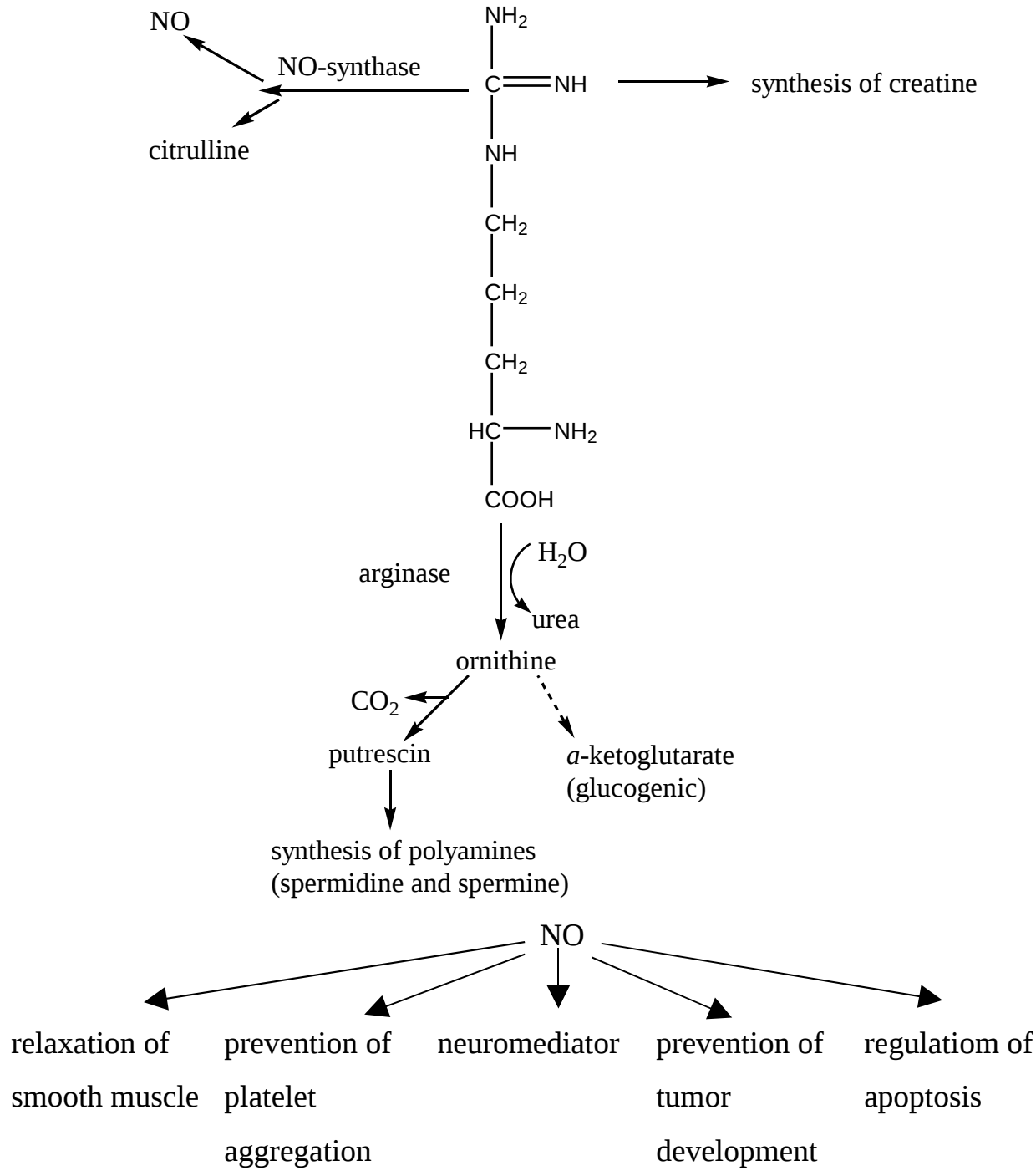
Glycinuria. The disease is characterized by excessive urinary excretion of glycine. It is linked with disturbance of glycine reabsorption.

Clinically: tendency to formation of oxalate stones in kidney, but amount of oxalate excreted in urine is normal. Plasma level of glycine is normal.

Primary hyperoxaluria. The cause is disturbance of glyoxilic acid conversion into N^{10}-formyl-FH_4.

Clinical symptoms: progressive bilateral calcium urolithiasis – oxalate stones formation in genitourinary tract. It is accompanied by recurrent infection of the urinary tract. Death occurs in childhood or early adult life from renal failure or hypertension.

Arginine metabolism



Tests for Self-control

1. C-terminal amino acids are removed by:
A. Dipeptidases
B. Aminopeptidases
C. Carboxypeptidases
D. Chymotrypsin
E. Pepsin

2. Analysis of gastric juice shows that the content of free hydrochloric acid is normal. Which of the below mentioned values (in mmol\L) corresponds to that?
A. 20-40
B. 10-20
C. 40-50
D. 30-50
E. 5-15

3. Pancreatic juice contains enzymes:
A. Trypsin, chymotrypsin, rennin
B. Chymotrypsin, elastase, pepsin
C. Carboxypeptidases, rennin, lipase
D. Elastase, carboxypeptidases, gelatinase
E. Chymotrypsin, trypsin, carboxypeptidases

4. Which of the below mentioned enzymes is activated by trypsin?
A. Pepsin
B. Aminopeptidase
C. Lipase
D. Amylase
E. Chymotrypsin

5. Patient was hospitalized with diarrhea after taking protein food. Physician believes the disturbance of protein digestion which results in their intensified putrefaction. Which of the below mentioned substances is the product of protein putrefaction in intestine?
A. Bilirubin
B. Lactate
C. Porphobilinogen
D. Cadaverin
E. Tryptophan

6. In newborn the curdling milk (the conversion of soluble caseins into insoluble paracaseins) is performed with participation of enzymes and calcium ions. Which enzyme participates in this process?
A. Secretin
B. Pepsin
C. Gastrixin
D. Rennin
E. Lipase

7. Point the biological role of biogenic amine, which is formed in decarboxylation of glutamate:

A. Coenzyme of complex enzymes

B. Activator of protein synthesis

C. Mediator of inhibition in CNS

D. Inhibitor of lipolysis

E. Inhibitor of gluconeogenesis

8. Point the biological role of histamine which is the product of histidine decarboxylation:

A. Activator of gastric juice secretion

B. Inhibitor of gastric juice secretion

C. Activator of bicarbonate secretion by pancreas

D. Inhibitor of bicarbonate secretion by pancreas

E. It has bactericidal effect

9. Which enzyme catalyzes the deamination of glutamate?

A. Glutamate dehydrogenase

B. γ-Glutamyl transferase

C. Glutamate decarboxylase

D. Glutaminase

E. Cystathionine-γ-lyase

10. Human's organism has a peptide, which formation is performed with participation of γ-carboxylic group of glutamic acid. What is the name of this peptide?

A.Vasopressin

B.Carnosine

C. Anserine

D. Oxytocine

E. Glutathione

11. Which substance is the acceptor of amine groups in reactions of transamination?

A. Argininosuccinate

B. α-Ketoglutarate

C. Lactate

D. Citrulline

E. Ornithine

12. In hepatitis, myocardial infarction the transaminase activity sharply increases in blood plasma of patients. Point the possible cause:

A. The increase of enzymes activity by hormones

B. Damage of cellular membrane and entering enzymes to the blood

C. Deficiency of pyridoxine

D. The increase of amino acids synthesis velocity in tissues

E. The increase of amino acids degradation velocity in tissues

13. Which is the process of ammonia detoxification in kidney?

A. Ammonia salts formation

B. Reductive amination

C. Indirect deamination
D. Urea synthesis
E. Synthesis of biogenic amines

14. Hyperargininemia and argininuria are observed in a 25-year-old patient. The urea level is decreased in blood and urine. Which enzyme deficiency is observed?

A. Glutamate dehydrogenase
B. Arginase
C. Ornithine carbamoyl transferase
D. Argininosuccinate synthetase
E. Tryptophan-5-monooxygenase

15. Olive green color appears after treatment of newborn child's urine by $FeCl_3$ solution. Which amino acid metabolism disturbance is observed?

A. Histidine
B. Cysteine
C. Phenylalanine
D. Glutamine
E. Lysine

16. In intestinal carcinoma about 60% of tryptophan is oxidized by means of serotonin pathway. Which vitamin requirement increases in that state?

A. Folic acid
B. Pantothenic acid
C. Pyridoxine
D. Riboflavin
E. Nicotinic acid

17. The suppression of phenylalanine to tyrosine conversion is observed in one of the inherited pathologies. Biochemical indicator of disease is the accumulation in organism of some organic acids, including:

A. Phenylpyruvate
B. Aspartate
C. Pyruvate
D. Lactate
E. Glutamate

Chapter 12. NUCLEIC ACIDS METABOLISM

12.1 Metabolism of Nucleotides

Purines and pyrimidines are dietarily **nonessential**. Humans and most other vertebrates can synthesize ample amounts of purine and pyrimidine nucleotides de novo (i.e, from amphibolic intermediates). Even when humans consume a diet rich in nucleoproteins, dietary purine and pyrimidine bases are not incorporated into tissue nucleic acids.

Degradation of nucleic acids in gastro-intestinal tract. Nucleic acids are released from ingested nucleoproteins by the action of gastric enzymes and of hydrochloric acid. Protein part of nucleoproteins is hydrolyzed by proteolytic enzymes to free amino acids. Nucleic acids are degraded to mononucleotides by pancreatic ribonucleases, deoxyribonucleases **and intestinal** polynucleotidases.

Nucleotidases and phosphatases hydrolyze mononucleotides to nucleosides, which either are absorbed or are further degraded by intestinal phosphorylase to purine and pyrimidine bases. Purine bases are oxidized to uric acid, which may be absorbed and subsequently excreted in the urine.

Biosynthesis of purine nucleotides. Three processes that contribute to purine nucleotide biosynthesis, listed in order of decreasing importance, are:

1. Synthesis from amphibolic intermediates (synthesis de novo) – main pathway.
2. Phosphoribosylation of purines } "salvage"
3. Phosphorylation of purine nucleosides } pathways

Synthesis de novo (main pathway) begins with formation of 5-phosphoribosyl-1-pyrophosphate (PRPP). Synthesis of PRPP involves transfer of pyrophosphate from ATP to carbon 1 of α-D-ribose-5-phosphate and is catalyzed by PRPP synthetase. Next 10 reactions lead to the formation of inosine monophosphate (IMP) from which both adenosine monophosphate (AMP) and guanosine monophosphate (GMP) are formed.

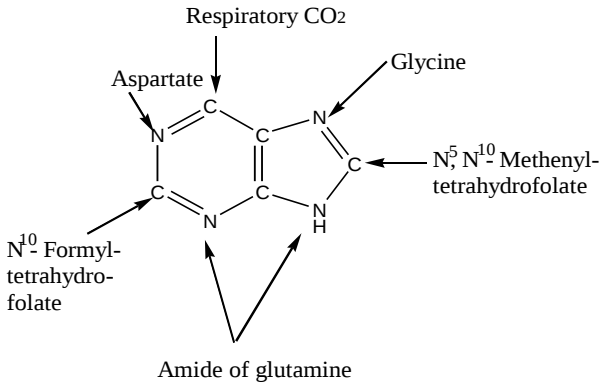


Figure 12.1.The sources of the nitrogen and carbon atoms of the purine ring. Atoms 4. 5. and 7 are derived from glycine.

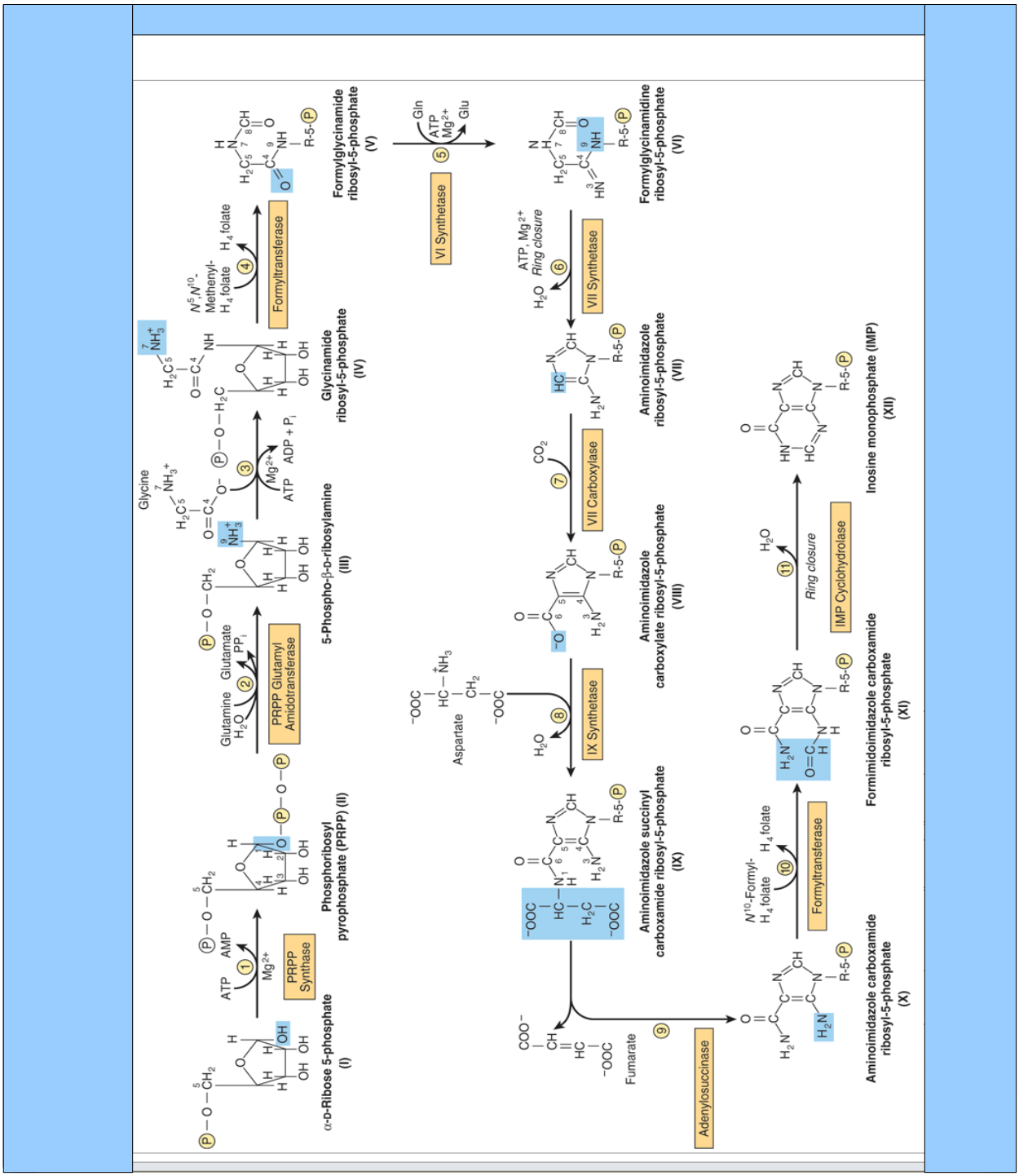


Figure 12.2. The pathway of de novo purine biosynthesis.

Conversion of IMP to AMP and GMP

Following synthesis of IMP, the pathway branches and two short reaction sequences lead to the formation of AMP and GMP.

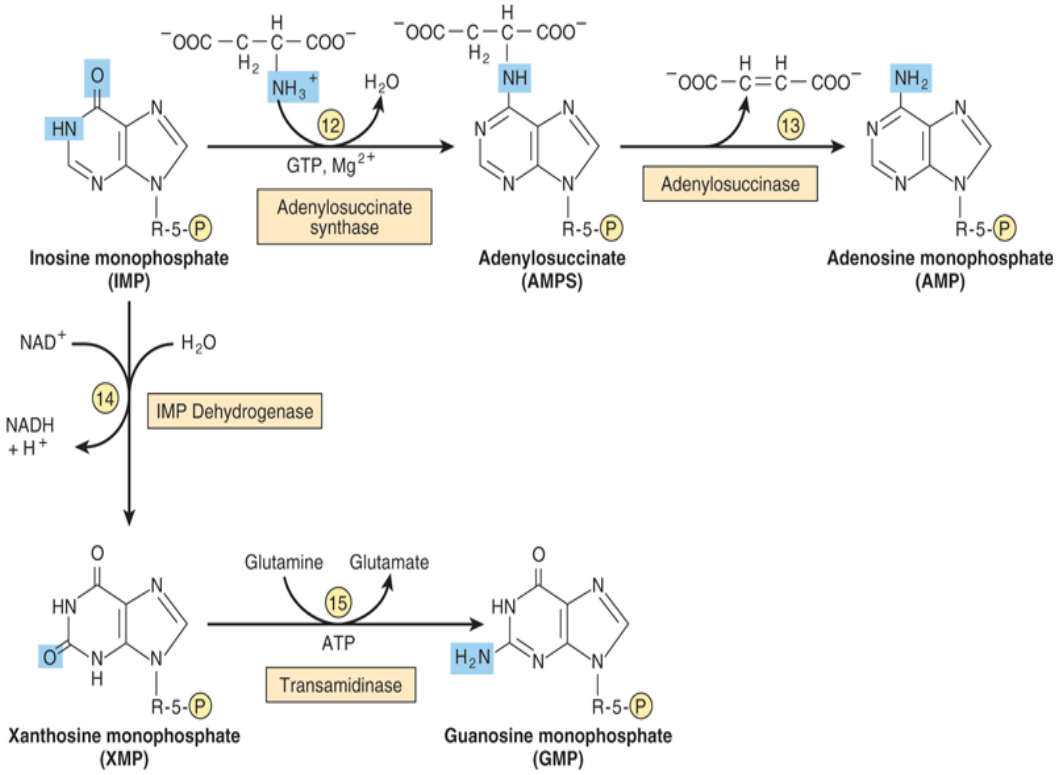
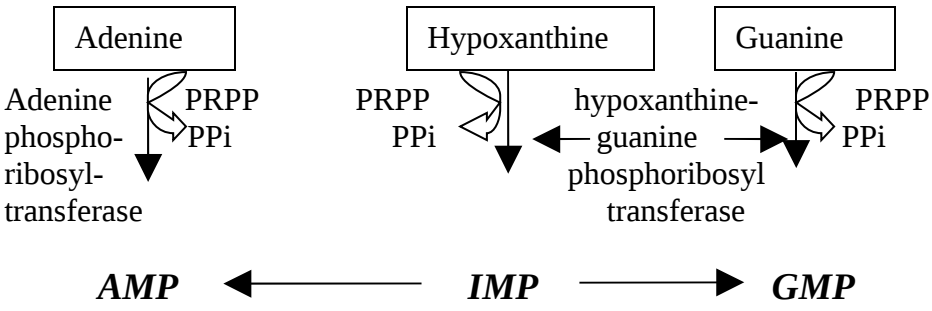


Figure 12.3. Conversion of IMP to AMP and GMP

Formation of Purine Nucleotides from Free Purine Bases



Complete deficiency of hypoxanthine – guanine phosphoribosyl transferase leads to development of *Lesch-Nyhan* syndrome.

Formation of Purine Nucleotides from Nucleosides

a) Adenosine — ATP → ADP — AMP (adenosine kinase)

Deoxyadenosine — dAMP

b) Deoxycytidine → dCMP
Deoxyadenosine (deoxycytidine kinase) dAMP
Deoxyguanosine d GMP

Mammalian liver, the major site of purine nucleotide biosynthesis, provides purines and their nucleotides for utilization by tissues incapable of their biosynthesis. For example, human brain has a low level of PRPP-glutamyl amidotransferase and hence depends, on part, on exogenous purines.

Conversion of Nucleoside Monophosphates into Nucleoside Triphosphates

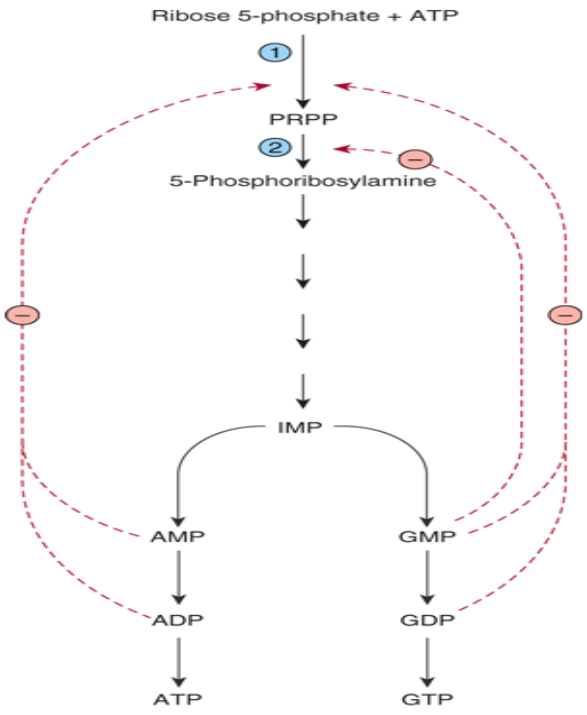
ATP *ADP* *ATP* *ADP*

Nucleoside → (nucleoside monophosphate kinase) Nucleoside → (nucleoside diphosphate kinase) Nucleoside

monophosphate diphosphate triphosphate

Conversion of AMP to ADP is analogic. Conversion of ADP to ATP is performed by oxidative phosphorylation, substrate level phosphorylation reactions and by adenylate kinase:

2ADP → *AMP + ATP*



Regulation of purine nucleotide synthesis is performed by means of feed-back mechanisms:

1 – PRPP-synthetase

2-PRPP- glutamyl amidotransferase

Figure 12.4. Control of the rate of de novo purine nucleotide synthesis.

Balance between adenylate and guanylate nucleotides is maintained by means of adenylosuccinate synthetase (adenylosuccinate synthase) (activator is GTP) and GMP-synthetase (transamidinase) (activator is ATP).

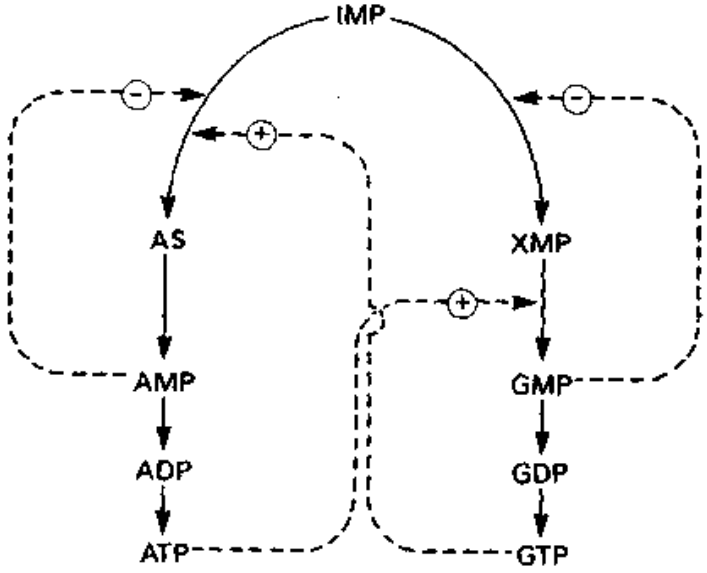


Figure 12.5. Regulation of the interconversion of IMP to adenosine nucleotides and guanosine nucleotides. Solid lines represent chemical flow, and broken lines represent both positive (+) and negative (-) feedback redulation.

Reduction of nucleotide diphosphates

Reduction of the 2'-carbon of purine and pyrimidine ribonucleotides, catalyzed by the ribonucleotide reductase complex, forms the deoxyribonucleoside diphosphates (dNDPs). The enzyme complex is active only when cells synthese DNA to prepare to cell division. Reduction of NDPs to dNDPs is subject to complex regulation, which achieves balanced production of deoxyribonucleotides for synthesis of DNA.

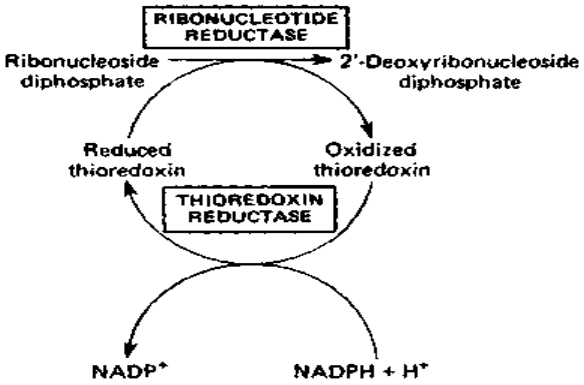


Figure 12.6. Reduction of ribonucleoside diphosphates to 2'-deoxyribonucleoside diphosphates.

Degradation of purine nucleotides

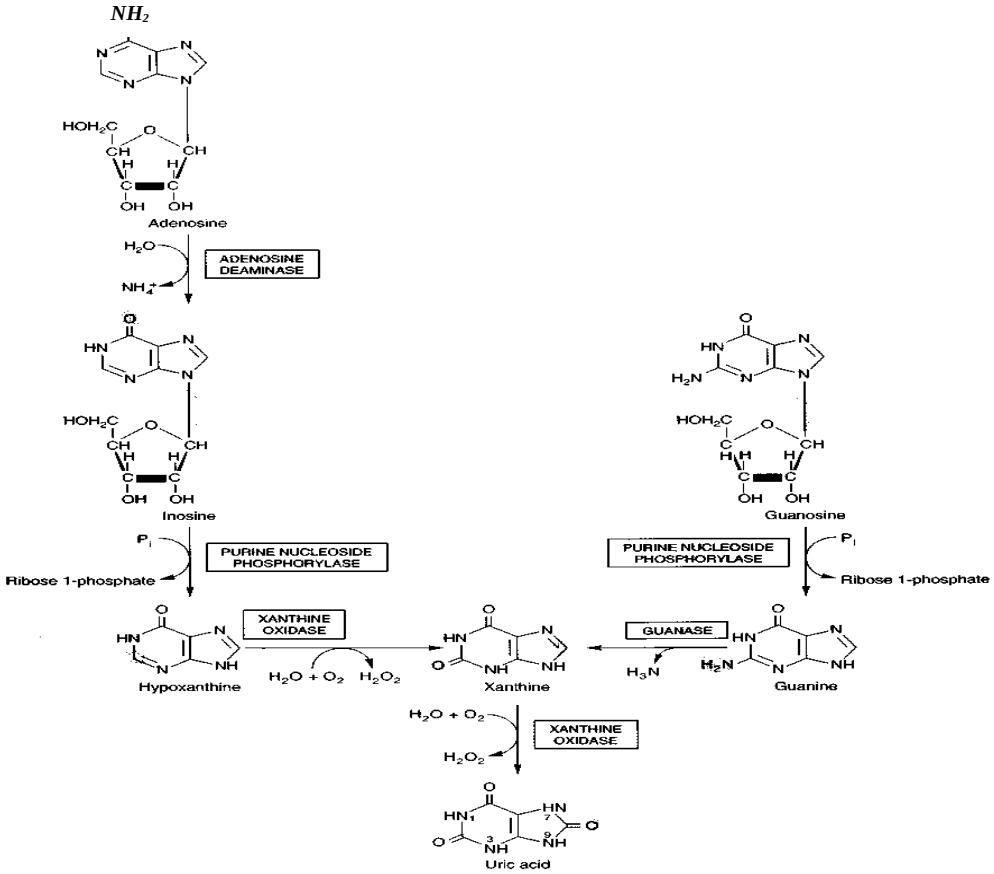
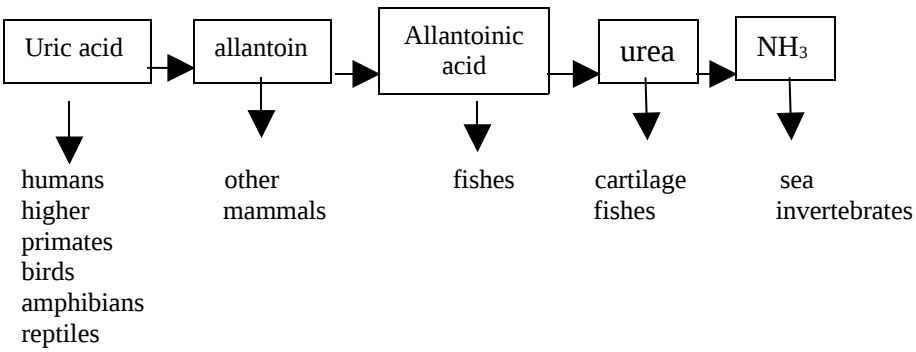


Figure 12.7. Degradation of purine nucleotides

Final product of purine degradation in human is uric acid. Humans lack uricase. Amphibians, birds, reptiles also lack uricase and excrete uric acid and guanine as end product of purines catabolism. In mammals other than higher primates the enzyme uricase cleaves uric acid, forming the highly water-soluble end product allantoin.



Uric acid is not only final product of purine metabolism, it:

- influences CNS;

- activates metabolism of prostaglandin precursors;
- decreases the insulin secretion;
- is antioxidant.

Uric acid

in blood: 149-405 μmol/L

in urine: 1.6-4.16 mmol/day

Disturbances of purine metabolism

Disturbances of purine nucleotide metabolism are accompanied either by hyperuricemia or hypouricemia.

Hyperuricemia may be linked either with increased production or with decreased excretion of uric acid.

Hyperuricemia

The primary hyperuricemia

is due to hereditary disturbances of purine nucleotide metabolism:

- Lesch- Nyhan syndrome;
- defect of PRPP synthetase.

The secondary hyperuricemia

accompanies some other diseases:

- Von Gierke's disease;
- diabetes mellitus;
- chronic diseases of kidney;
- metastasis and breakdown of tumors;
- hemoglobinopathies;
- pernicious anemia.

Hyperuricemia is obligatory manifestation of gout. Gout is metabolic disorder of purine catabolism. In hyperuricemia serum urate levels exceed the solubility limit. This leads to accumulation of sodium urate in soft tissues and joints. Crystallization of sodium urate in soft tissues and joints causes an inflammatory reaction, acute gouty arthritis, which can progress to chronic gouty arthritis. Also the intensive urates excretion with urine leads to the accumulation of these substances in kidneys and nephrolithiasis development.

Lesch - Nyhan syndrome is linked with the complete deficiency of hypoxanthine-guanine phosphoribosyl transferase. It is characterized by mental retardation, overproduction of uric acid with frequent uric acid lethiasis and a syndrome of self –mutilation (torture).

Von Gierke's disease is caused by deficiency of glucose-6-phosphatase. Hyperuricemia in this disease occurs secondarily to enhanced generation of the PRPP precursor ribose-5-phosphate. In addition associated lactic acidosis elevates the renal threshold for urate, elevating total body urates.

Allopurinol is used to decrease the uric acid level. It is irreversible inhibitor of xanthine oxidase.

Hypouricemia may be linked with deficiency of xanthine oxidase, with defect of mechanism of reabsorbtion of uric acid in renal channals, with influence of drugs which facilitate the excretion of uric acid in the urine.

Xanthinuria is linked with deficiency of xanthine oxidase, due either to a genetic defect or to severe liver damage. In severe xanthine oxidase deficiency patients may exhibit xanthinuria and xanthine lithiasis.

Pyrimidine nucleotide biosynthesis

Synthesis of the pyrimidines is less complex than that of the purines, since the base is much simpler. The first completed base is derived from 1 mole of glutamine, one mole of ATP and one mole of CO_2 (which form carbamoyl phosphate) and one mole of aspartate. An additional mole of glutamine and ATP are required in the conversion of UTP to CTP.

The carbamoyl phosphate used for pyrimidine nucleotide synthesis is derived from glutamine and bicarbonate, within the cytosol, as opposed to the urea cycle carbamoyl phosphate derived from ammonia and bicarbonate in the mitochondrion. The urea cycle reaction is catalyzed by carbamoyl phosphate synthetase I (CPS-I) whereas the pyrimidine nucleotide precursor is synthesized by CPS-II. Carbamoyl phosphate is then condensed with aspartate in a reaction catalyzed by the rate limiting enzyme of pyrimidine nucleotide biosynthesis, aspartate transcarbamoylase (ATCase).

The reaction of dTMP formation from dUMP is catalyzed by thymidylate synthase and requires N^5, N^{10}- methylene THF as coenzyme. During the transfer process, the methylene group of N^5, N^{10}- methylene – tetrahydrofolate is reduced to a methyl group, and the tetrahydrofolate carrier is oxidizet to dihydrofolate. For further synthesis to occur, dihydrofolate must be reduced to tetrahydrofolate in a reaction catalyzed by dihydrofolate reductase. Dividing cells, which are be necessity generating TMP and dihydrofolate, are especially sensitive to inhibitors of dihydrofolate reductase. *Methotrexate* (inhibitor of dihydrofolate reductase) and *5-fluorouracil* (inhibitor of thymidylate synthase) are used as anticancer drugs.

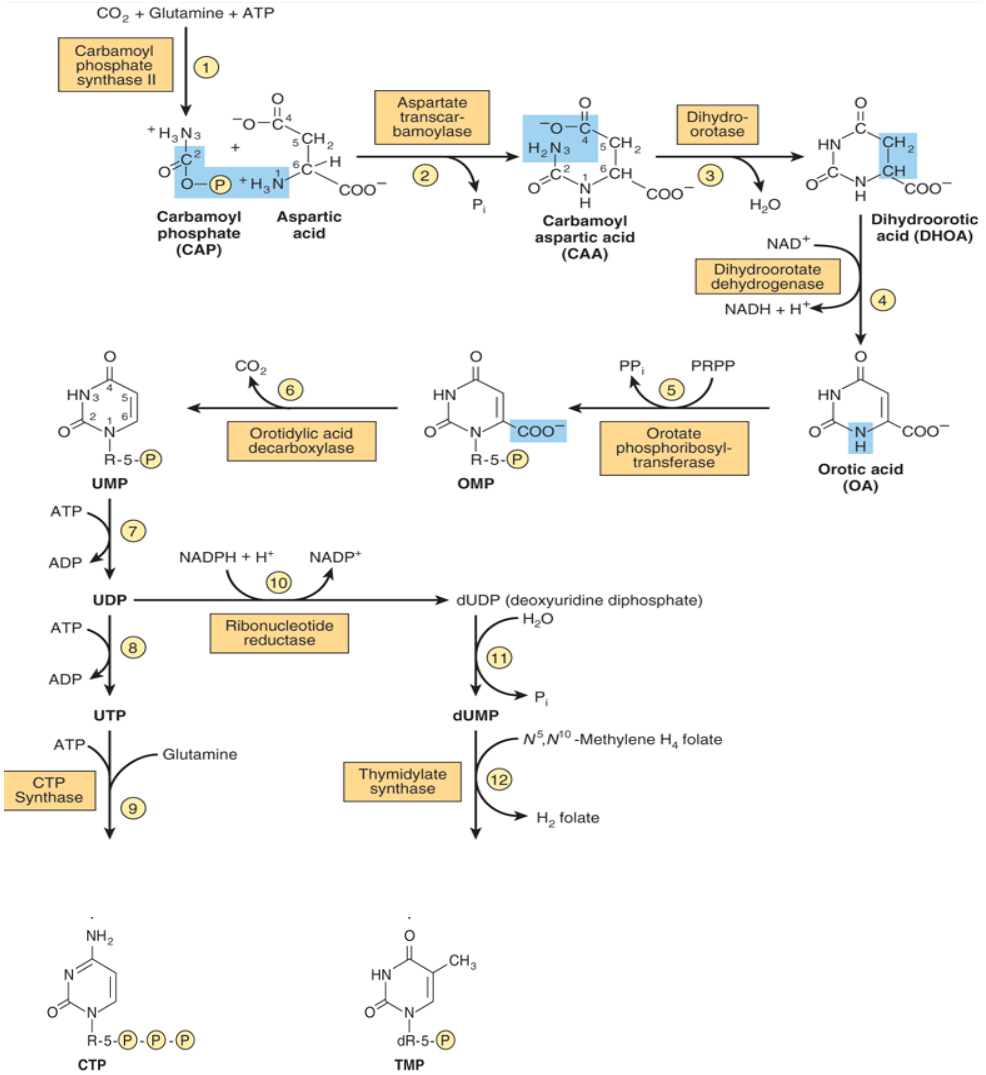


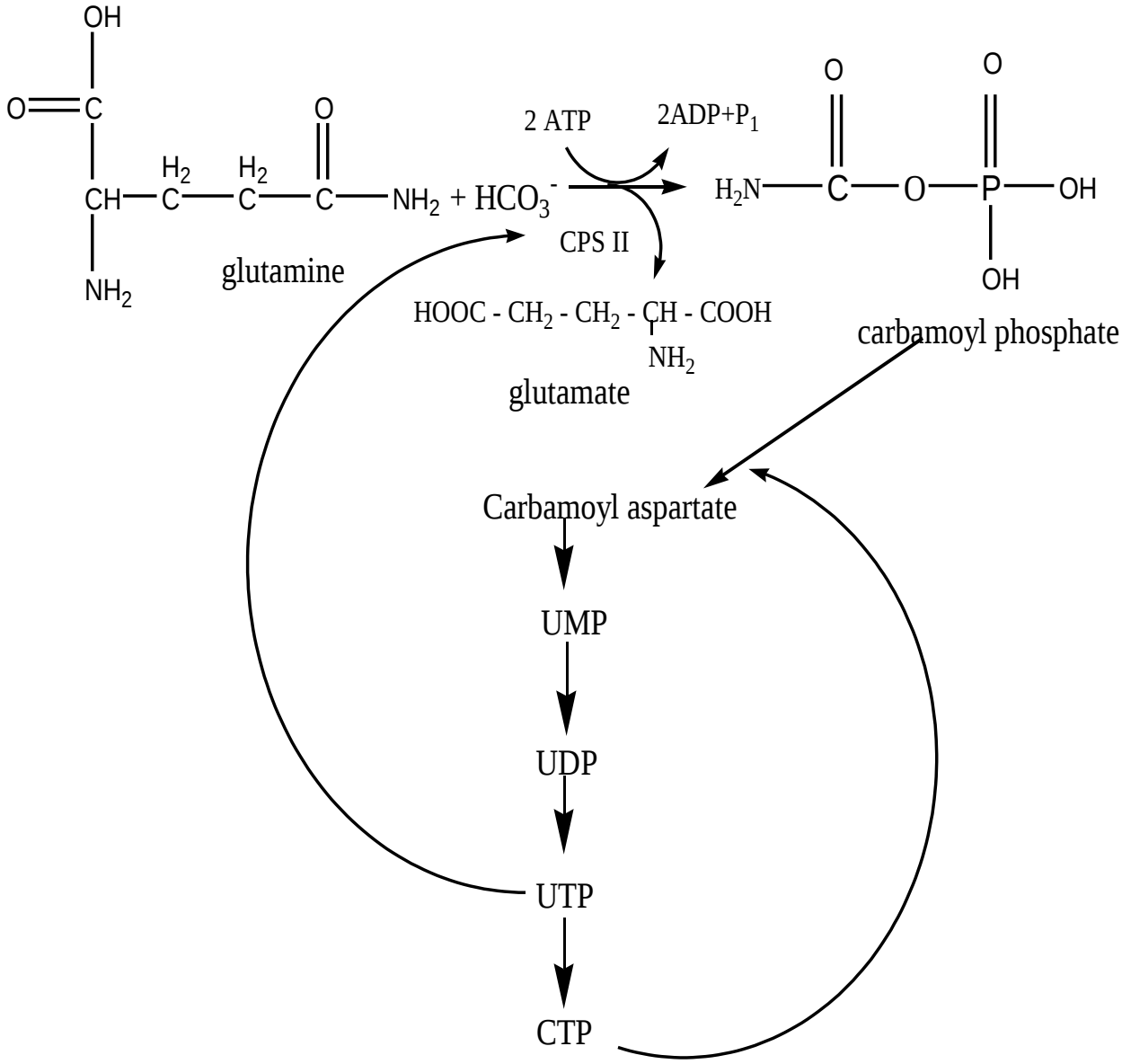
Figure 12.8. The biosynthetic pathway for pyrimidine nucleotides.

Five of the first six enzymes of de novo pyrimidine biosynthesis are organized as multifunctional polypeptides rather than as distinct enzymes. The sole exception

is dihydroorotate dehydrogenase. A 220 kDa polypeptide contains carbamoyl phosphate synthase, aspartate transcarbamoylase and dihydroorotase. The close association of these activities ensures that almost all of the carbamoyl phosphate is channeled to pyrimidine biosynthesis.

Uracil and cytidine ribo- and deoxyribonucleotides are salvaged. Salvage reactions convert two pyrimidine ribonucleosides (uridine amd cytidine) to their respective nucleotides. 2`-Deoxycytidine is phosphorylated by deoxycytidine kinase, an enzyme that also phosphorylates deoxyguanosine and deoxyadenosine.

Regulation of pyrimidine nucleotide synthesis.



The first two enzymes of pyrimidine nucleotide biosynthesis are sensitive to allosteric regulation. Carbamoyl phosphate synthase is inhibited by UTP and

purine nucleotides but activated by PRPP. Aspartate transcarbamoylase is inhibited by CTP and activated by ATP.

Mole for mole, pyrimidine biosynthesis parallels purine biosynthesis, suggesting coordinate comtrol. Several sites of cross-regulation characterize purine and pyrimidine nucleotide biosynthesis. The PRPP synthase reaction, which forms a precursor essential for the both processes, is subject to feed-back inhibition by both purine and pyrimidine nucleotides, and is activated by PRPP.

Catabolism of pyrimidines

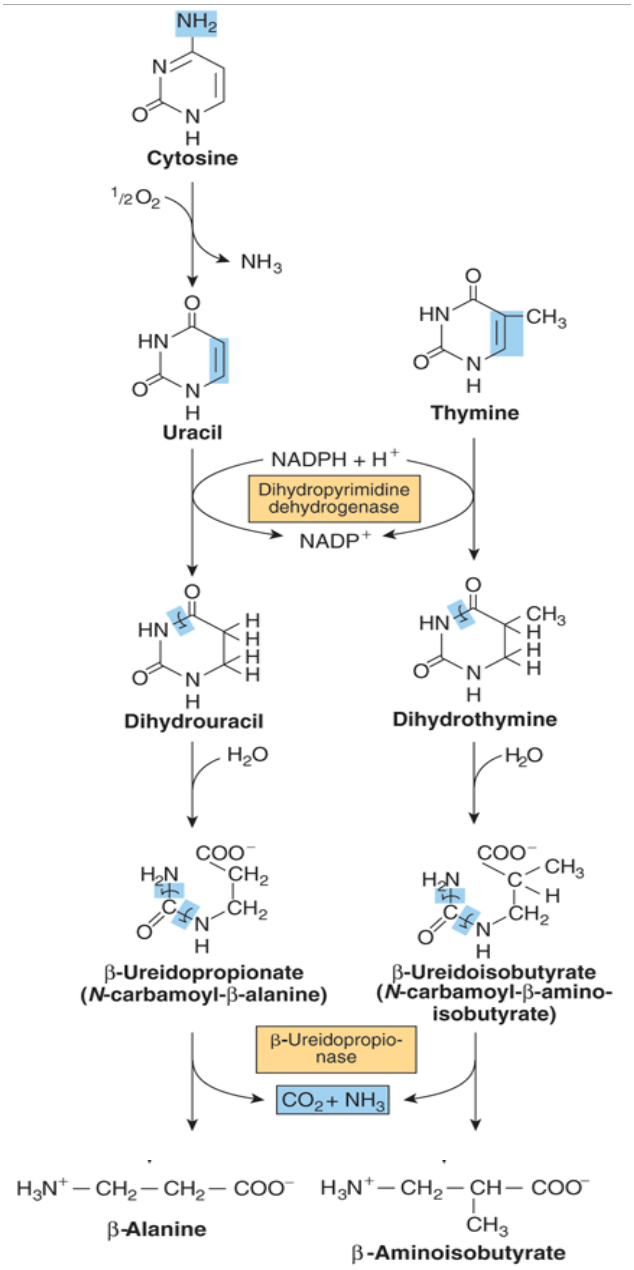


Figure 12.9. Catabolism of pyrimidines.

Pyrimidine nucleotide disorders

Orotic aciduria

1. Type I. Orotic aciduria reflects a deficiency of both orotate phosphoribosyl transferase and orotidylate decarboxylase. It is characterized by growth retardation, retardation of psycho-motoric development and megaloblastic anemia.

2. Type II. Orotic aciduria reflects a deficiency only of orotidylate decarboxylase. It is characterized by megaloblastic anemia. Oral uridine is used for treatment of both types of orotic aciduria because it inhibits the synthesis of carbamoyl aspartate.

3. Orotic aciduria may result from deficiency of urea cycle enzymes except carbamoyl phosphate synthetase I. The nonutilized substrate carbamoyl phosphate exits to the cytosol where it stimulates pyrimidine nucleotide biosynthesis.

4. Orotic aciduria may be caused by drugs for example allopurinol. Allopurinol is the substrate for orotate phosphoribosyl transferase and competes with the natural substrate orotic acid. In addition, the resulting nucleotide product inhibits orotidylate decarboxylase, resulting in orotic aciduria and orotidinuria.

12.2 Synthesis of Nucleic Acids.

12.2.1 DNA Replication

Much of the DNA in eukaryotic organisms is covered with a variety of proteins. These proteins and DNA form a complex structure, *chromatin.*

Chromatin consists of:

- very long double – stranded DNA molecules;
- nearly *equal* mass of small basic proteins termed *histones;*
- smaller amount of *nonhistone proteins* (mast of which are acidic and larger than histones);
- small quantity of RNA.

Chromatin consists of spherical particles called *nucleosomes.* Nucleosomes are composed of DNA wound around a collection of histone molecules. In the nucleosome, the DNA is supercoiled in a left handed helix over the surface of the disk-shaped histone octamer (histone core). Nucleosomes form fibril. Fibril is

probably further supercoiled with 6-7 nucleosomes per turn to form chromatin fiber.

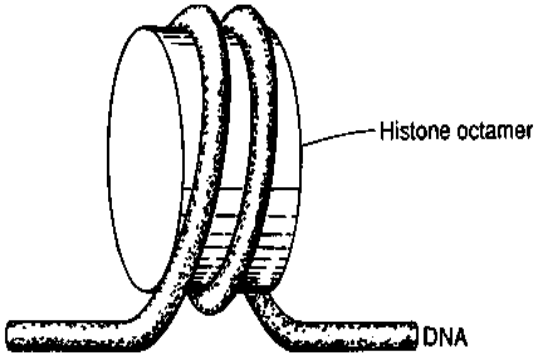


Figure 12.10. Model for the structure of the nucleosome, in which DNA is wrapped around the surface of a flat protein cylinder consisting of two each of histones H2A, H2B, H3, and H4. The 146 base pairs of DNA, consisting of 1.75 superhelical turns, are in contact with the histone octamer. This protects the DNA from digestion by a nuclease.

In animal cells, including human cells, the replication of DNA occurs during the *S-phase* of the cell cycle.

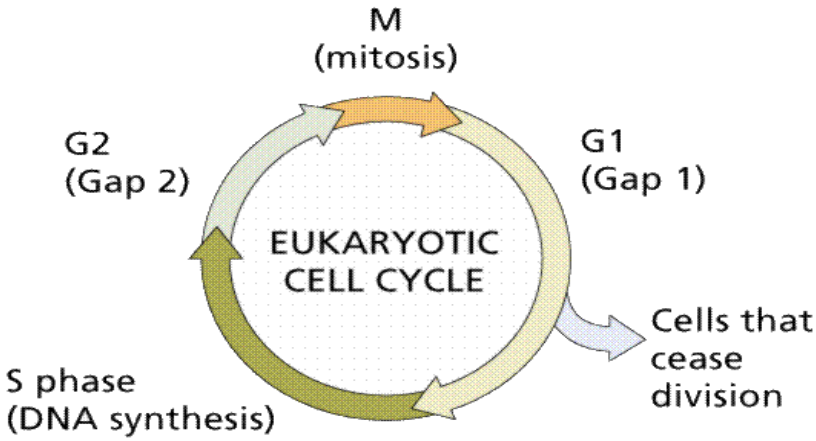


Figure 12.11**.** *Phases of cell cycle.*

S-phase is separated from the mitotic phase by presynthetic period (gap 1 – G_1) and postsynthetic period (gap 2 – G_2).

Regulation of cell cycle in eukaryotic organisms is performed by means of specific proteins – *cyclins*, concentration of which is changed during the cell cycle.

Cyclins activate cyclin-dependent protein kinases that phosphorylate substrates essential for progression through the cell cycle.

Features of DNA replication:

- Synthesis of DNA requires the primer.
- Biosynthesis of DNA is symmetrical process.

- It is performed by semiconservative mechanism.
- Elongation of each of the daughter strands can be accomplished only in the 5' to 3' direction.

Process of DNA replication is very complex. Many of enzymes and protein factors participate in this process.

$$\begin{array}{l} n_1\ d\,ATP \\ n_2\ d\,GTP \\ n_3\ d\,CTP \\ n_4\ d\,TTP \end{array} \xrightarrow[\text{DNA- polymerase}]{Mg^{2+}\ \text{DNA-template}} \begin{array}{l} D \\ N \\ A \end{array} \left[\begin{array}{l} d\,AMP\ n_1 \\ d\,GMP\ n_2 \\ d\,CMP\ n_3 \\ d\,TMP\ n_4 \end{array}\right] + (n_{1+}\ n_2 + n_3 + n_4)PP_1$$

Synthesis of DNA may be divided info 3 steps:

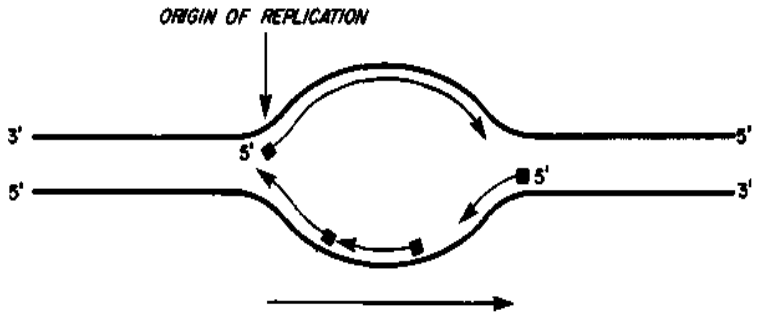
I. Initiation.

II. Elongation.

III. Termination.

Unit of the replication is replicon. It contains two points: starting or “ori” point (from “origin”) and ending or “ter” point (from “termination”). Prokaryotic DNA has only 1 replicon, eukaryotic DNA contains a lot of replicons.

I. Initiation is the stage of formation of replication fork and synthesis of primer. Synthesis of DNA begins in specific site as so called replication fork (or replication bubble). The entire mammalian genome replicates in approximately 9 hours. This requires the presence of multiple origins of DNA replication that occur in clusters of up to 100 of these replications units.



OVERALL DIRECTION OF REPLICATION

Figure 12.12. The process of semidiscontinuous, simultaneous replication of both strands of double-stranded DNA.

The following enzymes and proteins participate in the formation of replication fork:

- *Topoisomerase* (DNA-gyrase in prokaryotes) ruptures the phosphodiesteric bonds in DNA, provides negative supercoils.
- *Helicase*, which moves along the DNA and separates the strands using chemical energy from ATP and provides despiralization.
- *SSBP* (single strand binding proteins) stabilize the separated strands (they prevent the reverse complementary interaction of DNA strands).
- *Primase*. Synthesis of DNA requires the primer. Primer is a short segment of RNA (10-200 nucleotides) acting to trigger the DNA synthesis. Primer diminishes the appearance of errors during replication. Specific RNA – polymerase, named primase, catalyzes this synthesis.

II. Elongation includes a step involving the replication of both parental DNA strands and a step at which the fragments of newly–formed DNA strands bind to one another.

DNA polymerase is the basic enzyme which participates in this process. In addition to polymerizing activity DNA-polymerase has 3' → 5' exonuclease activity (as so called *proof reading activity*) which provides the removal of just added nucleotide with incorrect base. This mechanism reduces the amount of errors during replication. DNA polymerase makes about one error for every 10^6 to 10^8 bases added.

DNA polymerases of prokaryotes:

1. *DNA-polymerase III* is the basic enzyme of replication of DNA. It catalyzes the synthesis of newly formed DNA (velocity of polymerizing reaction is ~ 1000 nucleotides per 1 second).
2. *DNA-polymerase I* (in addition has 5' → 3' exonuclease activity) removes primer and replaces it with newly synthesized DNA (velocity – 10 nucleotides per 1 second).
3. *DNA-polymerase II* appears to have a highly specialized DNA repair function (velocity – 0.5 nucleotides per 1 second).

DNA polymerases of eukaryotes:

1. *DNA polymerases α- and δ-* are involved in the replication of nuclear chromosomes.
2. *DNA polymerases β- and ε-* participate in reparation of DNA.
3. *DNA polymerase γ* performs the replication of mitochondrial genome.

Synthesis of DNA is performed in direction 5'→ 3', but DNA chains are antiparallel, therefore one strand ("leading strand") is replicated in a continuous manner in the 5' to 3' direction and other strand "lagging strand" is replicated discontinuously (by Okazaki fragments).

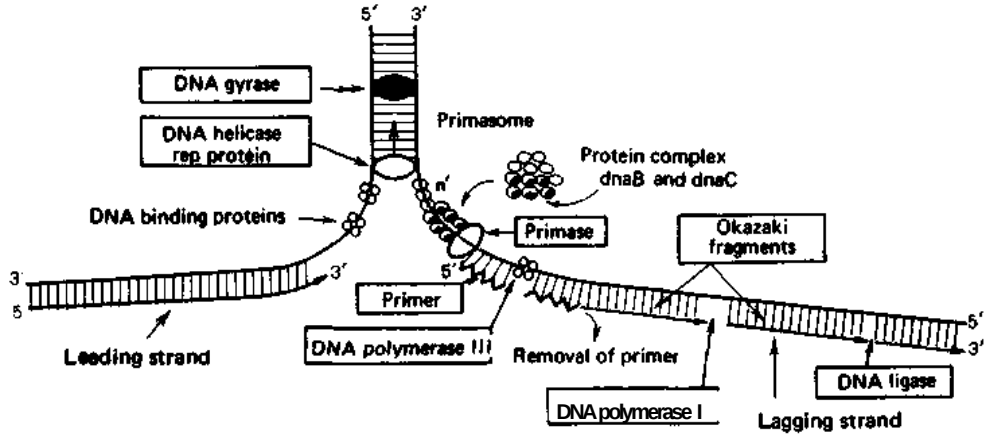


Figure 12.13. Main stages of DNA replication (schematic)

Then primers are removed (by DNA polymerase I in prokaryotes) and are replaced with DNA by the same enzyme. *DNA ligase* links fragments of DNA.

III. *Termination* of DNA synthesis occurs if the DNA template is informatively depleted.

12.2.2 RNA Synthesis (Transcription)

It should be noted that haploid genome of human's cell contains such amount of nitrogen bases which is enough to form 1.5 millions of genes. But really around of 100000 of proteins are synthesized in human's organism.

In every tissue between 10000 and 15000 genes are expressed. Different combinations of genes are expressed of each tissue and how this is accomplished is one of the major unanswered questions in biology.

Differences of cells during cell differentiation occur in result of stable repression of one genes and derepression of other ones. Molecular mechanisms of

this regulation are unknown. Thus in animal cell there are 2 types of regulation of **gene expression:**

I. Long-term regulation appears during cell differentiation. This induction and repression is frequently retained during life of many generations of cell (tissue – specific expression).

II. Short-term regulation or adaptive induction and repression, which occurs in changing concentration of specific regulatory elements – hormones, metabolites, etc.

Features of RNA synthesis

- Synthesis of RNA occurs de novo (without primer).
- Synthesis of RNA occurs assymmetrically.
- Transcription is conservative synthesis.
- Synthesis is performed in the 5' → 3' direction.

RNA synthesis involves:

I. Initiation

II. Elongation

III. Termination

I. Initiation. The enzyme RNA – polymerase is bound with specific sequences of DNA. These sequences are called promoter. Promoter of prokaryotic consists of 2 components:

- The first of them is located 35 bases to the left of the transcription start site. It is thought to be the initial site of σ-subunit binding.
- The second (AT –rich sequence) is located 10 bases to the left of the transcription start site. It is thought to ease the dissociation between the coding and noncoding strands.

This unique sequence (TATAAT) is also known as TATA-box or TATA-block. RNA-polymerase binds with TATA-box and is activated by phosphorylation with participation of 8 IF (initiation factors).

Binding RNA polymerase with promoter leads to local separation of 2 strands of DNA.

RNA polymerase initiates RNA synthesis. A single type of *RNA polymerase* is responsible for synthesis of mRNA, rRNA and tRNA in prokaryotes.

RNA polymerase of E.coli consists of the core enzyme (α_2 β β′) and sigma factor (σ).

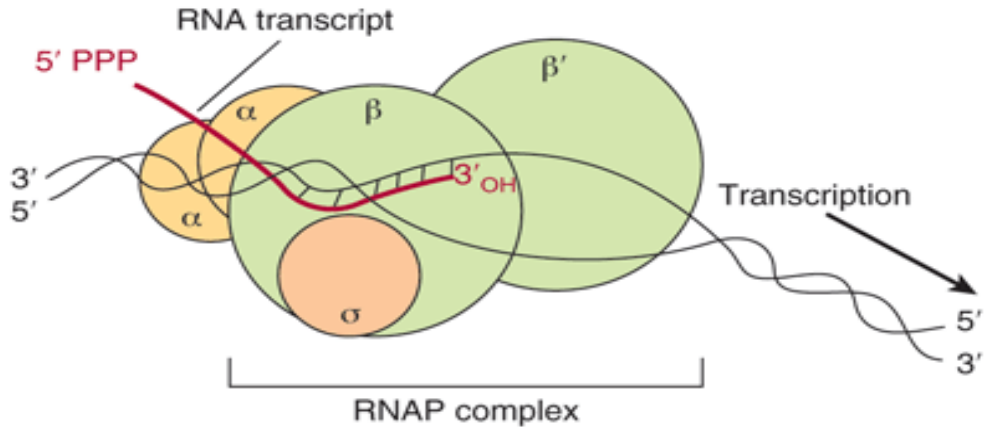


Figure 12.14. RNA polymerase (RNAP) catalyzes the polymerization of ribonucleotides into an RNA sequence that is complementary to the template strand of the gene.

Sigma factor helps the core enzyme recognition of the promoter region. Bacteria contain multiple σ factors, each of which acts as a regulatory protein that modifies the promoter recognition specifity of the RNA polymerase.

In eukaryotes several different enzymes are required to synthesize the different types of RNA.

Class of enzyme RNA-polymerase	Products	Principal localization
I (A)	rRNA (50-70%)	nuclear
II (B)	mRNA (20-40%)	nucleoplasmic
III (C)	tRNA and 5SRNA (10%)	nucleoplasmic

In eukaryotes RNA polymerases the function of sigma factors is assumed by a number of proteins as so called “factors of transcription”.

II. Elongation. RNA-polymerase moves in 3’→5’ direction of the coding strand of DNA and elongation of RNA molecule from 5’ to its 3’ end continues antiparallel to its template. The enzyme polymerizes the ribonucleotides in the specific sequence dictated by the template strand and interpreted by Watson-Crick

base-pairing rules. Pyrophosphate is released in the polymerization reaction. In both prokaryotes and eukaryotes, a purine ribonucleotide is usually the first to be polymerized into the RNA molecule.

III. Termination. Specific sequences of the DNA function as the signal for termination of the transcription process. Terminal sequences have inverted regions. Part of RNA which is synthesized on this template sequence has structure of hair-pin. Terminal signal is recognized by a termination protein, the rho (ρ) factor. After termination of synthesis of the RNA molecule, RNA - polymerase separates from DNA template.

Post-transcriptional modification of RNA (processing)

In prokaryotes the primary transcript of mRNA can serve as template for protein synthesis. The primary transcripts of rRNA and tRNA in prokaryotes undergo to processing.

Nearly all eukaryotic RNA primary transcripts undergo extensive processing.

The processing includes: capping, nucleolytic and ligation reactions (splicing), terminal additions of nucleotides and nucleotide modifications.

1. **Capping.** 7-methylguanosine cap is added to the 5'-end of transcript molecule. It occurs in the nucleus. Capping appears to be required:
   - for the formation of ribonucleoprotein complex necessary for the splicing reactions;
   - for transport of mRNA;
   - for initiation of translation;
   - it protects the 5' end of mRNA from attack by 5'→3' exonucleases.
2. **Splicing** (nucleolytic and ligation reactions). The most of genes of higher eukaryotes contain both amino acid coding portions (*exons)* and noncoding sequences (*introns).* Intron RNA sequences are removed and the exons of the transcript are appropriately spliced together in the nucleus.

A special structure the *spliceosome* is involved in conversion of the primary transcript into mRNA. Spliceosomes consist of:

- the primary transcript;
- five small nuclear RNAs (RNA serves as catalytic agent);
- proteins.

Alternative splicing provides an additional mechanism of gene expression regulation.

3. **Polyadenylation at the 3' terminal.** Poly A-tail is added at the 3'-end either in the nucleus or in the cytoplasma. The function of the poly (A) tail is unknown but it appears to protect the 3' end of mRNA from 3' $\rightarrow$5' exonuclease attack.
4. **Nucleotide modification.** 5-methylcytosine, N^6-methyladenine, hypoxanthine, dihydrouracil, pseudouridine may be formed. These reactions may be performed either in the nucleus or in the cytoplasm.

Regulation of gene expression on the level of transcription in eukaryotes

In addition to signals of basal expression which define the site of the beginning expression and frequency of initiation, DNA of eukaryotes has *signals of regulation of expression.*

1. *Enhancers and silencers.* These elements can either increase or decrease the rate of transcription initiation of eukaryotic genes. Some sequences bind only a single protein but the majority binds several proteins. Similarly, a single protein can bind to more than one element.
2. *Other elements* that mediate the response to various signals, including hormones, heat shock, metals and chemicals. For example, *hormone response elements* (for steroids, T_3, retinoic acid etc.).

Antibiotics-inhibitors of transcription

· *Actinomycin D* blocks the binding RNA – polymerase with DNA – template in eukaryotes.

· *Rifamycin* binds with β-subunit of RNA-polymerase of prokaryotes and blocks the initiation of transcription. It is used as antibacterial drug.

12.3 Molecular Mechanisms of Mutations. DNA Repair

Mutations are the changes of hereditary properties in result of quantitive and qualitative changing genome of organism. Depending of the type of changing structure of genetic apparatus of organism mutations are divided into:

1. *Genome* mutation - alteration of amount of chromosomes. There are polyploidia when the chromosome amount increases divisibly to gaploidic number and aneuploidia (losting or addition of one or some chromosomes). Down's disease is due to the appearance of additional 21 chromosome.

2. *Chromosome mutations* are structural alterations of chromosomes:

-*transposition* - transfer of fragment of DNA to other part of the same chromosome;

- *translocation* – transfer of fragment of one chromosome to other (non homologous) one;

- *inversions* – changing one sequence of genes to other inverted sequence;

- *deletion* – removal of fragment of DNA;

- *duplication* – duplication of some parts of DNA.

3.Gene mutations are disturbances of the sequence of nucleotides in the primary structure of DNA.

Gene mutations

point (deal with 1 nucleotide)

deal with the group of nucleotides

Point mutations are divided to:

- *Substitution:*

a) *transition* – changing one purine nucleotide to other purine nucleotide or changing one pyrimidine nucleotide to other pyrimidine nucleotide;

b) *transversion* – changing purine nucleotide to pyrimidine nucleotide, or pyrimidine nucleotide to purine nucleotide.

Substitution can lead to the changing sense of codon (missence mutations) or to conversion of codon into termination codon (nonsence mutation).

Subtype of missense mutations when the changing in gene structure doesn't lead to the change of general activity of enzyme, but shift its optimal conditions, is called "licky" mutations.

- *Deletion* – the removal of nucleotide.

- *Insertion* – the incorporation of nucleotide.

Deletion and insertion mutations lead to frame shift.

Systems of DNA repairing correct damage DNA.

Mismatch repair

Mismatch repair corrects errors made when DNA is copied.

- *Specific proteins* scan the newly synthesized DNA, using adenine methylation within a GATC sequence as the point of reference.
- *A GATC endonuclease* cuts the strand bearing the mutation at a site corresponding to the GATC.
- *An exonuclease* then digests this strand from the GATC through the mutation.
- This defect is then filled according to base pairing rules.

  Faulty mismatch repair has been linked to hereditary nonpolyposis colorectal cancer (HNPCC), one of most common inherited cancers.

Base excision-repair:

- *N – glycosidases* can recognize abnormal bases and remove the base itself from the DNA.
- This removal marks the site of the defect.
- Specific endonuclease excises the abasic sugar.
- The proper base is then replaced by a repair *DNA-polymerase β*, and a *ligase* returns the DNA to its original state.

Both *cytosine* and *adenine* bases in DNA spontaneously deaminate to form uracil and hypoxanthine, respectively.

Uracil, hypoxanthine, alkylated bases and base analogs can be removed and the DNA returned to its original informational content by means of base excision-repair.

Nucleotide excision:

- Specific *endonucleases* perform the hydrolysis of two phosphodiester bonds on the strand containing the defect.
- After the strand is removed it is replaced by means of DNA – polymerase.
- The ends are joined to the existing strands.

This mechanism is used to replace regions of damaged DNA up to 30 bases in length.

The damages of DNA by *ultraviolet* (UV) *light* (which induces the formation of cyclobutane *pyrimidine dimers)*, smoking (which causes formation of benzo and pyren – guanine adducts), ionization radiation, cancer chemotherapeutic agents and a variety of chemicals are repaired by a nucleotide excision-repair.

Xeroderma pigmentation is an autosomal recessive genetic disease. This defect involves the damage of the synthesis of *UV-specific endonuclease.* The clinical syndrome includes marked sensitivity of skin to sun light (ultraviolet) with subsequent formation of multiple skin cancers and premature death.

DNA double-strand break repair

High-energy radiation or oxidative free radicals can cause double-strand breaks in DNA, which are potentially lethal to the cell. Double-strand breaks also occur naturally during gene rearrangements. dsDNA breaks cannot be corrected by the excising the damage on one strand and using the remaining strand as a template for replacing the missing nucleotides. Instead, the double-strand breaks are repaired by one of two systems. The first is nonhomologous end-joining repair, in which the ends of two DNA fragments are brought together by a group of proteins that effect their relegation. This system does not require that, the two DNA sequences have the sequence homology. However, this mechanism of repair is error prone and mutagenic. Defects in this repair system are associated with a predisposition of cancer and immunodeficiency syndromes.

The second repair system, homologous recombination repair, uses the enzymes that normally perform genetic recombination between homologous

chromosomes during meiosis. This system is much less error prone than nonhomologous end-joining.

A third mechanism is recombination repair. The gap is closed by shifting the corresponding sequence from the correctly replicated second strand. The new gap that results is then filled by polymerases and ligases. Finally, the original defect is corrected by excision repair.

12.4 Recombinant DNA Technology. Application.

Recombinant DNA technology involves isolation and manipulation of DNA to make chimeric molecules.

Creating recombinant DNA

1. Obtaining definite gene:

- by means of chemical synthesis;

- by means of isolation of gene from genome (with utilization of restriction enzymes);

- by means of biological methods of gene synthesis with the aid of reverse transcriptase (revertase) on the template of mRNA.

2. *Preparing chimeric DNA molecules.*

- Restriction enzymes cut parts of cyclic DNA of plasmids. Sticky ends of DNA are linked with obtained gene.

3. Insertion of recombinant DNA to the host organism. Plasmids, phages cosmids are used as cloning vectors.

4. Cloning recombinant DNA. Cloning amplifies DNA.

Practical Applications of Recombinant DNA Technology:

1. Using recombinant DNA technology, human proteins can be produced in abundance for therapy (e.g., insulin, growth hormone, plasminogen activator).
2. Proteins for vaccines (e.g., hepatitis B) and for diagnostic tests can be obtained.
3. Recombinant DNA technology is used in the molecular analysis of disease.
4. Manipulation of the DNA to change its structure can be used to study the function of a certain fragments of DNA and to analyze how genes are regulated.

5. Chimeric DNA molecules are introduced into fertilized oocyte to make transgenic animals.

Tests for Self-control

1. Patient shows the pain in small joints, joints are enlarged. Urate level is increased in the blood serum. Which substances metabolism is disordered?

A. Amino acids
B. Disaccharides
C. Purines
D. Pyrimidines
E. Glycerol

2. Boy with hereditary Lesch-Nyhan's syndrome shows gout symptoms and neuro-psychic changes. Which metabolic pathway disorder leads to such signs development?

A. Purine nucleotide synthesis from free bases
B. Purine nucleotide synthesis from amino acids
C. Pyrimidine nucleotide synthesis from amino acids
D. Purine nucleotide break down
E. Pyrimidine nucleotide break down

3. Child shows delay of growth and psychic development, high amount of orotic acid is excreted with urine. This disease is observed as result of disorder of:

A. Pyrimidine nucleotide synthesis
B. Pyrimidine nucleotide break down
C. Purine nucleotide synthesis
D. Purine nucleotide break down
E. Convertion of ribonucleotides to desoxyribonucleotides

4. Joints are enlarged in the patient. Urate content is increased in the blood. Point the pathology.

A. Rickets
B. Scurvy
C. Pellagra
D. Caries
E. Gout

5. Which biochemical test should be performed to precise the diagnosis "gout"?

A. Examination of amino acid content in the blood
B. Examination of urea concentration in the blood and urine
C. Examination of creatinine content in the blood
D. Examination of uricase activity in the blood
E. Examination of uric acid level in the blood and urine

6. Synthesis of primer – RNA fragment – proceeds at the stage of:

A. Initiation
B. Elongation
C. Termination
D. Replication
E. Translation

7. At the patients with pigment xeroderma the skin is very sensitive to sunrise, cancer of skin can develop. The reason is hereditary deficiency of enzyme UV-endonuclease. This defect leads to disturbance of:

A. DNA replication
B. Transcription
C. DNA reparation
D. Translation
E. Reverse transcription

8. A women, 50 years old, adressed to the doctor with complanations on a pain in small joints of legs and arms. The joints are augmented and look like thickened knots. In blood serum the increased content of urates is found. The cause is failure of metabolism of:

A. Amino acids
B. Purines
C. Carbohydrates
D. Lipids
E. Pirimidines.

9. Allopurinol was administered to a patient with gout. Which pharmacological property of allopurinol makes therapeutic effect in this case?

A. Acceleration of pyrimidine nucleotides catabolism
B. Increase of velocity of nitrogen substances excretion
C. Competitive inhibition of xanthine oxidase
D. Inhibition of pyrimidine nucleotides reutilization
E. Acceleration of nucleic acid synthesis

Chapter 13. SYNTHESIS OF PROTEINS AND ITS REGULATION

13.1 Biosynthesis of Proteins

Translation takes place on ribosomes. On ribosomes the nucleotide sequence of mRNA is translated into the sequence of amino acids of the corresponding specific proteins.

Nucleic acids consist of 4 types of nucleotides and proteins consist of 20 types of amino acids. Therefore neither one nor two bases can specify all the amino acids. However, 64 kinds of amino acids can be specified by a three base code. The 64 combinations of three bases form genetic *code*.

Three nucleotides, which code 1 amino acid, are called *codon*. Three nucleotides in tRNA, which are complementary to any codon, are called *anticodon.*

Features of genetic code:

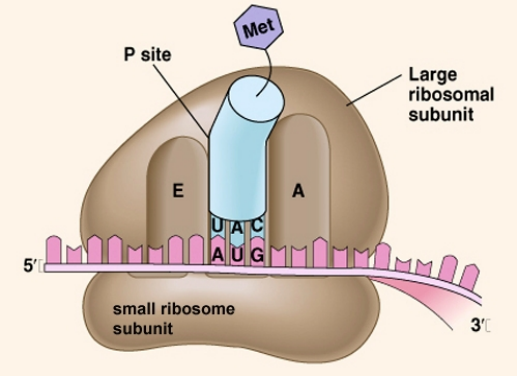
- *Degeneracy.* 61 codons represent 20 amino acids. Every amino acid except methionine and tryptophan is represented by several codons. This feature minimizes the effects of mutations.
- *Commalessnes.* Codons are arranged as a continuous structure. There is not one or more nucleotides between consecutive codons.
- *Universality*. In all the living organisms the genetic code is the same (in recent years it has been shown that the code of mitochondria of animals including human cells differs in 4 codons).
- *Unambiquity.* A given codon designates only one single specific amino acid.
- *Non-overlapping.* All codons are independent sets of 3 bases. No base functions as a common member of two consecutive codons.
- Out of the 64 theoretical codons only 61 are meaningful (sense codons). Three codons, namely UAA, UAG, UGA are “meaningless” (nonsense codons). These codons perform an important function - function of synthesis termination.

- Methionine codon (AUG) has additional information, if it occurs in the beginning mRNA chain. It participates in initiation of protein synthesis.

The translation requires:

- amino acids;
- t RNAs;
- amino acyl-tRNA-synthetases;
- m RNAs;
- ribosomes;
- factors of initiation, elongation and termination;
- ATP, GTP (sources of energy);
- ions of Mg^{2+}.

Ribosomes are nucleoproteins and contain 65% of rRNA and 35% of proteins.



Ribosomal functional sites:

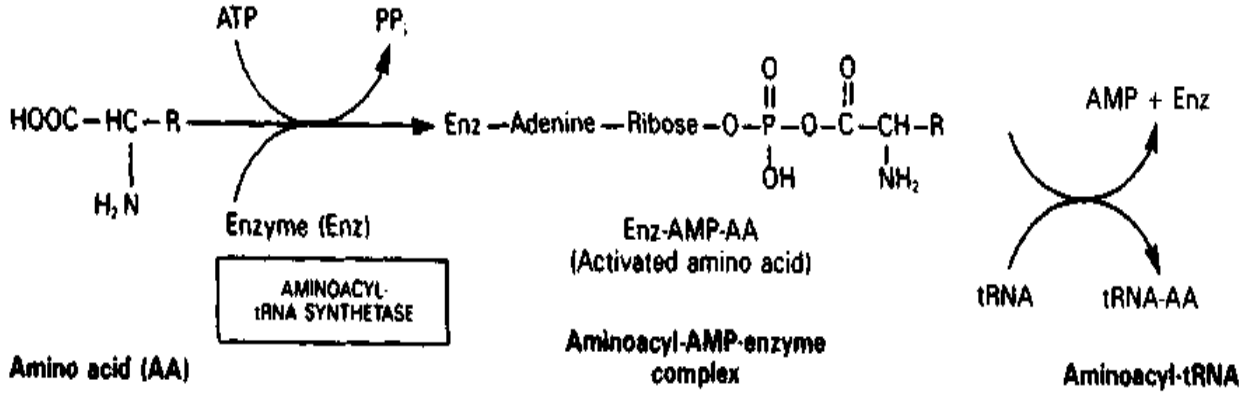
· mRNA –binding site. To combine with ribosome mRNA should contain CAP-sequence and initiation codon AUG.

· P-site } tRNA-binding
· A-site } sites

· GTP – binding site.

Activation of amino acids precedes the translation. The activation of free amino acids is effected by specific enzymes, amino acyl tRNA synthetases, in the presence of ATP. Activation reaction proceeds in two stages. Aminoacyl-tRNA-

synthetases have absolute specificity. They have 3 binding sites: for amino acid,



tRNA, ATP.

Translation consist of 3 stages:

I. Initiation

II. Elongation

III. Termination

I. Initiation is associated with the formation of initiation complex. Two initiation factors (IF-1A and IF-3) bind to the 40S subunit. This favours the dissociation of the 80S ribosome into its 40S and 60S subunits and prevents reassociation.

Initial amino acyl tRNA (the f-methionyl-tRNA in prokaryotes and methionyl tRNA in eukaryotes) and mRNA bind to 40S subunit; 40S initiation complex is formed. This process is accompanied with consumption of 3 molecules of ATP.

This complex is linked to 60S subunit with formation of initiation complex. The binding of the 60S ribosomal unit to the 40S complex involves the hydrolysis of GTP. This reaction results in release of the initiation factors bound to the 40S initiation complex and the rapid association of subunits.

II. Elongation is the stage of consecutive recognition of amino acids and their binding into polypeptide chain. It includes 3 steps:

a) Binding aminoacyl-tRNA to the A-site.

b) Peptide bond formation. Ribosomal enzyme peptidyl transferase transfers methionine and then peptidyl from P-site to A-site. Energy required for formation

of peptide bond is provided by means of hydrolysis of ester bond between amino acid and tRNA.

c. Translocation. The ribosome at translocation step is displaced along the mRNA strand in the towards its 3'-end by a distance of one codon. Peptidyl-tRNA is tranferred from the A-site to the P-site.

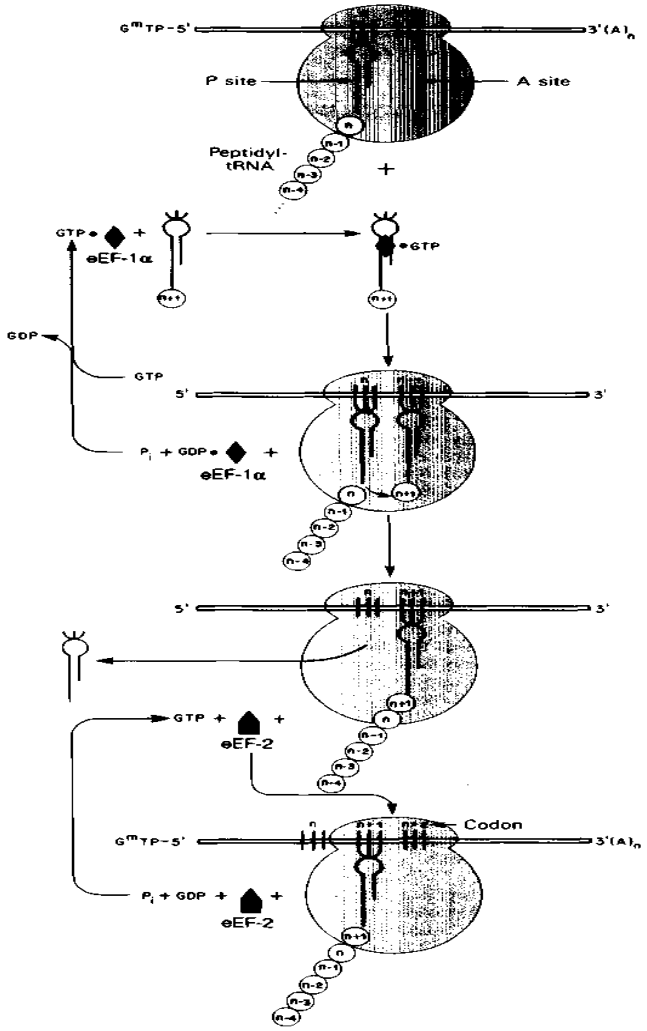


Figure 13.1. Stage of translation elongation

The energy requirements for incorporation of one amino acid into polypeptide chain include the hydrolysis of four high-energy phosphate bonds (2 of them - for activation of amino acid).

Many ribosomes can translate the same mRNA molecule simultaneously. Multiple ribosomes on the same mRNA molecule form a polyribosome, or polysome.

A single eukaryotic ribosome can incorporate as many as six amino acids per second; prokaryotic ribosomes incorporate as many as 18 amino acids per second.

III. Termination occurs when a nonsense codon (UAA,UAG,UGA) is recognized. The releasing factor (RF), in conjunction with GTP and the peptidyl transferase, promotes the hydrolysis of the bond between the peptide and tRNA occuping the P-site. This hydrolysis releases the protein and tRNA from P-site. Ribosome dissociates into 40S and 60S subunits and another cycle can be repeated.

Folding proteins.

Primary structures determine protein folding pathways. In cell polypeptides fold to their native conformations as they being synthesized, a process that normally requires only a few minutes. This is because all cells contain three types of accessory proteins that function to assist polypeptides in folding to their native conformations:

- peptidyl prolyl cis-trans isomerases;
- protein disulfide isomerases;
- molecular chaperons.

Protein disulfide isomerase is homodimeric eukaryotic enzyme. It catalyzes disulfide interchange reactions, thereby facilitating the shuffling the disulfide bonds in proteins until they achieve their native pairing.

Peptidyl prolyl cis-trans isomerase facilitates the formation of the cis peptide bonds preceding proline residues.

Molecular chaperones facilitate the formation of native conformation of protein molecules, prevent the improper folding and aggregation of proteins.

Molecular chaperons participate:

- in folding proteins to their native conformation;

- in assembling subunits to their quaternary structure;

- in translocation of proteins through membrane.

They include:

1. The heat-shock proteins, so named because the rate of synthesis of these ~ 70-kDa monomers greatly increases at elevated temperature. They function, in part, to reverse the denaturation and aggregation of proteins, processes that are all elevated at elevated temperatures. Their expression is increased when cells are

exposed to elevated temperature or other stress. The dramatic upregulation of the heat shock proteins is a key part of the heat shock response and is induced primarily by heat shock factor (HSF).

Heat-shock proteins are named according to their molecular weight. For example, Hsp60, Hsp70, Hsp90 refer to families of heat shock proteins on the order of 60, 70 and 90 kilodaltons in size, respectively. The small 8 kDa protein *ubiquitin,* which marks proteins for degradation, also has features of a heat-shock protein.

- The heat-shock proteins function as intracellular chaperones for other proteins. They play an important role in protein-protein interactions such as folding and assisting in the establishment of proper protein conformation and prevention of unwanted protein aggregation. Heat-shock proteins aid in transproting proteins across membranes whithin the cell.

- The heat-shock proteins act as “monitors” of the cells proteins, because they carry old proteins to the cell’s “recycling bin” (proteasome) and they help newly synthesized proteins to fold.

- Heat-shock proteins appear to serve a significant cardio-vascular role. Hsp90 binds both endothelial nitric oxide synthase and soluble guanilyl cyclase. Hsp20 plays a significant role in preventing platelet aggregation, cardiac myocyte function and prevention of apoptosis under ischemic injury, and skeletal muscle function and muscle insulin response.

- Extracellular membrane bound heat-shock proteins are involved in binding antigens and presenting them to the immune system.

Production of high levels of Hsp is triggered by exposure to different kinds of environmental stress conditions, suth as *infection, inflammation,* exercise, exposure of the cells to *toxins* (*ethanol, arsenic, trace metals* and *ultraviolet light* among many others), *starvation, hypoxia (oxygen deprivation) or water deprivation*. Consequently, the heat-shock proteins are also referred to as ***stress proteins***.

2. The shaperonins, large, multysubunit, cage like proteins that are universal components of bacteria, mitochondria, chloroplasts and possibly eukaryotes.

3. The nucleoplasmins acidic nuclear proteins whose presence is required for the proper in vivo assembly of nucleosomes from their component DNA and histones.

Many of proteins undergo to posttranslational modification**:**

- the removal of amino terminal amino acid residues by specific aminopeptidase;
- limited proteolysis (for example the conversion of prohormone into hormone);
- hydroxylation, glycosylation (for example, collagen);
- carboxylation of glutamic acid (for example, some factors of blood clotting).

13.2 Regulation of Protein Synthesis

Regulation of protein synthesis in prokaryotes

A general theory of protein synthesis regulation in prokaryotes was advanced by F.Jacob and J.Monod. According to the theory of Jacob and Monod, at least 3 types of genes, namely structural genes, regulatory genes and operator genes, are involved in bacterial protein synthesis. mRNA synthesis on the structural genes of the DNA molecule is controlled by a specific nucleotide sequence, named operator gene. The *operator* gene serves as a triggering mechanism for the functioning the structural genes. The formation of mRNA begins from the promoter (initiation site) and then proceeds along the operator and structural genes. A single gene or a group of neighbouring structural genes, encoded by one operator, represents a functional unit termed operon. The function of the operon, in its turn, is controlled by regulator gene by means of protein, which is called repressor. mRNA for synthesis of repressor is synthesized on regulator gene.

Regulation of protein synthesis by the induction mechanism. Repressor is synthesized in active form. It binds with operator gene. This leads to the blockage of mRNA synthesis and protein synthesis. If repressor binds with low-molecular compounds named inducers, or effectors, it is converted into inactive form. It can’t bind with operator gene. mRNA synthesis is allowed to proceed.

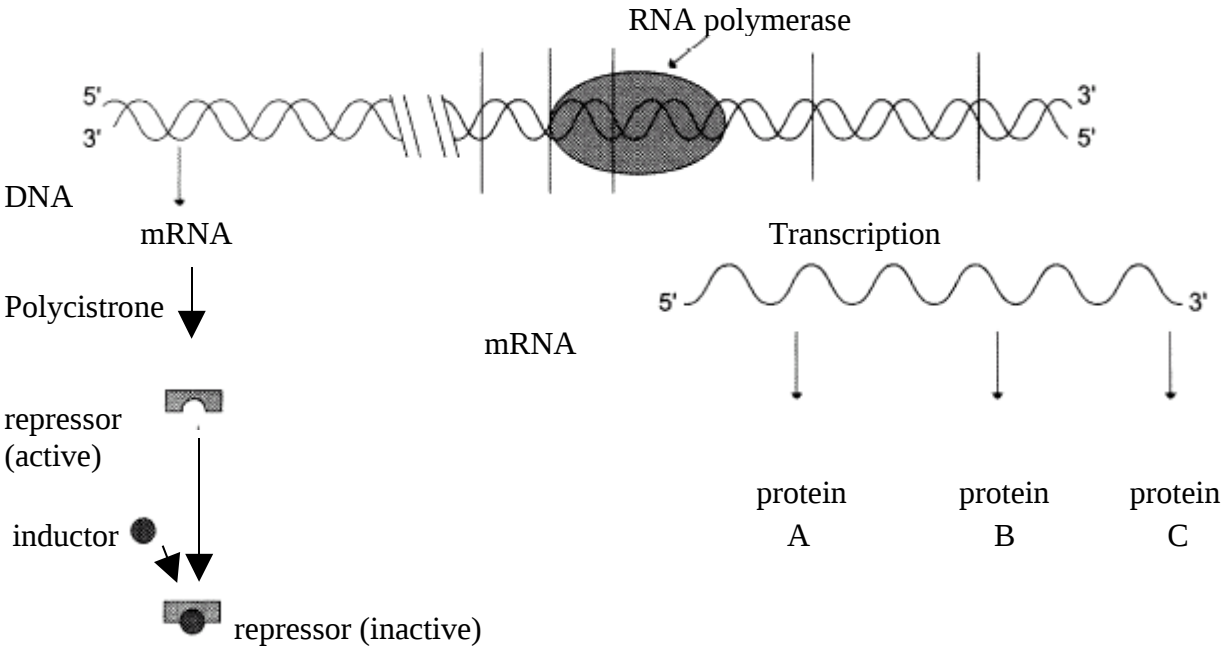


Figure 13.2. Scheme of protein synthesis regulation by the induction mechanism

Regulation by the repression mechanism. Repressor is synthesized in inactive form and therefore fail to inhibit the activity of the operator gene. If inactive repressor is linked with corepressor, it is converted into active form. It is able to bind with operator gene and inhibits the synthesis of mRNA and proteins.

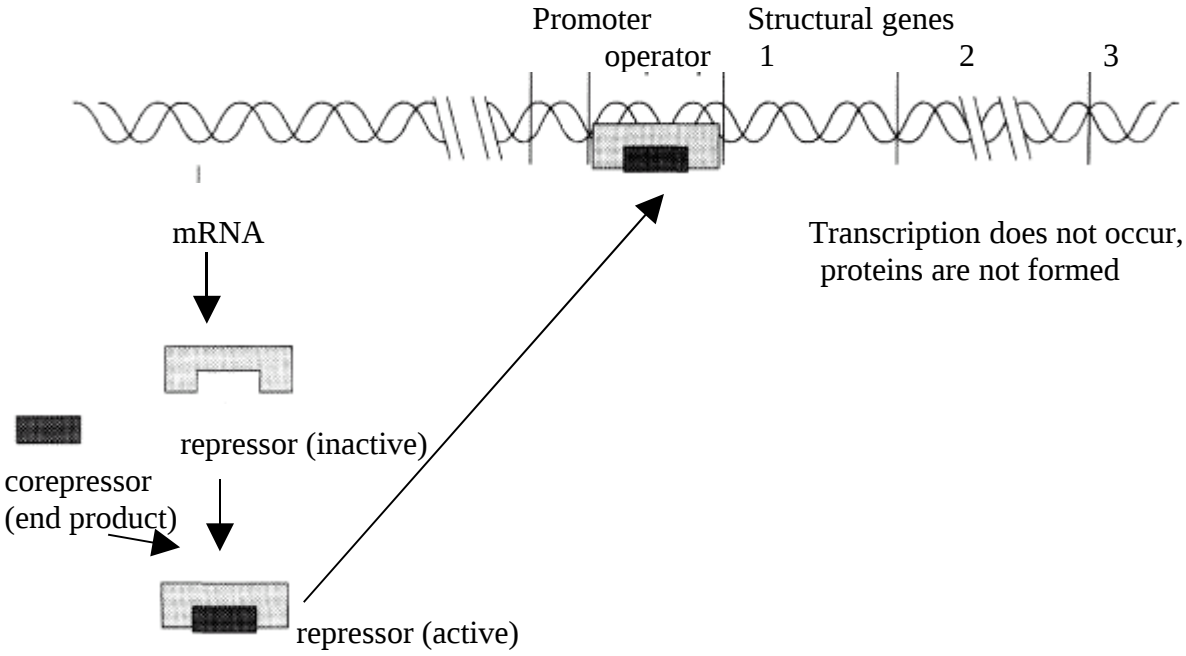


Figure13.3. Regulation of protein synthesis by repression mechanism (schematic).

Regulation of protein synthesis in eukaryotes

Cells of eukaryotes differ from prokaryotes by the following features:

1. The nuclear membrane of eukaryotic cells physically segregates gene transcription from translation, since ribosomes exist only in cytoplasm.

2. Eukaryotic cells are differentiated. Only part of genetic material is expressed in each cell. Different combinations of genes are expressed in each tissue.

3. The DNA in prokaryotic organism is generally not combined with proteins. Much of the DNA in eukaryotic organism is covered with a variety of proteins. These proteins and DNA form a complex structure, chromatin, that allows for numerous configurations of the DNA molecule and types of control unique to the eukaryotic organism.

Therefore mechanisms of regulation of protein synthesis are very complex. Regulation occurs at the level of transcription, nuclear RNA processing, mRNA stability, translation.

I. Regulation at the level of transcription

DNA of eukaryotes has signals of regulation of expression.

- *Enhancers and silencers.* These elements can either increase or decrease the rate of transcription initiation of eukaryotic genes.
- *Other elements* that mediate the response to various signals, including hormones, heat shock, metals, and chemicals.

Important mechanism of transcription regulation in eukaryotes is chemical modification of histones and nonhistones proteins (phosphorylation, methylation, acetylation, glycosylation).

II. *Regulation by alternative RNA processing*

Frequently the same primary transcript is processed differently on different tissues.

For example. Altertnative splicing and processing results in the formation of seven unique α-tropomyosin mRNAs in seven different tissues. It is not clear how these processing-splicing decisions are made or whether the steps can be regulated.

III. *Regulation of RNA stability*

In certain instances, mRNA stability is subject to regulation. This has important implications, since there is usually a direct relationship between mRNA amount and the translation of that mRNA into its cognate protein. Messenger RNAs exist in the cytoplasm as ribonucleoprotein particles. Some of these proteins protect the mRNA from digestion by nucleases, while others may, under certain conditions, promote nuclease attack. Certain effectors, such as *hormones*, *regulate* mRNA *stability* by increasing or decreasing the amount of these proteins. The 5'-cap structure in eukaryotic m RNA prevents attack by 5'-exonucleases, and the poly (A) tail prohibits the action of 3'-exonucleases.

IV. *Regulation of translation* is performed by means of phosphorylation-dephosphorylation of factors of translation initiation. IF-2 is one of two control points for protein synthesis in eukaryotic cells. Phosphorylation of α-subunit of IF-2 prevents fornation of 40S preinitiation complex and blocks protein synthesis.

Factor initiation 4F is inactivated by specific proteins. Phosphorylation of these proteins again by insulin or growth factors via the stimulation of serine protein kinase, results in the dissociation of these proteins and activation of 4F. By means of this pathway insulin causes a marked posttranscriptional increase of protein synthesis.

13.3 Inhibitors of Transcription and Translation. Their Utilization in Medicine.

1) Antibiotics-inhibitors of transcription: rifampycin, rifamycin.
2) Antibiotics-inhibitors of translation: puromycin, cycloheximide, tetracycline, streptomycin, chloramphenicol, erythromycin.

Mechanism of antibiotic action

Antibiotics which inhibit replication, transcription and translation in prokaryotes are used as antibacterial drugs. Antibiotic which inhibit replication, transcription and translation in eukaryotes are used as anticancer drugs.

- *Rifampycin* binds with β-subunit of the RNA polymerase to block the initiation of transcription.
- *Tetracycline* binds to the 30S subunit and inhibits binding amino acyl tRNA.
- *Streptomycin:*

a. It interfers with the binding fMet tRNA to ribosomes and thereby inhibits the initiation process.

b. It also leads to misreading mRNA.

- *Chloramphenicol* inhibits the peptidyl transferase activity of 50S subunit. Thus it inhibits the process of elongation.
- *Erythromycin* binds to the 50S subunit and inhibits translocation.

Antitumor antibiotics

- *Bruneomycin* inhibits DNA synthesis.
- *Rubomycin* inhibits DNA polymerase and DNA-dependent RNA-polymerase.

Puromycin effectively inhibits protein synthesis both prokaryotes and eukaryotes.

Cycloheximide inhibits peptidyl transferase in 60S subunit in eukaryotes

Puromycin and cycloheximide are not clinically usefull but have been important in elucidating the role of protein synthesis in the regulation of metabolic processes, particularly enzyme induction by hormones.

Tests for Self-Control

1. Amino acid during the amino-acyl-tRNA synthesis combines with:

A. Anticodon

B. Codon

C. 3` end of t RNA

D. 5` end of t PNA

E. 3` end of mRNA

2. Patient with inflammation is recommended to use erythromycin, which binds with 50S subunit of ribosome and blocks translocase. Inhibition of protein synthesis in prokaryotes occurs at the stage:

A. Termination

B. Amino acids activation

C. Elongation

D. Posttranslational protein modification

E. Initiation

3. Parts of DNA which contain the information about protein structure are called:

A. Introns

B. Exons

C. Hystones

D. Operons

E. Codons

4. Rifamycin, an antibiotic, which is used for tuberculosis treatment, influences some biochemical processes. Name it.

A. Inhibition of RNA-polymerase at initiation stage

B. Inhibition of DNA-polymerase at initiation stage

C. Inhibition of DNA-ligase

D. Inhibition of aminoacyl-RNA-synthetase

E. Inhibition of protein factors action in protein synthesis

Chapter 14 BLOOD

Blood is important biological liquid of organism. It performs a number of functions, which facilitate the integration of biochemical processes.

Major functions of blood:

- *Respiration* (transport of oxygen from the lungs to the tissues and CO_2 from tissues to lungs).
- *Transport function* (transport of different substances).
- *Excretion* (transport of metabolic products to the kidneys, lungs, skin and intestine for removal).
- Maintenance of the normal *acid-base balance* in the body.
- *Regulation of water balance* through the effects of blood on the exchange of water between the circulating fluid and the tissue fluid.
- *Regulation of body temperature* by means of distribution of body heat.
- *Defence* against infection by the white blood cells and circulating antibodies.
- Transport of *hormones* and regulation of *metabolism.*

14.1 Physico-chemical Properties. Buffer Systems of Blood. Acid-base Balance

Normally the average blood volume is *5200 ml* in men and *3900 ml* in women. The blood plasma accounts for about *55%* of the total volume. The *erythrocytes* constitute a major fraction of blood cells and account for *44%* of the total blood volume. Other blood cells account only *1%*.

The relative density of whole blood is 1,05 – 1,064, of blood plasma – 1,024 – 1,030, of blood cells – 1,08 – 1,097.

Viscosity of blood is 4-5 fold that of water. It is provided by means of high content of proteins and erythrocytes.

An essential physico-chemical characteristic of the blood is the osmotic pressure of blood plasma. It is provided by osmotic concentration that is by the sum total of all the blood particles per unit of volume. At a body temperature of 37^0C, the blood plasma osmotic pressure is about *7,6 atm. (768 – 818 kPa).*

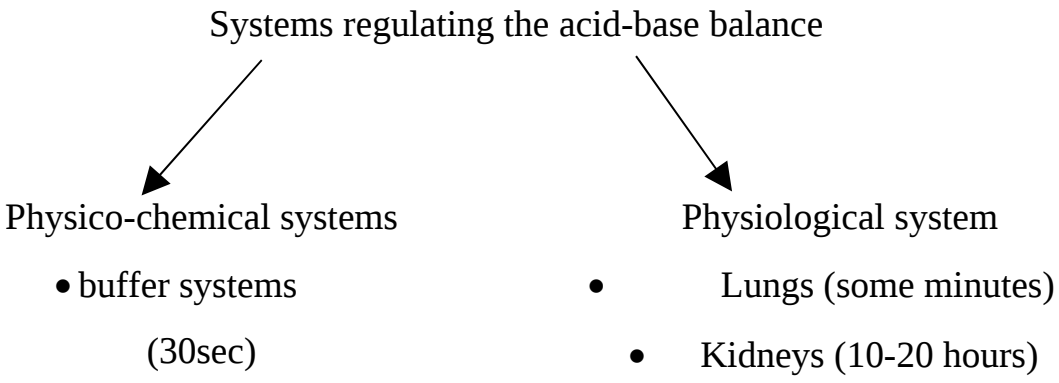
The major contributors to this value are NaCl and other low-molecular weight substances, contained in the blood. Osmotic pressure constancy provides the normal transport of substances from blood to tissues and back, promotes the stability of erythrocytes.

The part of osmotic pressure, which is provided by proteins, is called *oncotic (or colloid osmotic) pressure.* Oncotic pressure accounts for about 0,03 atm. (0,5% of osmotic pressure). But oncotic pressure is very important, as proteins can not penetrate through semipermiable membrane and therefore oncotic pressure facilitates the reverse stream of fluid to venous part of capillaries.

Acid-base balance and buffer systems of blood. Acid-base balance is the relation between concentrations of hydrogen and hydroxyl ions in liquids of organism. This balance is characterized by the hydrogen ion concentration (in nmoles per litre), or by the pH value which is the negative logarithm (to base 10) of hydrogen ion concentration.

Blood hydrogen ion concentration [H^+] is maintained with tight limits in health. *Normal levels* lie between 35 – 45 nmol/L (pH 7,45 – 7,35). Values greater than 120 nmol/l (pH 6,92) or less than 20 nmol/L (pH 7,7) are usually incompatible with life.

The total amount of hydrogen ion produced each day in this way is of the order of 60 mmoles. If all of this was be diluted in the extracellular fluid (~14 litres), [H^+] would be 4 mmol/L or 100 000 times more acid than normal. This just does not happen, as all the hydrogen ions produced are efficiently excreted in urine.



The „first line" of defence from changing pH is buffer systems. The buffer system is a conjugated acid-base pair composed of a donor and an acceptor of hydrogen ions (protons).

The acid-base balance of a buffer solution is described by the Henderson – Hasselbach equation:

$pH = pK_a + \log$ [proton acceptor]/[proton donor]

The major buffer systems of blood are bicarbonate, phosphate, protein, and especially hemoglobin systems.

1. ***The bicarbonate buffer system*** is powerful and perhaps the most controlable system of both the extracellular fluid and blood. Bicarbonate buffer system accounts for about 10% of the total buffering capacity of blood.

$$H_2CO_3 \qquad H^+ + HCO_3^-$$

proton donor — proton acceptor

$[HCO_3^-] = 27$ mmol/L

$[H_2CO_3] = 1{,}35$ mmol/L

$[HCO_3^-] / [H_2CO_3] = 20/1$

Bicarbonate buffer system functions as an effective medium regulator within a close range of pH 7,4. The limit to the effectiveness of the bicarbonate system is the initial concentration of bicarbonate. Only when all the bicarbonate is used up the system has no further buffering capacity. The acid-base status of patients is assessed by consideration of the bicarbonate system of plasma. The extracellular fluid contains a large amount of *bicarbonate* about *24 mmol/L*.

2. ***Phosphate buffer systems*** is a conjugated acid-base pair composed of ion $H_2PO_4^-$ (proton donor) and ion HPO_4^{2-}(proton acceptor)

$$H_2PO_4^- \leftrightarrow H^+ + HPO_4^{2-};$$

proton donor — proton acceptor

$[HPO_4^{2-}] / [H_2PO_4^-] = 4/1$

Phosphate buffer system accounts about 1% of the blood buffering capacity. Nonetheless, in the tissues, especially in kidneys, this system is a major one. This buffer is operative within a range of pH variation *from 6,1 to 7,7*.

3. ***Protein buffer system*** is of minor importance for maintaining the acid-base balance in the blood plasma as compared to other buffer systems. Proteins form a buffer system owing to the occurrence of acid and basic groups in their molecules:

Protein H $\leftrightarrow$ Protein + H^+

The protein buffer system of blood plasma is effective within a pH range *7,2 – 7,4.*

4. ***Hemoglobin buffer system*** is the most powerful buffer system of blood. This buffer system accounts for about 75% of the total buffering capacity of blood.

The involvement of hemoglobin in the control of blood pH is primarily associated with the function of hemoglobin in the transport of oxygen and carbon dioxide. The dissociation constancy of acidic hemoglobin groups is liable to variation depending on the degree of hemoglobin saturation with oxygen. Hemoglobin, on its uptake of oxygen, becomes a stronger acid (H^+ + HbO_2). By contrast hemoglobin without oxygen is a very weak organic acid (HHb).

Disturbanses of acid-base balance

Acidosis:	***Alkalosis:***
The hydrogen ion concentration in the blood is above the normal.	The hydrogen ion concentration in the blood is lower the normal.
Level of pH below *6,92* (↑[H^+] 120 nmol/l) causes death.	Level of pH above *7,7* (↓ [H^+] 20 nmol/l) is incompatible with life.
Acidosis → metabolic, respiratory	Alkalosis → metabolic, respiratory

Metabolic acidosis is the most common form of disturbed acid-base balance. It is associated with the accumulation of organic acids in the tissues and the blood.

Causes of metabolic acidosis:

- Renal disease (acute and chronic glomerulonephritis; acute and chronic pyelonephritis). The decrease of excretion of protons by kidney.

- Ketoacidosis (imperfect oxidation of lipids: in diabetes mellitus, starvation, fever; ketogenic diet).
- Lactate acidosis (imperfect oxidation of carbohydrates, lung diseases, cardiovascular diseases, different types of hypoxia).
- Certain causes of over dosage of poisoning (salicylate → lactate; methanol → formate; ethylene glycol →oxalate).
- Chronic diarrhea or intestinal fistula.

Clinical effects of metabolic acidosis

The compensatory response to metabolic acidosis is *hyperventilation* since the increased [H^+] acts as a powerful stimulant of the *respiratory centre.* The deep, rapid and gasping respiratory pattern is known as *Kussmaul* breathing. Hyperventilation is the appropriate physiological response to acidosis and it occurs rapidly.

A raised [H^+] leads to increased neuromuscular irritability. There is a hazard of arrhythmias progressing to cardiac arrest, and this is made more likely by the presence of hyperkaliemia which will accompany the acidosis.

Metabolic alkalosis is due to loss of a large amount of acid equivalents (for example, in noncontrolable vomiting), the accumulation of base equivalents in tissues (for example in tetany), a wrong correction for metabolic acidosis, high doses of glucocorticoids.

The condition may be due to:

- Loss of hydrogen ion in gastric fluid during vomiting.
- Ingestion of an absorbable alkali such as sodium bicarbonate
- Potassium deficiency.

In severe potassium depletion, often a consequence of diuretic therapy, hydrogen ions are retained inside cells to replace the missing potassium ions. In the renal tubule more hydrogen ions, rather than potassium, are exchanged for reabsorbed sodium. So, despite there being an alkalosis, the patient passes an acid urine. This is often referred to as a «paradoxical» acid urine, because in other causes of metabolic alkalosis urinary [H^+] usually falls.

Clinical effects of metabolic alkalosis

The clinical effects of alkalosis include hypoventilation, confusion and eventually coma. Muscle cramps, tetany and parasthesia may be consequence of a decrease in the unbound plasma calcium concentration which is a consequence of the alkalosis.

Recognizing primary metabolic acid-base disoders by inspecting the HCO_3^- concentration.

Blood gas results

$[H^+]$ elevated	**$[H^+]$ decreased**
↓	↓
Acidosis	**Alkalosis**
↓	↓
HCO_3^-↓	**HCO_3^-↑**
↓	↓
metabolic acidosis	**metabolic alkalosis**

Compensation in primary metabolic disorders

Metabolic acidosis

$$[H^-]\uparrow \propto \frac{PCO_2}{[HCO_3^-]\downarrow} \Rightarrow [H^-]\uparrow \propto \frac{PCO_2\downarrow}{[HCO_3^-]\downarrow}$$

acidosis develops — respiratory compensation occurs quickly

Metabolic alkalosis

$$[H^-]\downarrow \propto \frac{PCO_2}{[HCO_3^-]\uparrow} \Rightarrow [H^-]\downarrow \propto \frac{PCO_2\uparrow}{[HCO_3^-]\uparrow}$$

alkalosis develops — respiratory compensation occurs quickly

Respiratory acidosis develops because of a reduced minute respiratory volume (for example, in bronchiole asthma, edema of lungs, emphysema, atelectasis and traumatic asphyxia).

Respiratory acidosis may be acute or chronic. Examples of acute, and uncompensated, respiratory acidosis are

- choking;
- bronchopneumonia;
- acute exacerbation of asthma.

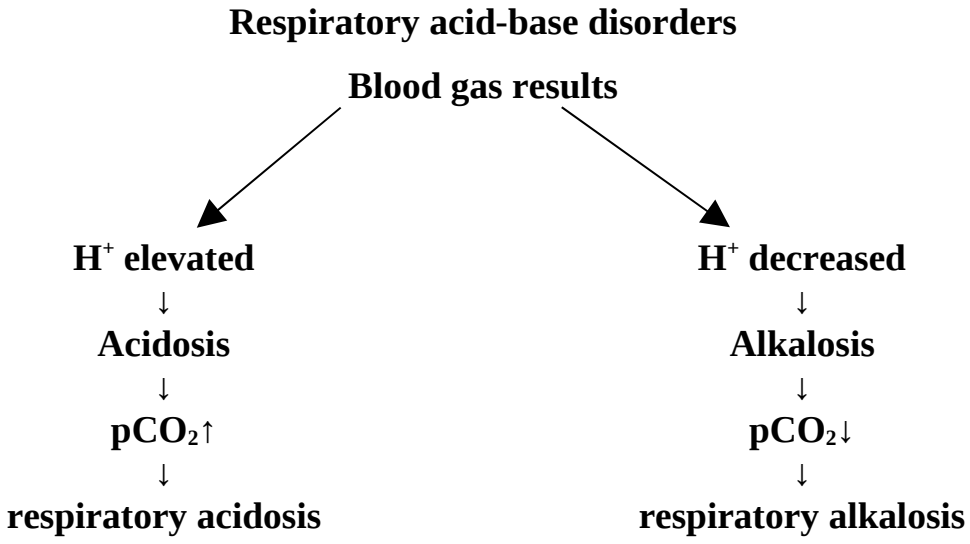
Chronic respiratory acidosis is usually a long-standing condition and is accompanied by maximal renal compensation. Examples of chronic respiratory disorders are:

- chronic bronchitis;
- emphysema.

Respiratory alkalosis arises from a sharply intensified pulmonary ventilation (in inhalation of pure oxygen, in compensatory dyspnea). Respiratory alkalosis is much less common than acidosis.

Examples are:

- hysterical over breathing;
- mechanical over ventilation;
- raised intracranial pressure or hypoxia, both of which may stimulate the respiratory centre.



Main indexes of acid-base balance

1. **Actual pH of the blood**, which is the negative logarithm (to the base ten) of the hydrogen ion concentration in the blood under physiological conditions (**7.35-7.45)**.
2. **Actual P CO_2 of the whole blood** which is the partial pressure of carbon dioxide in the blood under physiological conditions (**33-44 mm of Hg**).
3. **Actual bicarbonate (AB)** which is the bicarbonate ion concentration in the blood under physiological conditions (**27 mmol/L**).
4. **Standart bicarbonate (SB)** of the blood plasma which is the bicarbonate ion concentration in the blood plasma equilibrated by alveolar air at complete saturation with oxygen (**24-26 mmol/L**).
5. **Buffer bases (BB)** of the whole blood or of blood plasma, which is the buffering capacity index for the whole buffer system of the blood or blood plasma (**45-50 mmol/L).**
6. **Base excess (BE)** is the index for an excess or a deficiency of buffering capacities **(0±2,5 mmol/L).**

Measuring instruments of „Astrup" type or the home-made instruments of AZIV and AKOR types and appropriate nomograms (for example, Siggaard-Andersen nomogram) can be used to determine the above mentioned indexes of acid-base balance.

14.2 Chemical Composition of Blood

Blood plasma Proteins. Proteins account for 6,5-8,5% out of the total 9 – 10% of dry blood plasma residue.

Total proteins – 65 – 85 g/L

Albumins – 40 -50 g/L

Globulins – 20 – 40 g/L

Fibrinogen – 1,5-3,5 g/L

The most of blood serum proteins are synthesized in liver, but some of them are formed in other tissues. For example, γ-globulins are synthesized by

lymphocytes; peptide hormones are mainly secreted by endocrine glands; peptide hormone erythropoietin is formed by kidney cells. Almost all the blood plasma proteins, with the exception of albumin, are glycoproteins.

Functions of blood proteins:

- they take part in blood clotting,
- they provide viscous properties of blood,
- maintaining acid base balance,
- transport function,
- protective function,
- reserve of amino acids,
- regulation function,
- they maintain the oncotic pressure,
- maintaining a needed level of cations in blood.

Albumin level in blood plasma protein is 35-50 g/L. Albumins make up approximately 60% of the total plasma protein.

Functions of albumins:

- Albumins are responsible for 75 – 80% of oncotic pressure of human's plasma. The decreasing albumin concentration below 30 g/L leads to edema.
- Transport function. It transports free fatty acids, calcium, certain steroids hormones, bilirubin, copper, different drugs etc.

Globulins: are divided into α_1- globulins (3-6 g/L), α_2- globulins (4-9 g/L), β-globulins (6-11 g/l) and γ-globulins (7-15 g/L). They perform transport and protective functions.

α – Globulins:

- *Haptoglobin (*Hp) (is component of α_2– globulin fraction)

This glycoprotein binds extracorpuscular hemoglobin. The haptoglobin – hemoglobin complex can be absorbed by the macrophage system and can not pass the glomerulus of the kidney. Thus Hp prevents a loss of free hemoglobin by kidney and provides the conservation and reutilization of iron.

- *Ceruloplasmin* (α_2–globulin) is a blue, copper-containing (0,32%) glycoprotein found in mammalian blood plasma. It contains about 3% of total amount of copper in organism and more 90% Cu of blood plasma. Cerruloplasmin exhibits a weakly pronounced catalytic activity in the oxidation of ascorbic acid, adrenaline, dihydrophenylalanine and a number of other compounds, and ferrooxidase activity ($Fe^{2+} \rightarrow Fe^{3+}$).

It is antioxidant. In Wilson's diseases the concentration of ceruloplasmin in the blood plasma is significantly lowered which a major diagnostic test for this pathology.

- *α_1-Antitrypsin* can inhibit trypsin and other proteolytic enzymes. The level of trypsin inhibitors is increased in inflammatory processes, in pregnancy and in a number of other states of organism. In inflammatory process the level of α_1-antitrypsin increases in result of stimulation of its synthesis in hepatocytes. In acute pancreatitis the enchanced level of α_1-antitrypsin arises from delivery of active pancreatic proteinases. Deficiency of α_1-antitrypsin is associated with emphysema and one type of liver diseases (α_1-antitrypsin deficiency liver disease).

- *α_2- Macroglobulin* is a large plasma glycoprotein (720 kDa). It is inhibitor of serine-, thiol-, carboxy- and metal proteinases. Therefore it is involved in regulation of blood clotting, immunologic processes, inflammatory processes. In addition, it binds many *cytokines* (eg. platelet-derived growth factor, transforming growth factor-β, etc.) and is involved in targeting them toward tissues.

β-Globulins:

- *C – reactive protein* is able to form a precipitate with the somatic C-polysaccharide of pneumococcus C-reactive protein does not occur in the blood serum of healthy organisms. It is detected in many pathologic states attendant to inflammation and necrosis of the tissues. This protein is „acute phase” protein.

Table 14.1 Level and functions of some α-globulins

Group	Proteins	Concen-tration in blood serum, g/L	Functions
α_1-globulins	α_1-antitrypsin	1,9-3,5	Inhibitor of proteinases
	HDL	1,25-4,25 (in men)	Transport of cholesterol from tissues to liver
	Prothrombin	0,1	Factor II of blood clotting
	Transcortin	0,03	Transport of cortisol, corticosterone, progesterone
	Acid α_1-glycoprotein	1,0	Transport of progesterone
	Thyroxin-binding globulin	0,02	Transport of thyroxine and triiodothyronine
α_2-globulins	Ceruloplasmin	0,35	Transport of copper ions, oxidoreductase
	Haptoglobin	0,1-0,35	Binding hemoglobin
	α_2-Macroglobulin	2,5	Inhibitor of blood plasma proteinases
	Antithrombin III	0,3	Inhibitor of blood plasma proteinases
	Retinol binding protein	0,04	Transport of retinol
	Vitamin D binding protein	0,4	Transport of calciferol

- *Hemopexin* binds free heme.
- *Transferrin* is glycoprotein with a molecular mass of approximately 80 kDa. Transferrin plays a central role in the body's metabolism of iron, because it transports iron (2moles of Fe^{3+}per moll of transferrin) in the circulation to sites where iron is required, e.g. from the gut to the bone marrow and other organs.

Table 14.2 Level and functions of some β-globulins

Proteins	Concentration in blood serum g/L	Functions
LDL	3,5	Transport of cholesterol
Transferrin	3-4	Transport of Fe^{3+}
Transcobalamin	$2,5x10^{-9}$	Transport of vitamin B_{12}
Sex-steroid binding protein	$20x10^{-6}$	Transport of testosterone, estradiol
C-reactive protein	<0,01	Activation of complement

γ- globulins

• *Cryoglobulin* is absent in the blood serum of healthy individuals. It is found only in pathologic states. A specific feature of this protein is its solubility at a temperature 37°C and ability to form a precipitate or a gel in decreasing temperature to 4°C. It is detected in blood serum in myeloma, nephrosis, cirrhosis of the liver, rheumatism, lymphosarcoma, leukosis and other diseases.

• *Interferons are* specific proteins synthesized on the organism's cells invaded by virus. Interferon can inhibit viral multiplication in the cells however it has no effect on the viral particles that have been formed in the cell. Interferon is easy to leave the cell and to enter the blood stream in which it is carried over to tissues and organs. There are 3 types of interferons: IFN-α, IFN-β, and IFN-γ. IFN-α are mainty synthesized by leukocytes; IFN-β – by fibroblasts; IFN-γ by T- and B-lymphocytes.

• *Immunoglobulins (humoral antibodies)* are synthesized mainly in plasmocytes, specialized cells of B-cell lineage that synthesize and secrete immunoglobulins into plasma. All immunoglobulin molecules consist of 4 polypeptyde chains, which are linked by disulfide bonds: two identical light (L) chains and two identical heavy (H) chains. *L-chains* have molecular mass 23 000 Da. They are common to all the classes of immunoglobulins. They are two types: kappa (κ) and lambda (λ). A given immunoglobin molecule contains or two identical κ, or two λ chains.

Heavy chains have molecular mass 50 000 – 75 000 Da. Five types of heavy chains exist: α (alpha), γ (gamma), μ (mu), δ (delta), ε (epsilon). The type of H chain determines the class of immunoglobulin and thus its effector function. There are five immunoglobulin classes: IgG (γ-chains), IgA (α-chains), Ig M (μ-chains), IgD (δ-chains), IgE (ε- chains).

Every light and heavy chain consist of 2 segments: variable (V) and constant (C).The constant regions of immunoglobulin molecules are responsible for the class specific effectors functions of the different immunoglobulin molecules, e.g. complement fixation or placental transfer.

IgGs (7-20 g/L) and **IgMs** (0,5-2 g/L) are basic classes of immunoglobins. They realize humoral immune response on the incorporation of foreing antigens. IgMs participate in the primary immune response, they activate complement system. IgGs participate in the secondary immune response, activate a complement system. **IgG is the only** immunoglobin which is able to **pass placenta. IgA** (0,7-1,5 g/L) is antibody of other biological liquids and secretions (secretions of mucous, lungs). **IgD** (0,000001-0,0003 g/L) and **IgE** (0,02-0,02g/L) are minor components of blood serum. **IgE** participate in allergic reactions.

In clinical practice, there have been reported states characterized by alteration in both the total content of blood plasma proteins and the percentage of individual protein fractions.

Hypoproteinemia (a decrease in the total concentration of blood plasma proteins) is usually linked with the decreasing albumins. Hypoproteinemia occurs:

- in nephrotic syndrome;
- in liver disease (acute atrophy of the liver, toxic hepatitis, and other states);
- in a drastically increased permeability of the capillary wall,
- in protein deficiency (affected gastrointestinal tract, carcinoma, etc).

Paraproteinemia is the occurrence in the blood plasma of proteins, normally untypical to the healthy organism (for example, in myeloma). In the blood serum of patients with myeloma specific „myelomatous”proteins are detected.

Hyperproteinemia is a pathologic condition manifested by an increased content of blood plasma proteins.

It is caused by loss of liquid by organism (diarrhea in children; vomiting, due to an obstruction of the upper small intestine, or by extensive burns).

It is caused by an elevated level of γ-globulins.

Dysproteinemia is the changing ratio of individual protein fractions, while the total protein content in the blood serum is normal. *γ-Globulin* fraction is increased in chronic inflammation, chronic polyartritis etc. *α_2-Globulin* fraction is increased in acute infections, acute rheumatism.

The level of some proteins may be sharply raised in acute inflammatory processes and some other pathologic states (trauma, burns, myocardial infarction). Those proteins are called *«acute phase» proteins*, because they take part in development of inflammatory reaction of organism. Main inducer of the synthesis of the most acute phase proteins in hepatocytes is interleukin-1 liberated by mononuclear phagocytes. Haptoglobin, C-reactive protein, α_1-antitrypsin, acid α_1-glycoprotein, fibrinogen belong to proteins of acute phase.

Blood serum enzymes		
Secretory enzymes	***Indicator enzymes***	**Excretory enzymes**
• The are synthesized in liver, • The are normally secreted into blood plasma.	• They are typical to tissues. • In blood serum they are normally present only in trace amounts. • The affection of some tissues leads to the increasing indicator enzymes in blood.	• They are mainly synthesized in liver and pancreas • Under normal condition they are excreted in bile or with pancreatic juice.

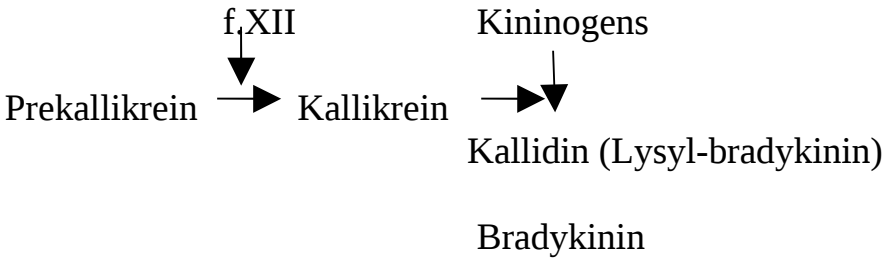
• They perform in blood physiological functions. Examples: choline esterase, enzymes of blood coagulation.	Examples: a) LDH_1, LDH_2 are typical to heart, LDH_4, LDH_5 – liver, muscles. The increase of LDH_1, LDH_2 indicates myocardial infarction. The increase of LDH_4, LDH_5 indicates liver diseases. b) *Creatine kinase* (isoenzymes –BB, MM, MB). MB form is characteristic for cardial muscle. A test for the creatine kinase isoenzyme activity is of particular diagnostic value in acute myocardial infarction, since the MB form is practically contained in the cardiac muscle. c) *Transaminases:* ↑AsAT indicates myocardial infarction; ↑AlAT indicates liver diseases.	• In many pathological processes the biliary secretion of these enzymes becomes impaired, and the activity of excretory enzymes in the blood plasma is observed to increase. Examples of these enzymes: Leucine aminopeptidase, alkaline phosphatase, lipase, amylase and other.

Kallikrein-kinin system

The initial components of this system are prekallikrein and kininogens. ***Kininogens*** are mainly synthesized in hepatocytes. There are high molecular kininogen (HMK: molecular mass = 120 kDa, concentration in blood plasma – 65-130 μg/ml) and low molecular kininogen (LMK: molecular mass = 65 kDa, concentration in blood plasma – 109-272 μg/mL).

Prekallikrein (precursor of blood plasma kallikrein, concentration in plasma – 295-580 nmol/L) is synthesized in hepatocytes. Plasma prekallikrein is activated

by trace amount of factor XIIa which is formed in adsorbtion of factor XII on the activation surface in the presence of HMK. Kallikrein converts kininogens into active kinins.



Kallikrein-kinin system plays the central role in activity regulation of proteolytic systems of blood plasma: coagulation, fibrinolysis, complement, kallikrein-kinin, renin-angiotensin. Especially important property of ***kinins*** is to liberate cytokines such as interleukin-1, tumor necrosis factor and many other mediators. Prostaglandins, leukotrienes, NO are frequently formed in the result of phospholipase A_2 activation by kinins. Bradikinin is potent vasodilator.

Half-life of kinins in plasma is about 20 sec. They are degraded by kininases: kininase I (carboxypentidase N), which is located in blood plasma; kininase II (angiotensin I converting enzyme) – membrane bound enzyme, which is located in vessel endothelium, mainly of lungs, kidney. Inhibitors of kinin formation (Gordox, Contrical) are used in clinical practice, because kinins play the important role in pathogenesis of inflammatory processes.

Non-protein Nitrogen Components of Blood. Azotemia

Total nitrogen of the blood includes a protein nitrogen and nonprotein nitrogen (or residual nitrogen):

N total = N prot. + N res.

N res. = 14,3 – 25 mmol/L.

Residual nitrogen of the blood includes: urea nitrogen (50%), amino acid nitrogen(25%), creatine nitrogen (5%), creatinine nitrogen, ammonia nitrogen, indican nitrogen, bilirubin nitrogen, uric acid nitrogen etc.

Ammonia level (25-40 μmol/L) increases in liver diseases, inherited disturbances of ornithine cycle. ***Urea*** level (3,3 – 8,3 mmol/L) increases in chronic

diseases of kidney, cancer of ureteral ducts, tuberculosis of kidney, some infectious diseases, sepsis and other. Its level decreases in liver diseases (hepatitis, cirrhoses), pregnancy, inherited disturbances of urea cycle. ***Creatinine*** (53-105 μmol/L) increases in retential azotemia, indicates the degree of chronic renal insufficiency. ***Uric acid*** level (149 – 405 μmol/L) increases in gout.

Azotemia is the increased level of residual nitrogen in blood.

Azotemia

Productive azotemia ***Retention azotemia***

Productive azotemia is observed in an excessive delivery of nitrogenous products to the blood as result of accelerated degradation of tissues proteins in different states: inflammation, wounds, extensive burns, cachexia and other states.

Retention azotemia is caused by incomplete urinary discharge of nitrogen containing products on their normal delivery to the blood stream.

Retention azotemia

Renal azotemia ***Extrarenal azotemia***

Renal retention azotemia is caused by reduced excretory function of kidney (reduced renal clearance). Urea is mainly responsible for the increased residual nitrogen level in renal retention azotemia. Urea constitutes 90% of residual nitrogen of blood instead of 50% in normal conditions.

Extrarenal retention azotemia may arise from an acute circulatory insufficiency, low arterial pressure, or reduced renal blood flow. Also, the frequent cause of extrarenal retention azotemia is an obstruction to the urine outflow from the kidney.

Nitrogen –free organic components of blood

Glucose – 3,3 – 5,5 mmol/L

Lactate – 0,33 – 0,78 μmol/L

Total lipids: whole blood – 1,0 – 7,2 g/L; plasma – 3,8 – 6,7 g/L.

Free fatty acids: plasma – 400 -800 mmol/L.

Phospholipids – 2,2 – 4,0 g/L.

Cholesterol (total content in blood): 3,63 – 6,48 mmol/l.

Ketone bodies: 0,1 – 0,6 mmol/l.

Hyperketonemia is observed in diabetes mellitus, starvation, thyrotoxicosis, infectious diseases. The increase of ***cholesterol*** concentration in blood plasma plays the major role in development of atherosclerosis and diseases which are associated with increased level of cholesterol, for example arterial hypertension, ischemic heart disease, insult.

Mineral substances of blood

Level of some inorganic substances in blood:

Bicarbonates – 20-28 mmol/L;

Na : plasma – 126 - 156 mmol/L; red cells -13,4 – 21,7 mmol/L;

K^+ :plasma – 3,8 – 5,1 mmol/L; red cells -79,3 – 99,7 mmol/L;

Cl - 96 – 104 mmol/L;

Ca - 2,12 – 2,6 mmol/L;

P - 0,65 – 1,6 mmol/L;

Mg - 0,74 – 0,95 mmol/L;

Fe - 9,0 – 32,2 μmol/L.

Sodium is a major osmotically active ion in the extracellular space. In *hypernatriemia*, a syndrome associated with the organism's hyperhydration is commonly observed to develop. The accumulation of excessive sodium in the blood plasma occurs in a specific renal disease know as parenchymatous nephritis, in patients with congenital cardiac insufficiency, also in primary (or true) and secondary hyperaldosteronism. *Hyponatremia* is accompanied by dehydration of the organism.

Potassium. The potassium level in the cells is much higher as compared to the extracellular space. *Hyperkalemia* is observed in acute renal insufficiency or in hypofunction of adrenal cortex. By contrast, an increased production of aldosterone by adrenal cortex leads to hypokalemia. The progressive hypokalemia leads to grave disturbances of cardiac performance. Occasionally, a decreased level

of potassium in the blood serum was observed as a side effect on administration of large therapeutic doses of adrenal cortex hormones to patients.

Calcium. In tumoral lesions of bone tissue, hyperplasia, or parathyroid adenoma, a marked increase of calcium level in the blood plasma is observed. The state of hypocalcemia is observed in hypoparathyrosis. The hypofunction of parathyroid gland results in a drastic drop of calcium concentration in the blood, with the eventual development into a convulsive state (tetany). Hypocalcemia is also observed in rickets, sprue, obstructive jaundice, nephroses, glomerulonephritis.

14.3 Respiratory Function of Erythrocytes. Hemoglobin Metabolism

14.3.1. Respiratory Function of Erythrocytes

Erythrocytes constitute about 44% of the total blood volume ($4,5\text{-}5x10^{12}$/L).

- The life of erythrocytes is **120 days**.
- New synthesized erythrocytes **contain ribosomes and elements of endoplasmic reticulum**.
- Mature erythrocytes **don't contain ribosomes, mitochondria, lysosomes, Golgi apparatus**.
- Synthesis of erythrocytes is regulated by **erythropoietin.** Erythropoietin is synthesized in kidney. It is liberated to blood in hypoxia and is transported to bone marrow.

Erythrocytes have unique and relatively simple metabolism:

- Main source of energy is glucose.
- Source of ATP is anaerobic glycolysis.
- The formation of 2,3-bisphosphoglycerate from 1,3- bisphosphoglycerate by Rappoport-Leubering shunt is very important for regulation of affinity of Hb to O_2.
- 5-10% of glucose are metabolized by means of pentose phosphate pathway. NADPH is necessary for reduction of glutathione. Deficiency of glucose-6-phosphate dehydrogenase is the cause of drug-induced hemolytic anemia

• Glutathione may be synthesized in erythrocytes. It is necessary for elimination of peroxides.

• Autooxidation of Hb results in formation of metHb (1,7% under normal conditions). This is accompanied by formation of O_2^- (superoxide radical). NADH-dependent methemoglobin reductase converts metHb to Hb.

• The synthesis of glycogen, fatty acids, proteins, nucleic acids does not occur in erythrocytes. Some lipids, for example cholesterol, may be exchanged with corresponding lipids of plasma.

• Erythrocytes have some enzymes of nucleotide metabolism.

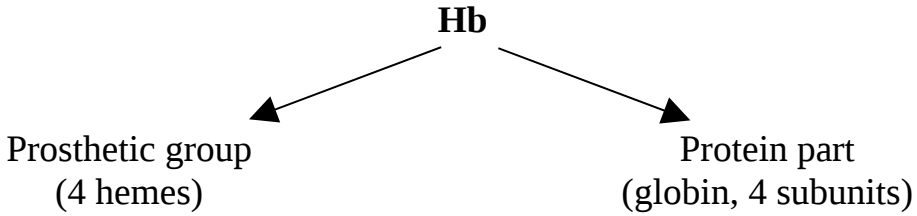
The main function of erythrocytes is the transport of gases. Hemoglobin constitutes 95% intracellular proteins of erythrocytes. Hemoglobin is the principal for transport in the blood of both oxygen and CO_2.

Structure and functions of hemoglobin

Functions:

- Respiratory
- Maintaining acid-base balance (buffer system)

Structure:



Heme is cyclic tetrapyrrole. Tetrapyrroles consist of four molecules of pyrrole linked in a planar ring by four α- methenyl bridges. Tetrapyrrole has 8 substitutions: 4 methyl groups, 2 - vinyl and 2 - propionate ones. One atom of ferrous iron (Fe^{2+}) is at the center of this planar ring.

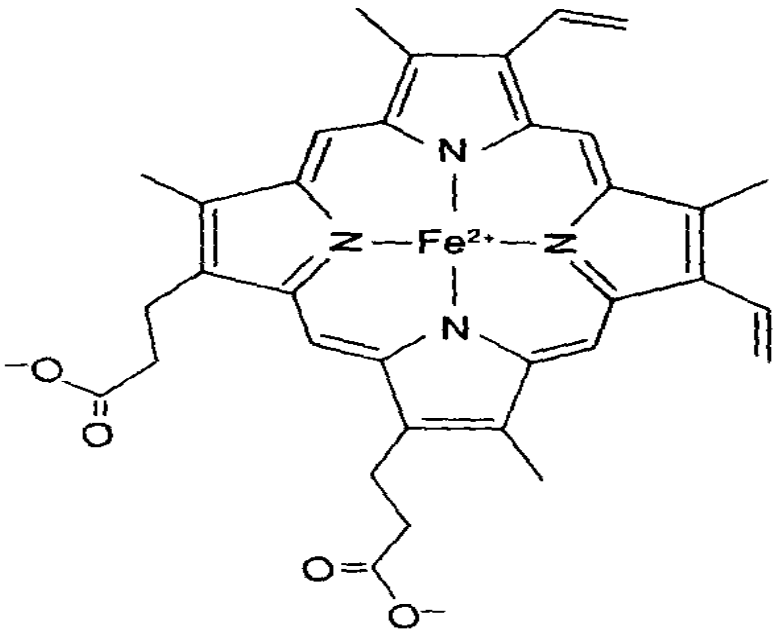


Figure 14.1. Heme structure

Globin part of HbA_1 is $\alpha_2\beta_2$ (α – 141 amino acid residues, β – 146 amino acid residues). *Secondary structure:* 75% of every polypeptide chain is α-helix. *Tertiary structure* is globule. Hydrophobic amino acid radicals are directed inside of protein molecule, hydrophilic amino acid radicals are directed outside. This facilitates the formation of hydrophobic heme pocket. This pocket defenses heme iron from oxidation. *Quaternary structure:* polypeptide chains of Hb are linked by means of hydrophobic interactions and salt bridges. Quaternary structure provides positive cooperative effect between subunits in binding O_2. Binding last molecule of O_2 occurs 300 times more readily, than the first one. Binding O_2 is accompanied by the rupture of salt bonds between four subunits. Subsequent O_2 binding is facilitated, since it involves a rupture of fewer salt bonds. Iron atoms of deoxyhemoglobin lie about 0,06 nm beyond the plane of the heme ring. On oxygenation the iron atoms move into the plane of the heme ring. This is accompanied by conformational changes and leads to rupture of salt bonds.

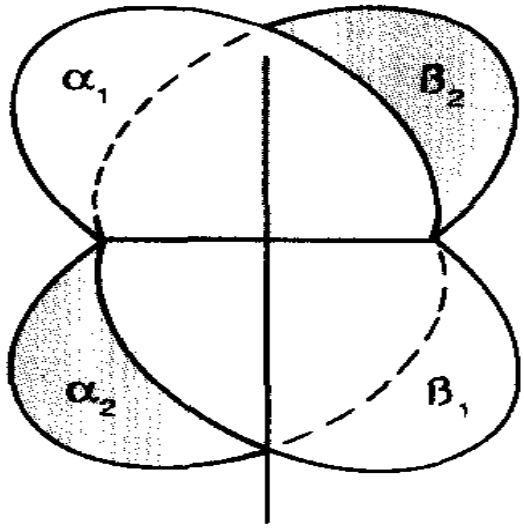


Figure 14.2. Quaternary structure of Hb

Quaternary structure of Hb provides its allosteric properties. The affinity of Hb to O_2 is regulated by low molecular mass ligands, for example by CO_2, 2,3-bisphosphoglycerate (2,3-BPG). 2,3-BPG is formed from the glycolytic intermediate 1,3- bisphosphoglycerate. 2,3-BPG decreases the affinity of Hb to O_2 by forming additional salt bridges.

This plays the important role in adaptative processes in hypoxia, in supplying of embryo by oxygen. 2,3-BPG regulates fetal hemoglobin in less extent, than adult one. Therefore fetal Hb has a higher affinity to oxygen than does HbA. This provides the transfer of oxygen from Hb of mother to Hb of embryo.

Heterogeneity of Hb

Ontogenetic	**Heterogenity of adults**	**Heterogenity, which is provided by mutations (abnormal Hb)**
1. Embryonal HbP is found in first three months of intrauterine life of the baby. • Gower I (ξ_4) • Gower II ($\alpha_2\xi_2$)	1. HbA ($\alpha_2\beta_2$)–90-95%. 2. HbA_2 ($\alpha_2\delta_2$)–2,5%. 3. HbF – 0,5% HbA_1c (glycosylated): in	Examples: • HbS (glutamic acid of 6-th position of β-chain is replaced by valine). • HbM (amino acid sequence is altered either in

2. HbF ($\alpha_2\gamma_2$)	normal individuals 3-	α- or β- chains: or α_{58}His →
3. HbA ($\alpha_2\beta_2$)	5%, in diabetus	α_{58}Tyr, or β_{63}His → β_{63} Tyr)
Newborns contain 80%	mellitus – 6 -15%	
HbF and 20% HbA		

Derivatives of Hb

1. Oxyhemoglobin (HbO_2). Oxygen adds to the hemoglobin heme via iron coordination bonds, the iron is in reduced state (Fe^{2+}). Factors, influencing the formation of HbO_2:

a) partial pressure of O_2 ($P O_2$) favours oxygenation;

b) partial pressure of CO_2 ($P CO_2$) favours dissociation;

c) temperature decreases the affinity of Hb to O_2;

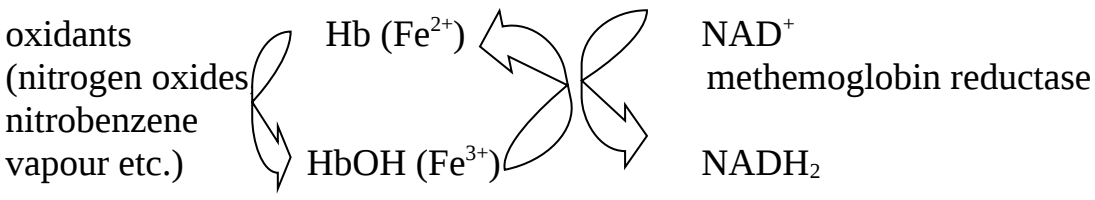
d) pH of the medium (acidosis favours liberation of O_2);

e) 2,3-bisphosphoglycerate diminishes the affinity of Hb to O_2.

2. Carboxy-Hb (HbCO). Oxygen adds to the hemoglobin heme via iron coordination bonds, the iron is in reduced state (Fe^{2+}). The affinity of Hb to CO is 210 times more than to O_2. Dissociation of HbCO is 30 times less than HbO_2. However, as the partial pressure of oxygen in the inspired air increases, CO is in part eliminated from its binding with hemoglobin.

3. *Carb-Hb ($HbCO_2$).* CO_2 combines with NH_2-group of globin. This is a normal and constant physiologic reaction and accounts for 2 to 10% of CO_2 transported by the blood.

4. *Methemoglobin (HbOH).* It is a derivative in which Fe is in the ferric state (Fe^{3+}). In normal healthy adult small amount of methemoglobin may be present (about 1,7% of total Hb). This is converted to normal Hb by methemoglobin reductase.



In vivo HbOH is produced by certain drugs or exposure to certain poisons which are oxidants. Injection of intravenous glucose or methylene blue helps to reduce methemoglobin (Fe^{3+}) to Hb (Fe^{2+}).

Other causes:

a) *Familial methemoglobinemia* is inherited disorder due to lack or absence of the enzyme *methemoglobin reductase*.

b) *Methemoglobinemia* may also be found in individuals with abnormal hemoglobin as HbM.

The increased formation of HbOH is used for the treatment of cyanide poisoning. The letal and toxic action of cyanides is provided by inhibition of cytochrome oxidase. Cyanides and HCN do not react directly with hemoglobin but they react with methemoglobin to form cyanmethemoglobin, which is not toxic.

In medical practice of common use is the analysis for blood pigment which is based on a study of spectral properties of hemoglobin heme or of its oxidized products – hemin or hematin, produced by treating hemoglobin with a dilute alkaline solution or with acetic acid in the presence of sodium chloride. Hematin reduced by ammonium sulphite in the presence of globin, produces a hemoglobin derivative *hemochromogen*. It exhibits a characteristic absorption spectrum. This method is widely used in forensic medical practice for examination of blood spots.

Transport of oxygen

Oxygen is continuously supplied to the tissue cells. The total requirement of O_2 is around 250 mL/minute in the resting state and more than ten times during vigorous exercise.

The requirement of O_2 to the tissue is fulfilled in two ways:

- oxygen in physical solution;
- by oxyhemoglobin.

A small amount of O_2 can be dissolved to form a solution (0,3 mL of O_2 per 100 mL of blood).

Most of O_2 is supplied to the tissues as HbO_2. One gram of Hb can carry 1,34 ml of O_2 at complete saturation. The hemoglobin concentration in the blood in healthy individual is 130-160 g/L. At the arterial blood the oxygen saturation of hemoglobin is 96%. Under these conditions the amount of O_2 which is linked with Hb is 19,3 ml of O_2 per 100 ml of blood.

Blood, which contains 150 g/L of Hb:

Arterial blood	***Venouse blood***
P O_2 95 mm of Hg	P O_2 40 mm of Hg
P CO_2 40 mm of Hg	P CO_2 46 mm of Hg
Hb: 97% of saturation by O_2	Hb: 75% of saturation by O_2

Oxygen supply to tissue cells is facilitated by high PO_2 levels in lungs. It is enhanced by the relatively high P CO_2 (Bohr effect), high acidity (low pH), high temperature in metabolically active tissues.

Transport of carbon dioxide

Carbon dioxide is transported from the tissues to lungs at the rate of about 180 ml/min:

- 6-7% of CO_2 is transported in a physically dissolved state,
- 3-10% in Hb CO_2;
- most CO_2 is transported in the bicarbonate form.

Carbon dioxide is supplied to erythrocytes. It is converted into H_2CO_3 under the influence of **capboanhydrase**. Deoxyhemoglobin is weak acid, it binds H^+. Accumulated bicarbonate anions (HCO_3^-) are transported from erythrocyte cell to plasma. They are exchanged for chloride ions (the chloride shift).

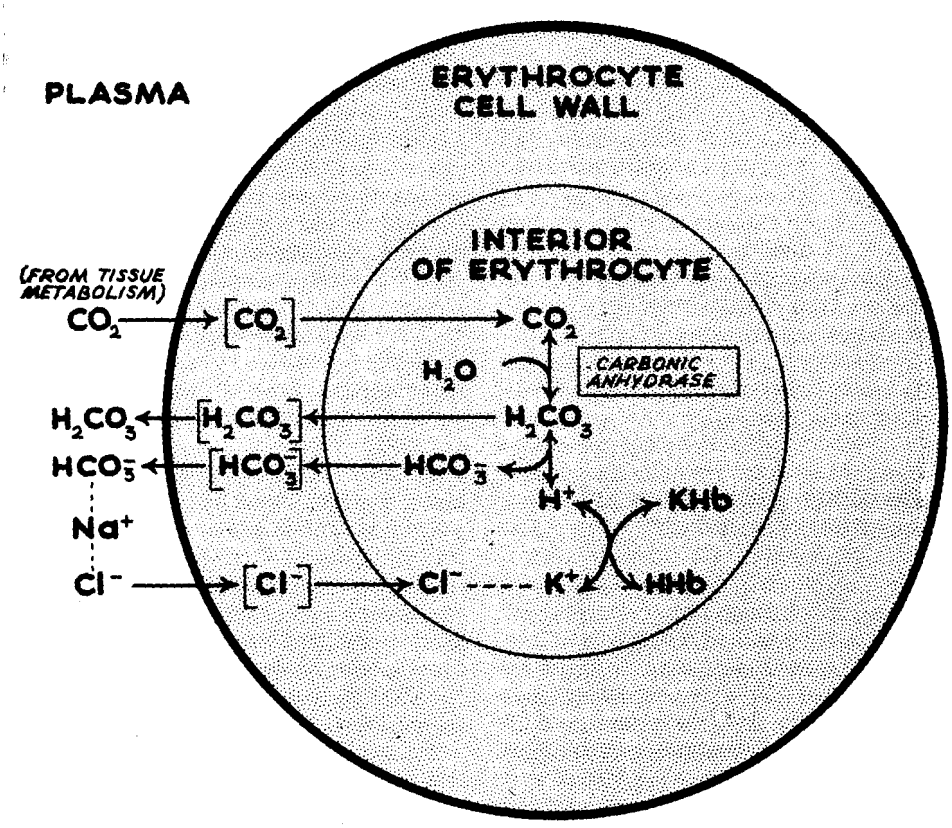


Figure 14.3. The chloride shift

14.3.2 Hemoglobin Metabolism

Hemoglobin Degradation. Types of Jaundices

The life of red cells is **120 days**. After this period of time, the erythrocytes suffer degradation to release hemoglobin. About **6g Hb** are renovated every day in adult human. This leads to releasing about **25 mg** of iron.

Free iron is toxic, but association with transferrin diminishes its potential toxicity and also directs iron to where it is required in the body.

The major organs responsible for the erythrocytolysis and hemoglobin breakdown are:

- liver,
- spleen,
- bone marrow.

The catabolism of heme from all the heme proteins is carried out in the microsomal fraction of reticuloendothelial cells by a complex enzyme system called **heme oxygenase.** This process starts with a cleavage of the α-methylene bridge between the rings I and II of the porphyrin ring system to produce

verdoglobin and carbon monoxide. Verdoglobin is spontaneously cleft into iron (ferric ion), globin and biliverdin. Biliverdin reductase reduces the methenyl bridge between pyrrole III and pyrrole IV to a methylene group to produce bilirubin.

The chemical conversion of heme to bilirubin by the reticuloendothelial cells can be observed in vivo as the purple color of the heme in a hematoma is slowly converted to the yellow pigment of bilirubin.

The further metabolism of bilirubin occurs primarily in the liver. It can be divided into 3 processes:

1. Uptake of bilirubin by liver parenchymal cells.
2. Conjugation of bilirubin in the smooth endoplasmic reticulum.
3. Secretion of conjugated bilirubin into the bile.

Bilirubin is hydrophobic molecule. Therefore bilirubin formed in cells of reticulo-endothelial system of spleen and bone marrow is transported to the liver by plasma albumin. Each molecule of albumin appears to have one high affinity site and one low affinity site for bilirubin. In **100 ml** of plasma approximately **25 mg** of bilirubin can be tightly bound to albumin. **1g** of Hb degradation yields **35 mg** of bilirubin. The daily bilirubin formation in human adults is approximately **250-350 mg**, deriving mainly from Hb, but also from various other hemoproteins such as cytochrome P450. Bilirubin which is not bound with albumin, is toxic, because it is hydrophobic. It can pass through blood-brain barrier and can form complexes with collagen of intercellular matrix and with lipids of membranes. The diminishing pH of blood decreases the affinity of albumin to bilirubin. Some drugs compete with bilirubin to bind with high affinity site of albumin.

In the liver, the bilirubin is removed from albumin and taken up by hepatocytes with a carrier-mediated saturable system. In smooth endoplasmic reticulum of hepatocytes bilirubin is converted to a polar form by adding glucuronic acid molecules to it. This process is called conjugation and is catalyzed by UDP-glucuronyl transferase. Secretion of conjugated bilirubin into the bile occurs by an active transport mechanism. Secretion of bilirubin is the rate limiting

stage of metabolism of bilirubin in liver. **Conjugation and secretion of bilirubin are induced by phenobarbital.**

Cells of reticulo-endothelial system of liver, spleen, bone marrow

Hb

heme oxygenase

O_2
$2NADPH+H^+$
$2NADP^+$
CO

Verdoglobin

Fe^{3+} globin

biliverdin (green pigment)

bilirubin reductase

bilirubin

↓

Blood bilirubin + albumin } indirect, free, unconjugated

↓

Liver bilirubin

UDP - glucuronic acid

UDP

UDP- glucuronyl transferase

bilirubin monoglucuronide (30%) + bilirubin diglucuronide (70%) } direct, conjugated

↓

Bile bilirubin glucuronides

↓

Intestine: terminal ileum and the large intestine

bilirubin glucuronide

↓ β-glucuronidase (bacterial enzyme)

bilirubin

↓

stercobilinogen
(urobilinogen)

↓

stercobilin [feces] 40 -280 mg/day

urobilin [urine] 4 mg/day

• Small amount of conjugated bilirubin enters to blood. This bilirubin is called direct, conjugated. Direct bilirubin is hydrophilic, therefore **only direct** bilirubin may be excreted in the **urine**.

• As the conjugated bilirubin reaches the **terminal ileum** and the large intestine, the glucuronides are removed by specific bacterial enzymes (β-glucuronidases).

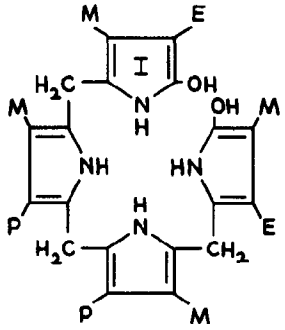
• Then bilirubin is finally reduced to stercobilinogen (urobilinogen) by influence of microflora enzymes.

• Part of it is absorbed, supplies to liver and then or is excreted with bile (large amount) or enters to blood and is excreted in the urine (small amount).

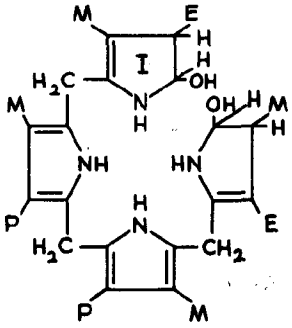
• Stercobilinogen, which is excreted with feces, is oxidized to stercobilin.

Total bilirubin of blood – 8,5 – 20 μmol/L

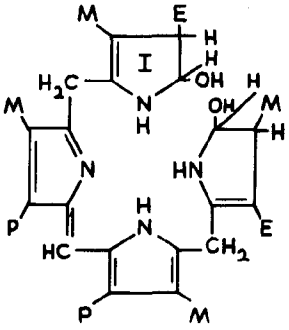
75% indirect **25% direct**



Mesobilirubinogen



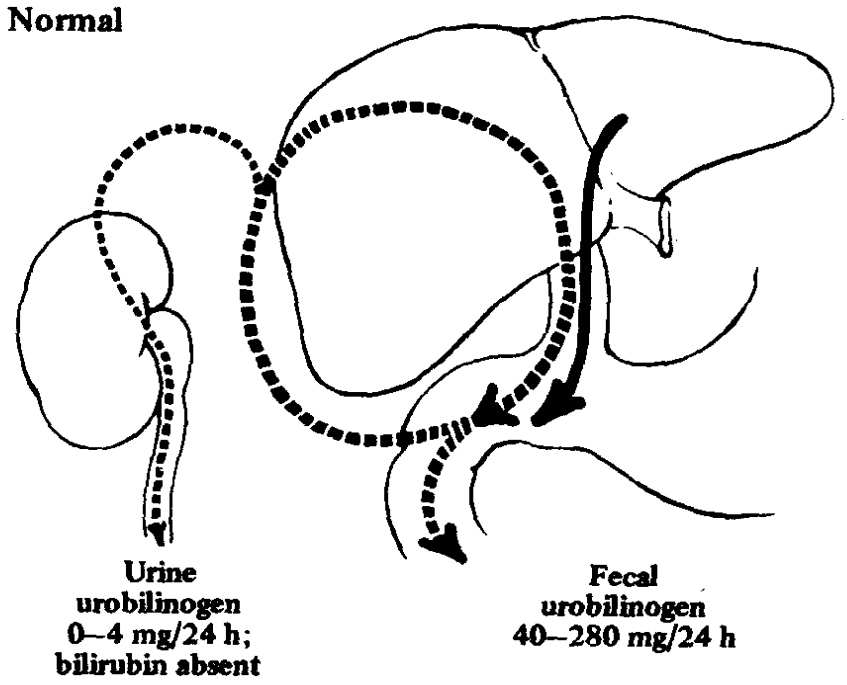
Stercobilinogen (L-urobilinogen)



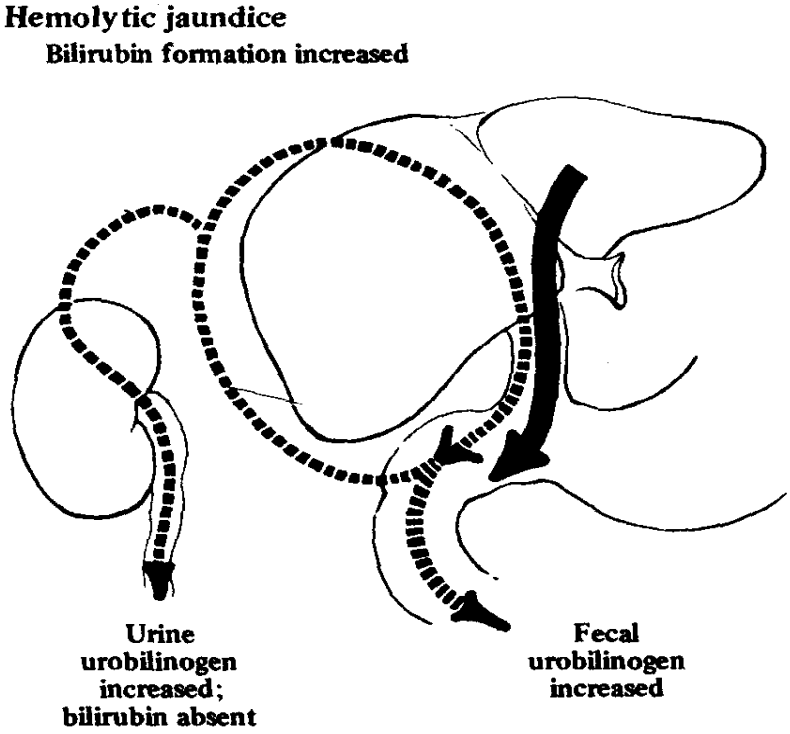
Stercobilin (L-urobilin)

Figure 14.4 Structure of some bile pigments.

Determination of bile pigments is very important for differential diagnosis of different forms of jaundice.



1. Hemolytic jaundice is caused by enhanced hemolysis (for example in drug hemolytic anemia, thalassemia, in incompatible blood transfusion). The synthesis of bilirubin is intensified. The liver is incapable to produce bilirubin glucuronides in adequate amount. Unconjugated bilirubin is accumulated in blood and tissues.



- Indirect bilirubin in blood is increased.
- Urobilun in urine is enhanced.
- Stercobilin in feces is also increased.

- Bilirubin in the urine **is absent**.

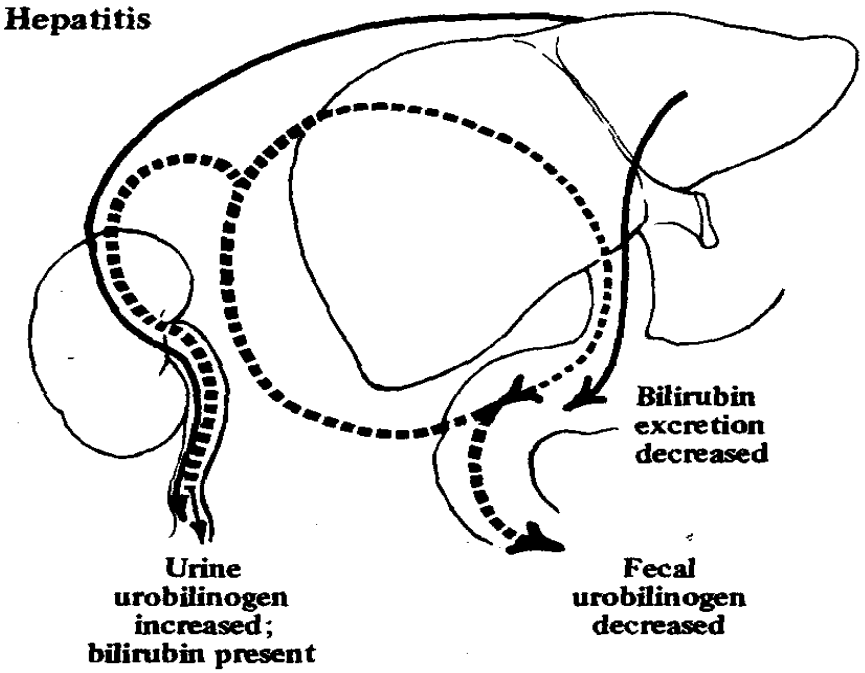
Neonatal physiological jaundice results from an accelerated hemolysis and an immature hepatic system for uptake, conjugation and secretion of bilirubin.

This leads to the increase of **indirect bilirubin** in blood.

If the concentration of bilirubin in blood exceeds the amount, which may be linked with albumin (more than **340 μmol/l**) bilirubin supplies to brain and causes its lesion, leads to development **kernicterus**.

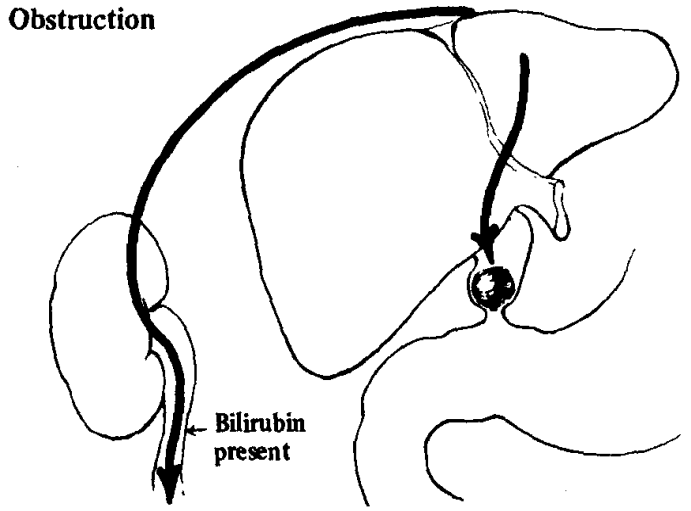
2. **Hepatic jaundice** is linked with damages of hepatocytes (in acute viral infections, chronic and toxic hepatitis). The liver cells undergo degradation, the excretion of conjugated bilirubin in the bile capillaries is disturbed, and this bilirubin is excreted directly in the blood stream:

- In blood both direct and indirect bilirubins are increased.
- Bilirubin is detected in the urine.
- The amount of stercobilin in feces is decreased.



3. **Obstructive jaundice** is developed in disturbance of bile excretion into duodenum. This may be caused by obturation of bile ducts, by tumor of pancreas, of bladder, of liver, of duodenum.

- Blood: The increase of direct bilirubin. Jndirect bilirubin is also increased, but in less extent.
- The decrease of stercobilin in feces (the complete obturation of bile ducts is manifested by absence of bile pigments in the feces – acholic stools).
- Bilirubin is present in urine.



	Urine urobilinogen	Fecal urobilinogen
COMPLETE		
Tumor Stricture Severe hepatitis (rarely)	Absent	Trace to absent
INTERMITTENT		
Stone	Fluctuates	Fluctuates

Congenital disturbances of bilirubin metabolism

I. Crigler – Najjar syndrome may be 2 types:

a. **Type I.** A rare autosomal recessive disorder. Primary metabolic defect is inherited absence of **UDP – glucuronyl transferase.** Indirect bilirubin increases in blood. It is usually fatal within the first 15 month of life, because kernicterus is developed.

b. **Type II** is linked with the decrease of UDP- glucuronyl transferase activity. Indirect bilirubin is enchanced in blood.

II. Gilbert's syndrome is a heterogenous group of disorders. It may be due to: a compensated hemolysis, a defect in the uptake of bilirubin by hepatocytes,

reduced UDP-glucuronyl transferase activity. It is characterized by the increase of indirect bilirubin.

III. Dubin-Johnson syndrome is caused by defect in hepatic secretion of conjugated bilirubin in bile. It is characterized by conjugated hyperbilirubinemia.

Synthesis of Hemoglobin

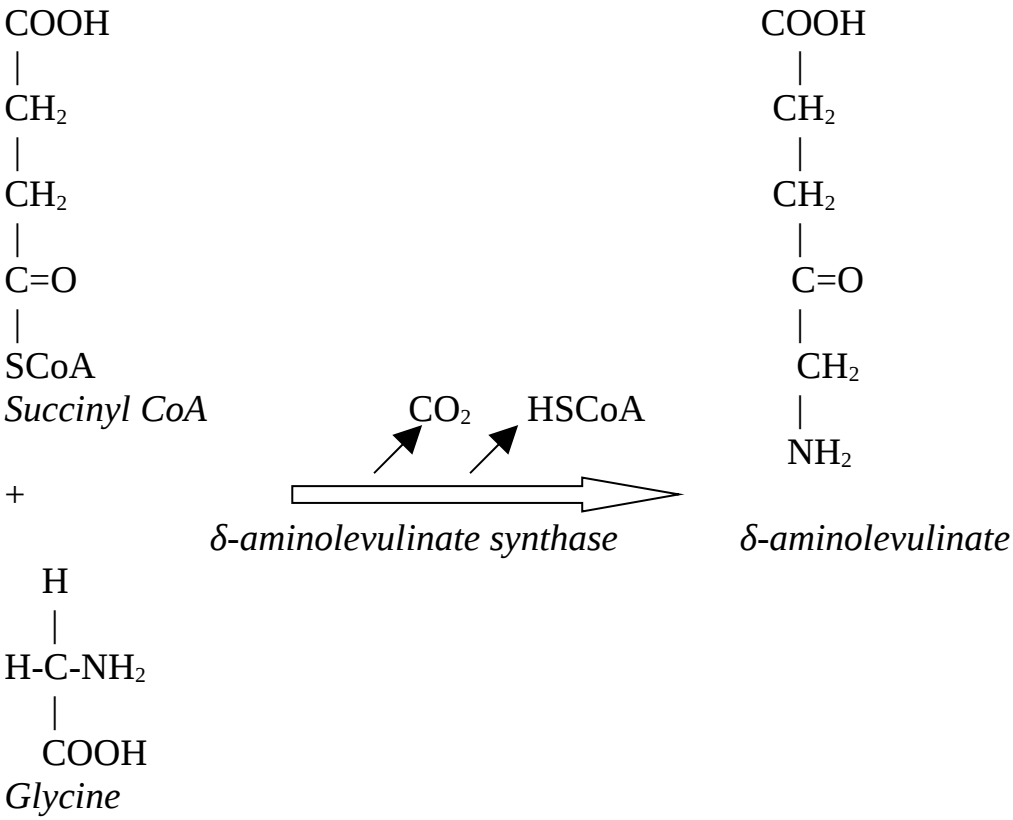
Synthesis of globin (protein part) and heme (prosthetic group) are necessary for formation of Hb.

Synthesis of globin is performed on ribosomes.

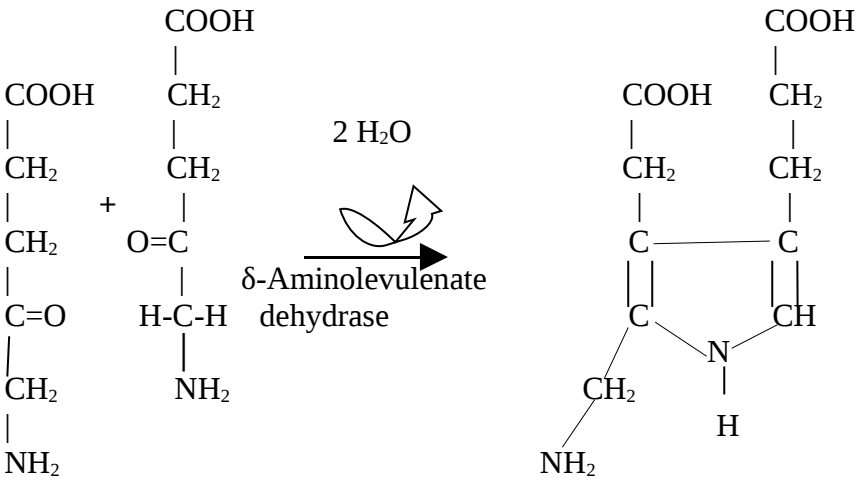
Heme is synthesized in all the tissues, but the most intensive synthesis of heme occurs in **bone marrow and in liver.** In bone marrow heme is necessary for synthesis of Hb in reticulocytes. In hepatocytes it is used for synthesis of cytochrome of P_{450}.

Synthesis of Heme

Mitochondria



Cytosol



Two molecules of *δ-aminolevulinate*

Porphobilinogen

4 porphobilinogen

4 NH_3 2 porphobilinogen deaminase

(uroporphyrinogen I synthase)

hydroxymethylbilane

Uroporphyrinogen III cosynthase 1

nonenzymatically

Uroporphyrinogen III

$4CO_2$

Coproporphyrinogen III

Uroporphyrinogen I

$4CO_2$

Coproporphyrinogen I

Mitochondria

Coproporphyrinogen III

$4H^+$ $2CO_2$

Protoporphyrinogen IX

$6H^+$

Protoporphyrin IX

Fe^{2+} 3 ferrochelatase

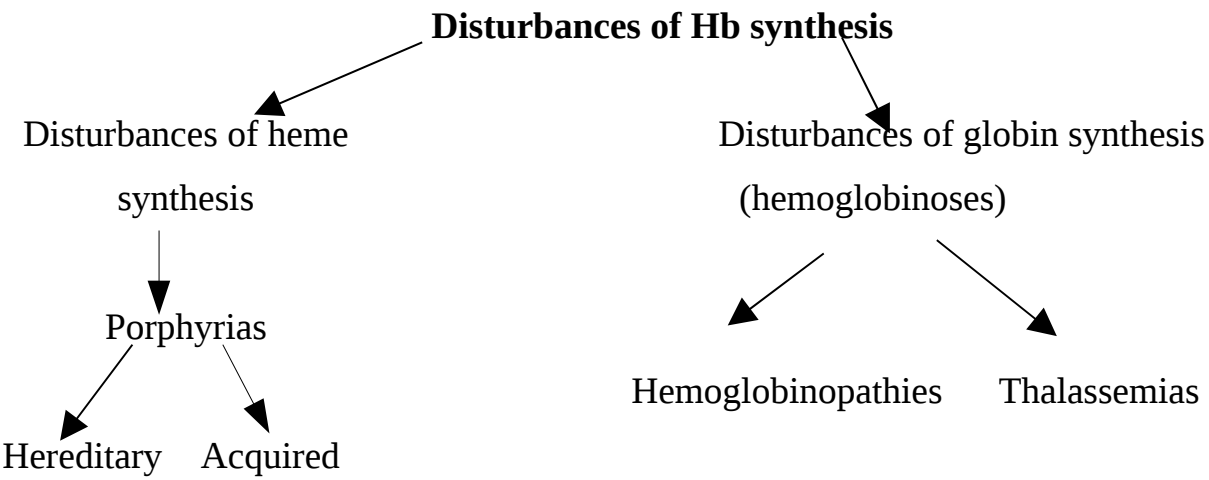
Heme

stimulates the synthesis of globin

Heme + Apoprotein

Hemoprotein

allosterically inhibits the activity of δ-aminolevulinate synthase



Hemoglobinopathies are due to a hereditary change in the primary structure of peptide chains, for example, in sickle cell anemia HbS (β-chain: 6 Glu → 6 Val).

Thalassemias are due to a disturbed synthesis of one of the normal hemoglobin chains. For example in β-chain thalassemia an excess of α-chains occurs which can combine with δ-chains producing an increase in Hb A_2 or with γ-chains producing an increase in HbF (15-60%).

Hereditary Porphyrias

1. ***Congenital erythropoietic porphyria (Hunter's disease)*** *i*s due to a deficiency of uroporphyrinogen III cosynthase. Patients with congenital erythropoietic porphyria excrete large quantities of the type I isomers of both uroporphyrinogen and coproporphyrinogen, which in the urine are spontaneously oxidized to uroporphyrin I and coproporphyrin I, both fluorescent red pigments. Urine is ussually red colored. Teeth and bones may be brownish or pink due to porphyrin deposition. Affected individuals exhibit abnormal sensitivity to light (photosensitivity) and development of skin lesions.

2. Intermittent acute porphyria (IAP) is due to a deficiency of porhobilinogen deaminase (uroporphyrinogen I synthase). Patients with IAP excrete massive quantities of porphobilinogen and aminolevulinate in the urine. Both of these compounds are colorless, but porphobilinogen upon exposure to light and air polymerizes spontaneously but slowly to form 2 colored compounds: porphobilin and porphyrin. The concentration of δ-aminolevulinate and porphobilinogen are increased. They are neurotoxins. Clinical symptoms are abdominal pain, neurophychiatric symptoms.

3. ***Protoporphyria*** is due to a deficiency of ferrochelatase. Photosensitivity is observed.

Acquired porphyrias are observed in iron deficiency anemia, in liver diseases, in exposure to toxic compounds.

Metabolism of Fe

The adult human organism contains 3-4 g of iron. Only 3,5 mg is in blood plasma.

- Hb – 68% of Fe
- Ferritin – 27% of Fe
- Myoglobin – 4% of Fe
- Transferrin – 0,1% of Fe
- Iron containing enzymes – 0,6% of Fe

Iron containing biomolecules perform 3 main functions:

1) electron transport (cytochromes, FeS-containing proteins);
2) transport and storage of O_2 (myoglobin, Hb);
3) oxidation – reduction enzymes.

Daily amount of food contains 15-20 mg of iron, but only about 10% of this amount is absorbed. Organism of adult human losses ~1 mg Fe/day.

Absorption of Fe. Iron is absorbed in the intestine (mainly in duodenum) as divalent ion (Fe^{2+}) after its release from protein complexes. The normal secretion of gastric juice is needed to optimal absorption of Fe; HCl of gastric juice liberates Fe from nonheme proteins.

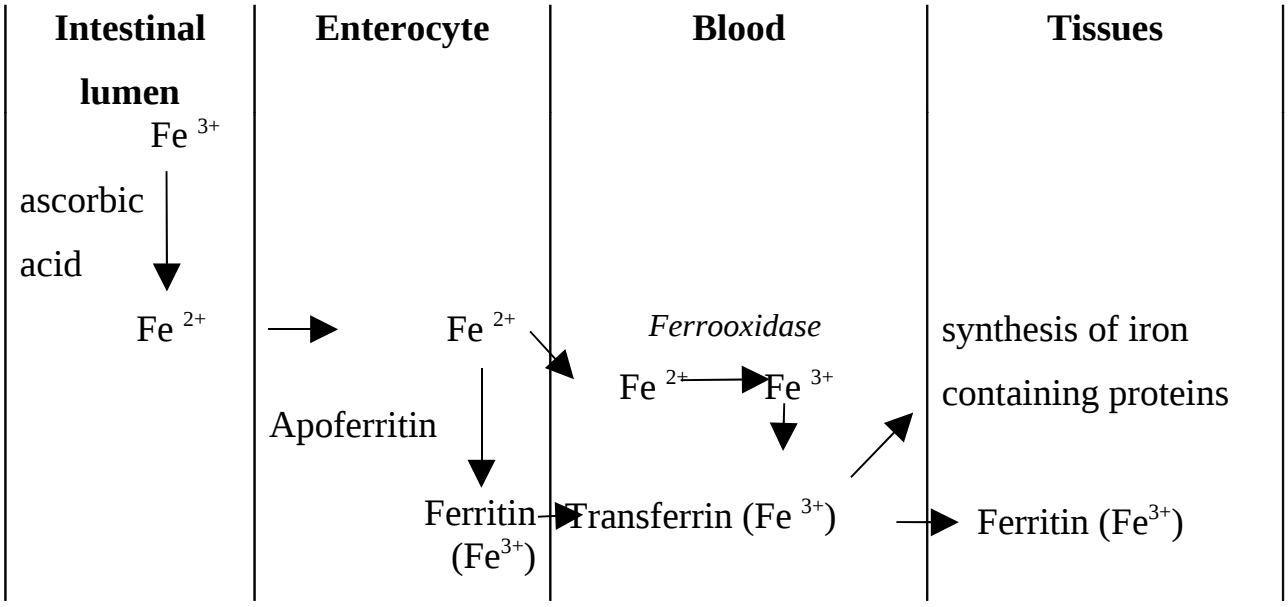
Ascorbic acid plays the important role in absorption and mobilization of Fe. This effect of vitamin C explains its antianemic action.

- Vitamin C and amino acids are able to form chelates with Fe which are absorbed.
- Vitamin C and glutathione reduce Fe^{3+} to Fe^{2+}, which is more soluble form of iron.

- Heme iron, which comes mainly from animal products, is effectively absorbed (about 20-30%).
- Non-heme iron, wich is present in plants, though ingested in larger amount than heme iron, is ineffectivelly absorbed (only to 5%).

The absorption of iron is inhibited by tea (60%) and coffee (35%).

Homeostasis of iron is provided by its entering from enterocytes to blood.



Quantity of iron, which is usually absorbed, is more than the requirement of organism. Iron entering from enterocytes to blood depends on the rate of apoferritin synthesis in enterocytes.

Apoferritin binds Fe and is converted into ferritin. This prevents the entering Fe into blood capillaries from cells of intestine. When the iron requirement is small, the rate of apoferritin synthesis is increased. Constant slicing cells of intestinal mucosa into intestinal lumen liberate the organism from excess of Fe. In deficiency of the Fe in organism apoferritin in enterocytes is practically not synthesized.

Iron transport by blood to the sites of hematopoiesis is performed by means of **transferrin**. Transferrin concentration in blood is 2,4 g/L. Under normal conditions only 1/3 (33%) of transferrin is saturated by Fe^{3+}. Transferrin binds only

oxidized form of Fe. Oxidation of Fe in blood is performed by ferrooxidase wich is called ceruloplasmin.

14.4 System of Hemostasis

System of hemostasis provides the formation of blood clot in damaged vessels and prevents intravessel activation of blood clotting system under physiologic conditions. Intravessel formation of thrombi (thrombosis) is pathologic procces. It is observed in severe disturbance of hemostasis system.

System of hemostasis **includes:**

1. Blood coagulation system.
2. Blood anticoagulation system
3. Fibrinolytic system of blood

Process of a hemostasis is distinguished into:

- Vessel-platelet hemostasis, which is provided by endothelium of vessels, platelets and erythrocytes.
- Coagulational hemostasis, which is provided by plasma coagulation factors.

There are four **stages of hemostasis**.

I. Constriction of the injured vessels to diminish blood flow to the injury (it is provided by constrictive substances, which are liberated from platelets, namely by serotonin, adrenaline).

II. Formation of temporary platelet aggregate at the site of injury.

III. Formation of a fibrin mesh that binds to the platelet aggregate, forming a more stable hemostatic plug.

IV. Partial or complete dissolution of hemostatic plug by plasmin.

Types of thrombi

1. **The white thrombus** is composed of platelets and fibrin and is relatively poor in erythrocytes. It forms at the site of an injury or abnormal vessel wall, particularly in areas where blood flow is rapid (arteries).

2. **The red thrombus** consists primarily of red cells and fibrin. It may form in vivo in areas of retarded blood flow or stasis (e.g. veins) with or without vascular

injury; or it may form at a site of injury or in abnormal vessels in conjuction with an initiating platelet plug.

3. A third type is a **disseminated fibrin deposit** in a very small blood vessels or capillaries.

All three types of thrombi contain fibrin in variable proportions.

14.4.1 Blood Coagulation System

Endothelial cells of blood vessels, platelets, erythrocytes, leukocytes, proteins of blood plasma, Ca^{2+}, platelets factors participate in blood clotting.

Endothelium of blood vessel wall performs the function of barrier between blood and vessel wall. Surface of endothelium has heparin sulfate and glycosaminoglycans, wich activate antitrombin III (main inhibitor of blood clotting system). Endothelial cells produce prostacyclins (which inhibit aggregation of platelets), NO (which causes local vasodilatation). They synthesize the activator of plasminogen, wich initiates a process of fibrin dissolvation (fibrinolysis). Endothelial cells have protein thrombomodulin, which binds thrombin. Endothelial cells have not only anticlotting action, but also procoagulant properties: they produce factor of platelet activation, inhibitor of plasminogen activators and tissue factor. Subepithelial layer of vessel wall is composed of collagen fibrils, elastin, proteoglycans and nocollagen proteins, including fibronectin and Willebrand's factor. Last is a stimulator of platelet aggregation after endothelium injury. Therefore endothelium plays the key role in regulation of hemocoagulation.

Platelets play the considerable role in process of coagulation. The main function of platelet is the formation of mechanical plugs during the normal hemostatic response to vascular injury. In the formation of a hemostatic plug, platelets must undergo three processes: (1) adhesion to exposed collagen on blood vessels, (2) release of the contents of their granules and (3) aggregation. Platelets have dense granules (which contain ADP, ATP, Ca^{2+}, serotonin) and α-granules (which contain platelet factor 4, β-thromboglobulin, platelet derived growth factor, fibrinogen, fibronectin), lysosomal granules (various hydrolytic enzymes). Many

specific compounds participating in blood clotting are related to trombocytes. They are called „platelet factors”.

Platelet factors of blood clotting: 1 – proaccelerin adsorbded on the surface of platelet (5% of total amount of blood proaccelerin is linked with thrombocytes); 2 – it stimulates the process of fibrinogen conversion to fibrin; 3 – thrombocytic thromboplastin (it is phospholipid of platelet membrane, which is released after the platelet destruction and utilized in the first phase of blood coagulation); 4 – heparin neutralising factor; 5 – thrombocytic fibrinogen; 6 – thrombosthenin ensures a retraction of clot; 7 – antifibrinolytic factor; 8 – activator of fibrinolysis; 9 – fibrin stabilizing factor; 10 – serotonin is vasoconstrictor; 11 – ADP is stimulator of platelet aggregation.

Erythrocytes take part in the formation of the primary platelet plug. In site of injury the damage of erythrocytes results in liberation of ADP, which is neccesary for aggregation of platelets. Erythrocytes perform transport function, because they adsorb plasma factors of clotting system on their surface. In addition to this erythrocytes constitute the mass of the primary hemostatic plug.

Leukocytes provide active surface for initiation of hemostasis process, release substances (some interleukins, tumor necrosis factor, endotoxin) which can induce reactions of blood clotting.

Factors of blood plasma

Factor I. **Fibrinogen** (glycoprotein) is synthesized in liver. On clotting fibrinogen is converted to fibrin that forms the supporting element of the clot.

Factor II. **Prothrombin** (glycoprotein) is synthesized in liver with participation of vitamin K. Its active form (thrombin) converts fibrinogen into fibrin.

Factor III. **Tissue thromboplastin** is lipoprotein fragment of cellular membranes. It acts as a cofactor for factor VII. It initiates extrinsic pathway of blood coagulation.

Factor IV. **Calcium ions** are required in all the phases of blood clotting.

Factor V and VI. **Proaccelerin and accelerin**. They are known as accelerator globulin (Ac G). The term „factor VI” is not used because accelerin is active form of proaccelerin (factor V). Proaccelerin is synthesized in liver, spleen and kidney. Factor Va is a cofactor in the activation of prothrombin by factor Xa.

Factor VII. **Proconvertin** is synthesized in liver with participation of vitamin K. It takes part in activation of factor X.

Factor VIII. **Antihemophilic globulin** A (AHG) is synthesized in liver. It is a cofactor in the activation of factor X by factor IXa. It is stabilized by Von Willebrand's factor.

Factor IX. **Christmas factor** or antihemophilic globulin B, plasma thromboplastin component (PTC), participates in the activation of factor X.

Factor X. **Stuart-Prower factor** is synthesized in liver with participation of vitamin K. It is one of the components of prothrombinase complex (Ca^{2+}, factors Va and Xa), which converts prothrombin into thrombin.

Factor XI. **Plasma thromboplastin antecedent** (PTA), Rozental's factor is formed in liver. It partucipates in the activation of factor IX.

Factor XII. **Hageman factor** is synthesized in liver. It is linked and nonenzymatically activated by collagen and negatively charged surfaces in the presence of *kininogen*. Factor XIIa activates the factor XI and initiates reactions of blood clotting. Along with the blood coagulation system, factor XIIa activates the kinine system and the systems of complement and fibrinolysis.

Factor XIII**. Fibrin stabilizing factor** (FSF), transglutaminase is synthesized in liver, stabilizes fibrin clot by covalent crosslinking.

High-molecular-weight kininogen (Fitzgerald factor) is synthesized in liver. It is the precursur of kinines (bradykinine and lisyl-bradykinine). Kinines activate factor XI.

Prekallikrein (Flethcher factor) is synthesized in liver. It activates factors VII, IX, converts kininogen into kinines. It is believed that factor XII in adsorbtion on surface undergoes the activation in the presence of *high-molecular-weight*

(HMW) kininogen. Small amount of XIIa activates plasma prekallikrein into kallikrein. Kallikrein is the most potent activator of factor XII.

Protein C (glycoprotein), is synthesized in liver with participation of vitamin K. Protein C in combination with protein S degrades factors Va and VIIIa, limiting their actions.

Protein S is synthesized in liver with participation of vitamin K. It acts as a cofactor of protein C.

Thrombomodulin (glycoprotein) is synthesized by endothelial cells. It binds protein C, which is then cleft by thrombin to yield activated protein C.

Von Willebrand factor is secreted by endothelial cells into the plasma. It stabilizes factor VIII and provides the binding platelets with subendothelium.

Role of vitamine K in blood clotting

Vitamin K participates in posttranslational modification of factors II, VII, IX, X and proteins C and S, all of which are synthesized in liver. Vitamin K is a cofactor of carboxylase wich catalyzes the conversion of glutamate (Glu) into γ-carboxyglutamate (Gla) residues in the amino terminal regions of these proteins.

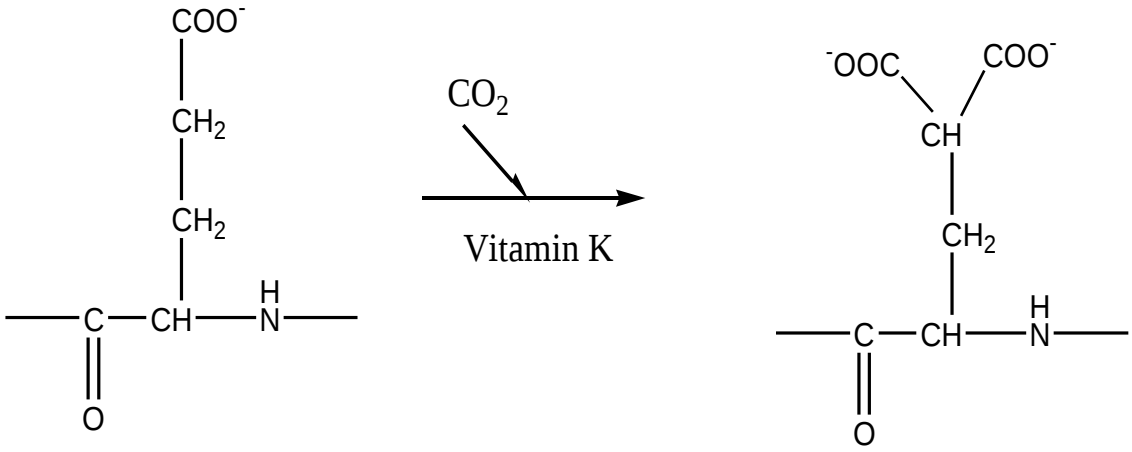


Figure14.5 Carboxylation of a glutamate residue catalyzed by vitamin K-dependent carboxylase.

Additional negatively charged carboxylic groups are necessary for binding Ca^{2+} by means of these factors. This binding provides interaction between molecules in reactions of coagulation, which occur in the presence of calcium ions.

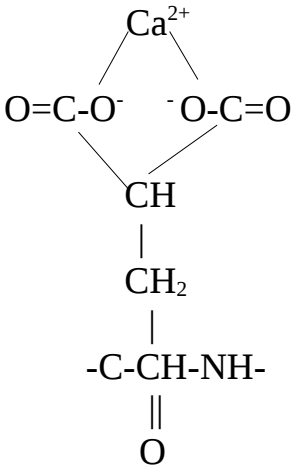


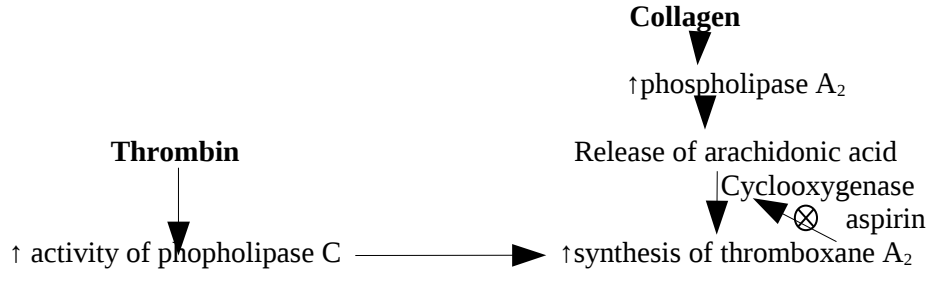
Figure 14.6. The chelation of calcium ion by the γ-carboxyglutamyl residue in clotting-factor proteins.

Vessel-platelet Hemostasis

Bleeding from capillaries and venules can be stoped by mechanisms of vessels-platelet hemostasis. Vessel-platelet hemostasis includes:

- Constriction of the injured vessels to diminish blood flow to the injury (it is provided by serotonin, adrenaline, noradrenaline).
- Adhesion of platelets on collagen fibers of endothelium. Platelet adhesion usually takes 3 – 10 sec.
- Aggregation of platelets to form platelet plug.

Platelets adhere to collagen via specific receptors on the platelet surface, including $\alpha_2\beta_1$ integrin in a reaction that involves Von Willebrand factor (this is a glycoprotein, secreted by endothelial cells into the plasma, which stabilizes factor VIII and binds to collagen and the subendothelium).



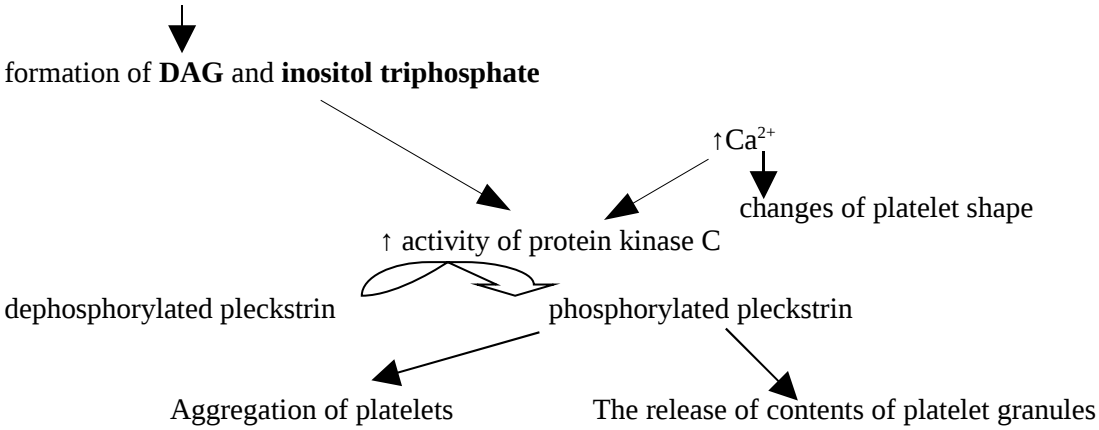


Figure 14.7. Activation of platelet aggregation

Reversed platelet aggregation begins nearly simultaneously with adhesion. The main stimulator of this process is ADP released from the damaged vessels and from the platelets and erythrocytes. Inducers of irreversible phase of platelet aggregation are thrombin, Ca, collagen.

Traces of thrombin are formed under the effect of tissue thrombinase which appears 5-10 sec following damage. Thrombin initiates platelet activation by interacting with its receptor on the plasma membrane. The interaction of thrombin with its receptor stimulates the activity of a phospholipase C in the plasma membrane. This enzyme hydrolyzes phosphatidyl inositol 4,5-diphosphate to form 2 second messengers: diacylglycerol (DAG) and 1,4,5-inositol triphosphate. DAG stimulates protein kinase C, which phosphorylates pleckstrin. Phosphorylation of this protein results in aggregation of platelets. Collagen-induced activation of a platelet phospholipase A_2 results in liberation of arachidonic acid from platelet phospholipids, leading to the formation of thromboxane A_2, which activates phospholipase C, promoting platelet aggregation.

Aspirin (acetylcalicylic acid) irreversibly acetylates and thus inhibits the platelet cyclooxygenase system. Platelets are very sensitive to aspirin; as little as 30 mg/d effectively eliminates the synthesis of thromboxane A_2. Aspirin also inhibits production prostacyclin PGI_2 (it opposes platelet aggregation and is vasodilator) by endothelial cells, but unlike platelets, these cells generate cyclooxygenase within a few hours. Therefore the overall balance can be shifted to

prostacyclin, opposing platelet aggeregation. Indications for treatment with aspirin include management of angina and evolving myocardial infarction, prevention of stroke and death in patients with transient cerebral ishemic attacks.

Hemostasis is provided by the vascular-thrombocytic reactions only in the microcirculatory vessels with low blood pressure.

In large vessels platelet thrombi cannot endure high pressure and are washed out. Hemostasis in such vessels can be achieved by the formation of fibrin thrombus.

Coagulation hemostasis is the cascade of enzymatic reactions, which results in the polymerization of fibrinogen with formation of fibrin thrombus. It involves three phases.

Phase I. Formation of prothrombinase complex (Ca^{2+}, factor Va and Xa). Factor Xa is generated by two mechanisms: **extrinsic pathway and intrinsic pathway.**

Extrinsic pathway is initiated at the site of tissue injury by means of tissue thromboplastin (factor III). Tissue factor acts as a cofactor in the factor VIIa. The activity of factor VIIa increases more than 15 000 times. Factor VIIa cleaves an Arg- Ile bond in factor X to produce the two chain serine protease, factor Xa. The formation of prothrombinase by means of extrinsic pathway takes 5-10 sec.

The intrinsic pathway for generating Xa. The initiation of the red thrombus in an area of restricted flow or in response to an abnormal vessel wall without tissue injury is carried out by the intrinsic pathway. The formation of prothrombinase by means of the intrinsic pathway is performed much more slowly (5-10 min).

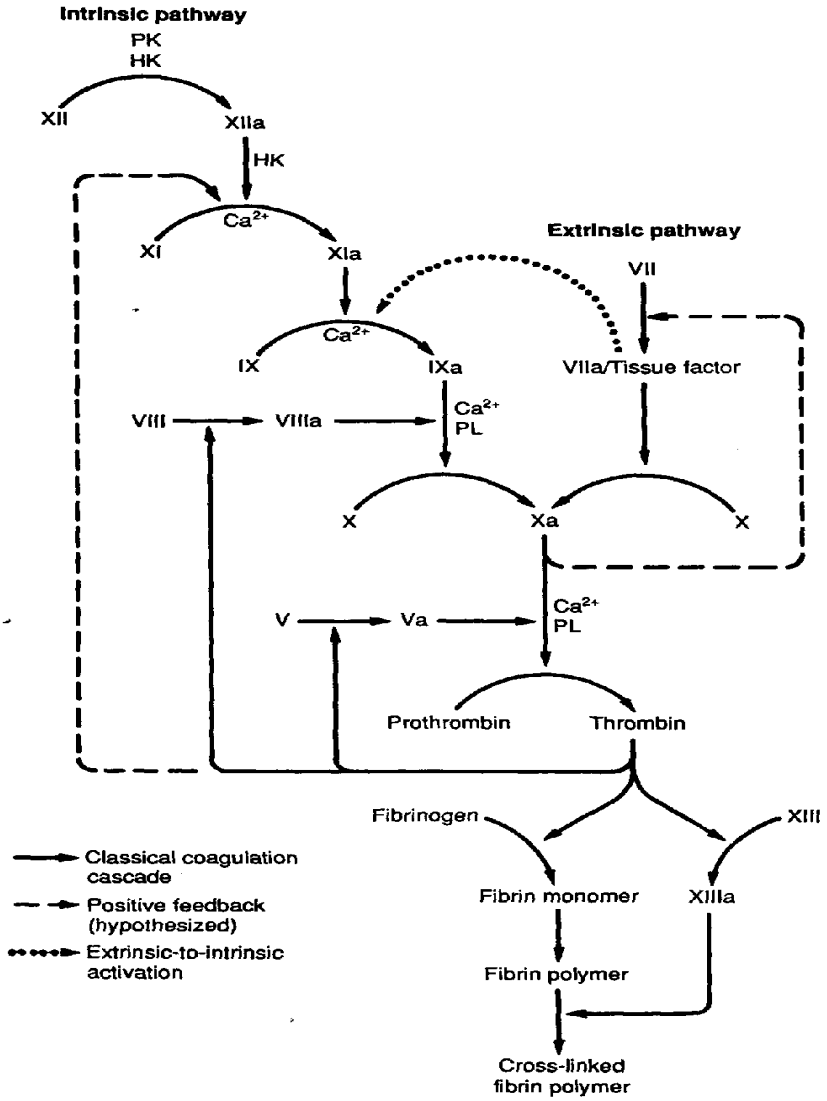


Figure 14.8. The pathway of blood coagulation.

This pathway commences with the „contact phase" in which precallikrein, kininogen, factor XII and factor XI are exposed to a negatively charged activating surface. Collagen on the exposed surface of blood vessel probably provides this site in vivo. The initial reaction is activation of Hageman factor when it comes in contact with the collagen fibres in the pressure of HMW kininogen. Factor XIIa performs the conversion of prekallikrein to kallikrein. Kallikrein catalyzes the conversion of high-molecular-weight (HMW) kininogen into bradykinin. Kallikrein is one of the effective activators of factor XII catalyzing its cleavage into two active forms – α-XIIa and β-XIIa. β-XIIa activates prekallikrein and α-XIIa activates factor XI. Thus factor XIIa (enzyme of the first stage of zymogen activation) is responsible for activation of its own zymogen.

Factor XIa in the presence of Ca^{2+} activates factor IX. Factor IX activates factor X. This reaction requires the assembly of components, called tenase complex, on the surface of activated platelets: Ca^{2+}, VIIIa, IXa, X.

For assembly of the tenase complex, the platelets must first be activated to expose the anionic phospholipids (phosphatidylserine and phosphatidyl inositol), that are normally on the internal side of the plasma membranes of nonactivated platelets. Factor VIII is not a protease precursor but a cofactor that serves as a receptor for factors IXa and X on the platelet surface. Factor VIII is activated by traces of thrombin to form active factor VIIIa which is in turn inactivated upon further cleavage by thrombin.

The final common pathway of blood clotting involves phases II and III.

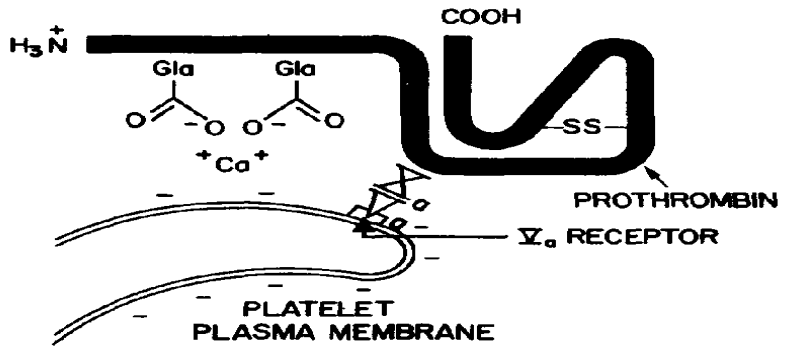


Figure 14.9. Diagrammatic representation of the binding of factor Va, Xa, Ca^{2+}, and prothrombin to the platelet plasma membrane.

Phase II. The activation of prothrombin to thrombin by means of thrombinase complex (factors Va, Xa, Ca^{2+}) in the presence of anionic phospholipids. Factor V has cofactor function when activated to factor Va by traces of thrombin, it binds to specific receptors on the platelet membrane and forms a complex with factor Xa and prothrombin.

Phase III. The conversion of fibrinogen to fibrin. This process has three stages:

- The conversion of fibrinogen to fibrin monomer under the action of thrombin.

- Spontaneous aggregation of fibrin monomer with the formation of fibrin polymer.
- Stabilization of fibrin polymer by forming peptide bonds between the amide groups of glutamine and the ε-amino groups of lysine residues (with participation of factor XIIIa).

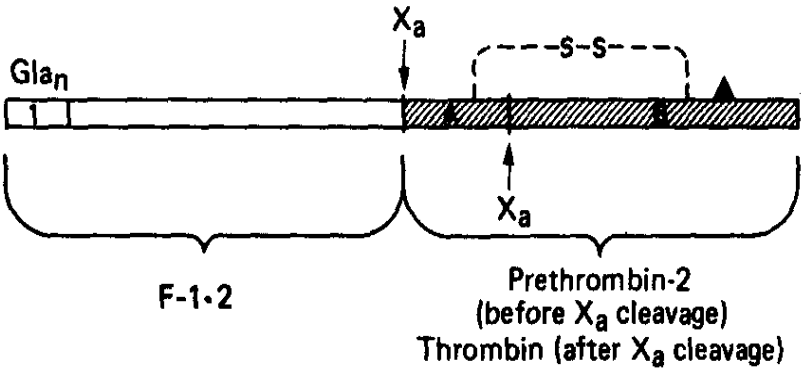


Figure 14.10. Diagrammatic representation of prothrombin. The amino terminus is to the left; region 1 contains all the Gla residues. The sites of cleavage by factor Xa are shown and the products named. The site of the catalytically active serine residue is indicated by ▲. The A and B chains of active thrombin (shaded) are held together by the disulfide bridge.

Factor XIII is also activated by thrombin. Fibrin clot undergoes the process of retraction by means of thrombocytic thrombosthenin and Ca^{2+}. Thrombostenin resembles actomyosin of skeletal muscles and consists of subunits A and M identical to actin and myosin. Thrombosthenin is ATP-ase and contracts at the expense of energy of ATP. In its contraction the clot shrinks to 25-30 % of its initial volume and becomes more firmly secured in the vessel. Retraction is completed 2-3 hours after clot formation.

The Most Spread Inherited Coagulopathies

- **Hemophilia A** is due to a genetically determined deficiency of factor VIII (X chromosome-linked disease). Individuals with hemophilia A lack only factor VIII clotting activity and have normal platelet adherence.
- **Von Willebrand's disease** (autosomal dominant disease) may be an inherited defect in a specific oligosaccharide moiety on the von Willebrand glycoprotein factor. The platelet adherence factor (Willebrand factor) also stabilizes factor VIII

procoagulant activity. The abnormal oligosaccharide may prevent normal platelet adherence and destabilize factor VIII.

- **Hemophilia B** (Christmas disease) is due to defect of factor IX synthesis
- **Hemophilia C** is due to a defect of factor XI synthesis.
- **A-(hypo)-fibrinogenemia** is due to deficiency or absence of fibrinogen in blood plasma (autosomal recessive disease).
- **Disfibrinogenemias** are due to abnormal molecules of fibrinogen.

14.4.2 Anticoagulant System

Although blood possesses a very powerful coagulative system it persists in the living organism in a liquid state. It is provided by anticoagulant and fibrinolytic systems.

Anticoagulants

Body anticoagulants are divided into two groups: the preexisting anticoagulants (the primary) and anticoagulants which are formed during coagulation and fibrinolysis (the secondary).

The primary anticoagulants:

- **Antithrombin III** is the most potent inhibitor of thrombin activity. It contributes approximatelly 75% of the antithrombin activity. Antithrombin III is greatly activated by the *heparin*. Antithrombin III is capable to inhibit the activity of thrombin, IXa, Xa, XIa and XIIa. Individuals with inherited deficiencies of antithrombin III are prone to develop frequent and severe widespead clots.
- **α_2-Macroglobulin** is responsible for 25% of the antithrombin activity of plasma. Its activity does not depend on heparin action.
- **α_1-Antitrypsin** is minor thrombin inhibitor. It inhibits not only thrombin but also factors Xa and XIa.
- **Heparin** is produced by basophils and connective tissue mast cells. The number of basophils is small, but all the body's mast cells have a mass of 1,5 kg. Heparin is anionic glycosaminoglycan, which binds to a specific cationic site of antithrombin III, inducing a conformational change that promotes the

binding antithrombin III to thrombin and other serine proteases. In addition, heparin supresses hyaluronidase activity, stimulates fibrinolysis, improves coronary circulation of blood. Anticlotting action of heparin may be supressed by cationic peptides (e.g., protamine).

The secondary anticoagulants are produced during blood clotting and fibrinolysis.

Fibrin (antithrombin I) adsorbs and neutralizes up to 90% of thrombin.

Thrombin is involved in an additional regulatory mechanism that operates in coagulation. It combines with thrombomodulin, a glycoprotein present on the surfaces of endothelial cells. The complex converts protein C to protein C_a. In combination with protein S protein C_a degrades factors Va and VIIIa, limiting their actions in coagulation. Products of fibrin degradation disturb fibrin-monomer polymerization, block fibrin polymer and suppress platelet aggregation.

The coumarin drugs (eg, warfarin, dicoumarin, neodicoumarin), which are used as anticoagulants, inhibit the vitamin K-dependent carboxylation of Glu to Gla residues in the amino terminal regions of factor II, VII, IX and X and also proteins C and S. These proteins, all of which are synthesized in the liver, are dependent on the Ca^{2+}-binding properties of Gla residues for their normal function in the coagulation pathways. The coumarins act by inhibiting the reduction of the quinone derivatives of vitamin K to the active hydroquinone forms. Coumarin drugs are widely used in the treatment of thrombotic and thromboembolic conditions.

Citrate salts (e.g. sodium citrate) are used as anticoagulants for blood conservation. Anticlotting action of sodium citrate is provided by binding citrate with Ca^{2+}.

14.4.3 Fibrinolytic System of Blood

The coagulation system is normally in a state of dynamic equilibrium in which fibrin clots are constantly laid down and dissolved. Fibrinolysis is performed by **plasmin.**

Plasmin is serine protease which is able to hydrolyze fibrinogen, fibrin, factors V and VIII, factors of complement and some polypeptide hormones. Plasmin circulates in the form of its inactive zymogen (plasminogen).

Activators of plasminogen of various types are found in most body tissues.

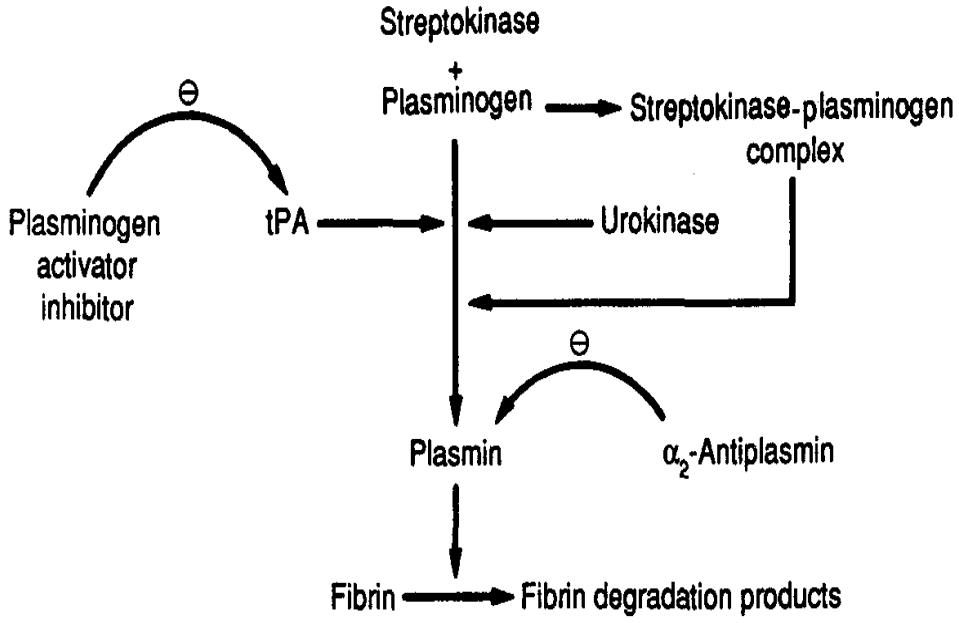


Figure 14.11. Scheme of sites of action of streptokinase, tissue plasminogen activator (t-PA), urokinase, plasminogen activator inhibitor, and α_2-antiplasmin (the last two proteins exert inhibitory actions). Streptokinase forms a complex with plasminogen, which exhibits proteolytic; this cleaves some plasminogen to plasmin, initiating fibrinolysis.

Tissue plasminogen activator (t-PA) is serine protease that is released into the circulation from vascular endothelium under conditions of injury or stress and is catalytically inactive unless bound to fibrin. Upon binding to fibrin, t-PA cleaves plasminogen within the clot to generate plasmin. Plasmin digests fibrin to form soluble degradation products and thus dissolves the clot.

Neither plasmin nor t-PA can remain bound to these degradation products. They are released into the fluid phase where they are inactivated by their natural inhibitors. There is considerable therapeutic interest in the use of t-PA, produced by recombinant DNA technology, to restore the patency of coronary arteries following thrombosis.

Urokinase is also activator of plasminogen, but it does not display the same high degree of selectivity for fibrin. Urokinase is secreted as prourokinase by

certain epithelial cells lining excretory ducts (e.g., renal tubules). It is probably involved in lysing any fibrin that is deposited in such ducts.

There are a number of disorders, including cancer and shock in which the concentrations of plasminogen activators increase. Certain bacterial products, such as **streptokinase**, are capable to activate plasminogen without of cleavage of its molecules. They may be responsible for the diffuse hemorrhage sometimes observed in patients with disseminated bacterial infections.

Excessive activation of fibrinolysis is prevented by **inhibitors of plasmin** (α_1-antitrypsin and α_2-antiplasmin). Plasmin in the fluid phase is rapidly inactivated by the fast-acting plasmin inhibitor, α_2-antiplasmin. Plasmin bound to fibrin is protected from α_2-antiplasmin. It remains active. Antiplasmin activities contributed by α_1-antitrypsin and α_2-antiplasmin may be impaired in cirrhosis of liver. *Aminocapronic acid* is used to stop bleeding due to increased fibrinolysis. Aminocapronic acid blocks activators of plasminogen and partialy inhibits action of plasmin.

Tests for Self-control

1. Which of the below mentioned pH values corresponds to normal pH in blood?

A. 7.25 - 7.31
B. 7.40 - 7.55
C. 7.35 - 7.45
D. 6.59 - 7.0
E. 4.8 - 5.7

2. Which mechanisms provide the pH stability of blood?

A. CO_2 removal by lungs
B. Buffer systems
C. Hydrogen ion secretion by kidney
D. Sodium reabsorption by kidney
E. All the above mentioned

3. Considerable losses of gastric juice in prolonged vomiting provide the development:

A.Respiratory acidosis
B. Metabolic alkalosis
C. Respiratory alkalosis
D.Metabolic acidosis

4. The content of total protein in blood plasma is normal. Which of the below mentioned parameters (g/L) corresponds to physiological norm?

A. 33-45
B. 50-60
C. 55-70
D. 65-85
E. 85-95

5. Which physico-chemical property of protein is the base of the method of electrochemical determination of blood protein spectrum?

A. Viscosity
B. Presence of charge
C. Ability to denaturation
D. Hydrophility and ability to swelling
E. Optical activity

6. Which level of residual nitrogen is normal for adults?

A. 14,3-25 mmol/L
B. 25-38 mmol/lL
C. 42,8-71,4 mmol/lL
D. 70-90 mmol/L

7. Which is the action of bradykinin on vessels?

A. Vasodilation
B. Vasoconstriction
C. The increase of blood pressure
D. Increasing blood clotting

E. The decrease of vessel wall permeability

8. C-reactive protein is revealed in blood serum:

A. After physical loading

B. In remission phase of disease

C. In lipid metabolism disturbances

D. In acute phase of inflammatory diseases

E. In diabetes mellitus

9. Substrates for the synthesis of pyrrol rings of porphyrin are:

A. Acetyl-CoA and glycine

B. Acetoacetyl-CoA and serine

C. Succinyl-CoA and serine

D. Succinyl-CoA and glycine

E. Malonyl-CoA and serine

10. The synthesis of heme is regulated by feed-back mechanism on the stage:

A. Incorporation of iron ion into protoporphyrin

B. Formation of δ-aminolevulinic acid

C. Condensation of porphobilinogen molecules

D. Formation of protoporphyrin III

E. Synthesis of porphobilinogen

11. Patient has high photosensitivity, lesions of skin, abdominal pain, neuropsychiatric disturbances. Urine becomes of red color when leaving for some period of time. Which diagnosis is the most probable?

A. Hemolytic jaundice

B. Pellagra

C. Alkaptonuria

D. Porphyria

E. Albinism

12. Which of the following statements about porphyrias is uncorrect?

A. Genetic disturbance of heme synthesis

B. They are divided into erythropoietic and hepatic

C. They are accompanied by the increased excretion of bile pigments with urine and feces

D. They are manifested by dermatitis and neuropsychiatric disturbances

E. Some symptoms are similar to produce by light

13. Skin, scleras and mucosa are of yellow color in patient. Urine has the color of dark beer, feces are acholic. The increased level of both direct and indirect bilirubin, the enhanced AlAT, LDH_4 and LDH_5 activities are revealed in blood. Bilirubin is found in the urine. Which is the type of jaundice?

A. Inherited

B. Hemolytic

C. Obstructive

D. Hepatic

E. Neonatal physiologic jaundice

14. Neurological abnormalities, skin jaundice, the increase of blood serum unconjugated bilirubin level were revealed in sick 10-years-old child. Which enzyme disturbed synthesis leads to development of Gilbert’s disease?

A. UDP-dehydrogenase

B. UDP-glucuronyltransferase

C. Glycerol kinase

D. Galactose-1-phosphate uridyltransferase

Chapter 15 IMMUNE PROCESSES

Immunity (from Latin “immunis” - free of burden) is the capacity of cell or organism to defense from living organisms and substances which have foreign genetic information.

The immune system is an organization of cells, tissues, organs, and molecules with specialized roles in defending against viruses, microorganisms, cancer cells, and nonself proteins (e.g., organ transplantants).

There are two fundamentally different types of immune responses to invading microorganisms and foreign material.

I. The nonspecific immune response is also known as ***nonspecific resistance*** and *innate or natural* immunity. It offers resistance to any microorganisms or foreign material encountered by the vertebrate host. It consists of cells and molecules that exist in the host prior to antigen exposure. The nonspecific immune response lack immunological memory.

II. The specific immune response is also known as ***acquired or specific immunity*** demonstrates the presence of a functional immune system that is capable of specifically recognizing and selectively elimination antigens moreover. Specific immune responses improve on repeated exposure to foreign agents such as viruses, bacteria and toxins.

Substances that are recognized as foreign and provoke immune responses are called *antigens.*

Pathogens are whole objects (bacterial cells, viruses, diet particles), which lead to pathologic changing after entering to organism.

Haptens are low-molecular-weight substances, which are not able to cause the immune response by themselves, but they acquire this ability after joining to immunogenic molecule.

The nonspecific and specific responses usually work together to eliminate pathogenic microorganisms and other foreign agents.

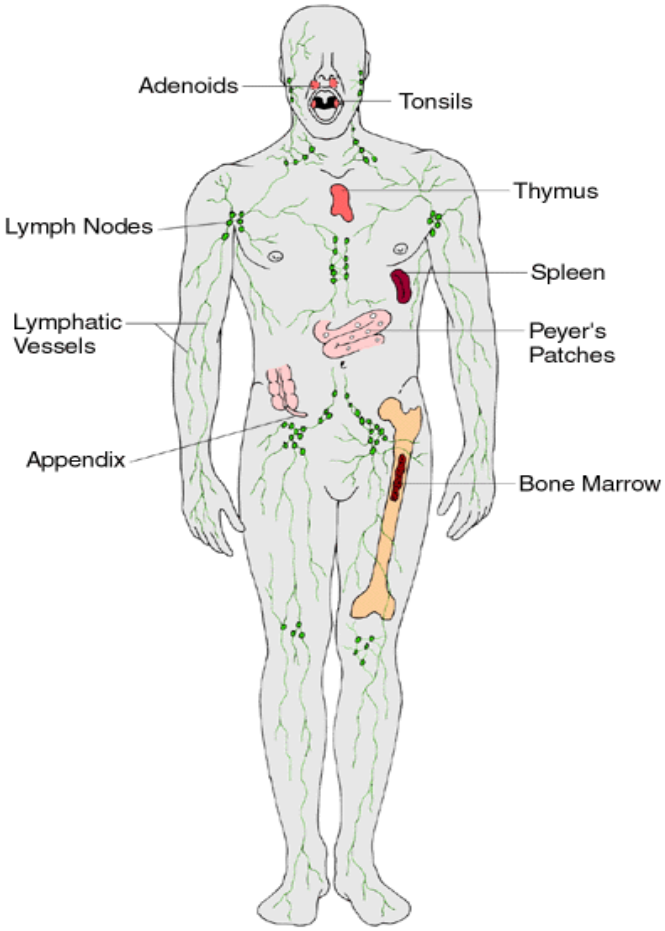


Figure 15.1 Organs and tissues of the immune system

Organs and tissues of the immune system are divided into primary and secondary lymphoid organs and tissues.

The primary lymphoid organs or tissues (the thymus and bone marrow) are where immature lymphocytes mature and differentiate into antigen-sensitive mature B and T cells. Immature undifferentiated lymphocytes are generated in bone marrow.

- The thymus is a lymphoid organ located above the heart. Precursor cells from the bone marrow migrate into the thymus to become mature T cells, and enter the blood stream.
- In birds the maturation of B cells occurs in bursa of Fabricius. In mammals *the bone marrow* is the site of B cell maturation.

The secondary organs and tissues of the immune system:

- the spleen;
- the lymph nodes;

- mucosal - associated tissues;
- gut – associated lymphoid tissue;
- skin – associated lymphoid tissue.

The secondary organs and tissues serve as areas where lymphocytes may encounter and bind antigen, where upon they proliferate and differentiate into fully mature, antigen specific effector cells.

The *spleen* is the most highly organized secondary lymphoid organ and the *lymph nodes* – the most highly organized tissue. Whereas lymph nodes are specialized for trapping microorganisms and antigens from local tissues, the spleen specializes in filtering the blood and trapping blood borne microorganisms and antigens. Once trapped by macrophages and dendritic cells, the pathogen is phagocytosed and antigens are presented to B and T cells, which become activated to carry out their immune functions. It is within the lymph nodes that B cells proliferate into antibody-secreting *plasma cells*.

Cells of the immune system

The main cells responsible for both nonspecific and specific immunity are the white blood cells called leukocytes. All of the leukocytes originate from pluripotent stem cells in the fetal liver and in the bone marrow, from which they migrate to other body sites, undergo further development, and perform their various functions.

Leukocytes are classified as *granular* (neutrophils, eosinophils, and basophils) and *agranular* (lymphocytes and monocytes). In addition to this, dendritic cells, mast cells, platelets, erythrocytes are also involved in immune response.

15.1 Nonspecific Immune Defense Mechanisms

Nonspecific immunity uses physicochemical harriers and involves inflammatory response. Inflammation involves the interaction of circulating blood cells, other cell types in the tissues and their secreted products (cellular and humoral factor of immunity).

Physical and mechanical barriers are the first line of defense against microorganisms. Skin and mucous membranes form a very effective mechanical

barrier to microbial invasion. These surfaces are colonized by normal microbiota, which by themselves provide a biological barrier against uncontrolled proliferation of foreign microorganisms. Many mucosal surfaces are bathed in specific antimicrobial secretions. For example cervical mucus, prostatic fluid, and tears are toxic to many bacteria. One of antibacterial substances is *lysozyme* (muramidase), an enzyme that lyzes bacteria.

15.1.1 Cellular Factors of Nonspecific Immunity

Cellular factors of nonspecific immunity *are:* neutrophils, eosinophils, basophils, monocytes, macrophages, natural killers, dendritic cells, erythrocytes.

Neutrophils *(microphages)* constitute up 50-75% of all blood leukocytes. Not more than 1% of total number of neutrophils circulates in the blood. Their main portion is accumulated in the tissues. The bone marrow has a reserve of neutrophils that exceeds the amount of circulating neutrophils by 50 times. They are discharged into the blood *immediately* the body requires them. The discharge of neutrophils into the tissues is called emigration. Neutrophils are able to phagocytosis, but less intensive than macrophages. They have two main types of granules: the primary and the secondary. The primary granules contain acid hydrolases, neutral proteinases (elastase, cathepsins, collagenase), cation proteins, myeloperoxidase, lysozyme. The secondary granules contain agents, which are active only in neutral and alkaline medium (alkaline phosphatase, lactoferrin, lysozyme). Coming into contact with live or dead bacteria, disintegrating cells of the own organism or with foreign particles neutrophils engulf, digest and destroy them at the expense of their own enzymes and bactericidal substances. One neutrophil is capable to phagocytosis of 20-30 bacteria but can die itself. Along with phagocytosis, neutrophils participate in other reactions directed to suppress microorganisms. The secondary granules function as secretory organelles. The antiviral action of neutrophils is expressed in production of interferon.

Eosinophils constitute 1-5% of blood leukocytes. They contain biologically active substances, which are able to phagocytosis and chemotaxis; enzymes histaminase, arylsulfatase B, phospholipase D.

Basophils constitute only 1% of blood leukocytes. Like the connective tissue mast cells, they produce histamine and heparin and are united in the group of heparocytes.

Monocytes make up 0-10% of blood leukocytes. They manifest the appreciable phagocytic and bactericidal activity. Monocyte phagocytizes up to 100 bacteria, while neutrophil only 20-30. They appear in the inflammatory lesion after neutrophils and display the highest activity in an acid medium in which neutrophils lose their activity. At the inflammatory lesion monocytes phagocytize bacteria, dead leukocytes, the abnormal cells of the inflamed tissue, clear the lesion and prepare it for regeneration, therefore they were called “body scavengers”.

After emigration into the tissues monocytes are converted into **macrophages**. The latter participate in formation of a specific immunity along with phagocytosis.

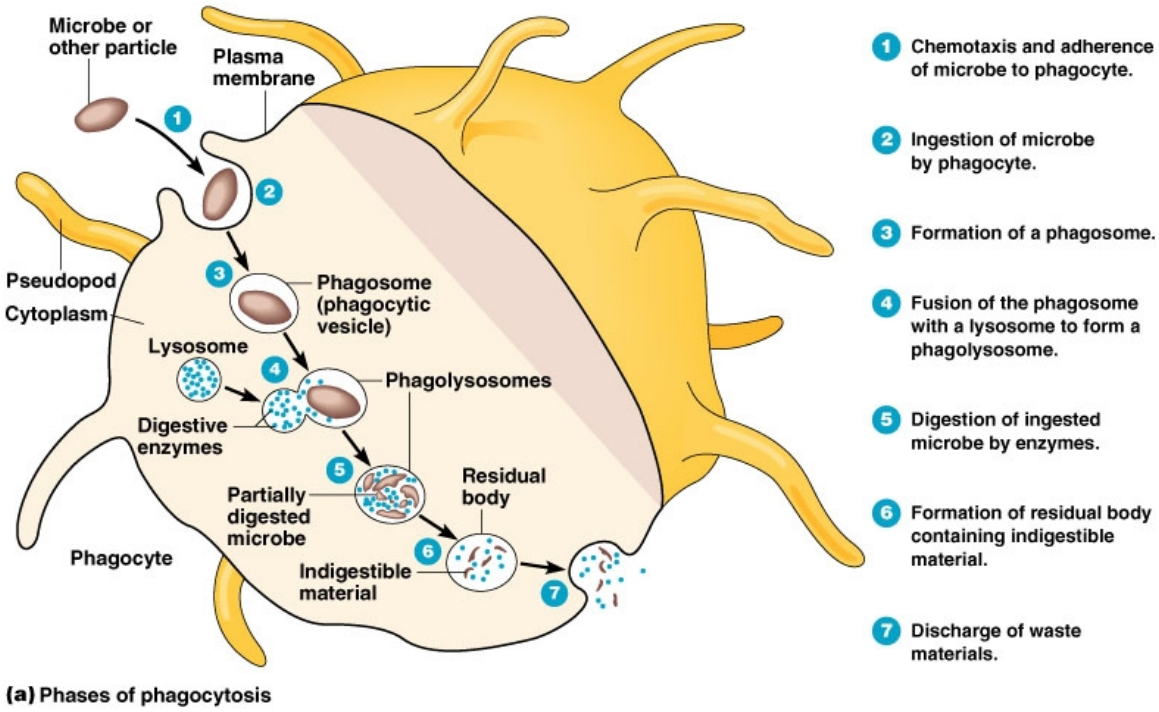


Figure 15.2 Phases of phagocytosis

Macrophages engulf foreign substances, digest and turn them into a specific compound which together with lymphocytes forms a specific immune response. Thus macrophages are *antigen-presenting cells*. Macrophages participate in inflammation and regeneration processes, in lipid and iron metabolism. They have

antitumour and antiviral effects, because they secrete lysozyme, complement, interferon, collagenase, plasminogen activator. Macrophages have receptors for antibodies and complement; these can coat microorganisms or foreign material and enhance phagocytosis. This enhancement is termed *opsonization.* Macrophages spread throughout the animal body and take up residence in specific tissues where they are given special names.

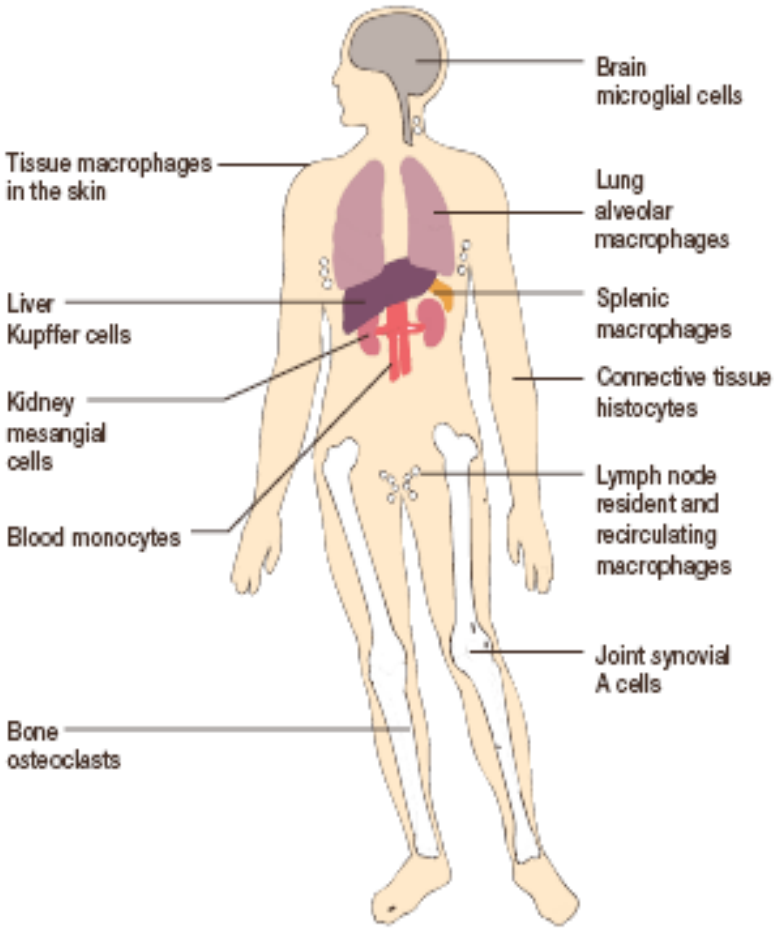


Figure: 15.3 The Monocyte-Macrophage System.

Natural killer cells (population of lymphocytes) are important in killing cell infected with either viruses or intracellular bacteria and destroying cancer cells.

Mast cells *are* bone marrow–derived cells found in connective tissue. They contain granules with histamine and other pharmacologically active substances that contribute to the inflammatory response. Mast cells, along with basophils, play an important role in the development of allergies and hypersensitivities.

Dendritic cells can recognize specific pathogen associated molecular patterns on microorganisms and play an important role in nonspecific resistance. After the

pathogen is recognized, it binds to the dendritic cell's pattern recognition receptors and then is phagocytosed. These cells are also stimulated by endogenous activators such as interferon, heat – shock proteins, and tumor necrosis factor (TNF) that are released in response to microbial infection. After stimulation, dendritic cells migrate to the blood stream or lymphatic system and present antigen to T cells. They stimulate proliferation and differentiation of antigen-specific T lymphocytes. Thus dendritic cells also play an important role in the specific immune response.

Platelets have cytotoxic properties. In activation they liberate biologically active substances (serotonin, adrenaline), produce free radicals, cathepsins and acid hydrolyses.

Erythrocytes are able to adsorb on their surface great amount of circulating antigens and target them to macrophages of spleen and liver.

15.1.2 Humoral Factors of Nonspecific Immunity

Humoral factors of nonspecific immunity are presented by groups of protein and polypeptides which are secreted by different cells of organism.

- ***Lysozyme***. Enzyme, which is synthesized and secreted by neutrophiles, monocytes and macrophages. It occurs in all the biological liquids of organism and provides their bactericidal properties. Lysozyme degrades murein (component of bacteria cellular wall), that leads to lysis of bacteria.
- ***Properdin*** takes part in alternative pathway of complement activation.
- ***The complement system*** consists of some 30 different proteins which are found in the blood. Components of the complement system are synthesized by the liver hepatocytes. But significant amounts are also produced by tissue macrophages, blood monocytes, and epithelial cells of genitourinal and gastrointestinal tract. Components of the complement system normally circulate as inactive precursors. When inflammatory reactions occur, the complement factors enter the infected tissues and take effect there.

Basic function of the complement system:

- *Chemotaxis*. Various complement factors (for example C5a) attract immune cells that can attack and phagocytoze pathogens.

- *Opsonization.* Certain complement factors (“opsonins”, for example C3b) bind to the pathogens and thereby mark them as targets for phagocytosing cells (e.g. macrophages).

- *Membrane attack.* Membrane attack complex (which is formed in complement activation) is deposited in the bacterial membrane, where it creates pores that lyse the pathogen.

Activation of the complement system is triggered by one of three routes, called *the* ***classic***, *the* ***alternative,*** *the* ***mannose-binding lectin (MBL)*** pathways.

The early components in the complement system are *serine proteinases*, which mutually activate each other through limited proteolysis: Factor C3 is central to the complement system.

Classic pathway of complement activation is triggered by interaction of the first component of complement system with complex Ag/antibody (IgG or IgM) on the surface of microorganisms. Fixation of C1 is provided by C1q subunit. This leads to activation of C1r subunit, which converts C1s subunit into active serine proteinase. Last one activates C4 into C4b which in turn cleaves C2 into C2a and C2b. C4b and C2a together form C3- convertase, which finally catalyzes the cleavage of C3 into C3a and C3b.

Alternative (properdin) pathway is triggered by interaction of bacterial antigens with protein of properdin system (which consists of three proteins: P - properdin, B- and D-serine proteinases).

Lectin pathway is homologous to the classical pathway but with the opsonin mannose-binding lectin, and ficolins, instead of C1q. This pathway is activated by binding MBL to mannose residues on the pathogen surface.

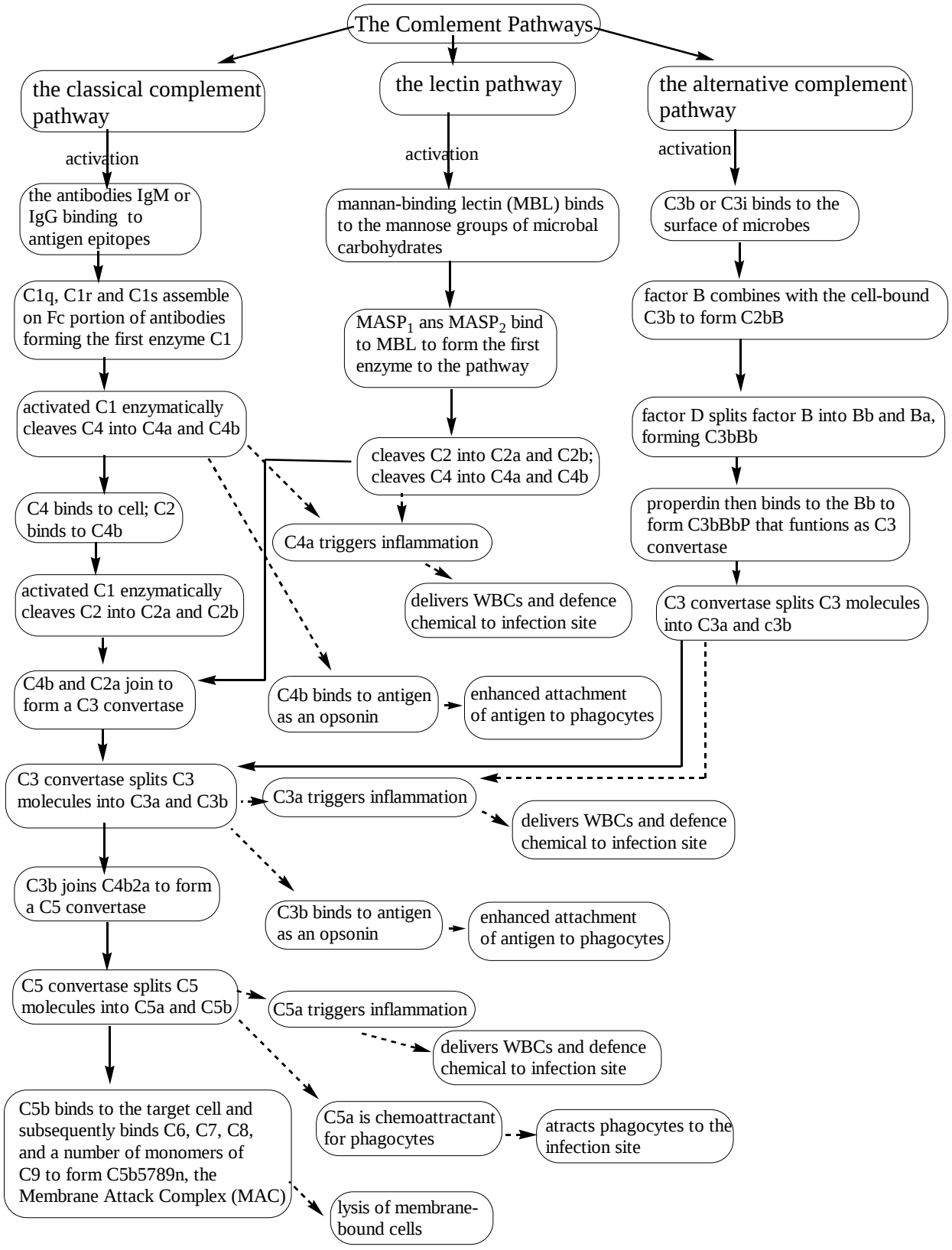


Figure 15.4 Complement system

In all cases an activation of C3-component leads to the removal of protease C3b, which activates terminal components of complement system (C5→C9)

leading to the formation of the ***membrane attack complex.*** C3b joints with C3 convertase (C4b2a complex) to make C5 convertase (C4b2a3b complex). C5 convertase cleaves C5 into C5a and C5b. Fragment C5b is fixated on the surface of foreign cell and forms complex C6, which joins C7, C8 and C9.

Regulation of the complement system. The complement system has the potential to be extremely damaging to host tissues, meaning its activation must be tightly regulated. The complement system is regulated by complement control proteins, which are present at a higher concentration in the blood plasma than the complement proteins themselves. Some complement control proteins are present on the membranes of self-cells preventing them from being targeted by complement. One example is CD59, also known as proteictin, which inhibits C9 polymerization during the formation of the membrane attack complex. The classical pathway is inhibited by C1-inhibitor, which binds to C1 to prevent its activation.

- ***Kallikrein – kinin*** system performs nonspecific designation of pathogens by means of Hageman factor. Factor XIIa converts prekallikrein into kallikrein. Kallikrein converts *kininogen* into *bradykinin.*

Bradykinin is the effector component of kallikrein – kinin system. It is able to cause local vasodilation and increasing vessel permeability. In addition to this bradykinin facilitates the liberation of neuromediator substance P.

- ***Proteins of acute phase:***

- ceruloplasmin effectively binds copper;
- haptoglobin performs the binding Hb;
- α_1 – antitrypsin
- α_2 – macroglobulin

} are inhibitors of proteinases

- fibrinogen forms fibrin thromb and prevents fast propagation of inflammatory process;
- mannose-binding lectin (MBL) interacts with mannose residues on the pathogen surface.

- ***Cytokines*** are soluble mediators of the inflammatory and immune response. They are produced by a variety of cells and tissues, are peptides or glycoproteins in nature, are active at concentrations between 10^{-9} – 10^{-15} molar. The majority have paracrine and autocrine effects. A few, however, are capable of acting on cells distant to their site of production. They show significant overlap in their functions. The cell types they act on may be multiple (pleiotropy).

Cytokines can be grouped into families:

- *Interferons (IFNs).* While IFN-α and IFN-β have role in protection against viral replication, IFN- γ play a significant role in regulation the specific immune response.
- *Interleukins (IL).* Currently there are 18 interleukins recognized, all of which participate in regulating the cells involved with both *nonspecific immune response and specific immune response.*
- *Chemokines* are a relatively recently described family of mediators that bring about chemokinesis – movement in response to chemical stimuli.

Cytokines are, however, more practically grouped by their *principal effect:*

- *proinflammatoty cytokines*: tumor necrosis factor (TNF)-α; IL-1β, IL-6; IL-8; IL-2; IL-15;
- *antinflammatory* cytokines: transforming growth factor (TGF)- β; IL-10;
- *immunostimulatory* cytokines: for cellular response – IL-2 and IL-8; for humoral, including allergic, responses – IL-4; IL-13; TNF-β and IL-10.

Table 15.1 Functions of basic interleukines

Interleukins	Cells of synthesis	Effects
IL-1	Macrophages; fibroblasts; keratinocytes; glial cells; endothelial cells.	It plays key role in regulation of immune competent cells and functional interaction between immune and neuroendocrine systems; stimulates a proliferation of T- and B- lymphocytes; is potent

		activator of inflammation; increases the synthesis of acute phase proteins in liver; stimulates the synthesis of prostaglandins and leukotrienes.
IL-2	T-helpers 1.	IL-2 stimulates the proliferation of T-lymphocytes and their differentiation to T-killers. It stimulates proliferation and differentiation of B-lymphocytes; enhances the function of monocytes and NK-cells; induces biosynthesis of some cytokines.
IL-3	Activated T-helpers.	It stimulates proliferation of stem hemopoietic cells.
IL-4	T-helpers 2, mast cells, basophils.	It stimulates proliferation of B-cells, inhibits cytotoxic activity of macrophages and T-lymphocytes; has antitumor activity.
IL-5	T-helpers 2, macrophages, monocytes.	It influences the differentiation and physiologic activity of eosinophils
IL-6	Different cells of immune system and connective tissue.	It induces growth and differentiation of B-lymphocytes, proliferation and differentiation of stem cells, stimulates the production of acute phase proteins, increases the expression of inflammatory process.
IL-7	T-lymphocytes, different cells of immune system and bone marrow.	It regulates proliferation and differentiation both T- and B-lymphocytes.
IL-8	T-lymphocytes, fibroblasts, monocytes.	It activates chemotaxis of neutrophils.
IL-9	T- lymphocytes.	It is believed that IL-9 is one of the

		apoptosis regulators. It has pleiotropic activity to different cell types.
IL-10	T-helpers, monocytes.	It suppresses the function of some populations of T-helpers and production of interferons and proinflammatory cytokines. It is potent antiinflammatory agent.
IL-11	Bone marrow stroma cells, fibroblasts.	It has pleiotropic action on different types of cells.
IL-12	Monocyte – macrophage system.	It increases activity of NK-cells, production of IFN and differentiation of T-helpers 1, but suppresses proliferation of T-helpers 2.
IL-13	Activated T-helpers.	It induces the synthesis of IgE, inhibits production of proinflammatory cytokines by monocytes (IL-1, IL-6, IL-8).
IL-14	T- lymphocytes.	It stimulates growth and differentiation of B-lymphocytes.
IL-15	Mononuclears of peripheric, blood, epithelial cells.	It stimulates the generation and differentiation of T-lymphocytes.
IL-16	T- lymphocytes.	Chemoattractant for lymphocytes, monocytes, eosinophils, macrophages.
IL-17	T-helpers.	It stimulates the secretion of IL-6, IL-8, granulocyte colony-stimulating factor.
IL-18	Monocytes, macrophages.	It increases the production of IFN-γ and enhances the activity of NK-cells.

Tumor necrosis factors (TNFs)

- *TNF-α* is produced by monocytes and macrophages, induces the synthesis of IL-1 and IFN-γ, has cytotoxic and cytostatic action.

TNF-β is produced by activated T-lymphocytes, plays the important role in antitumor and antiviral immunity.

Colony-stimulating factors are produced by activated T-lymphocytes, monocytes, fibroblasts, endothelial cells. They stimulate growth and differentiation of hemopoietic cells.

Transforming growth factors (TGFs) are produced by different classes of lymphocytes, platelets, placenta, some tumors. They stimulate the proliferation of fibroblasts, synthesis of collagen and fibronectin; take part in angiogenesis and wound reparation. They suppress the proliferation of T- and B-lymphocytes, activity of cytotoxic and killer cells.

Eicosanoids (prostaglandins and leukotrienes) are metabolites of arachidonic acid. They participate in inflammatory processes. Leukotrienes are initially formed in inflammatory processes. Synthesis of prostaglandins is activated later.

15.2 The Acquired (Adaptive) Immune System

The specific mechanisms of immunity are ensured by lymphocytes that create:

- specific humoral immunity;
- cellular immunity.

This immunity is provided by B-lymphocytes and T- lymphocytes.

Lymphocytes make up 20-40% of leukocytes. As distinct from all other leukocytes they don’t only penetrate through the tissues but can also return into the blood. Their life span is 20 and more years. Lymphocytes are the major cells of the specific immune system.

T-lymphocytes mature in the thymus gland; they can remain in the thymus, circulate in the blood, or reside in lymphoid organs such as the lymph nodes and spleen. *T-lymphocytes* are major effectors of cellular immunity. They take part in complex reaction of regulation of B- lymphocytes activity. They are divided into:

a) T-helpers. They induce proliferation, transformation and differentiation of B-lymphocytes into plasma cells.

b) T-suppressors are lymphocytes, which suppress the development of immune response, particularly, formation of antibodies.

c) T-killers are lymphocytes, which produce substances initiating the inflammatory reaction or destroy cells containing antigens.

B-lymphocytes reach maturity within the bone marrow, circulate in the blood, and also settle in various lymphoid organs.

B-lymphocytes are effectors of humoral immunity. They are precursors of *plasma cells (plasmacytes),* which produce antibodies.

15.2.1 Antibodies

Antibodies are soluble antigen receptors, which are formed by activated B cells (*plasma cells*) and released into the blood. Antibodies are an important part of the humoral immune defense system. They have no antimicrobial properties themselves, but support the cellular immune system in various ways:

- They bind to antigens on the surface of pathogens and thereby prevent them from interacting with body cells (***neutralization***).
- They link single-celled pathogens into aggregates (immune complexes), which are more easily taken up by phagocytes (***agglutination***).
- They activate the complement and thereby promote the innate immune defense system (***opsonization***).

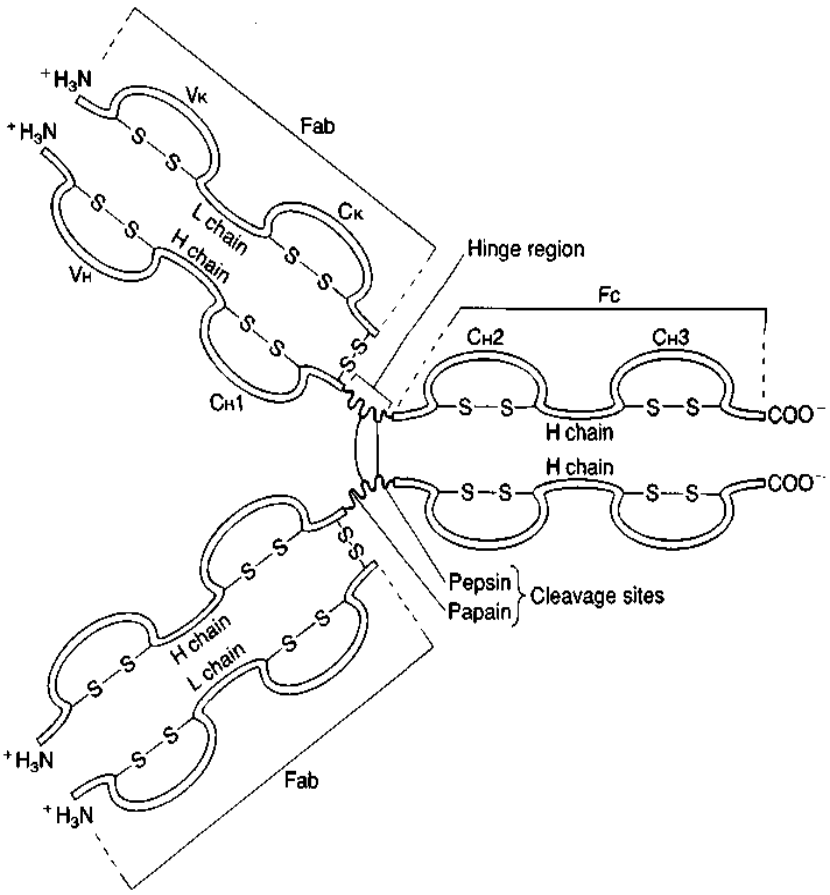


Figure 15.5 Simplified model for an IgG1 (κ) human antibody molecule showing the basic four-chain structure and domains (V_H, C_H, etc). V indicates the variable region. C indicates the constant region. Sites of enzyme cleavage by pepsin and papain are shown.

IgG, IgD and IgE are tetramers with the structure H_2L_2. By contrast, soluble IgA an IgM are multimers that are held together by disulfide bonds and additional J peptides (joining peptides).

Basic properties of antibodies:

1. *Specificity* is the ability to react only with one of antigens.
2. *Valency* is the capacity to simultaneous interaction with determinate amount of antigen determinants.
3. *Affinity* to specific antigen determinant.
4. *Avidity* – power of interaction with specific antigen determinant.

The antibodies have different tasks. *IgGs* and *IgMs* are basic classes of immunoglobulins. *IgMs* are the first immunoglobulins formed after contact with a foreign antigen. Their early forms are located on the surface of *B cells* while the later forms are secreted from *plasma cells* as pentamers.

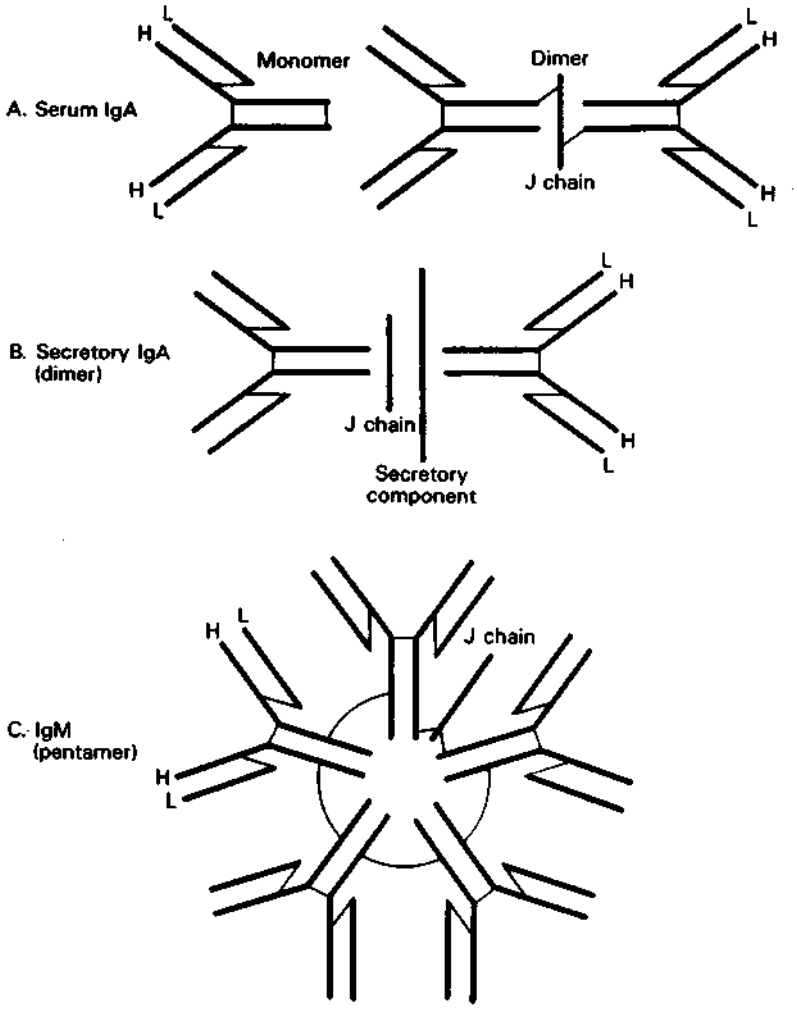


Figure 15.6 Highly schematic illustration of polymeric human immunoglobulins. Polypeptide chains are represented by thick lines; disulfide bonds linking different polypeptide chains are represented by thin lines.

Quantitatively, IgGs are the most important immunoglobulins. They occur in the blood and interstitial fluid. As they can pass the placenta with the help of receptors, they can be transferred from mother to fetus.

IgAs mainly occur in the intestinal tract and in body secretions.

IgEs are found in low concentration in the blood. As they can trigger degranulation of mast cells, they play an important role in allergic reactions.

The function of IgDs is still unexplained. Their plasma concentration is also very low.

15.2.2 Mechanisms Providing Variability of Antibodies

The wide ranges of immunoglobulins are produced by genetic recombination and additional mutations during the development and maturation of individual lymphocytes.

It is estimated that more than 10^8 different antibody variants occur in every human being. This variability affects both the heavy and the light chains of immunoglobulins.

There are three reasons for the extremely wide *variability* of antibodies:

1. *Multiple genes.* Various genes are available to code for the variable protein domains. Only one gene from among these is selected and expressed.
2. *Somatic recombination.* The genes are divided into several segments, of which there are various versions. Various combinations of the segments during lymphocyte maturation give rise to randomly combined new genes ("mosaic genes").
3. *Somatic mutation.* During differentiation of B cells into plasma cells, the coding genes mutate. In this way the "primordial" *germline genes* can become different *somatic* genes in the individual B cell clones.

There are three families of genes responsible for immunoglobulin molecule structure. Two families are responsible for the light chains (λ and κ chains) and one family for heavy chains. Each ***light chain*** is encoded by three distinct segments: the variable (V_L), the joining (J_L), and the constant (C_L) segments. For λ and κ light chains, there are approximately 300 variable (V_L) segments and five or six J_L segments. Lambda light chains are derived from fewer than ten C_L regions, whereas kappa light chains come from a single C_L segment. During the differentiations of a lymphoid B cell, a V_L segment is brought from a distant site on the same chromosome to a position closer to the region of the genome containing the J_L and C_L segments. This *DNA rearrangement* then allows the V_L, J_L and C_L segments to be transcribed as a single mRNA precursor and subsequently processed to generate the mRNA for a specific antibody light chain.

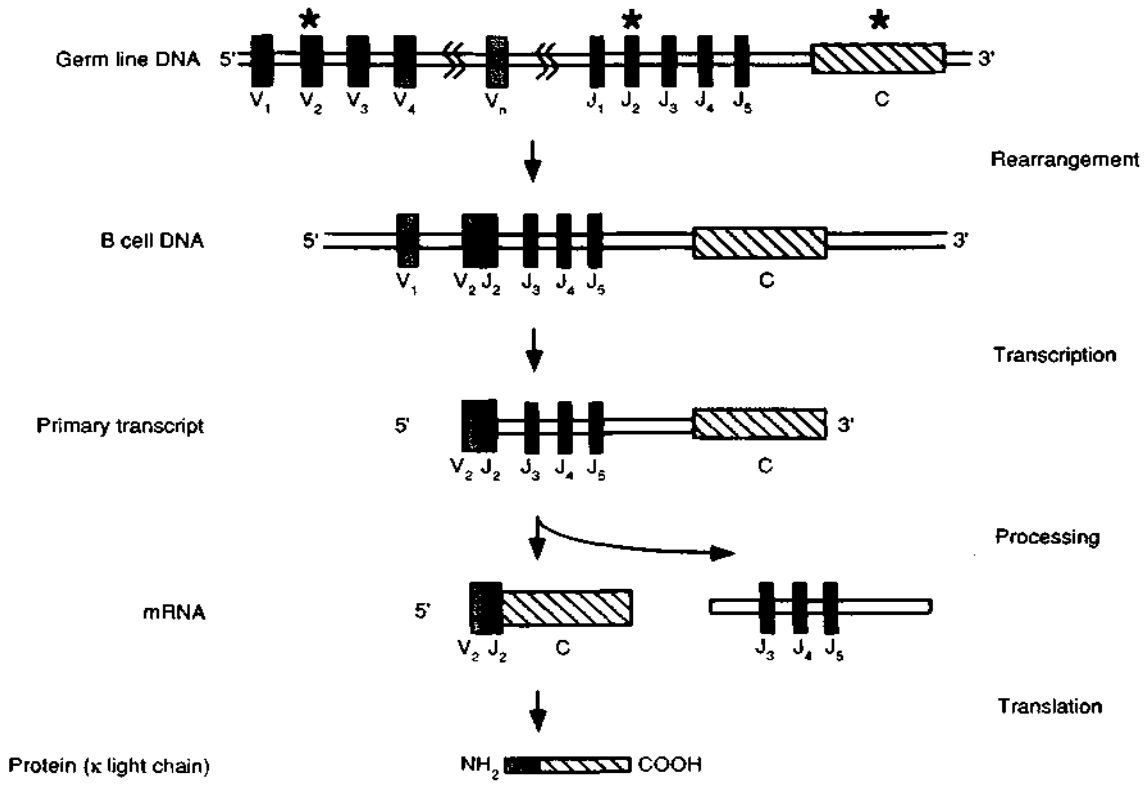


Figure 15.7 Recombination events leading to a V_2J_2 κ light chain.

The heavy chain is encoded by four gene segments: the V_H, the D (diversity), the J_H, and C_H DNA segments. In humans there are about 1000V_H segments, 12 or more D segments and four J segments. The variable region of heavy chain is generated by joining the V_H with D and J_H segments. The resulting V_H- D-J_H DNA region is in turn linked to a C_H segment of which there are nine. These C_H segments determine the class of immunoglobulin molecules.

15.3 Simplified Diagram of Immune Response

- Pathogens that have entered the body - e.g. viruses – are taken up by antigen – presenting cells (e.g., macrophages) and proteolytically degraded. ***Antigen-presenting cells*** (APCs) include: B-lymphocytes, macrophages and dendritic cells. The viral fragments produced in this way are then presented on the surfaces of these cells with the help of special membrane proteins (MHC proteins). The MHC proteins are named after the “major histocompatibility complex” – DNA segment that codes for them. Human MHC proteins are also known as HLA antigens (“human leukocyte – associated” antigens).

Their polymorphism is so large that it is unlikely that any two individuals carry the same set of MHC proteins, except for monozygotic twins. C*lass I MHC* proteins occur in almost all nucleated cells. They mainly interact cytotoxic T cells and are the reason for the rejection of transplanted organs.

Class II MHC proteins are found on all antigen-presenting cells in the immune system.

- The complexes of MHC proteins and viral fragments displayed on APCs are recognized by T cells. Binding leads to activation of T cell and its selective replication.

The proliferation of immune cells is stimulated by interleukins. For example, activated macrophages release IL-1, while T cells stimulate their own replication and that of other immune cells by releasing IL-2.

- Depending on their type, activated T cells have different functions. Cytotoxic ones are able to recognize and bind virus infected body cells or tumor cells. They then drive the infected cells into apoptosis or kill them with perforin. B lymphocytes, which as APCs present viral fragments on their surfaces, are recognized by *helper T cell.* Stimulated by interleukins, selective cloned replication of B cells then takes place. B cells carry antigen receptors matching those of the pathogen. They mature into plasma cells and finally secrete large amounts of soluble antibodies.

- Immunoglobulin M, a membrane protein of the surface of B lymphocytes, serves to bind free antigens to the B cells.

By contrast, *T cell receptors* only bind antigens, when they are presented by another cell as a complex with an MHC protein.

Interaction between MHC – bound antigens and T cell receptors is supported by co-receptors. This group includes *CD8,* a membrane protein that is typical in cytotoxic T cells. T helper cells use *CD4* as a coreceptor.

The abbrevation “CD” stands for “cluster of differentiation”. It is the term for a large group of proteins that are located on the cell surface and therefore can be identified by antibodies.

15.4 Disturbances of Immune System

Disorders of immune system fall into four main categories:

- Immunodeficiency disorders (primary or acquired)
- Autoimmune disorders (in which the body's own immune system attacks its owntissues as foreign matter)
- Allergic disorders (in which the immune system overreacts in response to an antigen)
- Cancers of the immune system.

Immunodeficiency states are disbalances of functioning some chains of cellular or humoral immunity.

The primary (inherited) and the *secondary (acquired) immunodeficiencies* are distinguished.

The primary immunodeficiencies are developed as result of disturbances of corresponding genome parts which are responsible for the realization of T- and B-system of immunity.

The primary immunodeficiencies are divided into:

1. B- cells insufficiency.
2. T- cells insufficiency.
3. The primary defects of phagocytosis.
4. Pathology of complement system.

Examples of the primary immunodeficiencies

Bruton's disease (agammaglobulinemia) is recessive disease linked with X – chromosome. It is characterized by sharply decreased activity of antibacterial immunity. The patients have considerably decreasing IgA and IgM (100 times as comparison to norme) and 10 fold decrease of IgG. Plasma cells are not found.

Disgammaglobulinemia is characterized by deficiency of one of the classes of immunoglobulins.

Loui-Barr's syndrome is manifested by neurologic disorders and pathologic vasodilation of conjunction and skin. Patients have decreased activity of cell immunity reactions, the absence of IgA and low level of IgG.

Di-George syndrome is inherited hypoplazia of thymus. It is an example of a primary T-lymphocyte disease. The thymus gland is where T-lymphocytes normally mature.

Chediak-Higashi syndrome and chronic *granulomatous disease* both involve the inability of neutrophils to function normally as phagocytes.

The secondary immunodeficiency states are due to the influence of the lymphotropic viral infections (acquired immunodeficiency syndrome), toxic factors, ionizing radiation.

HIV (human immunodeficiency virus) infection/AIDS (acquared immunodeficiency syndrome) is a disease that slowly and steadly destroys the immune system. It is caused by HIV, a virus that wipes out a certain types of lymphocytes called T-helper cells. Without T-helper cells, the immune system is unable to defend the body against normally harmless organisms, which can cause life-threatening infections in people who have AIDS. Newborns can get HIV infection from their mothers while in the uterus, during the birth process, or during breastfeeding. People can get HIV infection by having unprotected sexual intercourse with an infected person or from sharing contaminated needles for drugs, steroids or tattoo.

Immunodeficiencies caused by medications. Some medicines suppress the immune system. One of the drawbacks of chemotherapy treatment for cancer, for example, is that it not only attacks cancer cells, but other fast-growing, healthy cells, including those found in the bone marrow and other parts of the immune system. In addition, people with autoimmune disorders or who have had organ transplants may need to take immunosuppressant medications, which also can reduce the immune system’s ability to fight infections and can cause secondary immunodeficiency.

Autoimmune disorders. In autoimmune disorders, the immune system mistakenly attacks the body’s healthy organs and tissues as thouth they were foreign invaders. Autoimmune diseases include:

Lupus, a chronic disease marked by muscle and joint pain and inflammation (the abnormal immune response also may involve attacks on the kidney and other organs).

Juvenile rheumatoid arthritis, a disease in which the body's immune system acts as though certain body parts (such as the joints of the knee, hand and foot) are foreign tissues and attacks them.

Scleroderma, a chronic autoimmune disease that can lead to inflammation and damage of the skin, joints, and internal organs.

Ankylosing spondylitis, a disease that involves inflammation of the spine and joints, causing stiffness and pain.

Juvenile dermatomyositis, a disorder marked by inflammation and damage of the skin and muscles.

Allergic disorders occur when the immune system overacts to exposure to antigens in the environment. The substances that provoke such attacks are called allergens. The immune response can cause symptoms such as swelling, watery eyes, and sneezing, and even a life-threataning reaction called anaphylaxis. Medications called antihistamines can relieve most symptoms.

Allergic disorders include:

Asthma, a respiratory disorder that can cause breathing problems, frequently involves an allergic response by the lungs. If the lungs are oversensitive to certain allergens (like pollen, molds, animal dander, or dust mites), it can trigger breathing tubes in the lungs to become narrowed, leading to reduced airflow and making it hard for a person to breathe.

Eczema is an itchy rash also known as *atopic dermatitis*. Although atopic dermatitis is not necessarily caused by allergic reaction, it more often occurs in children and teens who have allergies, hay fever, or asthme or who have a family history of these conditions.

Allergies of several type scan occur in children and teens. Environmental allergies (to dust mites, for example), seasonal allergies (such as hay fever), drug allergies (reaction to specific medications or drugs), food allergies (such as to

nuts), and allergies to toxins (bee stings, for example) are the common conditions people usually refer to as allergies.

Cancers of the immune system. Cancer occurs when cells grow out of co0ntrol. This also can happen with the cells of the immune system. Lymphoma involves the lymphoid tissues and is one of the more common childhood cancers. Leukemia, which involves abnormal overgrowth of leukocytes, is the most common childhood cancer.

Disorders of immunoglobulins include increased production of specific classes of immunoglobulins or even specific immunoglobulin molecules.

Tests for Self-control

1. The examination of several classes of immunoglobulins in newborns can be used as diagnostic test to verify the fetal infection. Which class of immunoglobulins can pass through placenta?

A. Ig M
B. Ig A
C. Ig G
D. Ig E
E. Ig D

2. Immune reactions of organism are provided by high-specific interaction “antigen-antibody”. Such specificity of immunoglobulins depends on their molecular structure. Immunoglobulins are:

A. Lipoproteins
B. Metalloproteins
C. Chromoproteins
D. Glycoproteins
E. Nucleoproteins

3. Immune system by means of cellular and humoral mechanisms provides the distinguishing, binding and destroying of antigens. The main classes of blood immunoglobulines, which realize humoral immune response, are:

A. Ig A and Ig E
B. Ig G and Ig M
C. Ig D and Ig A
D. Ig A and Ig M
E. Ig E and Ig D

4. In fever development the increase of “acute phase” proteins (ceruloplasmin, fibrinogen, C-reactive protein) is characteristic. Which mechanism of this is possible?

A. Proliferate action of IL-2 to T-lymphocytes
B. Damage action of temperature to organism cells
C. Degranulation of tissue basophils
D. Stimulating influence of IL-1 to hepatocytes

5. Factors of nonspecific immunity are:

A. Complement system
B. Interferon
C. Lysozyme
D. All above mentioned
E. Nothing from above mentioned

6. Immunoglobulins are synthesized by:

A. T-lymphocytes
B. Neutrophyls
C. Plasmacytes
D. Macrophages
E. All the above mentioned

7. Complement can combine:
A. IgM and IgG
B. IgA
C. IgD
D. IgE
E. Nothing from above mentioned
8. IgA takes part in the following reactions:
A. Local immunity
B. Bacteria neutralizing
C. Comlement binding
D. Local immunity and bacteria neutralizing
E. All the above mentioned
9. IgE takes part in following reactions:
A. Local immunity
B. Allergy reactions
C. Comlement binding
D. Primary immune responce
E. All the above mentioned
10. Plasmacytes are formed from:
A. B-lymphocytes
B. T-lymphocytes
C. Macrophages
D. Fibroblasts
E. Nothing from above mentioned

Chapter 16 BIOCHEMISTRY OF KIDNEY. METABOLISM OF WATER AND MINERALS

16.1 Biochemistry of Kidney

In the adult human organism the mass of the two kidneys is about 300g. The primary function of the kidney is to provide for a constancy of the internal medium of the organism.

Two zones are distinguished in the renal tissue:

- the outer, or cortical, zone colored brown-red;
- the inner, or medullary, zone colored lilac-red.

The basic functional unit of renal parenchyma is the nephron. In humans, the two kidneys number about 2 million nephrons.

The nephron of mammals consists of:

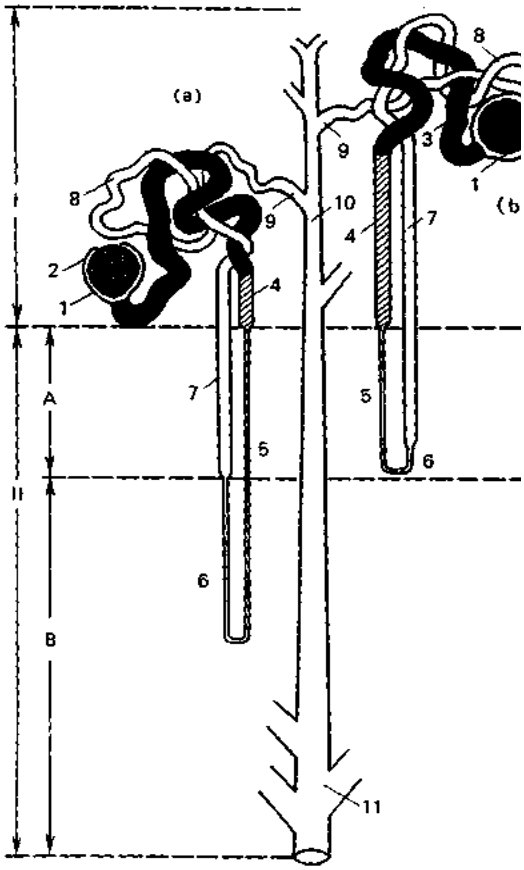


Figure 16.1 Schematic representation of the structure and topography of juxtamedullar (a) and cortical (b) nephrons: I- cortical tissue; II – medullary tissue; A- outer zone of medullary tissue; B – inner zone of medullary tissue; 1-glomerulus; 2-Bowman's capsule; 3-proximal convoluted tubule; 4-proximal straight tubule; 5-descending thin limb of the loop of Henle; 6-ascending thin limb of the loop of Henle; 7- ascending thick limb of the loop of Henle; 8- distal convoluted tubule; 9- junctional tubule; 10 – collecting duct; 11- papillary duct (of Bellini)

In the nephron three major processes occur:

-filtration at the glomerulus;

-tubular reabsorption;

-tubular secretion.

16.1.1 Glomerular Filtration

- Glomerular filtration is a passive process.
- The total renal blood flow is about 1300 ml/min in adult human males.
- In health, the mean filtration rate is 125 ml/min.
- The filtration rate is determined by the filtration pressure:

FP=CP - (OP+Caps P),

where FP - filtration pressure

CP – capillary pressure

OP – oncotic pressure

Caps P – intracapsular pressure.

- In health FP ~ 30 mm Hg.
- Capillary pressure within the kidney is dependent not so much on the arterial pressure as on the **lumen ratio** of the ″afferent″ and ″efferent″ glomerular arterioles. The efferent arteriole is narrower (by about 30% in diameter) than the afferent arteriole. The lumen ratio regulation for these arterioles is effected primarily by **the kinin system.**
- The primary urine, practically devoid of proteins, is produced by ultrafiltration of the blood plasma into the lumen of Bowman's capsule. In health, proteins as colloid particles are incapable of penetrating the capsular cavity of the glomerulus through the capillary wall. Approximately 180 L of primary urine is produced. The pores in the glomerular basal membrane, which are made up of type IV collagen, have an effective mean diameter of 2,9 nm. This allows all plasma components with a molecular mass of up to about 15kDa to pass through membrane. At increasing masses, molecules are progressively held back; at masses greater than 65 kDa, they are completely enable to enter the primary urine. This

applies to almost all plasma proteins – which in addition, being anions, are repelled by the negative charge in the basal membrane.

16.1.2 Reabsorption and Secretion

- Only 1% of the total fluid, filtrated in the glomerulus, is converted into urine.
- 99% of water, sodium chloride, hydrocarbonate ions, amino acids, 93% of potassium ions and 45% of urea are reabsorbed in the renal tubules.
- The cells of the proximal segment of the nephron reabsorb glucose, amino acids, vitamins and electrolytes, 6/7 of the fluid constitutive of the primary urine is also subject to reabsorption in the proximal tubules. In the tubule, organic substances (e.g., glucose, amino acids, lactate and ketone bodies) are recovered by secondary active transport. There are several group-specific transport systems for resorbing amino acids, with which hereditary diseases can be associated (eg, *cystinuria*, *glycinuria* and *Hartnup's disease*).
- Additional regulated reabsorption of water, Na^+ and Cl^- occurs in the distal tubules. These processes are controlled by hormones (*aldosterone*, *vasopressin*).
- In the distal tubules potassium, ammonium, hydrogen ions may be secreted into the lumen of the nephron.
- Renal clearance is used as a quantitative measure of renal function. It is defined as the plasma volume cleared of a given substance per unit of time. *Inulin*, a fructose polysaccharide with a mass of 6 kDa that is neither actively excreted nor reabsorbed but it freely filtered, has a clearanse of 120 mL/min in healthy individuals.
- Sodium ions penetrate from the tubular lumen into the cell by passive transport and they are transported from cells to the extracellular fluid by means of Na^+-pump. About 80% of the ATP energy in channel's cells is spend on the Na^+- pump.
- The water uptake in the *proximal* segments is effected passively, assisted by the active absorption of sodium ions.

16.1.3 Functions of Kidney

They include:

- the regulation of water and salt balance;

- the maintenance of acid-base balance;
- the maintenance of osmotic pressure;
- the removal of final products of metabolism;
- metabolic function;
- hormonal function.

Metabolic function. Specific features of renal tissue metabolism. Kidney uses at least 8-10% of the total oxygen consumed by the human organism.

Concentrating urine and transporting it through membranes are processes that require large amounts of energy. The kidneys therefore have very high energy demands.

In the proximal tubule, the ATP needed is obtained from oxidative metabolism of *fatty acids, ketone bodies,* and several *amino acids*. To a lesser extent, lactate and glycerol are also used. In the distal tubule and Henle's loop, *glucose* is the main substrate for the energy metabolism.

The endothelial cells in the proximal tubule are also capable of *gluconeogenesis.* The substrates for this are mainly carbon skeletons of amino acids. Their amino group is used as ammonia for buffering urine.

Enzymes for peptide degradation and the amino acid metabolism occur in the kidneys at high levels of activity (e.g., amino acid oxidases, amine oxidases, glutaminase).

The first stage of creatine synthesis is performed in the renal and pancreatic tissues. Glycine amidinotransferase (or arginine-glycine transamidinase) catalyzes this reaction. ***The observation*** of this enzyme in the blood may be linked either to a renal disease or to pancreonecrosis.

Hydroxylation of 25-hydroxycholecalciferol occurs in kidney.

Hormonal function. The kidney plays an important role as an incretory (endocrine) organ.

The juxtaglomerular cells, located in the region of the vascular pole of the glomerulus, produce rennin. Rennin, through the agency (see water-salt

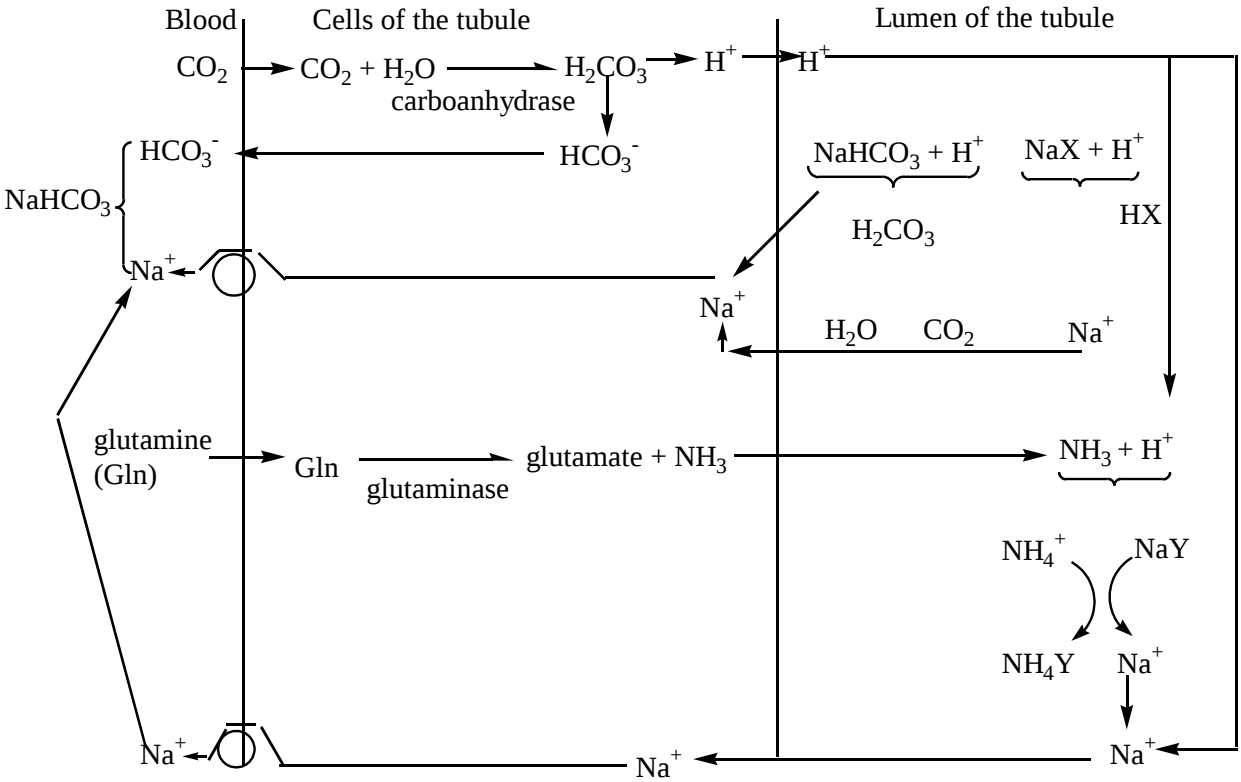
metabolism) of angiotensin, exerts influence on the blood pressure of the whole organism, and on the production of aldosterone and ADH.

The kidney also elaborates *erythropoietin* which stimulates the red blood cell production (erythropoiesis). Erythropoietin is a glycoprotein hormone. Its biosynthesis in the kidney is activated in a number of stress states – hypoxia, loss of blood, shock etc.

The kidney produces prostaglandins, which are capable of influencing the responsiveness of the renal cells to the action of certain hormones.

Role of kidney in maintenance of osmotic pressure is provided by means of rennin-*angiotensin* system (see "Regulation water salt metabolism"). In addition to this, kidney is the target of aldosterone and ADH action.

Renal mechanisms for maintaining the acid-base balance



3 main mechanisms:

1. Secretion of hydrogen ions; and formation and conversion of carbonic acid.

2. Sodium ions reabsorption and conversion of disubstituted phosphates to monosubstituted phosphates. In blood monosubstituted-to-disubstituted phosphate ratio is 1:4; in glomerular filtrate 9:1; in urine of the distal segment of the nephron 50:1.

$\underline{NaH_2PO_4}$ = $\underline{1}$ (blood) = $\underline{9}$ (glomerular filtrate) = $\underline{50}$ (urine)
Na_2HPO_4 4 1 1

3. Renal production of ammonia and its use instead of other cation for neutralization of acid equivalents and their urinary discharge.

16.1.4 Physico-chemical Properties of Urine

Daily urine (diurnal diuresis): 1000 – 2000 ml (1500 ml)

Pathologic state: < 500ml and > 2000 ml

Polyuria (increased excretion of daily urine):

- after large fluid intake;
- after dietary intake of nutrients stimulating diuresis (eg. water melon and pumpkin).

Pathologic polyuria:

- in renal diseases (chronic nephritides and pyelonephritides),
- diabetes mellitus,
- diabetes insipidus (up to 15 liters).

Oligouria (diminished excretion of daily urine):

- in insufficient fluid intake,
- in febrile state,
- vomiting,
- toxicosis,
- acute nephritis.

Anuria is complete or nearly complete suppression of urinary excretion:

- in severely affected renal parenchyma,
- ureteral obstruction.

In health, the urinary excretion is larger in the day time than at night. The diuretic day-to-night ratio varies from 4:1 to 3:1. In certain pathologic states (early stages of cardiac decompensation, cystopielitis) the passage of urine is larger at night than during the day. This state is known as *nycturia*.

Color. Normal urine is yellow. *Colorless* urine is under diabetes insipidus, excessive drinking, taking diuretics. *Red ("meat slops")* color is caused by

hematuria. Hematuria can be renal (as symptom of glomerulonephritis, trauma, nephrolytiasis) and extrarenal (under cystitis and uretritis). Red color of urine is also observed at porphyria and hemoglobinuria. *Orange* color is observed after taking several vitamins. *Red-violet* color occurs after beet eating. *Green* color is the sign of increasing putrefaction processes in intestine, after ingestion of rhubarb. *Color of dark beer* is examined under hepatitis. *Blue* color is observed after ingestion of methylene blue or as a sign of Hartnup disease. *Black* urine is present in patients with alkaptonuria.

***Odour*.** Normal urine shows the characteristic smell or smell of "meat broth". *Ammonia* odour occurs under hyperammoniemia. *Aceton* smell is observed in diseases which are accompanied by accumulation of ketone bodies. *"Mouse or mould"* smell shows phenylketonuria. *"Maple syrup"* or *"beer yeasts"* smell is the sign of leucinosis (maple syrup urine disease). *"Cabbage"* or *"cat's urine"* smell occurs under tyrosinemia. The smell of *rotted fish* is present under trimethylglycinuria or dymethylaminuria. *"Dirty socks"* odour occurs under isovaleric aciduria.

***Transparency*.** Normal urine is transparent. *Muddy* urine is observed at pyuria, hematuria, proteinuria, crystalluria, bacteriuria.

Density. The relative urine density in the adult human is liable to diurnal variation within a fairly wide range **(from 1,002 to 1,035).** Most commonly the urine density ranges from **1,017 to 1,020**. *Low density:* in diabetes insipidus. *High density:* in acute nephritis, in diabetes mellitus.

Isostenuria – urine density is equal to that of the primary urine, or ultrafiltrate (~1,010). It is observed only in a severe renal insufficiency. The isostenuria indicates a disturbed concentration function of the kidney. This state has been recorded in chronic nephrites, "contracted kidney".

***pH*.** The urine usually has a slightly acidic pH value (pH5,3 – 6,5). However, the pH value is strongly affected by metabolic status. After ingestion of large amount of plant food, it can increase to over 7. In meat rich diet it is acidic.

The decrease of pH is observed in febrile states; diabetes mellitus; in starvation

The increase of pH is observed in cystitis, pyelitis, curative intake of alkaline mineral water.

16.1.5 Chemical Composition of Urine under Normal and Pathologic Conditions

The dense substances of the urine (~ 60g) are represented by both organic and inorganic components.

Organic components

Urea: about 30g per day (80 – 90% of total amount of nitrogen). Urea content increases in urine in protein–rich food, febrile state, tumor, hyperthyroidism, diabetes mellitus. Urea content decreases in urine in severe affected liver, in renal diseases.

Creatinine. For each individual, the daily excretion of creatinine is a fairly constant parameter primarily reflective of the organisms muscular mass. The normal content of creatinine in the urine: men – 8,8 – 17,7 mmol/day; women 7,1 – 15,9 mmol/day. Increased amount of creatinine in the urine is observed: in meat diet, syndrome of prolonged crushing, smashing of soft tissues, hard muscle work, fever, pneumonia. Decreased content of creatinine in the urine is observed in chronic nephritis with uremia, muscle atrophy, kidney degeneration, leukemia, senile age. Endogenous creatinine clearance is used to examine for impairment of glomerular filtration. At normal levels of creatinine, this metabolite is filtered at the glomerulus but neither secreted nor reabsorbed by the tubules. Hence its clearance gives the glomerular filtration rate.

Amino acids **(**about 1,1g). The amount of amino acids excreted in free form is strongly dependent on the diet and on the efficiency of liver function. Amino acid derivative are also found in the urine: *hippuric acid*, a detoxification product of benzoic acid (0,7g/day); *indican*, a detoxification product of indol (47-56 μmol/ day).

Uric acid. Normal value is *1,6 – 4,16 mmol/day*. Its content in the urine increases in *leukemia, gout, hepatitis, after therapy by certain steroid hormones.*

The main **inorganic components** of the urine are *cations* Na^+, K^+, Ca^{2+}, Mg^{2+} and NH_4^+ and *anions* Cl^-, SO_4^{2-}, and phosphate as well as traces of other ions. In total, Na^+ and Cl^- represent about two – thirds of all the electrolytes in the final urine. Calcium and magnesium occur in the feces in even larger quantities. The amount of the various inorganic components of the urine also depends on the composition of the diet. For example, in acidosis there can be a marked increase in the excretion of ammonia (normal value – 30-60 mmol/day). Excretion of Na^+, K^+, Ca^{2+}, Cl^-, phosphate via the kidneys is subject to hormonal regulation. Shift in the concentrations of the *physiological components* of the urine and the appearance of *pathological urine components* can be used to diagnose diseases.

Pathologic components of urine occur in the normal urine at a very low level escaping their determination by routine analytical techniques. These compounds are: proteins, glucose, acetone (ketone bodies), bile pigments, blood, creatine.

Proteinuria
- **renal** is observed in organic lesion of the nephron; in increased size of the pores of the glomerular filter; reduced blood flow rate in the glomeruli;
- **extrarenal** is associated with lesioned urinary tracts, with affected prostate.

Blood
- **hematuria** (in the form of red blood cells)
  - **renal** (main symptom of acute nephritis)
  - **extrarenal** (in inflammation or traumatism of the urinary tract)
- **hemoglobinuria** (as dissolved blood pigment)

Glucosuria may be alimentary and it is also observed in emotional stress; in diabetes mellitus; in hyperfunction of adrenal cortex; in renal diseases. Congenital fructosuria, congenital galactosuria (see: carbohydrate metabolism disturbances).

Ketonuria is observed: in diabetes mellitus (>150g/day); in starvation; in thyrotoxicosis; subarachnoidal hemorrhage; craniocerebral injury; infectious diseases.

Bilirubinuria is observed**:** in obstructive jaundices; in hepatic jaundices.

Porphyrins. In health, the urine contains type I porphyrins in very small amount (to 300 μg). The increased amount of porphyrins is observed in hepatic diseases; pernicious anemia; in congenital erythropoietic porphyria.

Only traces of **creatine** occur in the urine under normal conditions. **Creatinuria** may be physiologic and pathologic (see chapter 11).

16.2 Metabolism of Water and Minerals

Metabolism of water and minerals is the aggregate of water and electrolyte supply, distribution and excretion processes in organism. This is tightly linked with organic substances metabolism.

Classifications of chemical elements, which are found in the organism:

Classic classification by V.I.Vernadsky:

Macroelements: Na, K, Ca, P, Cl, S. Mg

Microelements *(trace elements):* Fe, Cu, Zn, Mn, Se, Mg, I, Co, F, Br, Sr, Li, etc.

Ultramicroelements: Hg, Au, Ni, Ti, Ra, etc.

Modern classification:

Biogenic elements (more than 70)

macrobiogenic	oligobiogenic	microbiogenic	ultramicrobiogenic
O, C, H, N, P, Ca	K, Na, Cl, S, Mg, Fe	Zn, Mn, Cu, Co, Br, F, I	B, Li, Al, Si, Sn, Cd, As, Se, Ti, Va Cr, Ni etc.

Each element has to be supplied in organism in optimal concentration. Decreased supply results in diminishing biochemical process intensivity. Increased supply leads to toxicosis.

Mineral substance functions:

- Plastic function (they are structural elements of bone tissue (Ca, P, Mg, F, etc.).
- They provide osmotic homeostasis (Na, Cl, etc.).
- They participate in maintaining acid – base balance (Na, K, P).
- Electrogenic function (they take part in formation of rest and action potentials).
- They participate in coupling excitation and contraction processes in muscles (Ca^{2+}).
- They are included in vitamin structure (Co).
- They are included in hormone structure (I) and facilitate to formation of storage form of insulin (Zn).
- They play an important role in erythropoiesis (Fe, Cu, Co).
- They participate in blood clotting (Ca).
- They are activators and cofactors of enzymes (Mg ~ 300 enzymes, Zn> 200 enzymes, etc.).
- They participate in oxygen transport and reservation (Fe).
- They are second messengers of hormones (Ca).
- Energy function (as component of ATP, creatine phosphate).

16.2.1 Water Metabolism

Structure features: Water molecule is dipole. Hydrogen bonds are formed between water molecules. This explains high boiling temperature and high thermal capacity of water.

Biological role of water:

1. Water is universal dissolvent. Water polarity provides good solubility of different substances and electrolyte ionization in water. Water is chemically inert, therefore substances dissolved in it keep their chemical and biological properties.

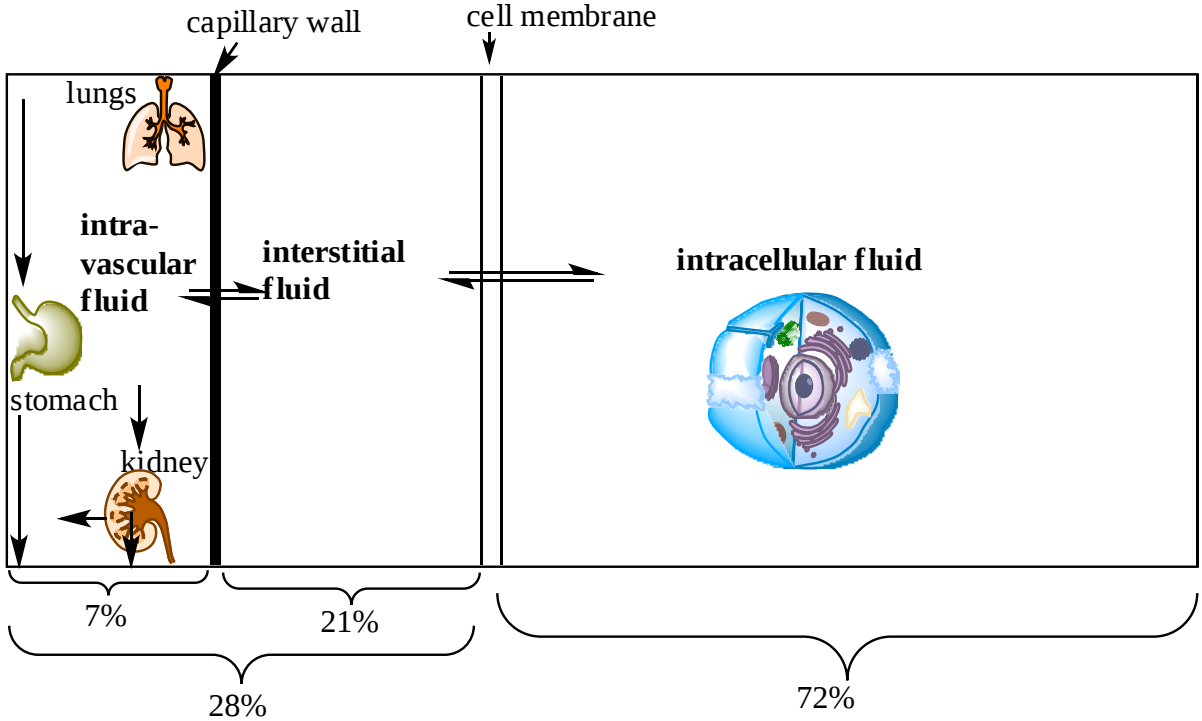
2. Water plays an important role in supporting unique structure and functions of cell organelles.
3. Water is obligatory component of biochemical processes. All the reactions in the organism are performed either in presence, or with participation of water.
4. Water performs transport function.
5. It participates in supporting osmotic pressure.
6. Water is an important thermoregulation factor.

Water content in organism

The total amount of body water is about 60 – 65% in adults, and 72% in newborns. The proportion of weight as water declines with age and with increased body fat content. By the age of 60 men have 51,5% of water and women 45,5% of it.

Body water distribution

Body water is distributed between 2 main compartments: ***intracellular*** and ***extracellular***. Intracellular water comprises 70-72 % of total body water of normal healthy adults. Extracellular water includes that of interstitial fluid and that of plasma, lymph, cartilage, etc.



Major differences in composition between intracellular and extracellular fluids are the following:

1. Potassium is the principal cation within cells, whereas sodium predominates in extracellular fluid.
2. Because of many phosphorylated organic compounds are present within cells, phosphate is the primary intracellular anion, chloride replaces it in extracellular fluids.
3. Finally, the intracellular protein concentration is higher than that of blood plasma.

Water balance

Water is essential nutrient factor. A loss of 12-25% of water leads to death. In a normal healthy person, total body water volume remains remarkably constant, fluctuating less than 1% of body weight per day, and this constancy is maintained in spite of large variations in water intake.

Daily water intake is 2,5-3 l. It depends on age, occupation, climate, diet, etc.

Water need is higher in children, than in adults, namely: in children it is 100-150 g per kg of weight; in adults it is 30-50 g per kg.

Water sources:

1. Exogenic water is 85%. This is food and drinking water.
2. Endogenic water (15%). This is metabolic water.

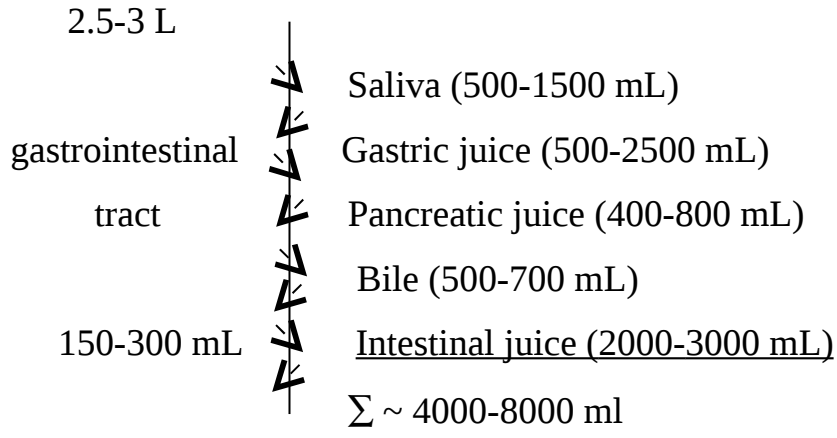
The oxidation of 100 g of each carbohydrate, protein and fat yields 55.6; 41.3 and 107.1 g of water, respectively, but the total amount of metabolic water is quite small (200-400 ml per day) relative to that ingested in food or drink.

Water losses:

Water is required to replace fluid lost through the skin, lungs and gastrointestinal tract and to accompany renal excretion of urea, salts and other osmotically active substances.

The amounts of these obligatory losses vary significantly with climate, activity level, state of health, and diet. Hot temperature, dry climate, vigorous physical activity and fever increase water losses from the skin and lungs.

Great amount of water is secreted into gastrointestinal tract with juices. Water secreted into the gastrointestinal tract is usually reabsorbed, but diarrhea and other intestinal diseases can result in very large water losses and organism dehydration.



Total urine volume generally depends on water intake, but a minimum amount of water – an obligatory volume – is required to accompany the excretion of osmotically active substances especially urea, sodium chloride.

Table 16.1Average daily intake and output of water in a normal adult human

Water intake (ml)			Water output (ml)		
Source	Obligatory	Facultative	Source	Obligatory	Facultative
Drink	650	1000	Urine	700	1000
Water of food	750		Skin	300-500	
Metabolic water	350		Lungs	400	
			Feces	150-300	
Subtotal	1750	1000	Subtotal	1750	1000
Total	2750		Total	2750	

16.2.2 Regulation of Water-salt Metabolism

Regulation of water-salts metabolism come to supporting:

- constant osmotic pressure;
- constancy of total water volume in organism and its distribution between different fluid spaces;

- constancy of ionic composition;
- acid-base balance.

Constancy of ionic composition is provided by means of systems of active transport.

Distribution of water between fluid spaces of the organism is generally determined by physico-chemical mechanisms.

This process is influenced by the following factors:

1. *Osmotic pressure.* Gradient of molar concentrations between fluid spaces of organism is motive force of water current between them. Water will be transferred to water space with greater molar concentration.
2. *Oncotic pressure.* Decreasing protein content in blood plasma leads to edema.
3. *Hydrodynamic pressure in vessels* (is created by heart work).
4. *Permeability* of cell membranes.
5. *Active biological transport of ions.*

Regulation of total water volume constancy and osmotic pressure of blood is performed by neurohumoral way.

Osmotic pressure of extracellular fluid in greater extent depends on [NaCl], therefore base mechanism of osmotic pressure regulation is linked with the change of excretion rate or water, or sodium chloride.

Regulation of extracellular fluid volume is performed by simultaneous change of excretion rate both water and sodium chloride.

Supply of water to the organism depends on the thirst sense. Center of thirst is located in dorsal and central nucleus of hypothalamus. The water excretion by kidneys is regulated by neurohumoral way with participation of antidiuretic hormone.

Antidiuretic hormone (vasopressin) is synthesized in special neurons of the hypothalamus from which it is transported to the posterior pituitary and is secreted directly in blood. This is nonapeptide. Vasopressin stimulates the contraction of the muscular tissue of blood vessels (the vasopressory action). However, its major function is water balance control. It stimulates the reabsorption of water in the

renal tubules through the adenylyl cyclase system and increasing hyaluronidase activity.

Vasopressin secretion is stimulated by increasing osmotic pressure and by considerable decreasing volume of extracellular fluid. It should be noted, that the system of osmotic regulation functions in very limited range: a change of osmolality by 1% only leads to vasopressin secretion, which corrects this change. With the blood volume, it must be decreased about 7-15 % before similar response reaction is arisen. If both system get opposite signals (for examples, the loss of blood under hyponatremia conditions), a “volume” regulation prevails over osmotic regulation.

In pathology, for example, in atrophy of posterior pituitary, ***diabetes insipidus*** develops, a diseased state manifested by an excessive urinary water discharge.

Vasopressin has important value for restoration of total volume of fluid in the organism. But increased reabsorption of water without sodium by vasopressin is little effective in restoration of extracellular fluid because 2/3 of reabsorbed water enters into intracellular space.

The regulation of sodium concentration is necessary to support the constancy of extracellular fluid volume. This regulation is performed by aldosterone and natrouretic factor. In kidney aldosterone increases the sodium ions reabsorption (together with chloride) in distal tubules. This leads to a delay of sodium chloride in organism. ***In hyperaldosteronism*** the surplus delay of sodium chloride leads to increasing osmotic pressure. This is a signal to vasopressin secretion. Vasopressin enhances water reabsorption in the kidneys. Accumulation of sodium chloride and water is observed. Extracellular fluid volume is increased. Under supporting normal osmotic pressure, blood pressure is increased.

The primary regulators of aldosterone production by the glomerulosa cells are the *renin-angiotensin system* and potassium. Sodium, neural regulation, ACTH, adrenoglomerulotropin (isolated from pineal gland) are also involved.

Main mechanism of the regulation of the aldosterone secretion is ***renin-angiotensin system.*** Renin is proteolytic enzyme, which is synthesized in

juxtaglomerular cells of the renal afferent arteriol. They are sensitive to blood pressure change, to change of Na^+ and Cl^- concentration in the renal tubular fluid. Any combination of factors, that decreases fluid volume or decreases NaCl concentration, stimulates rennin release. Renal sympathetic nerves that terminate in the juxtaglomerulas cells mediate the central nervous system effects on rennin release (through the β-adrenoreceptors).

Renin is able to convert angiotensinogen (α_2-globulin, produced by liver) into decapeptide angiotensin I. *Angiotensin-converting enzyme*, a glycoprotein, removes two carboxyl terminal amino acids from the decapeptide angiotensin I to form angiotensin II. Various nonapeptide analogs of angiotensin I and other compounds act as competitive inhibitors of converting enzyme and are used for treating *renin-dependent hypertension*. These are reffered to as angiotensin converting enzyme inhibitors. Converting enzyme also degrades *bradikinin*, a potent vasodilator; thus, this enzyme increases blood pressure in two distinct ways.

Angiotensin II increases blood pressure by causing vasoconstriction of the arteriole and is a very potent vasoactive substance. It is the potent stimulator of aldosterone production. It causes thirst. Angiotensin II inhibits renin release from the juxtaglomerular cells by feed-back mechanism.

Sodium uretic factor is synthesized by cells of auricle of the heart.

1. It stimulates excretion of sodium ions.
2. It shows vasodilatory effect.
3. It inhibits aldosterone synthesis.
4. It inhibits renin release.

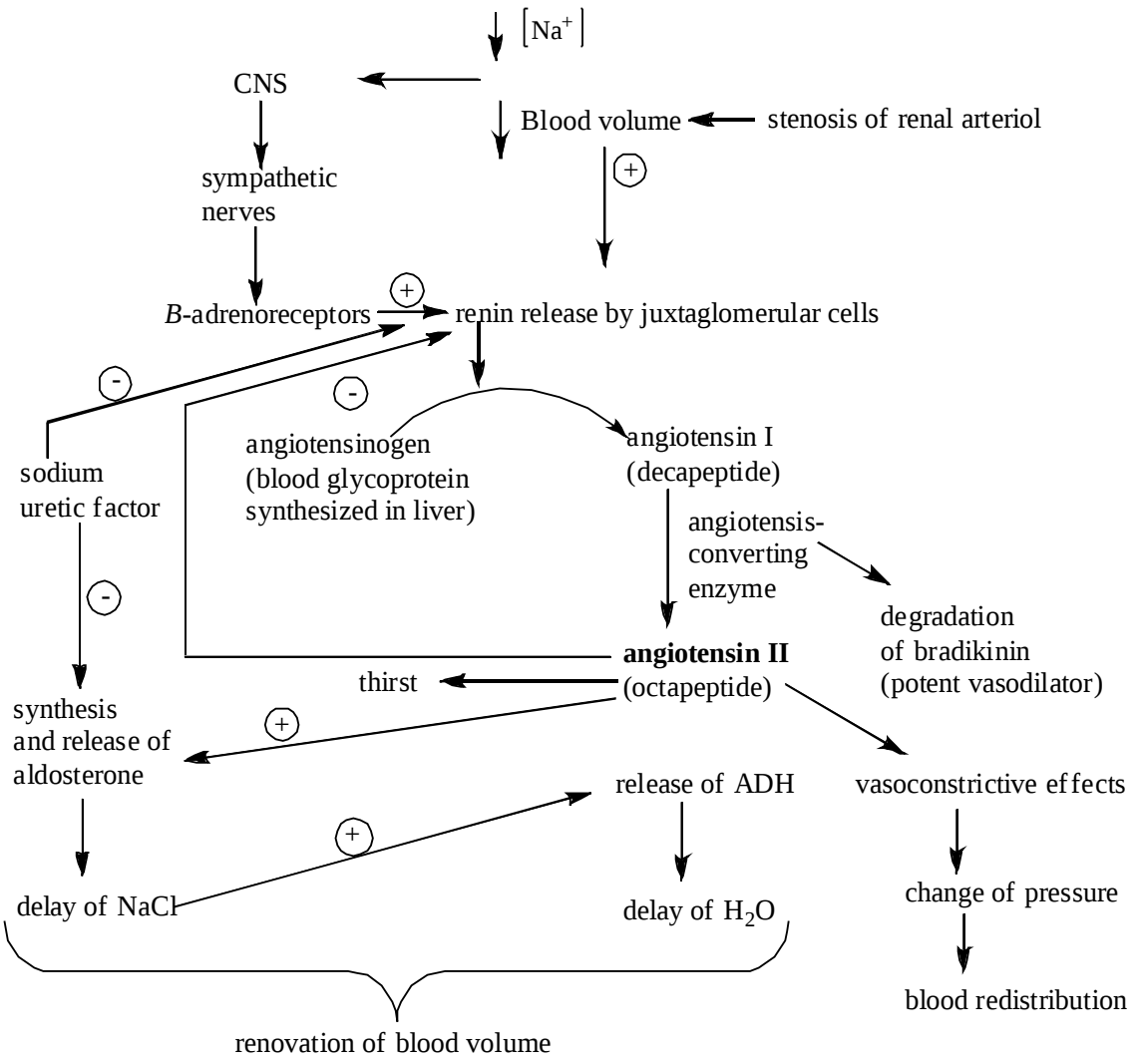


Figure 16.2 Participation of renin-angiotensin system in mechanism of blood volume renovation and mechanism of renal hypertension development

Supporting of pH constancy

Acid-base balance is the relation between concentrations of hydrogen and hydroxyl ions in liquids of organism. Under normal conditions pH of blood is 7.35-7.45; pH of intracellular liquid is lower, than extracellular one, and pH value inside cells of different types may be different, but constant for the given type of cells. pH in different compartments of one type cell may be different. Difference of pH inside cells of various types and in various compartments of the given type of cells is explained by the features of metabolism, by mechanisms of active transport, by election permeability of membranes. Regulation of acid-base balance is achived by physico-chemical (buffer systems) and physiological mechanisms (lungs, kidney).

16.2.3 Role of Some Minerals

Most of minerals (sodium and potassium are notable exception) form salts and other compounds that are relatively insoluble, they are not readily absorbed, and most ingested minerals are excreted in feces. Mineral absorption often requires specific carrier proteins; the synthesis of these proteins serves as an important mechanism for control of mineral levels in the body. Transport and storage also require specific binding to carrier proteins.

Excretion of most minerals is accomplished by kidneys, but many minerals are also secreted into the digestive juice and bile and are excreted with feces.

Calcium. The human body contains more calcium than any of other essential minerals – about 1-1.5kg g in 70-kg adults.

Functions:

- Plastic function. About 99% of the total amount of Ca is in bones and teeth. Most skeletal calcium is deposited as a form of hydroxyapatite, $Ca_{10}(PO_4)_6(OH)_2$, but bone also contains considerable amounts of noncrysralline calcium phosphates and carbonates as well as a small amount of other salts. Because bone is constantly being remodeled, its mineral levels reflect the equilibrium between daily deposits and withdrawals. As much as 700 mg calcium may enter and leave the bones every day.
- It is necessary for blood clotting.
- Ca^{2+} takes part in coupling of excitation and contraction in muscle.
- It provokes initial mediator secretion during synaptic excitation.
- It is necessary for adequate functioning of membrane channels.
- It is the “second messenger” that mediates cellular responses to a wide range of stimuli similar to the regulatory actions of cyclic nucleotides. The action of calcium appears to be mediated by intracellular receptor protein, calmodulin that binds calcium ions when their concentration increases in response to a stimulus. Calmodulin has been found to be present in every nucleated cell type examinated. When Ca^{2+} is bound to calmodulin it modulates the activities of a great variety of

enzymes, including those involved in cyclic nucleotide metabolism, protein phosphorylation, secretory function, muscle contraction, glycogen metabolism.

Phosphorus

- This participates in bone formation (50% all the phosphorus in organism).
- Phosphorus takes place in maintaining acid-base balance.
- It is also an integral compound of nucleic acids, nucleotides, nucleotide coenzymes, phospholipids, some proteins, 2.3-bisphosphoglycerate.
- It participates in formation of macroergic phosphate bonds (ATP, creatine phosphate etc.).

Ca and P metabolism regulation

Many hormones influence the calcium and phosphorus metabolism. Parathyroid hormone, calcitonin and hydroxylated forms of cholecalciferol are main of them (see chapter “Hormones”).

Normal plasma contains the equivalent of 9-11 mg of calcium per deciliter (2,25-2,75 mmol/L). The symptoms of calcium deficiency include tetany and related muscle and neurologic disorders. Low serum Ca^{2+} levels occur in vitamin D deficiency, hypoparathyroidism or renal insufficiency. The net negative Ca^{2+} balance leads to rickets in children or osteomalacia in adults. High serum Ca^{2+} levels accompany clinical disorders such as hyperparathyroidism, vitamin D intoxication, sarcoidosis, and cancer. *Osteoporosis*, which mainly occurs in women following the menopause, is based (at least in part) on a reduction in *estrogen* levels. Estrogens normally inhibit the stimulation of osteoblast differentiation by osteoblasts. If the effects of estrogen decline, osteoclasts predominate and excess bone removal occurs.

Sodium

Sodium is the cation (Na^+) of the extracellular fluid. The concentration of Na^+ in blood plasma is 126-152 mM, in erythrocytes 13,4-21,7mM. 1-3.5 g of Na is required daily for adults. Infants need 0.1-0.5 g and children 0.3-2.5 g daily.

Functions of sodium:

- Maintaining and supporting normal water balance and distribution of water in the organism.
- Na^+ is also important in the maintenance of osmotic pressure of body fluids and thus in protection against excessive fluid loss.
- It is largely associated with chloride and bicarbonate in the regulation of acid-base equilibrium.
- It participates in formation of resting membrane potential and regeneration of excitation potential.
- It participates in supporting normal neuromuscular excitability.
- It facilitates formation of conformation of enzyme molecule which is needed to precise orientation of catalytic groups.
- It intensifies proteins swelling and increases the amount of water bound with them.

Every 24 hours approximately 2500 mmol of sodium are filtered by the kidneys. However, due to tubular reabsorption less than 1% of this sodium appears in the urine (100-200 mM/day). Approximately 80% of the filtered sodium are reabsorbed in the proximal tubules with equivalent amount of water. The reabsorption of sodium in the distal tubules is 5 times less than in the proximal ones. The reabsorption in the distal tubules takes place contrary to a concentration gradient and is regulated by aldosterone (see above).

Hyponatremia may be relative and absolute. Relative hyponatremia is caused by excessive drinking or excessive parenteral infusion of hypotonic solutions. Absolute hyponatremia is caused by diuretic medication, intensive sweating, prolonged vomiting, intestinal fistula, in hypoaldosteronism.

Hypernatremia may also be relative and absolute. Relative hypernatremia is observed in hyperosmolar dehydration, in limited liquid intake. Absolute hypernatremia is developed in excessive Na intake, in primary hyperaldosteronism (Conn’s syndrome), in secondary hyperaldosteronism, in chronic nephritis. The secondary hyperaldosteronism is caused by hyperproduction of renin. This mechanism is observed in hypertonic disease, in cardiovascular insufficiency, etc.

Potassium

Potassium is the major intracellular cation. The concentration of potassium is 3.8-5.4 mM in blood serum and 79.8-99.3 mM in erythrocytes.

Functions:

- It participates in formation of resting membrane potential.
- It favors the activation of some enzymes. It is needed for the synthesis of proteins, ATP, glycogen.
- Potassium potentiates the function of parasympathetic nervous system and the action of acetylcholine on the nerve terminals in muscles.
- It is involved in supporting acid-base balance.
- It plays an important role in cardiac function.

Hypokalemia is observed in insufficient potassium intake, in changing redistribution of potassium between intracellular and extracellular fluids and in increased loss. Insulin facilitates the supply of potassium in cells, therefore hypokalemia is observed after infusion of insulin to patients with Diabetes mellitus. Loss of K^+ in gastro-intestinal diseases, renal and exstrarenal polyuria, diuretic therapy, primary and secondary hyperaldosteronism, metabolic and respiratory alkalosis causes hypokalemia. Hypokalemia is accompanied by muscle hypotonia, weakness, paresthesia, change of contractive function of miocardium, tachycardia. Potassium deficiency results in changes of electrochemical gradient of cell membranes of myocardium, a decrease of potential difference and depolarization of membrane.This leads to increasing muscular excitability. Low resting membrane potential causes slow enhancing and low amplitude of action potential. Therefore excitation irradiation is reduced. Myocardium insufficiency develops.

Hyperkalemia. The mechanism for excretion of potassium in normal persons is so effective that it is difficult to produce hyperkalemia simply increasing the oral intake. Hyperkalemia however may occur after rapid intravenous infusion of potassium salts. Hyperkalemia is also caused by excretion disturbance and by sudden release of potassium from the intracellular space. Decreased excretion of

potassium is observed in renal failure, in hypofunction of adrenal cortex (Addison's disease). Damage of body cells from any cause results in release of cell contents including K^+ into extracellular fluid. Crush injuries with damages to large volumes of muscle tissue, massive hemolysis are examples. In ketoacidosis there is substantial loss of intracellular K^+ to the extracellular fluid. If ketoacidosis presents for a long time, there will be major depletion of total body K^+. In hyperkalemia the changes in myocardium are observed. Bradicardia, arrhythmia, blockade, asystole occur.

16.2.4 Water and Mineral Metabolism Disorders

Disturbances of water and mineral metabolism are distinguished into water and electrolyte imbalance (dishydrations) and disturbances of metabolism of separate minerals.

Water imbalance is distinguished into:

1. Dehydration:
- primary (hyperosmolar, pure water depletion);
- secondary (hypoosmolar, pure salt depletion);
- mixed (isoosmotic, water and salt depletion).
2. Water intoxication (hyperhydration):
- total hyperhydration;
- intracellular hyperhydration;
- extracellular hyperhydration.

Primary dehydration (pure water depletion)

Causes:

- inadequate water intake (coma, dysphagia etc.);
- in infants great amount of water practically without electrolytes may be lost through lungs in hyperventilation, fever, acidosis;
- excessive loss of water by kidney in diabetes insipidus.

Osmotic pressure in extracellular space is increased. Water flows from intracellular space to extracellular space. Intracellular dehydration develops.

Symptoms: thirst, oligouria, hyperosmia, azotemia due to oligouria, hypernatremia.

Secondary dehydration (hypoosmolar)

Causes: excessive sweating (mainly NaCl depletion); vomiting, diarrhea, duodenal fistules, cholera, etc.; Addison's disease; vigorous use of diuretics.

From hypoosmolar extracellular space water flows to cells. This leads to intracellular edema.

Symptoms: thirst is absent; dryness of skin; decreasing turgor of skin; headache; collapse.

Mixed dehydration (isoosmolar) occurs: in bleeding; peritonitis; exudates; in burns.

The volume of both extracellular and intracellular fluids is decreased.

Water intoxication (hyperhydration)

Total hyperhydration occurs due to excessive water intake or insufficient water excretion. Water is accumulated in all water spaces.

Causes: severe stagnant cardiovascular insufficiency; hypersecretion of ADH following administration of anesthetics for surgery, administration of narcotic drugs or in stress (including any surgery); excess of aldosterone (Conn's syndrome); excessive parenteral administration of fluids.

Symptoms: headache, nausea, depression.

Intracellular hyperhydration.

Causes: infusion of hypotonic solutions; excessive drinking; insuficient excretion of fluid in nephropathies.

Extracellular hyperhydration (edema syndrome) due to the accumulation of water in interstitial fluid.

Causes: reduction in colloid-osmotic pressure; increase of hydrostatic pressure; disturbances of functioning the heart; allergic and inflammatory processes.

Disturbances of metabolism of separate minerals can be **primary** and **secondary** ones.

Primary disturbances are caused by deficiency or excess of any minerals in diet. Examples: endemic goiter (deficiency of I), fluorosis (excess of F).

Secondary disturbances of mineral metabolism may be caused:

- by insufficient amount of protein-carrier (for example, Addison-Biermer disease);
- by lack of apoenzyme (for example, insufficiency of sulfite oxidase – Mo containing enzyme – leads to mental retardation);
- by hormonal disbalance (for example, hypofunction of adrenal cortex – Addison’s disease, hyperaldosteronism – Conn’s syndrome).

Tests for Self-control

1. In the patient the average daily output of water is lower than its intake. Which disease can lead to that state?

A. Renal disease
B. Hepatitis
C. Pancreatitis
D. Infectious diseases
E. Myocardial infarction

2. The development of Addison-Biermer's disease (pernicious hyperchromic anemia) is due to a deficiency of vitamin B_{12}. Choose metal which is included to composition of this vitamin:

A. Zink
B. Cobalt
C. Molybdenum
D. Magnesium
E. Iron

3. Which hormone influences the blood sodium and potassium levels?

A. Calcitonin
B. Histamine
C. Aldosterone
D. Thyroxine
E. Parathyroid hormone

4. The patient complains of thirst and polyuria. The urine analysis revealed: daily diuresis - 10 L; urine density - 1.001 (normal – 1.012-1.024). Which disease causes the indexes?

A. Diabetes mellitus
B. Steroid diabetes
C. Thyrotoxicosis
D. Acromegaly
E. Diabetes insipidus

5. Which is the normal blood calcium level (in mmol/L)?

A. 1.50-1.75
B. 1.75-2.00
C. 2.25-2.75
D. 3.0-4.5
E. 0.65-1.60

6. Daily water requirement for adults is:

A. 30-50 ml/kg
B. 75-100 ml/kg
C. 75-80 ml/kg
D. 100-120 ml/kg

7. Goiter is a disease which is widely spread in some biogeochemical areas of the earth. Which element deficiency causes this disease?

A. Iron

B. Iodine
C. Zinc
D. Copper
E. Cobalt

8. Kidneys make all functions excepting:
A. Excretion of final products of metabolism
B. Regulation of water-salt metabolism
C. Keeping osmotic pressure
D. Regulation of blood pressure
E. Breakdown of urea to CO_2 and H_2O

9. Organ specific enzyme for kidneys is:
A. Lactate dehydrogenase
B. Succinate dehydrogenase
C. Aspartate aminotransferase
D. Transamidinase
E. Creatinephosphokinase

10. Ammonia content in urine is important index of acid-base balance of organism. Ammonia amount increases both under respiratory and metabolic acidoses. It is connected with following enzymes stimulation in the renal epithelial cells under acidosis:
A. Glutaminase
B. Krebs cycle
C. Carboanhydrase
D. ATP-ase
E. Hyaluronidase

Chapter 17. BIOCHEMISTRY OF LIVER. BIOTRANSFORMATION OF XENOBIOTICS

Weighing 1,5 kg, the liver is one of the largest organs in the human body. Although it only represents 2 – 3 % of the body mass, it accounts for 25 – 30% of oxygen consumption.

Liver plays the central role in regulation and integration of metabolism. *Hepatocytes* make up 90% of the cell mass of liver. They are in close contact with the blood, which enters the liver from the portal vein (more than 70%) and the hepatic arteries (30%), flows through capillary vessels known as sinusoids, and is collected again in the central vein of the hepatic lobes. Hepatocytes are particularly rich in endoplasmic reticulum as they carry out intensive protein and lipid synthesis. The cytoplasm contains granules of glycogen. Between hepatocytes there are bile capillaries through which bile components are excreted.

17.1 Functions of Liver

- ***Uptake*** of nutrients supplied by intestine via the portal vein.
- Biosynthesis of endogenous compounds and storage, conversion and degradation of them into excretable molecules (***metabolism***). In particular, the liver is responsible for the biosynthesis and degradation of almost all plasma proteins.
- ***Supply*** of the body with metabolites and nutrients.
- ***Detoxification*** of toxic compounds by biotransformation.
- ***Excretion*** of substances with the bile.

17.2 Role of the Liver in Carbohydrate Metabolism

The liver plays the important role in supporting glucose concentration constancy in blood. This is provided by the following mechanisms:

- The liver takes up glucose and other monosaccharides from the plasma. Transporters in the plasma membrane of hepatocytes allow insulin-independent transport of glucose and other sugars in both directions. The liver has the enzyme *glucokinase,* which has higher K_m (10mM) as compared with hexokinase (0,01 – 0,1 mM). This enzyme can react by increasing activity in response of the enhance of glucose content in portal vein after food intake.

- Glucose is then either stored in the form of polysaccharide *glycogen* or converted into fatty acids. When there is a drop of the blood glucose level, the liver releases glucose again by breaking down glycogen. In contrast to muscle, the liver possesses the enzyme *glucose-6-phosphatase*, which can release glucose from glucose-6-phosphate. Therefore glycogen of liver is used by not only this organ but also by other tissues and organs.
- If the glycogen store is exhausted, glucose also can be synthesized by *gluconeogenesis* from lactate, glycerol or the carbon skeletons of amino acids. Regeneration of glucose (up to 250 g per day) mainly takes place in the liver. The tubule cells of the kidney are also capable of carrying out gluconeogenesis, but due to their much smaller mass, their contribution only represents around 10% of total glucose formation.
- Fructose and galactose are mainly metabolized by the liver, which channels them into glycolysis.
- The process of glucose utilization is also intensive in liver:
  - metabolites of glycolysis and acetyl-CoA are used for biosynthesis of TAGs;
  - NADPH, which is formed in pentose phosphate pathway, is used for the synthesis of fatty acids and cholesterol;
  - ribose-5-phosphate is used for synthesis of nucleic acids.

17.3 Role of Liver in Lipid Metabolism.

The liver is the most important site for the formation of fatty acids, fats (triacylglycerols), cholesterol and the only site for the synthesis of ketone bodies. Most of these products are released into the blood. In contrast, the triacylglycerols synthesized in adipose tissue are stored there.

- Lipid metabolism in the liver is closely linked with carbohydrates and amino acids metabolism. When it is a good supply of nutrients in the resorptive state, the liver converts glucose via acetyl-CoA into *fatty acids*. Fatty acids are converted into *fats* and *phospholipids*. Together with apoproteins they are packed into VLDLs and then released into the blood by exocytosis.

- The most of cholesterol (250 – 500 mg/day, 50 – 80 %) is synthesized in the liver. It is transported to tissues by means of LDLs. LDLs are mainly formed in blood stream from VLDLs. Small amount of LDLs is synthesized immediately in liver. Some cholesterol is required for the synthesis of *bile acids*. The liver also contributes to the cholesterol metabolism by taking up from the blood and breaking down lipoproteins that contain cholesterol and cholesterol esters (HDLs, IDLs, LDLs).
- The synthesis of ketone bodies is located in liver. Acetoacetate and β-hydroxybutyrate are alternative fuel for extrahepatic tissues (skeletal muscles, heart, kidneys). Brain also uses ketone bodies in long starvation. Acetone cannot be metabolized and is exhaled via the lungs or excreted with urine.

17.4 Role of Liver in Protein Metabolism

- The liver controls the plasma level of amino acids. Excess amino acids are broken down. In patients with severe liver insufficiency level of amino acids in blood is 21mmol/L instead 2,9 – 4,3 mmol/L.
- The carbon skeleton of amino acids enters intermediary metabolism and serves for glucose synthesis or energy production.
- The liver is the only organ with complete complex of enzymes for urea synthesis. Disturbances of functioning urea cycle lead to accumulation of ammonia in blood and tissues. Brain neurons are the most sensitive to such pathological situation. This is manifested by development of liver encephalopathy and coma.
- Synthesis of choline, creatine, hydroxylation of phenylalanine occurs in the liver.
- Most of the plasma proteins (albumins, 13 – 18 g/day; 80 % of globulins, factors of blood clotting, fibrinolytic systems of blood: II, V, IX, X, XI, XII, XIII fibrinogen, antithormbin, antiplasmin) are synthesized in the liver.

17.5 Bile Formation Function of Liver

Bile is an important product released by hepatocytes. It promotes the digestion of fats from food by emulsifying them in small intestine. The emulsifying components of bile, apart from phospholipids, mainly consist of *bile acids* and *bile*

salts. The bile also contains bile pigments, free cholesterol, which are excreted by this way. The cholesterol excreted with the bile is poorly water-soluble. Together with phospholipids and bile acids it forms micelles, which keep it in solution. If the proportions of phospholipids, bile acids and cholesterol shift, *gallstones* can arise. These mainly consist of precipitated cholesterol (cholesterol stones), but can also contain Ca^{2+} salts of bile acids and bile pigments.

17.6 Storage Function

The liver not only stores energy reserves and nutrients for the body, but also certain mineral substances (including iron) and vitamins (A, D, K, folic acid, B_{12}).

17.7 Biotransformation Function

Steroid hormones and bilirubin as well as drugs, ethanol, and other xenobiotics are taken up by liver and inactivated and converted into highly polar metabolites.

Phase I reactions include:

- ***Hydrolytic cleavage*** of esters, peptide bonds. For example, hydrolysis of acetylsalicylic acid.
- ***Oxidation.*** Hydroxylation, epoxide formation, sulfoxide formation, dealkylation, deamination. For example, benzene is oxidized into phenol, and toluene (methylbenzene) is oxidized into benzoic acid.
- ***Reductions.*** Reduction of carbonyl, azo- or nitro-compounds, dehalogenization.

Most oxidation reactions are catalyzed by *cytochrome P450 systems.*

Cytochrome P450 systems. During the first phase of biotransformation in the liver substances, that are weakly chemically reactive, are enzymatically hydroxylated. This makes it possible for them to be conjugates with polar substances.

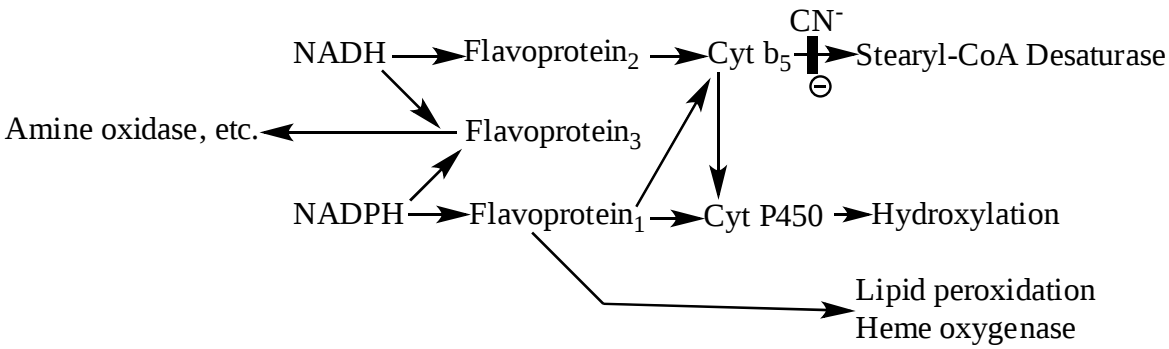


Figure 15.1 Cytochrome P450 transport chain.

Cytochrome P450 systems are also involved in many other metabolic processes – eg, the biosynthesis of steroid hormones, bile acids, eicosanoids, as well as the formation of unsaturated fatty acids. The liver's reddish-brown color is mainly due to the large amounts of P450 enzymes it contains.

Cytochrome P450-dependent monooxygenases catalyze reductive cleavage of molecular oxygen (O_2). One of the two oxygen atoms is transferred to the substrate, while the other is released as a water molecule. The necessary reducing equivalents are transferred to the actual monooxygenase by an FAD-containing auxiliary enzyme from the coenzyme NADPH + H^+.

Cytochrome P450 enzymes occur in numerous forms in the liver, steroid-producing glands, and other organs. The substrate specificity of liver enzymes is low.

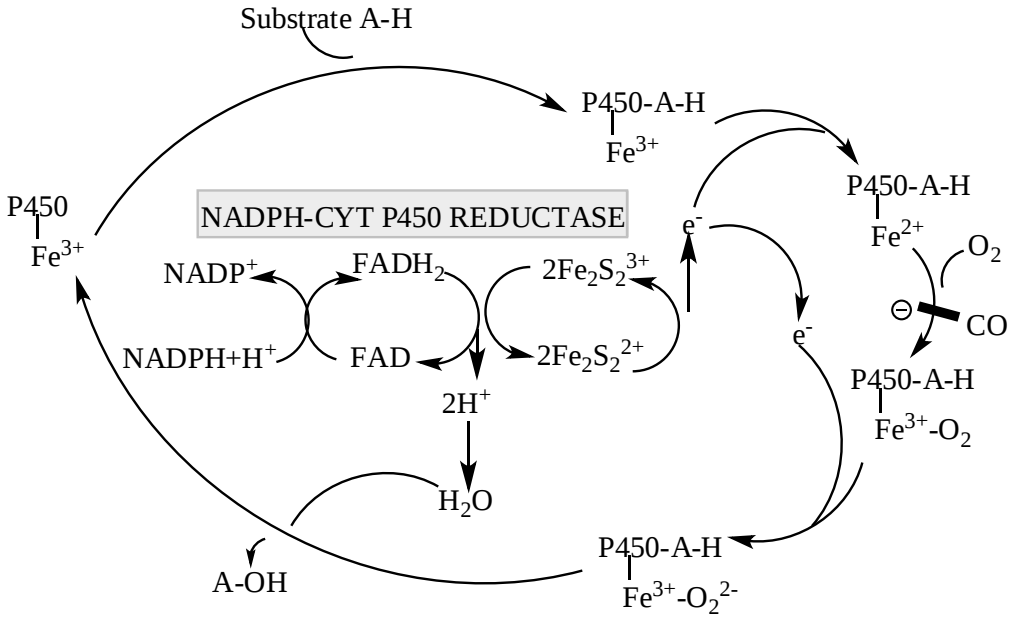


Figure 15.2 Cytochrome P450 monooxygenase cycle in microsomes.

Endogenous substances as steroid hormones are inactivated with participation of cytochrome P450 monooxygenase system. Among the substances metabolized by this system are benzpyrene, aminopyrene, aniline, morphine, benzphetamine. The degradation of ethanol in the liver is also partly catalyzed by cytochrome P450 enzymes (“the mitochondrial ethanol-oxidizing system”). The effects of alcoholic drinks and medical drugs can sometimes be mutually enhancing – even sometimes to the extent of becoming life-threatening.

Many drugs such as Phenobarbital have the ability to induce the formation of microsomal enzymes and cytochrome P450.

Mutations of cytochrome P450 genes in humans are associated with severe metabolic disorders. For example, mutation in gene CYPIBI leads to enzymatic defect in catabolism of aldosterone and to development of arterial hypertension. Mutation of gene CYP24AI leads to anomalous accumulation of Ca in internal organs in result of endogenous hypervitaminosis D_3.

Phase I reactions usually reduce the biological activity or toxicity of a substance (“detoxification”). However, some substances only become biologically active as a result of the interconversion reactions or become more toxic after interconversion than initial substances (“toxification”).

For example, the mutagenic effect of benzpyrene is based on this type of interconversion in the liver.

Phase II reactions (conjugate formation). These reactions couple their substrates (bilirubin, steroid hormones, drugs, and products of phase I reactions) via ester or amide bonds to highly polar negatively charged molecules. The enzymes involved are transferases, and their products are known as *conjugates.*

- Conjugation with ***glucuronate*** as O- or N-glucuronide. The coenzyme for this reaction is UDP-glucuronic acid. Example: conversion of bilirubin into bilirubin glucuronide (mono- or di-).
- ***Conjugation with sulfate*** (“active sulfate” is phosphoadenosyl phosphosulfate - PAPS). For example: formation of indican from indol.

- ***Conjugation with glycine.*** For example, benzoic acid is conjugated with glycine to form more soluble and less toxic hippuric acid.
- ***Conjugation with glutathione.*** A number of potentially toxic electrophilic xenobiotics (such as certain cancerogens) are conjugated to the nuclephilic GSH in reactions that can be represented as follows:

R + GSH → R-S-G,

where R = an electrophilic xenobiotic. The enzymes catalyzing these reactions are called *glutathione S-transferases* and are present in high amounts in liver cytosol and in lower amounts in other tissues.

- ***Reactions of methylation and acetylation***. For example, the inactivation of norepinephrine by methylation of phenolic OH group (donor of methyl group is SAM – S-adenosyl methionine). The reaction intensivity of sulfanilamide acetylation is index of drug biotransformation in human's organism.

In contrast with unconjugated compounds, the conjugates are much more water-soluble and capable to be excreted.

To detoxify ***heavy metals,*** the liver contains ***metallothioneins***, a group of cysteine-rich proteins with a high affinity for divalent metal ions such as Cd^{2+}, Cu^{2+}, Hg^{2+},and Zn^{2+}. These metal ions also induce the formation of metallothioneines via a special metal-regulating element (MRE) in the gene's promoter.

17.8 Ethanol Metabolism

The major site of ethanol degradation is the liver, although the stomach is also able to metabolize ethanol.

Most of ethanol is initially oxidized by ***alcohol dehydrogenase*** to form ***acetaldehyde***. A further oxidation, catalyzed by ***aldehyde dehydrogenase***, leads to acetate. Acetate is then converted with the help of acetate-CoA ligase to form acetyl-CoA.

In addition to cytoplasmic alcohol dehydrogenase, ***catalase*** and inducible "***microsomal ethanol-oxidizing system***" also contribute to a lesser extent to ethanol degradation.

The rate of ethanol degradation in the liver is limited by ***alcohol dehydrogenase activity***.

The ***calorific value*** of ethanol is 29,4 kJ/g. Alcoholic drinks – particularly in alcoholics – can therefore represent a substantial proportion of dietary energy intake.

Toxicity of ethanol

Ethanol is rapidly distributed throughout the body. A large amount is taken up by muscles and brain.

In the brain, ethanol is deposited in membranes and influences receptors for neurotransmitters. The effect of GABA is enhanced, while that of glutamate declines.

High ratio of $NADH/NAD^+$ facilitates the conversion of pyruvate into lactate, acetoacetate into β-hydroxybutyrate, biogenic amines into alcohols, the shifting acid-base balance to acidosis. Accumulation of lactate increases renal threshold for uric acid. The increase [lactate]/[pyruvate] ratio leads to gluconeogenes isinhibition and to hypoglycemia development.

Ethanol inhibits the metabolism of some drugs in liver (for example, barbiturates), because it competes with them for P450.

High ethanol consumption over many years leads to liver damage. Ethanol-related high levels of $NADH+H^+$ and acetyl-CoA in the liver lead to increased synthesis of neutral fats and cholesterol. However, since the export of the form VLDLs is reduced due to alcohol, storage of lipids occurs (***fatty liver***). The increase in the fat content of the liver (from less than 5% to more than 50% of the dry weight) is initially reversible. But in chronic alcoholism hepatocytes are increasingly replaced by connective tissue. When ***liver cirrhosis*** occurs the damage of the liver finally reaches an irreversible stage, characterized by progressive loss of liver functions.

Acetaldehyde, which is about 15 times more toxic than alcohol, enters into condensation reactions with biogenic amines to form endogenous alkaloids. Acetaldehyde together with dopamine leads to the formation of salsolinol as well

as tetrahydropapaveroline and β-carbolines. They bind with opiate receptors, causing hallucinations, stimulation of “enjoyment” centre. This explains the development of pathologic attraction to alcohol.

Competitive inhibition of acentaldehyde dehydrogenase (for example, disulfiram) blocks the breakdown of acetaldehyde. The simultaneous intake of alcohol and disulfiram leads to increased acetaldehyde levels in the blood. This results in perspiration, tachycardia, nausea, vomiting and even severe circulatiry failure.

Tests for Self-control

1. The decrease of blood residual nitrogen level was revealed in the patient with liver insufficiency. The diminishing blood nonprotein nitrogen is due to:

A. Urea
B. Ammonium
C. Amino acids
D. Bilirubin
E. Uric acid

2. Wilson's disease (hepatolenticular degeneration) is accompanied by the decrease of:

A. Fibrinogen
B. Transferrin
C. Albumin
D. C-reactive protein
E. Ceruloplasmin

3. The detoxification of natural metabolites and xenobiotics is disturbed in the patient's liver. The decrease of which chromoprotein activity can be reason of this?

A. Cytochrome b
B. Hemoglobin
C. Cytochrome oxidase
D. Cytochrome P450
E. Cytochrome c_1

4. Fat dystrophy of liver is examined in the patient. The disturbance of which substance synthesis can lead to such pathology?

A. Cholic acid
B. Urea
C. Phosphatidic acid
D. Tristearylglycerin
E. Phosphatidylcholine

5. Tabun, zarin, fluorodiisopropyl phosphate (phosphororganic substances) are poisons of neuro-paralytic action. Which of the mentioned enzymes is inhibited by phosphororganic substances?

A. Cytochrome P450
B. Phospholipase A_2
C. Angiotensin converting enzyme
D. Tyrosine aminotransferase
E. Acetylcholine esterase

Chapter 18. NERVOUS TISSUE

Functions of nervous system

I. Communication and integration of processes, which occur in organism.

II. Psychic activity (perception, reservation and reproduction of information; memory, thinking, consciousness).

Nervous tissue accounts 2,4% of body weight:

- brain – 1400g;
- spinal marrow – 50g;
- craniocerebral nerves – 10g;
- peripheral nerves – 150g;

18.1 Features of Biochemical Composition

- High content of H_2O, protein, phosphorus, lipids (complex lipids)
- Small amount of neutral fats and free fatty acids
- Constancy of chemical composition

	Grey substance	**White substance**
H_2O	85%	70%
Protein	51% of dry residue	33% of dry residue
Lipids	~ 35% of dry residue	~ 61% of dry residue

Proteins (~ 40% of dry residue)

have a high heterogeneity

Simple:

• neuroalbumins;

•neuroglobulins;

• neuroscleroproteins; (structural supportive proteins: neurocollagens, neuroelastins, neurostromatins)

Conjugated:

• lipoproteins;

• proteolipids (are the most abundant in myelin);

• phosphoproteins;

• glycoproteins;

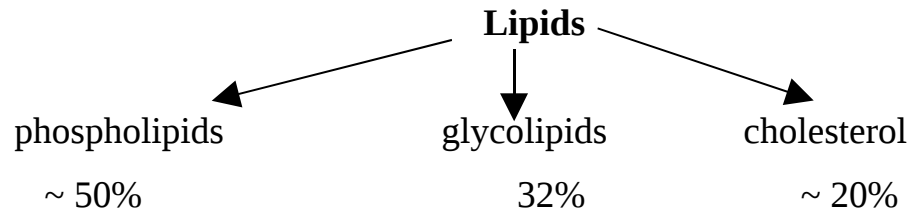
• nucleoproteins.

Specific Proteins of Nervous Tissue

- S-100 protein (Moore protein) occurs chiefly in the neuroglia. It contains a large number of Glu and Asp residues. Its mediation in the learning and memorizing processes cannot be excluded.
- 14-3-2 protein is located mostly in the neurons. Specific functions are unclear.

Peptides of nervous tissue play the important role in formation of behavior learning and memory, neuroendocrine regulation etc.

Lipids constitute about 50% of dry residue (grey substance – 35%, white substance – 60%, myelin – 70%)



Dominant element of white *substance is myelin sheath* (accounts ~ 50% of white substance weight). *Myelin* is electrical insulator main function of which is a facilitation of nervous impulse propagation. *Demyelinization* leads to development of *cerebrospinal sclerosis. Demyelinization* of nerves is observed in hereditary disorders of carbohydrate metabolism, protein metabolism (phenylketonuria), lipids metabolism (Tay-Sach's disease, Gausher’s disease, Niemann-Pick's disease).

Lipid composition of nervous tissues is constant. Changes of lipid composition of nervous tissues indicate the pathology.

18.2 Features of Metabolism in Nervous Tissue

- Intensive metabolic processes.
- Autonomicity and specificity of metabolism, which is provided by blood – brain barrier that performs regulatory and protective functions.

Features of Carbohydrate Metabolism

- The major substrate for brain tissue respiration is glucose. Total quantity of glucose in brain is about 750 mg. Glucose is oxidized by the brain tissue at a rate

of 75 mg/min, therefore the amount of glucose stored in the brain is sufficient only for 10 min.

- Small amount of glycogen [0,1% - 2,5 – 4,5 μmol/g (based on glucose)]. Degradation of glycogen in brain tissue is performed by phosphorolysis.
- Brain has high dependence on glucose supply. A severe drop in the bllod glucose level – as can occur after insulin over dosage in diabetics for example - rapidly leads to a drop in the ATP level in the brain.
- Over 90% of utilizable glucose is oxidized in the brain tissue to H_2O and CO_2 via tricarboxylic acid cycle mechanism.
- Pentose phosphate pathway is present in all the cells of brain tissue. It provides NADPH for synthesis of fatty acids and steroids.

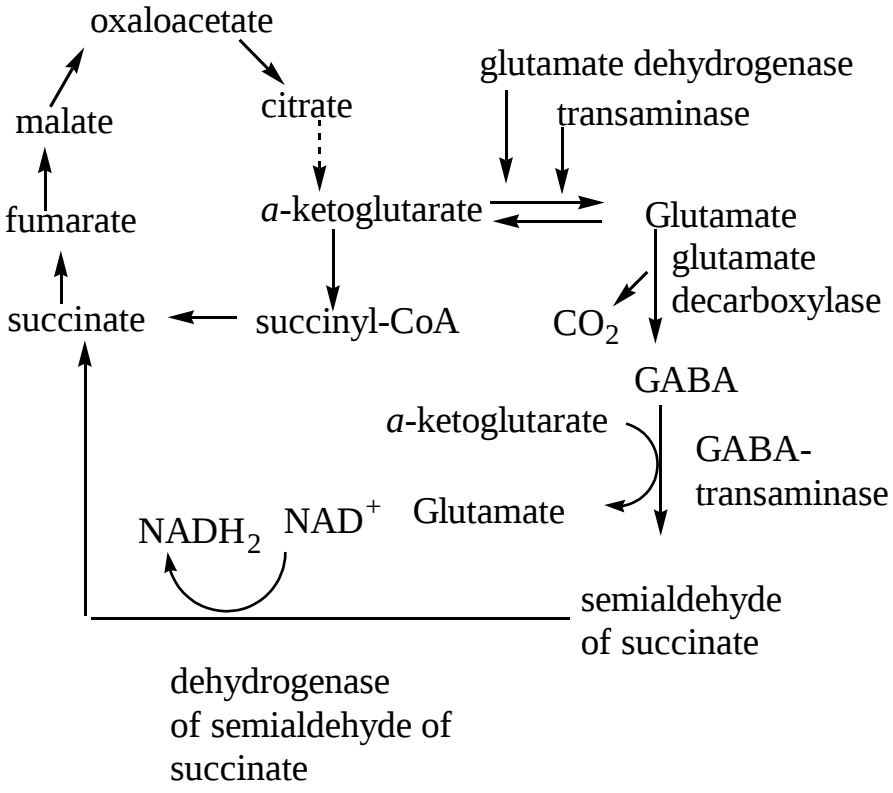
Features of Lipid Metabolism

- Synthesis of fatty acids is intensive in brain.
- In childhood, the brain tissue is also a site for the cholesterol biosynthesis. As the mature age has been reached the cerebral synthesis of cholesterol sharply diminished.
- Brain is capable to synthesize all the fractions of phospholipids.
- β-Oxidation of fatty acids is practically absent in brain. All the acetyl-CoA, which is necessary for Krebs cycle, for synthesis of cholesterol, acetyl choline, is formed from *glucose*.
- In long starvation brain is able to use ketone bodies as energy source.

Features of Protein and Amino Acid Metabolism

- Metabolism of amino acids and their derivatives in nervous tissue is very important for differentiation of nervous cells in growing organism and for supporting structure and functions of mature nervous tissue.
- Defficiency of proteins in food in early childhood is especially negative for development of brain. Irreversible disturbances of mental activity occur.
- Glutamate, aspartate and their derivatives account about 75% of the amino acids of brain.

- In nervous tissue glutamate may be formed:
  - by transamination of α-ketoglutarate with amino acids;
  - by reductive amination of α-ketoglutarate.
- Major mechanism of ammonia detoxification in brain is the formation of glutamine.
- Glutamate and aspartate are excitatory mediators, therefore the formation of glutamime and asparagine is not only mechanism of temporary detoxification of NH_3 in brain, but also mechanism of inactivation of excitatory amino acids.
- Glutaminase is present in brain. This enzyme plays the important role in regulation of glutamate content in nerve endings.
- Glutamate is the precursor of inhibitory mediator γ-aminobutyric acid (GABA). Synthesis and degradation of GABA is additional “loop” in Krebs cycle which is called GABA shunt:



- In brain there are enzymes of urea synthesis, beginning from citrulline, but enzyme of synthesis of citrulline from ornithine is practically absent.

Features of Energy Metabolism

- Glucose is normally the only metabolite from which the brain is able to obtain adequate amounts of ATP.
- Glucose (90%) is oxidized by aerobic pathway.
- Bioenergetics of brain is characterized by considerable dependence on oxygen supply. Although the brain accounts for 2-3% of the body mass, the uptake of oxygen by brain is 20-25% of the total requirement of organism. In children under the age of 4 years, the cerebral consumption of oxygen reaches even 50%.
- In a suddenly ceased supply of oxygen the brain can “exist” only within a short (slightly over one minute) period of time at the expense of the labile phosphate reserve. The interruption of oxygen supply even for 10-15s produces a disbalance in the nerve cell energetics, which in the whole organism is manifested by a syncopal state.
- The intensity of renewal of energy-rich phosphate compounds in the cerebral tissue is very high. ATP and creatine phosphate contents are characterized by considerable constancy.
- In the state of anesthesia, respiration is suppressed, the concentrations of ATP and creatine phosphate are elevated, and the level of inorganic phosphate is lowered. Consequently, the cerebral consumption of energy-rich compounds becomes reduced.

18.3. Synaptic Signal Transmission

Structure of Nerve Cells

Nerve cells (neurons) are easily excitable cells that produce electrical signals and can react to such signals as well. Their structure is markedly different from that of other types of cells. Neurons are able to receive signals via *dendrites* and to pass them on via *axons*. The axons, which can be up to 1m long, are usually surrounded by *Schwann cells*, which cover them with a lipid-rich myelin sheath to improve their electrical insulation.

The transfer of stimuli occurs at the *synapses*, which link the individual neurons to each other as well as linking neurons functional to muscle fibers.

Neurotransmitters are released in response to electrical signals in order to excite neighboring neurons (or muscle cells). It is estimated that each neuron in the brain is in contact via synapses with approximately 10000 other neurons.

Resting Potential and Action Potential

A. Resting potential

A characteristic property of living cells is the uneven distribution of positively and negatively charged ions on the inside and outside of the plasma membrane. At rest, the membrane potential in most cells is – 60 to – 90 mV. It mainly arises from the activity of Na^+/K^+ transporting ATPase (Na^+/K^+ ATPase), which occurs on practically all animal cells. It "pumps" three Na^+ ions out of cells in exchange for two K^+ ions. Some of the K^+ ions, following the concentration gradient, leave the cell again through *potassium channels.* As the protein anions that predominate inside cell cannot follow them, and inflow of Cl^- ions from the outside is not possible, the result is an excess of positive charges outside the cell, while anions predominate inside it.

An *equilibrium* potential exists for each of the ions involved. For K^+ ions, the resting potential lies in the of a membrane potential, while for Na^+ ions it is much higher at + 70mV. At the first opportunity, Na^+ ions will therefore spontaneously flow into the cell. The occurrence of action potentials is based on this.

Nerve cell membranes contain ion channels for Na^+, K^+, Cl^-, and Ca^{2+}. These channels are usually closed and open briefly to let ions pass through. They can be divided into channels that are regulated by membrane potentials ("voltage-gated" – e.g., fast Na^+ channels) and those regulated by ligands ("ligand-gated" – e.g., nicotinic acetylcholine receptors).

B. Action potential

Action potentials are special signals that are used to transmit information in the nervous system. They are triggered by chemical stimuli (or more rarely electrical stimuli). Binding of a neurotransmitter to an ionotropic receptor results in a brief local increase in the membrane potential from – 60mV to about + 30mV. Although the membrane potential quickly returns to the initial value within a few

milliseconds at its site of origin, the depolarization is propagated because neighboring membrane areas are activated during this time period.

- The process starts with the opening of voltage-gated Na^+ channels. Due to their high equilibrium potential Na^+ ions flow into the cell and reverse the local membrane potential (***depolarization)***.
- The Na^+ channels immediately close again, so that the inflow of positive charges is only very brief.
- Due to the increase in the membrane potential, voltage-dependent K^+ channels open and K^+ ions flow out. In addition, Na^+/K^+ ATPase pumps the Na^+ ions that have entered back out again. This leads to ***repolarization*** of the membrane.
- The two processes briefly lead to the charge even failing below the resting potential (***hyperpolarization).*** The K^+ channels also close after a few milliseconds. The nerve cell is then ready for re-stimulation.

Conduction of the action potential on the surface of the nerve cell is based on the fact that the local increase in the membrane potential causes neighboring voltage – gated ion channels to open, so that the membrane stimulation spreads over the whole cell in the form of a ***depolarization.***

Synaptic Signal Transmission

All chemical synapses function according to a similar principle. In the area the synapse, the surface of the signaling cell (*presynaptic membrane)* is separated from the surface of the receiving cell (*postsynaptic membrane*) only by a narrow *synaptic cleft.*

When an *action potential* reaches the presynaptic membrane, *voltage – gated* Ca^{2+}channels integrated into the membrane open and trigger *exocytosis* of the neurotransmitter stored in the presynaptic cell.

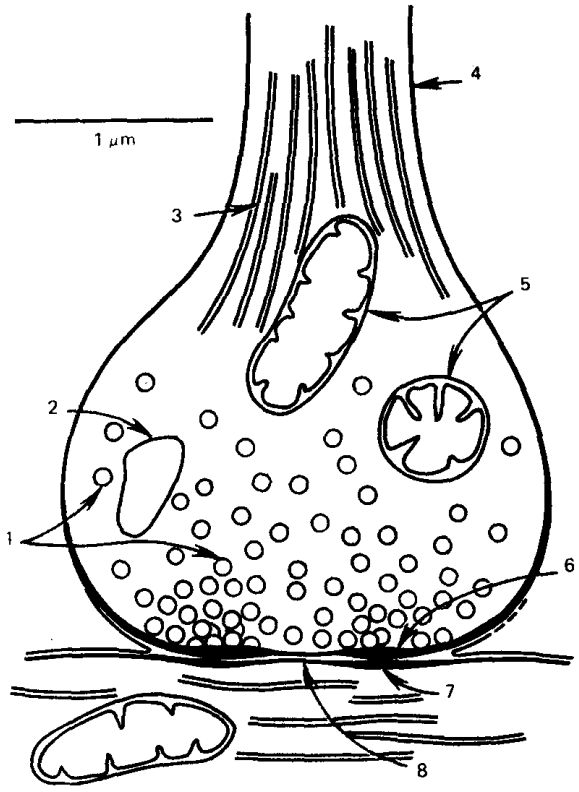


Figure 18.1 Schematic representation of a synapse (after Metzler):
1, synaptic vesicles; 2, lysosome; 3, microfibrils (neurofibrils); 4, axon; 5, mitochondria; 6, dense region of presynaptic membrane; 7, dense region of postsynaptic membrane; 8, synaptic cleft (about 20 nm wide)

The transmitters that are released diffuse through the synaptic cleft and bind on other side to *receptors* on the postsynaptic membrane. These receptors are integral membrane proteins that have binding sites for neurotransmitters on their exterior. A considerable number of receptors for neurotransmitters are already know and new are continuing to be discovered. They are classified into two large groups according to their mode.

Receptors

Ionotropic receptors are ligand-gated ion channels. When they open as a result of the transmitter's influence, ions flow is due to the membrane potential. If the inflowing ions are cations (Na^+, K^+. Ca^{2+}) *depolarization* of the membrane occurs and an action

Metabotropic receptor. After binding of the transmitter they interact with *G proteins*, which in turn activate or inhibit the synthesis *of second messengers.* Finally, second messengers activate or inhibit *protein kinases*, which phosphorylate cellular proteins

potential is triggered on the surface of the postsynaptic cell. This is way in which stimulatory transmitters work (e.g. acetylcholine and glutamate). By contrast, if anions flow in (mainly Cl^-) the result is *hyperpolarization* of the postsynaptic membrane, which makes the production of a postsynaptic action potential more difficult. The action of inhibitory transmitters such as glycine and GABA is based on this effect.

and thereby alter the behavior of the postsynaptic cells (signal transduction).

Synaptic transmission efficiency may be modulated. There are two basic types of neuromodulation: presynaptic and postsynaptic. Presynaptic autoreceptors to glutamate, serotonin, dopamine, GABA, histamine, adrenoreceptors, muscarine receptors are known. In addition to this presynaptic heteroreceptors exist which are sensitive to mediators releasing by other neurons. Postsynaptic modulation may also be autoregulation and heteroregulation.

18.4. Biochemistry of Neuromediators

Fife basic criteria for typical mediators:

- They are synthesized in neurons.
- They are accumulated in synaptic vesicles.
- They are released by portions in response of nerve impulse.
- They have receptors on postsynaptic membrane.
- They are quickly inactivated.

Mediators have different biochemical structure:

· Acetylcholine

· Amino acids and their derivatives: glutamate, aspartate, glycine, taurine, β-alanine, GABA, dopamine, norepinephrine, epinephrine, serotonin.

· Neuropeptides: substance P, endorphins, enkephalins, N-acetyl-aspartyl-glutamate etc.

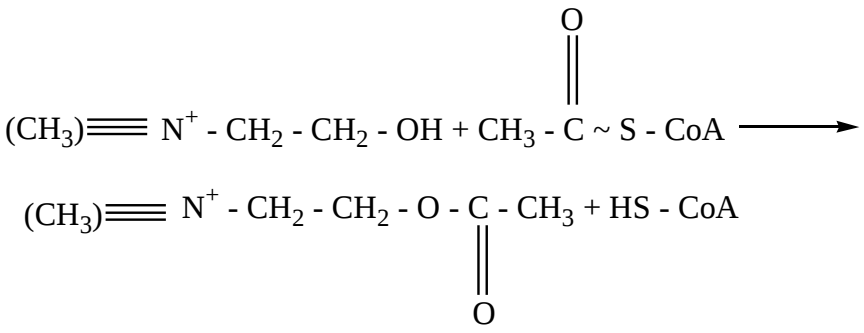
· Purine nucleosides: adenosine.

· Gases: NO.

Characteristic of Some Mediators

Acetylcholine (ACh) was the neurotransmitter first discovered, at the beginning of the 20th century.

Acetylcholine is *synthesized* from acetyl-CoA and choline in the cytoplasm of the presynaptic axon.



It is stored in synaptic vesicles, each of which contains around 1000-10000 ACh molecules.

After it is released by exocytosis ACh travels by diffusion to the receptors on the postsynaptic membrane. Catalyzed by *acetylcholinesterase* hydrolysis of ACh to acetate and choline immediately starts in the synaptic cleft, and within a few milliseconds the ACh released has been eliminated again. The cleavage product choline and acetate are taken up again by the presynaptic neuron and reused for ACh synthesis.

Acetylcholine is mainly excitatory mediator and rarely inhibitory one (for example in synapses of vagus nerve on the myocardial fibers). Acetylcholine acts at neuromuscular junctions, where it triggers muscle contraction, and in certain parts of the brain and in the autonomous nervous system.

It binds to two types of receptors: nicotinic and muscarinic ones.

The *nicotinic ACh receptor* responds to the *alkaloid nicotine* contained in tobacco (many of physiological effects of nicotine are based on this). The nicotinic receptor is ionotropic.

The muscarinic ACh receptors (of which there are at least five subtypes) are metabotropic. The muscarinic ACh receptors influence the cAMP level in the postsynaptic cells (M_1, M_3, and M_5 increase it, while subtypes M_2 and M_4 reduce it). Their name is derived from alkaloid *muscarine,* which is found in the fly agaric mushroom (Amanita muscaria) for example.

Cholinergic synapses of brain contain mainly muscarinic receptors. In spinal marrow ACh is neuromediator in synapses formed by α-motoneurone on the Renshow cells. In vegetal nervous system ACh serves neuromediator in all the preganglial nervous endings of sympathetic and parasympathetic nervous systems (including adrenal medulla) through nicotinic cholinoreceptors; in all the postganglial parasympathetic nerves, postganglial sympathetic nerves of sweat glands through muscarinic receptors.

Neuromuscular junction. Muscle contraction is triggered by motor neurons that release the neurotransmitter acetylcholine. It diffuses through the narrow synaptic cleft and binds to *nicotinic* ACh receptors on the plasma membrane of the muscle cell (the sarcolemma), thereby opening the ion channels integrated into the receptors. This leads to an inflow of Na^+, which triggers an action potential in the sarcolemma.

The action potential propagates from the end plate in all directions and constantly stimulates the muscle fiber.

Substances that block the serine residue in the active center of *acetylcholinesterase – e.g., the neurotoxin E605* and other *organophosphates* – prevents ACh degradation and thus cause prolonged stimulation of the postsynaptic cell. This impairs nerve conduction and muscle contraction.

Curare, a paralyzing arrow-poison, used by South American Indians, competitively inhibits binding of ACh to its receptor.

*Prozerin, galactamine (*reversible inhibitors of acetylcholinesterase) are used in clinical practice in myasthenia, atony of intestine and bladder to increase the activity of acetylcholinergic transmission.

Excitatory Amino Acid (Glutamate and Aspartate)

Glutamate is the main stimulatory transmitter in the CNS. More than half of the synapses in the brain are glutamatergic. Glutamatergic and glutamatereceptive neurons are widely spread practically in all the regions of mammal brain that indicates the considerable role of this type of mediation in brain action.

Excitatory amino acids (glutamate and aspartate) and their receptors take part in the formations of such fundamental processes of nervous functioning as synaptic plasticity, long-term potentiation, which is in the base of neuronals memory, supporting of seizure threshold, regulation of muscle tonus.

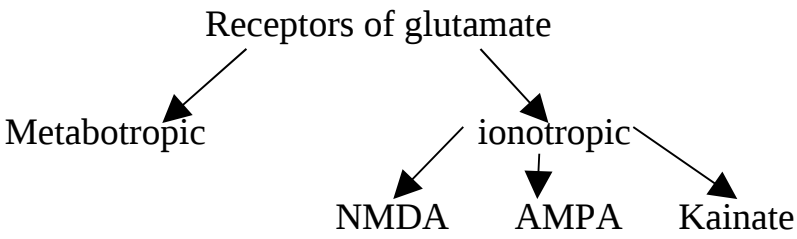
But hyperfunction of mediatory system of excitatory amino acids results in irreversible degeneration of brain neurons. Superactivation of glutamate receptors is the main cause of excessive entering Ca^{2+} to cells, which leads to their damage and death.

System of excitatory amino acids is involved to pathogenesis of seizure states, including epilepsy, to neurological disorders such as Alzheimer's and Huntington's diseases.

The function of glutamate as a stimulatory transmitter in the brain is the cause of what is known as the "Chinese restaurant syndrome". In sensitive individuals, the monosodium glutamate used as a flavor enhancer in chinese cooking can raise the glutamate level in the brain to such an extent that transient mild neurological disturbances can occur.

Postsynaptic glutamate receptors are classified into ionotropic and metabotropic ones.

Ionotropic receptors are coupled with ionic channels. Metabotropic receptors realize the effects of mediators by means of G-proteins and systems of the second messengers.



NMDA – receptors are activated by glutamate, aspartate and N-methyl-D-aspartate. Activation of NMDA – receptors leads to entering extracellular calcium to cells through the Ca^{2+}-channels. AMPA – receptors are activated by glutamate, DL-α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid. Kainate receptors are activated by glutamate and kainate. Activation of AMPA and kainate receptors is accompanied by depolarization of membrane through opening Na^{+}-channels. This leads to opening potential – depending Ca^{2+} channels and increasing intracellular concentration of Ca.

Metabotropic receptors are activated by glutamate. Activation of these receptors results in the formation of diacylglycerol and inositol triphosphate (the second messengers which lead to the increasing Ca concentration by means of Ca mobilization from intracellular depots).

Inhibitory amino acids (GABA, glycine, taurine, β-alanine).

Glycine is an inhibitory neurotransmitter with effects in the spinal cord and in parts of the brain.

GABA is the most important inhibitory transmitter in CNS. GABA acts through ionotropic channels, which are coupled with Cl^{-} channels.

In addition to the neurons, which use Glu and GABA as transmitters, *neuroglia is also involved* in the metabolism of these substances. Since glutamate and GABA as transmitters must not appear in the extracellular space in an unregulated way, the cells of the neuroglia supply “glutaminergic” and “GABAergic” neurons with the precursor glutamine (Gln), which they produce from glutamate with the help of *glutamine synthetase.* GABA neurons and glutamate neurons initially hydrolyze glutamine with the help of *glutaminase* to form glutamate again. The glutamate neurons store this in vesicles and release it when stimulated. The GABA neurons continue the degradation process by using *glutamate decarboxylase* to convert glutamate into the transmitter GABA.

Both types of neurons take up their transmitter again. Some of it also returns to the neuroglia, where glutamate is amidated back into glutamine. Glutamate can also be produced again from GABA (GABA shunt is characteristic of the CNS).

Taurine is formed by decarboxylation of cysteinic acid (cysteine derivative). Functions of taurine in nervous system:

- neurotransmitter (central function);
- trophic factor in development of CNS;
- supporting structural integrity of membranes;
- regulation of Ca^{2+} transport;
- osmolyte;
- neuromodulator, neuroprotector.

Receptors to taurine are metabotropic. They are coupled with phospholipase C (PlC) through inhibitory G-protein. Activation of taurine receptors by taurine would lead to inhibition of PLC activity resulting in reduction in IP_3 formation and hence IP_3-mediated release of Ca^{2+} from the internal pools.

Catecholamines

Biosynthesis of catecholamines starts from tyrosine. Hydroxylation of the aromatic ring initially produces DOPA. The reaction catalyzed by tyrosine hydroxylase is rate limiting. This reaction uses the unusual coenzyme tetrahydrobiopterin (THB). Decarboxylation of DOPA yields *dopamine*, an important transmitter in the CNS. In dopaminergic neurons, catecholamine synthesis stops at this point.

Hydroxylation of dopamine leads to *norepinephrine (noradrenaline).* Noradrenaline acts in the autonomic nervous system and certain areas of the brain. In humans brain noradrenergic neurons are mainly located in locus coeruleus, hippocampus, and considerable part of cortex. Functional role of noradrenaline is associated with supporting level of neuro-psychic reaction activity, with formation of cognitive and adaptive processes.

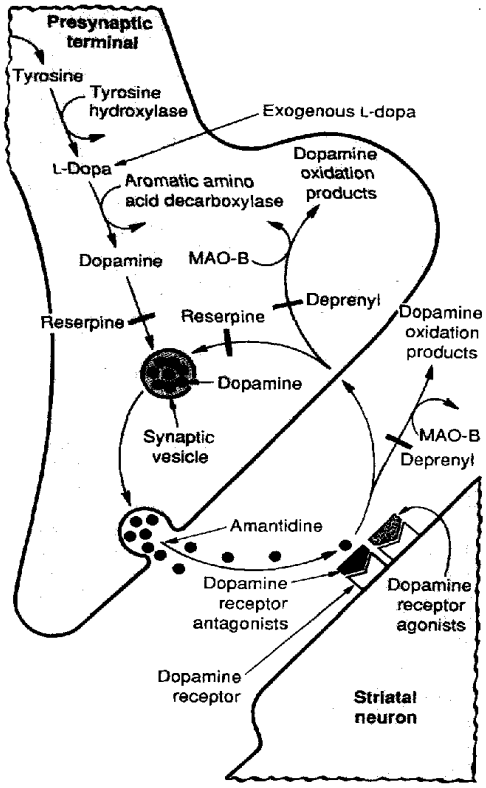


Figure 18.2 Schematic diagram illustrating the synthesis, the release and inactivation of dopamine. The sites of actions of drugs (L-dopa, deprenyl, amantadine, and dopamine receptor agonists [eg, bromocriptine]) that are used to treat parkinsonism are also shown.

Finally, N-methylation of norepinephrine yields *epinephrine (adrenaline).* S – Adrenosylmethionine is the donor of methyl group. Epinephrine mainly shows hormonal activity, but it is also used as a transmitter by some neurons.

The physiological effects of the catecholamines are mediated by a large number of different receptors that are of particular interest in pharmacology.

There are at least four types of adrenoreceptors: α_1 -, α_2 -, β_1- and β_2-. They have different biochemical, physiological and pharmacological properties.

β- Adrenoreceptors (β_1- and β_2-) are coupled with adenylyl cyclase through G*s*- proteins; α_2 – adrenoreceptors are coupled with adenylyl cyclase through G_i- proteins. α_1- Adrenoreceptors are coupled with phosphatidyl inositol messenger system. There are at least five classes of dopamine receptors. These produce their effector actions by affecting adenylyl cyclase positively or negatively or by

affecting other signaling systems (phospholipase C and polyphosphoinositide cycle).

Reuptake of catecholamines occurs. This is achieved by a high affinity transporters (which use ATP) present in the presynaptic membrane. The recycled catecholamines can again be incorporated into synaptic vesicles and reused as transmitters.

Degradation of catecholamines can occur within the synaptic cleft or, following reuptake, within the presynaptic terminal. Monoamine oxidase β (MAO-B) is present in the outer membrane of mitochondria within the presynaptic terminal and also present in the synaptic cleft. MAO-B and MAO-A are distinguished from each other by their preferences for different substrate and by their different susceptibilities to various inhibitors. Dopamine is converted to 3.4-dihydroxyphenylacetate, noradrenaline – to dihydroxymandelic acid. The latters are respectively converted to homovanilinic and 3-methoxy-4-hydroxy-mandelic acids by the action of catechol-0-methyl-transferase (COMT).

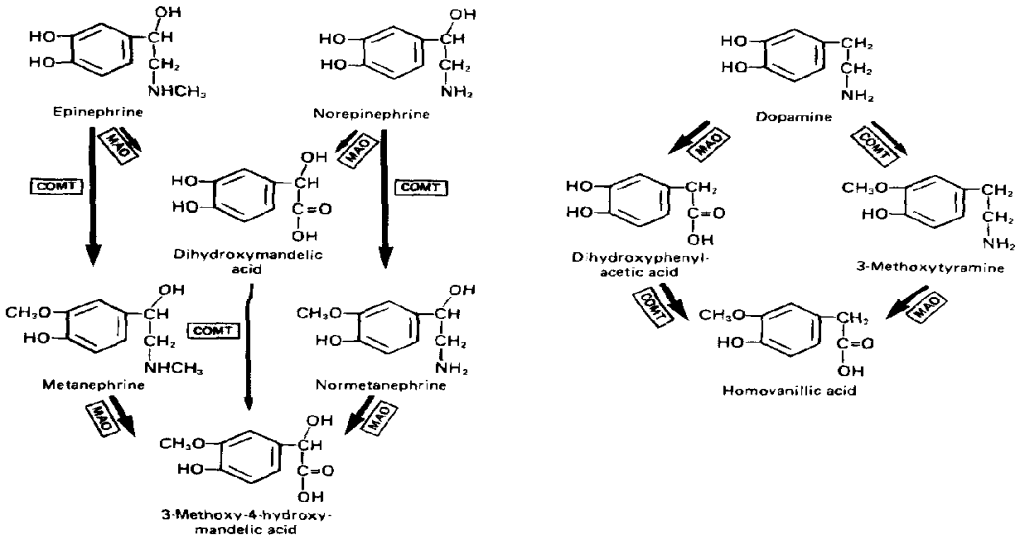


Figure 18.3 Metabolism of catecholamines by catechol-O-methyltransferase (COMT) and monoamine oxidase (MAO).

The development of schizophrenia, alcoholism, depressive states, Parkinson's disease is linked with disorders of dopamine metabolism and dopamine receptors functions.

Serotonin (5-hydroxytryptamine). Serotonin has many types of receptors: 5-HT_1, 5-HT_2, 5-HT_3, 5-HT_4 and subtypes (particularly 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1C}). They are metabotropic ones.

Serotonin participates in regulation of psycho-emotional reactions, sexual behavior, in control of physiologic sleep cycles. It is “neuromediator of good feeling”.

Disturbances of serotonin metabolism are linked with pathogenesis of depressive states, schizophrenia, alcoholism, narcomany.

Opioide peptides

Endorphins, dynorphins and enkephalins act as “endogenous opiates” by producing analgetic, sedative and euphoriant effects in extreme situations.

Drugs such as morphine and heroin activate the receptors for these peptides. The receptors to them are divided into some subtypes: μ-(mu), δ-(delta), κ-(kappa), σ- (sygma), ε-(epsylon). The highest density of opiate receptors was found in limbic zone of brain.

Disturbances of mediator metabolism in brain lead to development of neuropsychiatric disorders.

Schizophrenia. Genetically determined hyperactivity of dopaminergic system of mesocortical and mesolimbic tracts is the most considerable factor in pathology of schizophrenia.

Parkinson’s disease is characterized by tremor, bradikinesia, rigidity, and postural instability. Parkinson’s disease is caused by degeneration of the cells of the substantia nigra, resulting in a deficiency of dopamine in the nigrostriatal system.

The diminishing of dopamine raises the ratio acetylcholine to dopamine in cells of nigrostriatal system because levels of acetylcholine are not so affected. This imbalance contributes to the various disorders of movement found in Parkinson’s disease.

Dopamine itself does not cross the blood-brain barrier so it cannot be used in treatment of Parkinson’s disease. Injection of levodopa (L-DOPA), which is the

precursor of dopamine, has good therapeutic effect. But most of the administered DOPA is decarboxylated in the periphery and only 1% reaches the brain. Therefore levodopa is administered with carbidopa that inhibits the activity of peripheral DOPA – decarboxylase but does not cross the blood - brain barrier and so does not inhibit the conversion of levodopa to dopamine in the brain.

Epilepsy is linked with prevalence of excitatory processes over inhibitory ones. The increase of glutamatergic and the decrease of GABA-ergic transmissions play the important role in development of this disease.

Alzheimer's disease is characterized by loss of memory, by progressive decline of intellectual functions. Some 2 million people in the USA suffer from Alzheimer's disease. The basic pathologic picture is of degenerative process characterized by the loss of cells in certain areas of the brain (e.g, the cortex and hippocampus). Amyloid β protein is accumulated. Amyloid plaques and neurofibrillary tangles are key features of Alzheimer's disease. The amyloid cascade hypothesis postulates that deposition of amyloid β protein, derived from amyloid precursor protein, is an initial event in the causation of the disease and that amyloid β protein, or fragments of it, are neurotoxic, leading to formation of the neurofibrillary tangles and other features. However, the possibility that deposition of amyloid β protein, is secondary in most cases to other event has not been excluded.

Hungtington's disease is characterized by brief involuntary movements (chorea) and progressive demenzia. Neurons in the corpus striatum (caudate nucleus and putamen) are most affected. They are partly replaced by glial cells (gliasis). Studies of mechanisms of cell injury and death in Huntington's disease have shown that one major factor is excitation of the NMDA class of glutamate receptors.

The action of psychotropic drugs is directed on regulation and correction of:

- the rate of synthesis and degradation of mediators;
- the processes of neuromediator deposition in synaptic vesicles;
- the release of mediator to synaptic cleft;

- binding neuromediators with postsynaptic receptors;
- the processes of mediator reuptake.

Neuroleptics are drugs which are used for treatment of psychoses, mainly schizophrenia. They are mainly antagonists of dopamine receptors (D_2; D3; D_4). These drugs are derivatives of phenotiazin (aminazin) and butyrylphenol (haloperidol).

Antidepressants stimulate monoaminergic neurotransmission increasing synaptic concentration of noradrenaline and serotonin. According to mechanism of action antidepressants are divided into two groups:

- inhibitors of monoamine reuptake (imipramine, amitriptyline);

- MAO inhibitors (iproniazid - irreversible inhibitor; pirazidol - reversible inhibitor);

Anxiolytics (tranquilizers) are the group of substances which have sedative effect on the nervous system. *Benzodiazepins* belong to them. Neurochemical mechanisms of central pharmacologic effects of benzodiazepins are linked with their interaction with GABA receptors (suptype $GABA_A$) of brain and stimulation of inhibitory effect. The most wide spread of them are lorazepam, diazepam, oxazepam.

Tests for Self-control

1. Point biogenic amines which are mediators of inhibition:
A. Dopamine
B. Histamine
C. Serotonin
D. γ-aminobutyric acid
E. Taurine
2. Which is the main process of ammonia detoxification in nervous tissue?
A. Transamination
B. Urea synthesis
C. Formation of dicarbonic acid amides
D. Ammonia salts formation
E. Biogenic amines synthesis
3. Toxicity of ammonia (especially for brain) is due to its capacity to disturb the functioning of Krebs cycle as result of the removal from cycle of:
A. Malate
B. Citrate
C. α-Ketoglutarate
D. Succinate
E. Fumarate
4. GABA (γ-aminobutyric acid), which belongs to inhibitory mediators, is he product of glutamate decarboxylation. Which vitamin prescription has the sense in convulsion states due to the decreased formation of GABA?
A. B_9
B. B_6
C. B_1
D. B_5
E. B_2
5. Psycho-pharmacologic drugs with antidepressive action inhibit oxidative deamination of noradrenaline and serotonin in mitochondria of brain by means of inhibition of:
A. Monoamine oxidase
B. Cytochrome oxidase
C. Pyruvate dehydrogenase
D. Aldolase
E. Succinate dehydrogenase
6. Which of the following substances belong to as so called “inhibitory amino acids”?
A. Histidine, tyrosine
B. Glutamate, glutathione
C. Aspartate, asparagine
D. Proline, lysine
E. GABA, glycine

Chapter 19 MUSCLE TISSUE

19.1 Function and Chemical Composition

Muscle is the major biochemical transducer (machine) that converts potential (chemical) energy into kinetic (mechanical) energy.

Effective transformation of chemical energy into mechanical one is provided by the following conditions:

- Constant supply of chemical energy.
- Regulation of muscle contractility.
- Regulation by the nervous system.
- Availability of the system to return to its original state.

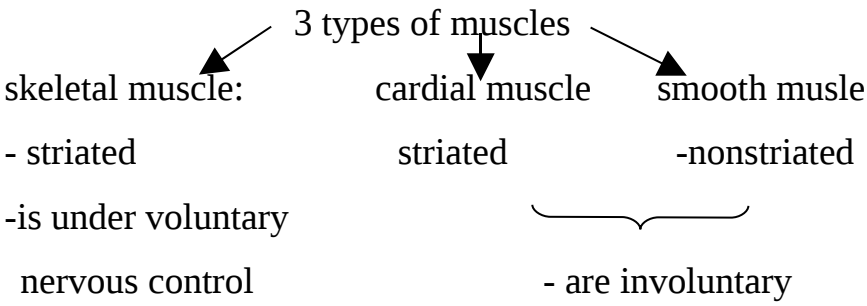
The muscular tissue accounts:

at birth – less than 25% of body mass;

in the young adult – more than 40%;

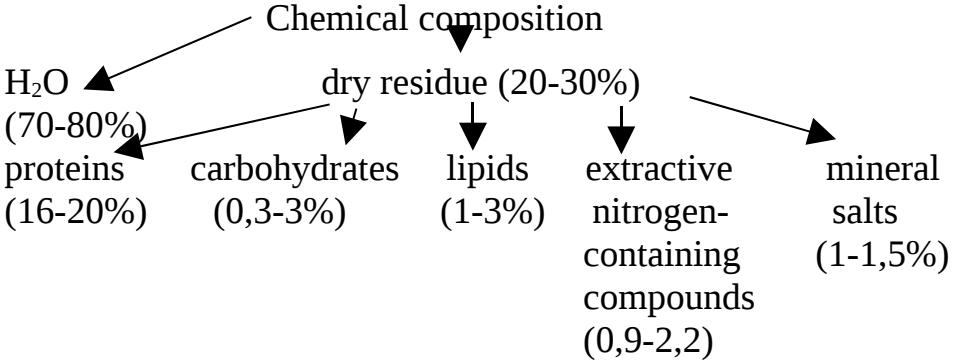
in the aged adult – less than 30%.

Three types of muscular tissue are distinguished:



Functions

- The major function of muscle is to provide the mobility of the organism via the alternating acts of muscular contraction and relaxation.
- Thermoregulation.



Muscle proteins

Sarcoplasmic proteins	**Myofibrillar proteins**	**Proteins of stroma**
Myogen – this is a group term. The proteins of myogen group include enzymes, for example, glycolytic enzymes, etc. *Myoglobin* - heme containing protein. Its level is different in red and white muscle. It creates reserve of O_2 for tissue respiration. *Globulin X* - fraction of proteins with properties of globulins. *Myoalbumin.* It is in the greatest amount in muscles of embryons and in smooth muscles	*Myosin* contributes 55% of muscle protein by weight and forms the thick filaments. It is an assymetric hexamer which consists of 4 light (L) and 2 heavy (H) chains. The heavy chains are twisted into a long α -helix (the “tail” of the molecule). One end of each heavy chain (N-terminal end) has a globular “head”. Every globular “head” is linked with 2 L-chains. Myosin has ATPase activity (namely, globular “head” of H-chains of myosin). *Actin* accounts for about 20% of myofibrils. It is the component of thin filaments. Two forms for actin are known. Actin G-actin (globular actin) F-actin (fibrillar actin)	*Collagen* – fibrillar protein; accounts about 22% of total muscle protein. It binds muscle fibers. *Elastin* covers muscle fibers.

At physiologic ionic strength and in the presence of Mg^{2+}, actin is polymerized noncovalently to form an insoluble double helical filament called F-actin.

Actomyosin is a complex formed by combination of myosin with F-actin in the course of muscle contraction.

Tropomyosin is a fibrous molecule that consists of two chains, α - and β -, that attach to F-actin in the groove between its filaments.

Troponin is composed of three subunits: Tp-I, Tp-T, Tp-C

Tp-T binds to tropomyosin as well as to the other two troponin components.

Tp-I inhibits the F-actin - myosin interaction and also binds to the other components of troponin.

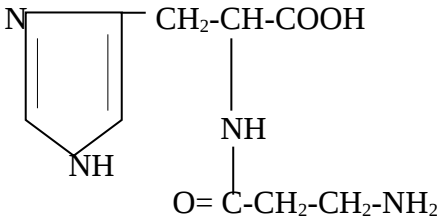
Tp-C is a calcium -binding polypeptide that is structurally and functionally analogous to calmodulin

Table 19.1 Some accessory protein of muscle

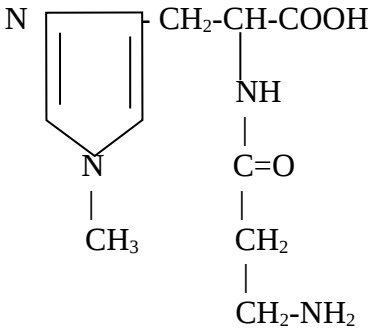
Protein	Location	Comment of function
Titin	Reaches from the Z line to the M line.	Largest protein in body. Role of relaxation in muscle.
Nebulin	From Z line along length of actin filaments.	May regulate assembly and length of actin filaments.
α-Actinin	Anchors actin to Z lines.	Stabilizes actin filaments.
Desmin	Lies alongside actin filaments.	Attaches to plasma membrane (plasmalemma).
Dystrophyn	Attached to plasmalemma.	Deficient in Dushenne muscular dystrophy.

Non-protein nitrogen-containing extractive compounds

- **carnosine** } dipeptides
- **anserine**



Carnosine
(β-alanyl - histidine)



Anserine (N-methylcarnosine)

Functions of these dipeptides:

- They enhance amplitude of muscle contraction prior decreased by tiredness. Imidazole containing peptides have been shown to increase work efficiency of ionic pumps of muscle cells.
- They buffer the pH of anaerobically contracting skeletal muscle.
- Carnosine and anserine activate myosin ATPase activity.

- **Creatine** transfers high potential phosphoryl groups from mitochondria in cytoplasma.
- **Creatine phosphate** is a reservoir of high potential phosphoryl groups.
- **ATP** is the immediate source of energy for muscle contraction.

- **Glutamine**
- **Glutamate**

} constitute about 75% of all the amides and amino acids

- **Other amino acids**

Nitrogen – free components:

- **Lipids** – TAG, phospholipids, cholesterol

reservic fat components of membranes

- **Carbohydrates:**

glycogen (about 1% of dry residue).

- **Minerals** (1-1,5% of dry residue): K^+, Na^+, Ca^{2+}, Mg^{2+}, Cu^{2+}, Mn^{2+}, Zn^{2+},Cl – and others.

19.2 Features of Metabolism

Carbogydrate metabolism

- Active processes of aerobic and anaerobic oxidation of glucose.
- Gluconeogenesis is absent.
- Glucose-6-phosphatase is absent
- Glucose-lactate and glucose-alanine cycles play the important role in providing muscle cells by glucose.

- Features of glycogen metabolism:

- Receptors to glucagons are absent in skeletal muscles.

- Glucose-6-phosphatase is absent in muscles, therefore muscular glycogen is used only in muscle cells.

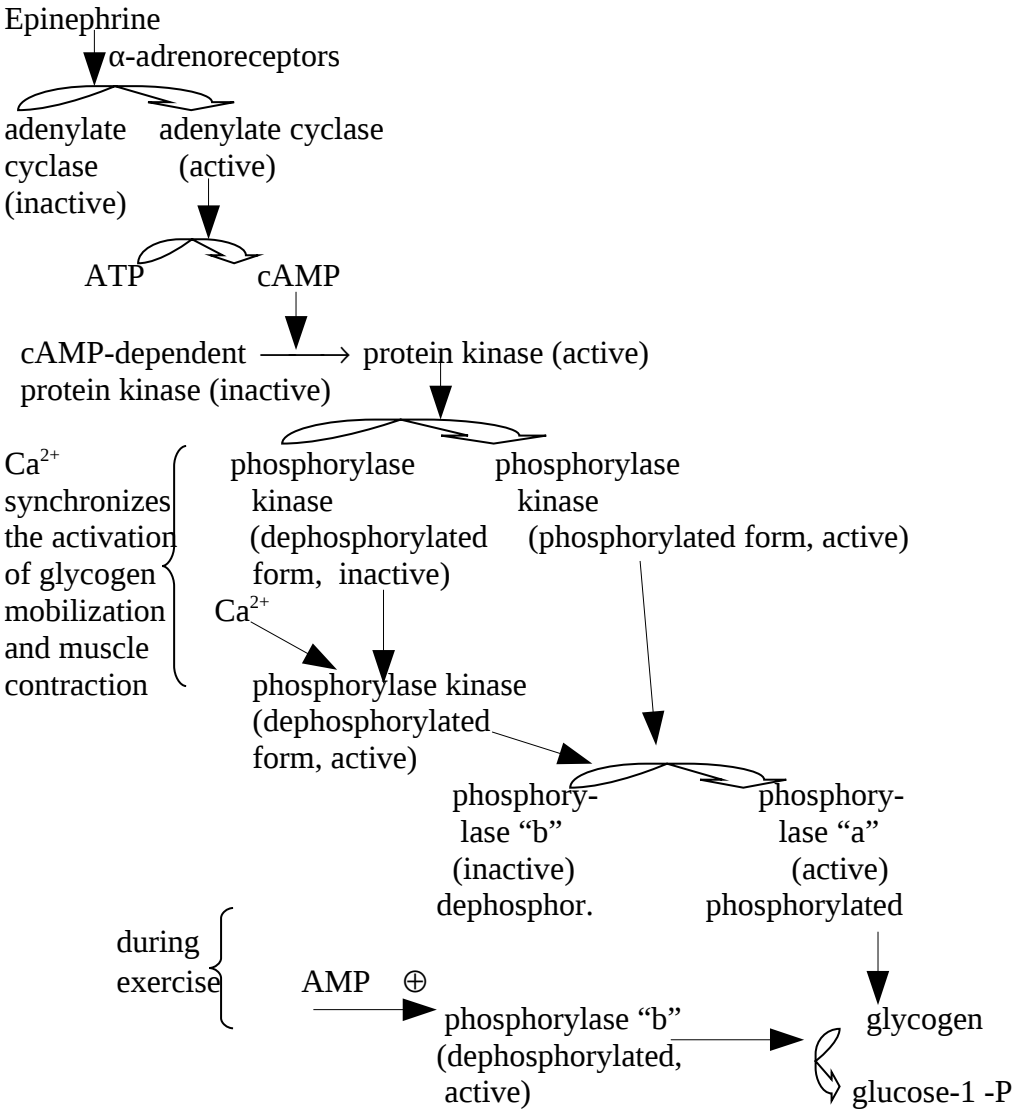


Figure 19.1 Scheme of glycogen mobilization regulation in muscle

- Phosphorylase kinase in muscle is activated not only by means of phosphorylation, but also by Ca^{2+} ions. It consists of 4 subunits. β -Subunit is identical to calmodulin. This subunit is bound with $4Ca^{2+}$ ions and this leads to activation of phosphorylase kinase. Thus **Ca^{2+} synchronizes** the activation of phosphorylase with muscle contraction.

- Phosphorylase of muscle is also activated by two pathways: by phosphorylation and by allosteric regulation of dephosphorylated phosphorylase by means of AMP (this occurs during exercise, when the level of AMP rises, providing, by this mechanism, fuel for the muscle).

- Phosphorylase of muscle is linked with pyridoxal phosphate, wich is needed for stabilization of enzyme; 70-80% of vitamin B_6 is linked with phosphorylase of muscles.

Lipid metabolism

During long muscle work β -oxidation of fatty acids and ketolysis are intensive in muscles. About 70% of O_2, absorbed by cardiac muscle, is used for oxidation of fatty acids.

Protein metabolism

- Proteins of skeletal muscle are a reserve of amino acids (especially, in starvation).
- In muscles catabolism amino acids (especially with branched chains) is intensive. Activity of glutamate dehydrogenuse is low and therefore amino acids are mainly deaminated by transamination. Amino groups of amino acids, which are deaminated, are used for the synthesis of adenylate nucleotides. Excess of amino groups is transported from muscles to liver as alanine (pyruvate-alanine cycle).

Features of metabolism in red and white skeletal muscles

Red (slow, aerobic) muscles contain many of myoglobin and mitochondria. Their metabolism is aerobic, and they maintain relatively sustained contractions.

White (fast, anaerobic) muscles. They contain few mitochondria. They derive their energy from anaerobic glycolysis and exhibit relatively short durations of contraction.

Features of metabolism in cardiac muscle

Cardiac muscle is contracted 100000 times per day (7200 L of blood). Cardiac muscle, like skeletal muscle, is striated. Its metabolism is aerobic. Main fuel is fatty acids. Glucose, pyruvate, lactate are also used as fuel.

19.3 Energy Sources for Muscular Activity

Constant energy source for the contraction – relaxation cycle of muscle is ATP. The amount of ATP in skeletal muscle is only sufficient to provide energy for contraction for 1-2 seconds, so that ATP must be constantly renovated.

I. Creatine phosphate constitutes a major energy reserve in muscle. It prevents the rapid depletion of ATP by providing a readily available high-energy phosphate, which can be used to regenerate ATP from ADP. Creatine phosphate is formed from ATP and creatine at times when the muscle is relaxed and demands for ATP are not so great.

II. Adenylyl kinase reaction

Adenylyl kinase catalyzes formation of one molecule of ATP and 1 of AMP from two molecules of ADP.

$$2ADP \rightarrow ATP + AMP$$

This reaction is coupled with the hydrolysis of ATP by myosin ATPase during muscle contraction. Degradation of AMP leads to formation or IMP+NH_3 or adenosine. Adenosine acts as vasodilator, increasing the blood flow and supply of nutrients to muscle. Adenosine is then converted into inosine +NH_3.

AMP activates phosphofructokinase I, thus increasing the rate of glycolysis in rapidly exercising muscle, such as during a splint.

Role of adenylyl kinase reaction:

- Reserve mechanism of fast formation of ATP.

- Formation of AMP that allosterically activates phosphorylase “b” (activation of glycogen mobilization in muscle) and phosphofructokinase I (activation of glycolysis).

III. Glycolysis

Glycolysis starts 20 s after the onset of maximally intense muscular load and reaches a peak within 40-80s.

Blood glucose and muscle glycogen are used. Under intense muscular work, the rate of glycolysis to produce lactic acid in the muscular tissue may be as high as -1,2g/kg and over.

Fatigue of muscle during exercice is a phenomenon that almost everyone has experienced. The primary cause is accumulation in muscle tissue not of lactate (due to anaerobic glycolysis) but rather of protons. This fact has been demonstrated by infusing lactate observing that fatigue does not necessarily follow. Increase of protons (decreased pH) can affect the function of muscle in a number of ways, including the following: (1) lowering the Vmax of PFK1, (2) lessening the release of Ca^{2+} from the sarcoplasmic reticulum, (3) lessening the activity of the actomyosyn ATPase, (4) possibly by affecting the conformation of some of the muscle proteins involved in contraction.

A large portion of lactic acid is delivered, in the blood stream, to the liver to be resynthesized to glucose and glycogen.

Glycolysis is the major source of energy for muscle contraction under intensive muscular work.

The major sources of energy in the 100-m sprint are creatine phosphate (first 4-5 seconds) and then anaerobic glycolysis, using muscle glycogen as the source of glucose.

IV. Aerobic oxidation of glucose and fatty acids (oxidative phosphorylation)

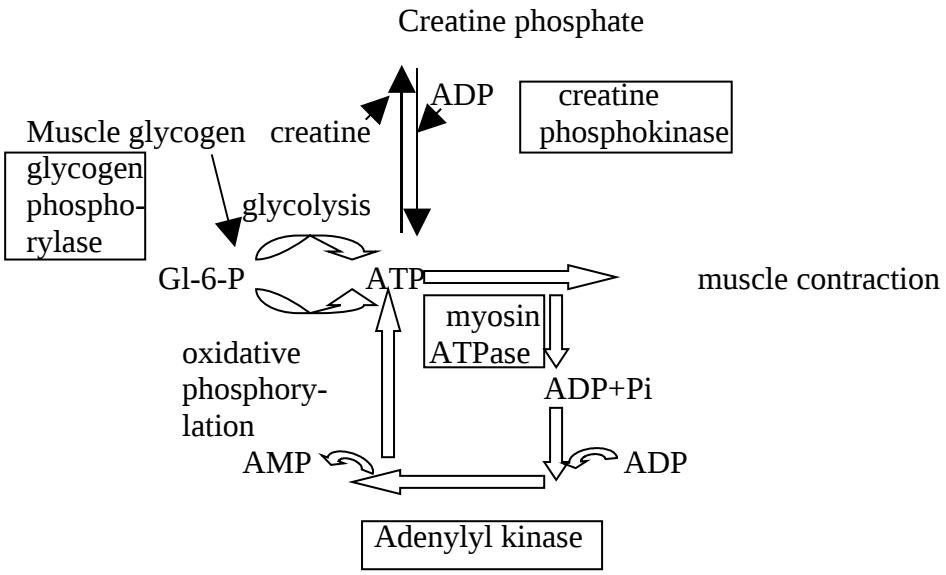
Under long-lasting exercise, when the muscular load is usually moderate, the aerobic pathway for ATP resynthesis is very important.

In the marathon aerobic metabolism is the principal source of ATP. The major fuel sources are blood glucose and free fatty acids, largely derived from the breakdown of triacylglycerols in adipose tissue, stimulated by epinephrine. Hepatic glycogen is degraded to maintain the level of blood glucose.

Muscle glycogen is also a fuel source, but it as degraded much more gradually than in a sprint.

It has been calculated that the amounts of glucose in the blood, of glycogen in the liver, of glycogen in muscle, and triacylglycerol in adipose tissue are sufficient to supply muscle with energy during a marathon for 4 minutes, 18 minutes, 70 minutes and approximately 4000 minutes, respectively.

Sources of ATP in muscle



19.4 Mechanism of Muscle Contraction

General characteristic of muscle fiber

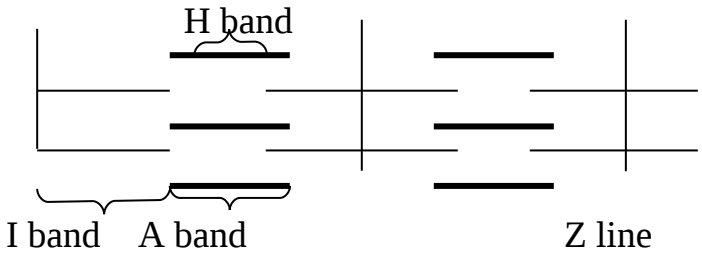
Striated muscle is composed of muscle fibers. ***Muscle fiber*** is multinucleated cell surrounded by an electrically excitable plasma membrane - the ***sarcolemma***.

An individual muscle fiber cell contains many ***myofibrils*** arranged in parallel, embedded in intracellular fluid termed ***sarcoplasma***. This fluid contains glycogen, the high–energy compounds ATP and phosphocreatine, and the enzymes of glycolysis.

Myofibrils are constructed of two types of longitudinal ***filaments*** – *thick* and *thin*.

Thick filaments contain chiefly the protein myosin. ***Thin filaments*** contain the proteins actin, tropomyosin and troponin.

Functional unit of myofibril is ***sarcomer. The sarcomer*** is defined as the region between two Z lines and is repeated along the axis of a fibril at distances of 1500-2300 nm, depending on the state of contraction. It includes actin filaments anchored in the Z lines and myosin filaments.



I-bands (isotropic bands) – they are formed by thin filaments

A-bands (anisotropic bands) – myosin and actin filaments overlap.

H-band – part of A-band, in which actin and myosin filaments don′t overlap.

According to the sliding filament hypothesis suggested in the 1950s by Henry Huxley and G. Henson and by Andrew Huxley and R. Nieberherske, the contraction of the microfibrils is achieved by parallel penetration of the actin and myosin filament sets into each other, that is, the filaments, when they are activated, start sliding along each other, which results in the contraction of the muscle.

- Ca^{2+} plays a central role in regulation of muscle contraction, in coupling excitation and contraction in muscles.
- In resting muscle sarcoplasma, the concentration of Ca^{2+} is 10^{-7} to 10^{-8} mol/L. This concentration is maintained by means Ca^{2+}-ATPase. Ca^{2+} is pumped into the sarcoplasmic reticulum. The sarcoplasmic reticulum is a network of fine membranous sacs. Inside the sarcoplasmic reticulum, Ca^{2+} is bound to a specific Ca^{2+} - binding protein, designated calsequestrin. The sarcoplasma is surrounded by an excitable membrane (the T tubule system) composed of tranverse (T) channels closely associated with the sarcoplasmic reticulum.

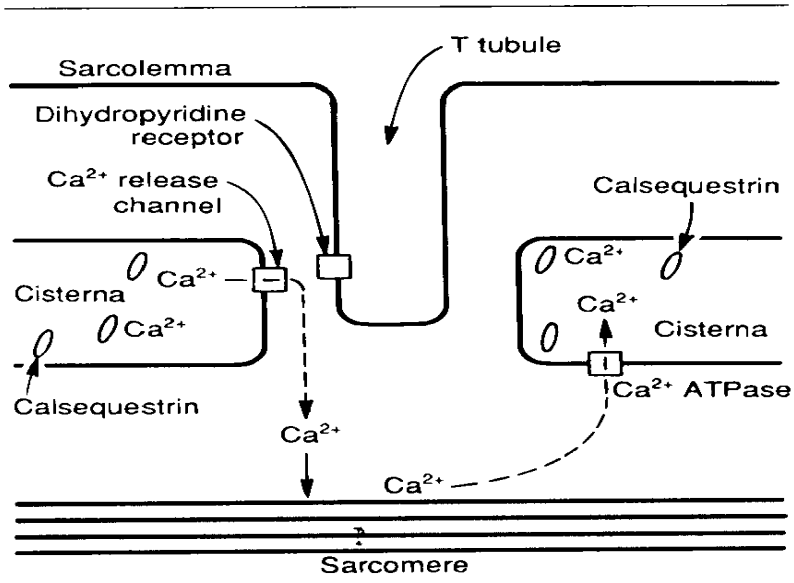


Figure 19.2 Diagram of the relationships among the sarcolemma (plasma membrane), a T tubule and two cisternae of the sarcoplasmac reticulum of skeletal muscle

- When the sarcolemma is excited by a nerve impulse the signal is transmitted into T tubule system. Ca^{2+} release channel opens, Ca^{2+} is released from sarcoplasmic reticulum into the sarcoplasma.
- The concentration of Ca^{2+} in the sarcoplasma rises rapidly to 10^{-5} mol/l.
- Ca^{2+} is linked with TpC. The TpC-4Ca^{2+} interacts with TpI and TpT to alter their interaction with tropomyosin.
- Tropomyosin alters the conformation of F-actin so that the myosin head can interact with F-actin.
- In the relaxation phase of muscle contraction, the S-1 head of myosin hydrolyses ATP to ADP and Pi, but these products remain bound. The resultant ADP-Pi-myosin complex is in a so-called high-energy conformation. (Figure 19.3,1)
- Interaction of myosin head-ADP-Pi with F-actin leads to formation the actin-myosin-ADP-Pi complex. (Figure 19.3, 2)
- Formation of this complex promotes the release Pi and ADP. (Figure 19.3,3)

- Release of ADP is accompanied by a large conformational change in the head of myosin and leads to turning a head by 45°, pulling actin about 10nm toward the center of the sarcomer. This is the power stroke.
- The myosin is now in a so-called low-energy state, indicated as actin-myosin.
- Another molecule of ATP binds to the S-I head, forming an actin-myosin-ATP complex. (Figure 19.3, 4)
- Myosin-ATP has a low affinity for actin and actin is thus released. (Figure 19.3, 5) This last step is a key component of relaxation and is dependent upon the binding of ATP to the actin-myosin complex.
- If new signal is absent, Ca^{2+} is pumped into the sarcoplasmic reticulum through the action of Ca^{2+}-ATPase. Ca^{2+} concentration falls below 10^{-7} mol/L. TpC-4Ca^{2+} loses its Ca^{2+}. Troponin, via its interaction with tropomyosin, inhibits futher myosin head – F-actin interaction. Relaxation occurs.

If intracellular levels of ATP drop (e.g., after death), ATP is not available to bind the S-I head, actin does not dissociate and relaxation does not occur. This is the explanation for rigor mortis, the stiffening of the body that occurs after death.

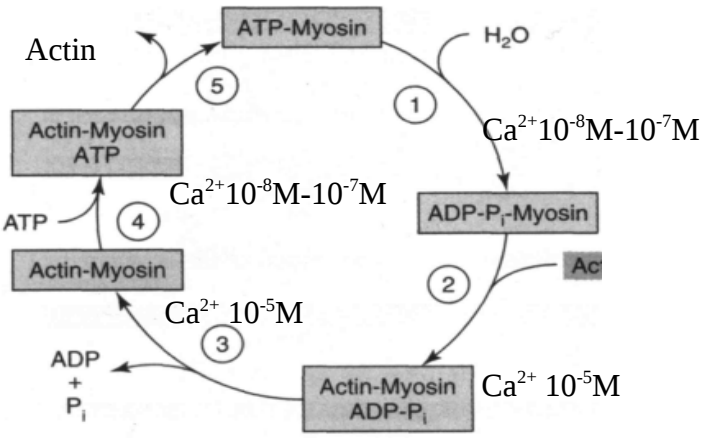


Figure19.3 The hydrolysis of ATP drives the cyclic association and dissociation of actin and myosin in five stages described in the text

Diminished formation of ATP such as might occur in ischemia, has two major effects:

1) The Ca^{2+}-ATPase (Ca^{2+} pump) in the sarcoplasmic reticulum ceases to maintain the low concentration of Ca^{2+} in the sarcoplasm. Thus, the interaction of the myosin heads with F-actin is promoted.

2) The ATP-dependent detachment of myosin heads from F-actin cannot occur, and rigidity (contracture) sets in.

Calculations have indicated that the efficiency of contraction is about 50%; that of the internal combustion engine is less than 20%.

Features of cardial muscle contraction

The general picture of muscle contraction in the heart resembles that of skeletal muscle.

Features:

- Cardiac muscle exhibits intrinsic rhythmicity and individual myocytes communicate with each other because of its syncytial nature.
- The T tubular system is more developed in cardiac muscle, whereas the sarcoplasmic reticulum is less extensive. Cardiac muscle thus relies on extracellular Ca^{2+} for contraction.
- Cyclic AMP plays a more prominent role in cardiac than in skeletal muscle. It modulates intracellular levels of Ca^{2+} and response of troponin-tropomyosin regulatory complex to it through the activation of protein kinases.
- That′s why catecholamines induce contraction in cardiac muscle.
- Level of ATP and creatine phosphate in cardiac muscle is lower than in skeletal muscle. ATP is mainly synthesized by oxidative phosphorylation therefore cardiac muscle is especially sensitive to oxygen deficiency. Main substrate for oxidation is fatty acids.

19.5 Features of Smooth Muscle Contraction

There are two general mechanisms of regulation of muscle contraction: actin-based and myosin-based.

Actin-based regulation occurs in striated muscle. Myosin-based regulation occurs in smooth muscle. In all the systems, Ca^{2+} plays a key regulatory role.

- Smooth muscle contains actin, myosin and tropomyosin
- They do not have the troponin system.
- Myosin-actin interaction is mediated by a light – chain (p-light-chain).
- Dephosphorylated form of p-light-chain inhibits myosin-actin interaction.
- Phosphorylated form of p-light-chain facilitates the formation of myosin–actin complex and activation of myosin ATPase (the phosphate on the myosin light chains may form a chelate with the Ca^{2+} bound to the tropomyosin –TpC-actin complex, leading to an increased rate of formation of cross-bridges between the myosin heads and actin).

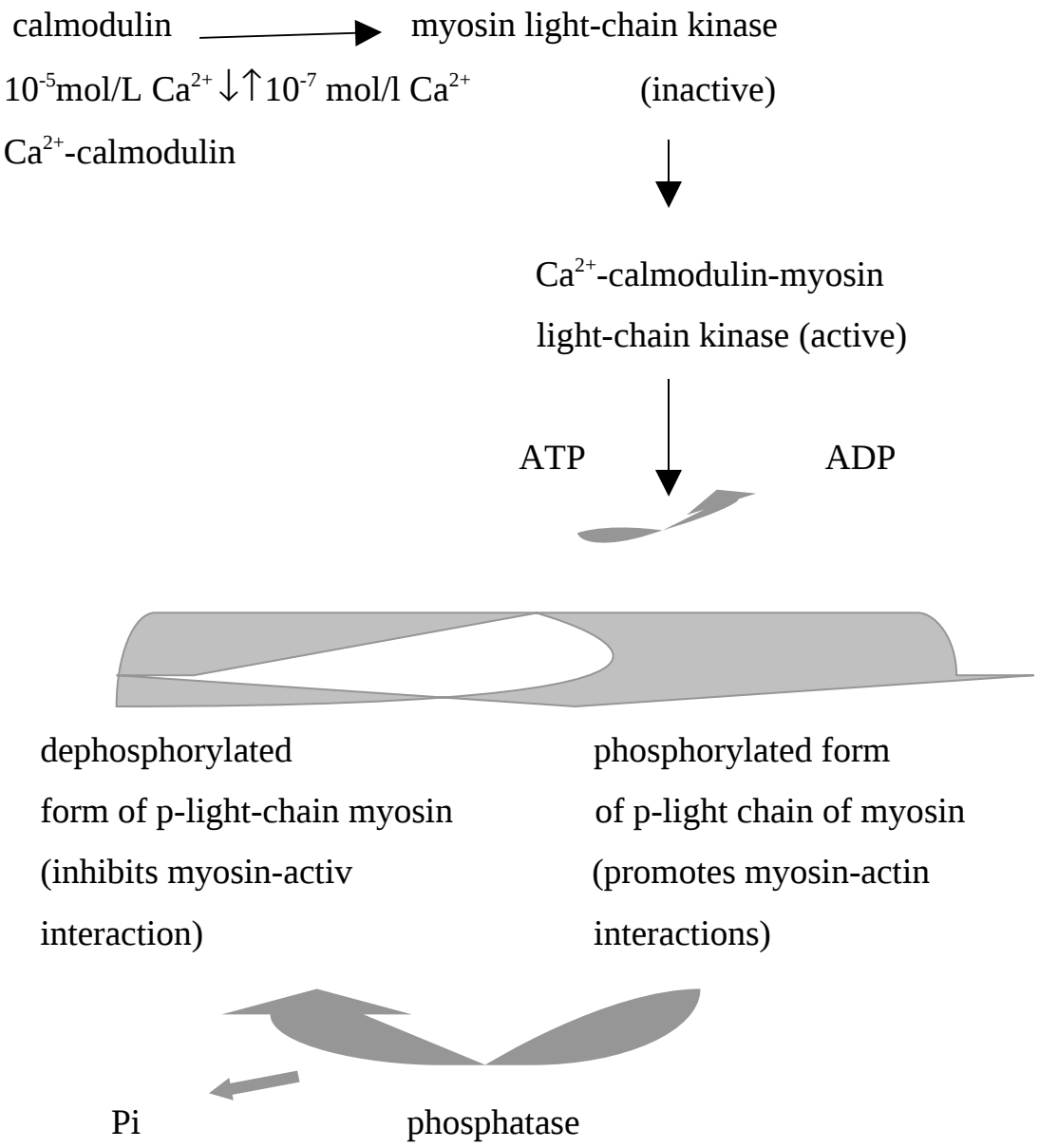


Figure 19.4 Regulation of smooth muscle contraction by Ca^{2+}

- Phosphorylation of light chains of myosin is performed by myosin light-chain kinase.
- Myosin light-chain kinase is activated by calmodulin-4Ca^{2+} complex.
- In its turn, myosin light-chain kinase may be phosphorylated by cAMP-dependent protein kinase. The phosphorylated myosin light-chain kinase exhibits a significantly lower affinity for calmodulin – Ca^{2+} and thus is less sensitive to activation.

Accordingly, an increase of cAMP dampens the contraction response of smooth muscle to a given elevation of sarcoplasmic Ca^{2+}. This molecular mechanism can explain the relaxing effect of β -adrenergic stimulation on smooth muscles.

β-Adrenergic receptors activate adenylyl cyclase. They are located in smooth muscle of bronchi, gastrointestinal tract, urinary bladder. Therefore epinephrine relaxes smooth muscle of bronchi, gastrointestinal tract and urinary bladder.

Catecholamines have vasoconstrictive effects through α_1 -receptors, which are coupled to processes that alter intracellular calcium concentration or modify phosphatidylinositide metabolism (or both).

- Relaxation of smooth muscle occurs when sarcoplasmic Ca^{2+} concentration falls below 10^{-7} mol/L.
- Ca^{2+} dissociates from calmodulin, which in turn dissociates from the myosin light-chain kinase, inactivating the kinase.
- Light chain is dephosphorylated by light chain protein phosphatase.
- Dephosphorylated light chain of myosin inhibits the binding myosin heads to F-actin and the ATP-ase activity.
- Myosin head detaches from the F-actin in the presence of ATP, but it cannot reattach because of the presence of dephosphorylated p-light chain; hence, relaxation occurs.

Role of nitric oxide in relaxation of smooth muscle of blood vessels

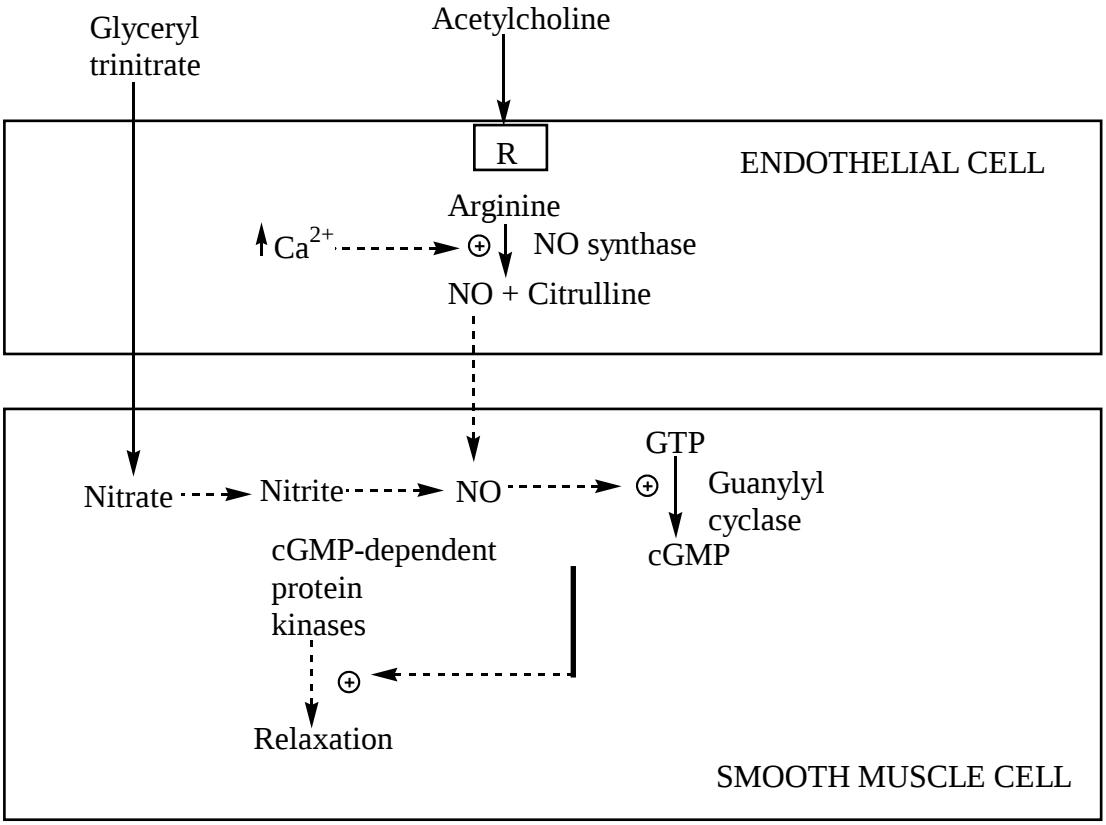


Figure 19.5 Role of NO in relaxation of blood vessel smooth muscle

- Acetylcholine is a vasodilator that acts by causing relaxation of the smooth muscle of blood vessels.
- However, it does not act directly on smooth muscle.
- Acetylcholine initially interacts with the endothelial cells of small blood vessels via receptors.
- The receptors are coupled to the phosphatidyl inositol messenger system, leading to the intracellular release of Ca^{2+} through the action of inositol triphosphate.
- Ca^{2+} activates NO synthase.

$$\text{Arginine} \xrightarrow{\text{NO synthase}} \text{citrulline} + \text{NO}$$

NO has a very short half-life (approximately 3-4 seconds) in tissues because it reacts with oxygen and superoxide. The product of the reaction with superoxide is

peroxynitrite ($ONOO^-$), which decomposes to form the highly reactive $OH^\bullet$ radicals.

- NO diffuses into adjacent smooth muscle, where it leads to activation of guanylyl cyclase, formation of cGMP, stimulation of cGMP-dependent protein kinases, and subsequent relaxation.

The important coronary artery vasodilator nitroglycerin, widely used to relieve angina pectoris, is converted into NO.

19.6 Biochemical Changes in Muscle in Pathology

A common feature of most diseased muscles (in progressive muscular dystrophy, neurotic atrophy of the muscle, tenotomy, polymyositis, certain avitaminoses, and other states) is a sharply diminished concentration of myofibrillar proteins and an increase in the concentration of stromatic proteins and some of sarcoplasmic proteins.

Along with a change in the fractional composition of muscle proteins, a decrease of ATP and creatine phosphate concentrations are also observed in the affected muscle.

In progressive muscular dystrophy and other states associated with the decay of muscular tissue, the common symptom is an alteration in the phospholipid composition of the muscle: the concentrations of phosphatidylcholine and phosphatidylethanolamine are decreased; the concentrations of sphingomyelin and lysophosphatidylcholine tend to rise. Creatinuria is also observed.

Duchenne-type muscular dystrophy is due to mutations in the gene, located on the X chromosome, encoding the protein dystrophin. It has an incidence of approximately 1:3500 live male births. It affects young boys, which first show loss of strength in their proximal muscle, leading to a waddling gait, difficulty in standing up, and eventually very severe weakness.

Disturbed metabolism of cardiac muscle in myocardial ischemia. The myocardial ischemia is manifested by decreased oxidative phosphorylation and increased anaerobic metabolism. The early enhancement of glycogenolysis and glycolysis at the initial stage of ischemia is provided by higher concentration of

catecholamines. But glycogen reserve becomes very soon depleted, and the glycolysis is retarded because of intracellular acidosis which produces an inhibitory effect on phosphofructokinase.

The cellular concentrations of ATP and creatine phosphate are drastically diminished because of impaired oxidative phosphorylation in the mitochondria.

An early manifestation of this pathologic state is impaired membrane permeability. This leads to an escape of ions and enzymes from the cell.

Compositional alterations in myocardial proteins are manifested by a sharp drop in myofibrillar protein concentrations and by accumulation of stromatic proteins. Fatty infiltration of cardiac muscle is observed.

Damage of myocytes is indicated by determination of CK, LDH, AsAT, glycogenphosphorylase (GP) catalytic concentrations, myoglobin, troponin T (TnT) and I (TnI) levels in blood. The determination of isoenzymes CK-MB and LDH_1, GP-BB; ratio between CK-MB and troponine is specific for damage of only cardiomyocytes (but no myocytes of skeletal muscles).

Glycogen phosphorylase BB is the most sensitive test for diagnostic of ***myocardial infarction*** at 3 – 4 hours after stenocardia attack. It stabilizes to 48 hours after damage.

Myoglobin examination is used to diagnose the repeated myocardial infarction, because it is the earliest test and is quickly removed from blood. Myoglobin has the molecular mass 18 kDa, therefore it can pass through filtration barrier. It quickly increases and decreases in blood.

In spite of organ specificity the determination of AsAT is used for diagnostic of myocardial infarction. Its activity increases in blood serum 4 – 6 hours after damage and stabilizes 3 – 7 days.

CK-MB is widely used as early indicator of myocardial infarction and is more specific as AsAT.

Activity of LDH_1 increases to 24 hours after damage and stabilizes more slowly verses to CK and AsAT.

Myocardial isoforms of cTn T and cTn I are used as specific markers of cardiomyocytes damage. The determination od Tn T provides the diagnostic of myocardial infarction both in early and late periods.

Cardiomyopathies may be nonheritable and inherited ones. The causes of inherited cardiomyopathies fall into two broad classes:

1) disorders of cardiac energy metabolism, mainly reflecting mutations in genes encoding enzymes or proteins involved in fatty acid oxidation and oxidative phosphorylation;

2) mutations in genes encoding proteins involved in affecting myocardial contraction such as myosin, tropomyosin, troponin, and dystrophin.

Mutations in the genes encoding these latter proteins cause familial hypertrophic cardiomyopathy. Patients with familial hypertrophic cardiomyopathy can show great variation in clinical picture. This in part reflects genetic heterogeneity.

Tests for Self-control

1. A 46 year woman complains of progressing Duchenne-type muscular dystrophy. Which enzyme activity changing is diagnostic test in this case?

A. Glutamate dehydrogenase
B. Lactate dehydrogenase
C. Pyruvate dehydrogenase
D. Creatine phosphokinase
E. Adenylyl kinase

2. Which myofibril protein performs both structural and enzymatic functions?

A. Actin
B. Myosin
C. Troponin I
D. Troponin T
E. Troponin C

3. Specific diagnostic sign of muscular dystrophy is increased excretion with urine of

A. Creatinine
B. Creatine
C. Proteins
D. Indican
E. Bilirubin

4. Point isoforms of LDH, concentration of which increase in blood plasma of patients with myocardial infarction:

A. LDH_1 and LDH_2
B. LDH_3 and LDH_4
C. LDH_3
D. LDH_4 and LDH_5
E. LDH_5

Chapter 20. BIOCHEMISTRY OF CONNECTIVE TISSUE

20.1 General Characteristic of Connective Tissue, Functions

The connective tissue accounts for about 50% of body mass. The loose connective tissue of subcutaneous fat, hard bone and teeth, tendons and fasciae, skin and stroma of parenchymatous organs, neuroglia and peritoneum – all these are grouped under the common name “connective tissue”.

All the diverse forms of connective tissue, despite their morphological distinctions, have the number of common properties:

- They are developed from mesynchyma.
- They consist of cells and extracellular matrix. The extracellular matrix takes more space than the cell elements.
- Functional properties of different forms of connective tissue are mainly provided by physicochemical properties of the extracellular matrix.

Functions of connective tissue:

- Trophic function.
- Protective (protective role of skin integuments; glycosaminoglycans; phagocytosis; antibody production).
- Plastic (participation in regenerative processes, erythropoiesis).
- Mechanic (it forms stroma of organs, aggregates them, forms fasciae).

Cells of connective tissues are mainly presented by fibroblasts and their varieties (osteoblasts, chondroblasts, etc.), macrophages, plasmacytes, mast cells.

Fibroblasts are the main and the most widely spread cell form of connective tissue. The functions of fibroblasts are: production of proteoglycans, glycoproteins; formation of collagen, reticulin and elastin fibers; regulation of metabolism and structural stability of these elements. Specialized tissue forms of fibroblasts are distinguished: chondroblasts, osteoblasts, keratoblasts, odontoblasts, etc.

Extracellular matrix contains three major classes of biomolecules:

- Structural proteins (collagen, elastin, fibrillin).
- Specialized proteins (fibronectin, laminin).

- Proteoglycans, which consist of glycosaminoglycans (GAG) and core proteins.

General functions of extracellular matrix:

- It participates in proliferation and differentiation of cells and in formation of tissues.
- It participates in adhesion of cells.
- It supports form of cells and organs.
- It provides the mechanical solidity of tissues

20.2 Features of Chemical Composition of Connective Tissue.

In connective tissue the intercellular medium takes more space than the cell elements. The specific fibrillar structures – collagen, elastin and reticulin fibres are characteristic of connective tissue. They ramify through the interstitial substance.

Collagen is the major component of most connective tissues. It accounts for 25% of total proteins of the adult human organism, of 6% of body mass.

It is able to formation of insoluble fibrils. Structure of collagen depends on type of tissue and corresponds to its specialization. About 19 distinct types of collagen have been identified in human tissues. They are formed by 30 different polypeptide chains, which are encoded by 30 genes.

Table 20.1 Collagen types

Type	Polypeptide chains	Distributions
I	$[\alpha1(I)]_2\ \alpha2$	Skin, bone, tendon
II	$[\alpha1(II)]_3$	Cartilage
III	$[\alpha1(III)]_3$	Blood vessels, fetal skin
IV	$[\alpha1(IV)]_3$	Basement membrane

Structural unit of collagen fibrils is tropocollagen. Tropocollagen molecule consists of three polypeptide chains.

Each polypeptide subunit or alpha chain has about 1000 amino acid residues. It is twisted into a left-handed helix of three residues per turn. Three of these α-chains are then wound into a right-handed super helix, forming a rod-like molecule 1.4 nm in diameter and about 300 nm long. Glycine residues occur at every third position of the triple helical portion of the α-chain.

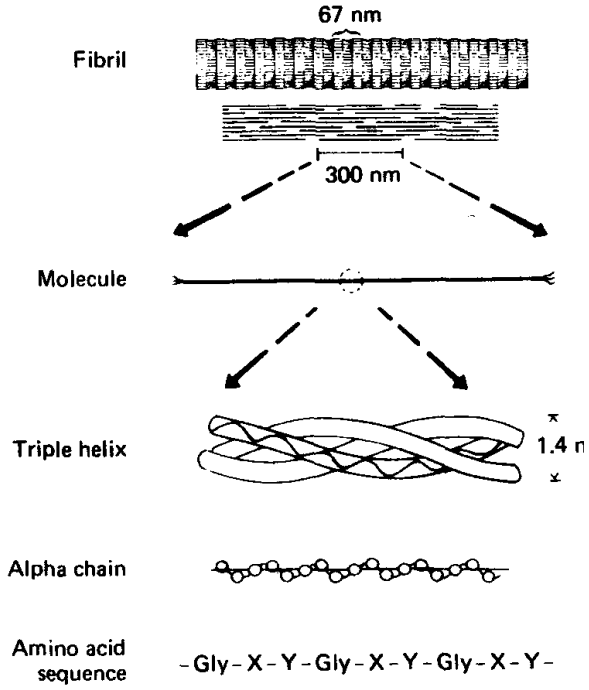


Figure 20.1. Molecular features of collagen structure from primary sequence up to the fibril.

This is necessary because glycine is the only amino acid, which may be accommodated in the limited space of the central core of the triple helix. This repeating structure, represented as $(Gly – X - Y)_n$, is an absolute requirement for the formation of the triple helix. While X and Y can be any other amino acids, about 100 of the X position are proline and about 100 of the Y position are hydroxyproline. Proline and hydroxyproline confer rigidity of the collagen molecule.

Collagen fibers are further stabilized by the formation of covalent cross-links, both within and between the triple helical unit. These cross-links form through the

action of lysyl oxidase. The stable, covalent cross-links are important for the tensile strength of the fibers.

Elastin is a connective tissue protein that is responsible for properties of extensibility and elastic recoil in tissues. Elastin is present in large amounts in tissues that require these physical properties, e.g. lung, large arterial blood vessels, and some elastic ligaments. Smaller quantities of elastin are also found in skin, ear cartilage, and several other tissues.

In contrast to collagen, there appears to be only one genetic type of elastin, although variants arise by differential processing of the hnRNA for elastin.

Native elastin fibrils are formed by small practically spheric molecules, which are combined by means of cross-links. Elastin molecules have about 800 amino acid residues. Most of them are hydrophobic ones (alanine, valine, leucine, proline). Glycine constitutes about 1/3 of all the amino acid residues in elastin. Elastin has many of lysine residues, which participate in the formation of cross-links unique to elastin (desmosines – from 4 lysine residues and lysylnorleucine – from 2 residues).

Fibrillin is a large glycoprotein (about 350 kDa) that is a structural component of microfibrils. Fibrillin is secreted into the extracellular matrix by fibroblasts and is incorporated into the insoluble microfibrils, which appear to provide scaffold for deposition of elastin.

Fibronectin is a major glycoprotein of the exracellular matrix, also found in a soluble form in plasma. It consists of two identical subunits, each of about 230 kDa, joined by two disulfide bridges near their carboxyl terminals. Fibronectin is organized into functional domains (at least seven); functions of these domains include binding heparin and fibrin, collagen, DNA, and cell surfaces. The fibronectin receptor interacts indirectly with actin microfilaments. Via the interaction with its cell receptor, fibronectin plays an important role in the adhesion of cells to the extracellular matrix. It is also involved in cell migration, by providing a binding site for cells and thus helping them to steer their way through the extracellular matrix.

Laminin is a major protein component of renal glomerular and other basal laminae. It has binding sites for type IV collagen, heparin, nidogen, and integrins on cell surfaces. Main functions of laminin are provided by its ability to bind cells. It can influence the growth, differentiation and migration of cells.

Entactin, also known as "***nidogen***", is a glycoprotein, which binds to laminin and is a major cell attachment factor.

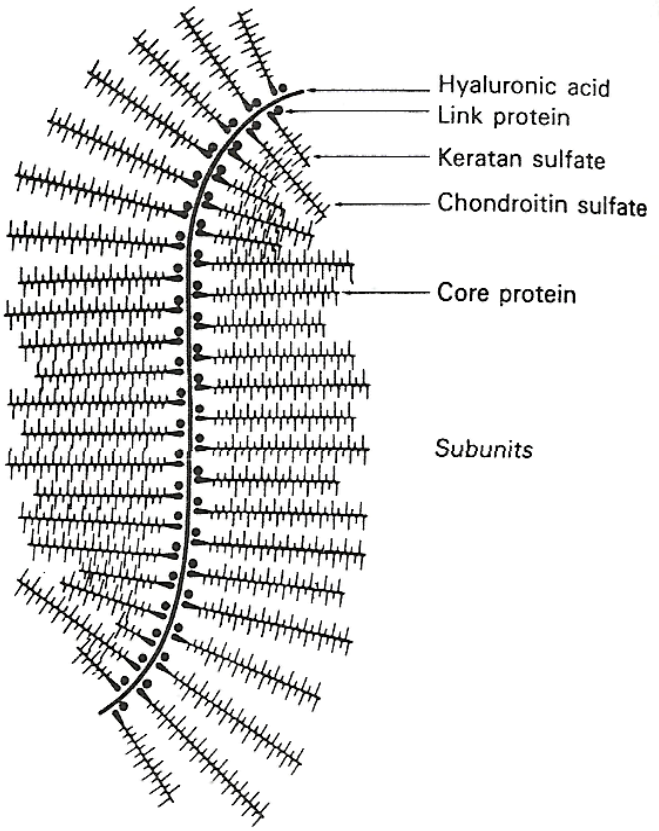


Figure 20.2 Schematic representation of the proteoglycan aggrecan

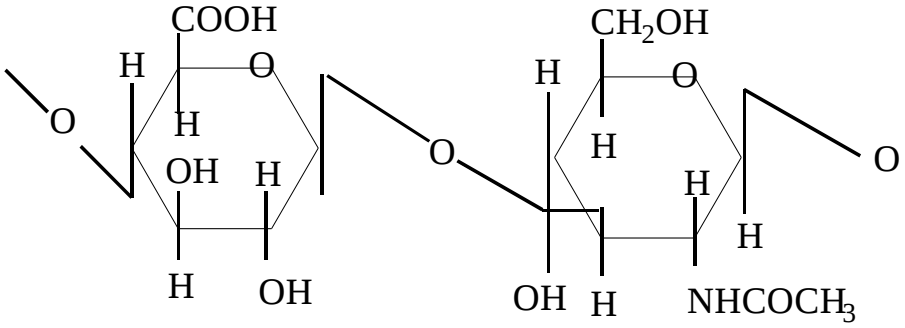
Proteoglycans form base of intercellular substance of connective tissue. They consist of protein part (5-10%) and glycosaminoglycans (GAG) (90-95%). Glycosaminoglycans are linked with protein part by covalent bonds through OH-group of serine, threonine or NH_2-group of asparagine. The proteins bound covalently to GAG are called "core protein".

Proteoglycans vary in tissue distribution, nature of core protein, attached GAG, and function. In the extracellular matrix proteoglycans are associated with each other and also with the major structural components of the matrix, collagen and elastin. These interactions are important in determining the structural

organization of the matrix. Some of proteoglycans interact with certain adhesive proteins, such as fibronectin and laminin.

There are at least seven glycosaminoglycans: hyaluronic acid, chondroitin sulfate, keratin sulfates I and II, heparin, heparan sulfate, and dermatan sulfate. GAG is an unbranched polysaccharide made up of repeating disaccharides, one component of which is always an amino sugar, either D-glucosamine or D-galactosamine. The other component of the repeating disaccharide (except in the case of keratan sulfate) is an uronic acid, either D-glucuronic acid or its 5'-epimer, L-iduronic acid. With the exception of hyaluronic acid, all the GAGs contain sulfate groups.

The structural formula of the repeating disaccharide unit of *hyaluronic acid* molecule:



D-glucuronic acid residue N-acetyl glucosamine residue

The GAGs present in the proteoglycans are polyanions and hence bind polycations and cations such as Na+ and K+. This latter ability attracts water by osmotic pressure into the extracellular matrix and contributes to its turgor. GAGs form also gel at relatively low concentrations, therefore proteoglycans can act as sieves, restricting the passage of large macromolecules into the extracellular matrix.

Hyaluronic acid is widely distributed among various animal tissues, including synovial fluid, the vitreous body of the eye, cartilage, and loose connective tissues. Hyaluronic acid is especially high in concentration in embryonic tissues and is thought to play an important role in permitting cell migration during morphogenesis and wound repair. Its ability to attract water into the extracellular

matrix and to form gel provides the role of hyaluronic acid in regulation of tissue permeability. Gel-like structure of hyaluronic acid solution provides the function of synovial fluid in joints as grease which decreases the rubbing of joint surfaces.

Chondroitin sulfates (chondroitin 4-sulfate and chondroitin 6-sulfate) are prominent components of cartilage. They differ by physico-chemical properties and distribution in different types of connective tissue. Chondroitin 4-sulfate is mainly located in cartilage, bones, sclera, aorta. Chondroitin 6-sulfate prevails in tissue of tendons, skin, heart valves, intervertebral discus.

Dermatan sulfate predominates in derma, is also present in arteria, sclera, cornea.

Keratan sulfates don’t contain uronic acids. Keratan sulfate I and dermatan sulfate are present in the cornea. They lie between collagen fibrils and play a critical role in corneal transparency. Keratin sulfate II is GAG of cartilage and bones.

Heparin is heteropolysaccharide, which consists of disaccharide fragments that contain residues of sulfated D-glucuronic or L-iduronic acids and N-acetylglucosamine. It differs from other GAGs by localization in tissues and by functions. Heparin is intracellular component of mast cells. Heparin is an important anticoagulant. It binds with factors IX and XI but its most important interaction is with plasma antithrombin III. The binding of heparin to antithrombin III greatly accelererates the ability of the latter to inactivate serine proteinases, particularly, thrombin. Heparin activates lipoprotein lipase. It can bind specifically to lipoprotein lipase present in capillary walls, causing a release of this enzyme into the circulation. Thus it facilitates the hydrolysis of TAG of chylomicrons and VLDL.

Heparan sulfate is similar to heparin, but has more N-acetyl and less N- and O-sulfate groups. It is mainly located on the surface of endothelial cells and thrombocytes. It is also found in the basement membrane of the kidney, along with type IV collagen and laminin, where it plays a major role in determining the charge selectiveness of glomerular filtration. Negative charges of heparin sulfate and of

sialic acid–containing glycoproteins present in the lamina prevent the passing of albumin across the glomerulus although albumin is smaller than the pores in the glomerular membrane.

Features of chemical composition of bone. Bone contains both organic and inorganic material. The organic matter is mainly protein. Type I collagen is the major protein comprising 90—95% of the organic material. Type V collagen is also present in small amount, as are a number of non collagen proteins, some of which are relatively specific to bone (*osteocalcin*, *bone sialoprotein).* ***Osteocalcin*** is the ***marker*** to estimate the relation between bone deposition and resorption processes. Specific features of chemical composition of bone are high concentrations of citrate. About 90% of citrate of human's organism is located in bone.

The inorganic component is mainly crystalline hydroxyapatite ($Ca_{10}[PO_4]_6[OH]_2$), along with sodium, magnesium, carbonate and fluoride. Approximately 99% of the body's calcium is contained in bone. Hydroxyapatite confers on bone the strength and resilience required by its physiologic roles.

Features of chemical composition of cartilage. General biochemical feature of different types of carticages is the high amount of lipids and sulfated GAGs, mainly *chondroitin sulfates and keratan sulfates.* Moreover matrix contains unsulfated GAG – hyaluronic acid. It plays the important role in the formation supermolecular structure of matrix.

Type II collagen is the principal protein of *cartilage* and a member of other minor types of collagen (V, VI, IX, X, XI) are also present.

Biosynthesis by cells of *type II collagen* is regarded to be the reliable marker of chondrogenic differentiation of *hyaline* cartilage (the main type of cartilage).

In addition to these components, *elastic* cartilage contains *elastin* and fibroelastic cartilage contains type I collagen.

Cartilage contains a number of proteoglycans, which play an important role in its compressibility.

Aggrecan is the *m*ajor proteogycan. It contains several GAGs (hyaluronic

acid, chondroitin sulfate, and keratan sulfates), link and core proteins. The core protein has three domains: A, B and C. The hyaluronic acid binds noncovalently to domain A of the core protein and to the link protein. Keratan sulfate chains are located in domain B; chondroitin sulfate chains are located in domain C. Both of these types of GAGs are covalently bound to the core protein.

The other proteoglycan *chondronectin* is involved in the attachment of type II collagen to chondrocytes.

20.3 Features of Metabolism in Connective Tissue

Synthesis of intercellular matrix components is mainly performed by fibroblasts. Small amounts of them are synthesized by endothelial, muscle and nervous cells.

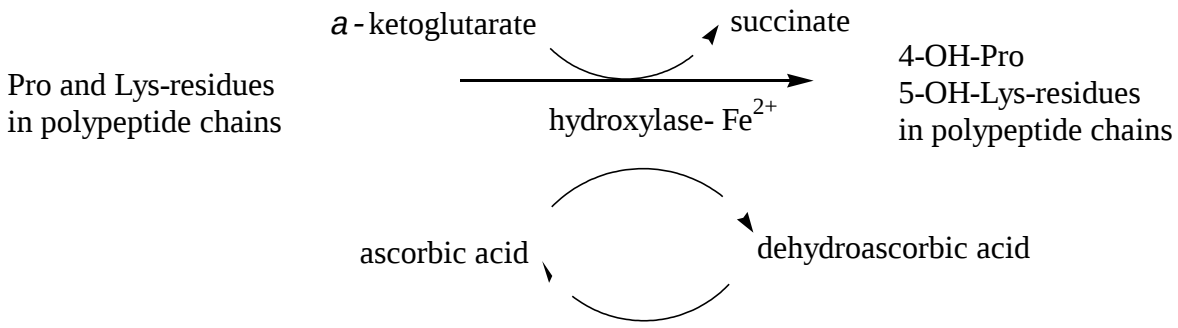
These polymers are synthesized as precursors, which undergo to maturation in intercellular substance.

Synthesis of collagen consist of the following stages.

I. Synthesis of polypeptide chains of preprocollagen on ribosomes. Preprocollagen contains a leader or signal sequence that directs the polypeptide chain into the vesicular space of the endoplasmic reticulum.

II. Intracellular posttranslational modifications:

- Cleavage of signal peptide.
- Hydroxylation of some prolyl and lysyl residues; glycosylation of some hydroxylysyl residues.



Vitamin C is needed to supporting Fe in bivalent state.

Hydroxylation of proline is needed for formation of stable triple helical structure of collagen. *Hydroxylation of lysine* is needed for formation of covalent linkages

between collagen molecules during formation of collagen fibrils. Hydroxylysine residues are sites of glycosylation.

- Formation of intrachain and interchain S-S-bonds in extension peptides.
- Formation of triple helix

The procollagen molecule contains extension peptides at both its amino and carboxyl terminal ends. Both extension peptides contain cysteine residues. While the amino terminal propeptide forms only intrachain disulfide bonds, the carboxyl terminal propeptides form both intrachain and interchain disulfide bonds. Formation of these disulfide bonds assists in the formation of the three collagen molecules to form the triple helix.

Role of extension peptides:

- They facilitate the reciprocal orientation and formation of triple helix.
- It is possible, that they participate in translocation of procollagen through membrane.
- They prevent premature formation of collagen fibers.

III. Secretion from the cell by way of the Golgi apparatus.

IV. Extracellular posttranslational modifications

- Cleavage of amino and carboxyl terminal propeptides. Extracellular enzymes called procollagen amino- and carboxy-proteinases remove the extension peptides at the amino and carboxyl terminal ends, respectively.
- Assembly of collagen fibers in quarter – staggered alignment.

When the propeptides are removed, the triple helical collagen molecules, containing approximately 1000 amino acids per chain, spontaneously assemble into collagen fibers.

- Oxidative deamination of **ε**-amino groups of lysyl and hydroxylysyl residues to aldehydes.
- Formation of intra- and interchain cross-links and formation of mature collagen fibers.

The same cells that secrete collagen also secrete fibronectin, a large glycoprotein present on all surfaces, in the extracellular matrix, and in the blood. Fibronectin binds to

aggregating precollagen fibers and alters the kinetics of fiber formation in the pericellular matrix. Proteoglycans heparan sulfate and chondroitin sulfate may be associated with fibronectin and procollagen. Type IX collagen, a minor collagen type from cartilage, contains attached proteoglycan chains. Such interactions may serve to regulate the formation of collagen fibers and to determine their orientation in tissues.

Elastin is synthesized as a soluble monomer called "tropoelastin". Some of the prolines of tropoelastin are hydroxylated to hydroxyproline, though hydroxylysine and glycosylated hydroxylysine are not present

After secretion from the cell, certain lysyl residues of tropoelastin are oxidatively deaminated to aldehydes by lysyl oxidase. Three of these lysine-derived aldehydes with an unmodified lysine form desmosine. Desmosines are cross-links unique to elastin. After the formation of these links elastin becomes insoluble. It is extremely stable and has a very low turnover rate.

Synthesis of proteoglycans. Protein part of proteoglycans as other secretory proteins is synthesized on polyribosomes bound to endoplasmic reticulum. Peptide chain pierces through membrane and grows in endoplasmic reticulum. Here the synthesis of GAGs begins. The first reactions of GAG formation occur in endoplasmic reticulum. Most of the later steps in the biosynthesis of GAG chains and their subsequent modifications occur in the Golgi apparatus. The enzymes catalyzing sulfation are designated sulfotransferases and use 3’-phosphoadenosine-5’-phosphosulfate (PAPS) as the sulfate donor.

These Golgi–located enzymes are highly specific, and distinct enzymes catalyze sulfation at different positions on the acceptor sugars. An epimerase catalyzes conversions of glucuronyl to iduronyl residues.

The proteoglycans are included to secretory granules.

Catabolism of connective tissue occurs in ground substance under the influence of specific enzymes – collagenase, elastase, proteases, glycosidases. These enzymes are produced by the same cells of connective tissue which participate in the synthesis of these polymers. Collagen is relatively metabolically

stable. However, its breakdown is increased during starvation and various inflammatory states. Collagen is cleft by collagenase. Formed fragments are water soluble and are easely denaturated, their peptide bonds are able to be hydrolyzed by different peptidehydrolases. Amount of hydroxyproline in blood and urine reflects the balance between the rates of collagen and hydroxyproline catabolism.

Excretion of hydroxyproline is increased in hyperparathyroidism, Paget's disease, hereditary hyperhydroxyprolinemia.

Features of metabolism in bone. Bone is a dynamic structure that undergoes continuing cycles of remodeling, consisting of resorption followed by deposition of new bone tissue. Approximately 4% of compact bone is renewed annually in the typical healthy adult, whereas approximately 20% of trabecular bone is replaced.

The major cell types in bone deposition and resorption are o*steoblasts* and *osteoclasts. Osteocytes* are descended from osteoblasts; they also appear to be involved in maintenance of bone matrix.

Osteoblasts synthesize most of proteins found in bone and various growth factors and cytokines. They are responsible for deposition of new bone matrix and its mineralization. Osteoblasts control mineralization by regulating the passage of calcium and phosphate ions across their surface membranes. Membranes contain *alkaline phosphatase*, which is used to generate phosphate ions from organic phosphates.

Alkaline phosphatase is the *marker* of osteoblast activity. Alkaline phosphatase contributes to mineralization but in itself is not sufficient. Type I collagen participates in processes of mineralization. The result of collagen interaction with mineral substances is the perfect biological structure, which has high mechanic strength and high phisiologic activity. Bone collagen differs by occurence of very stable crorss-links, which provide the specific type of packing the molecular aggregates of bone collagen that ensures the condifions for mineralization.

Acidic phosphoproteins, such as bone sialoprotein, act as sites of nucleation. There proteins contain sequences (e.g., poly-Asp and poly-Glu) that bind calcium

and may provide an initial scaffold for mineralization. Certain proteoglycans and glycoproteins can also act as inhibitors of nucleation.

Osteoclasts are multinucleated cells derived from pluripotent hematopoietic stem cells. An apical membrane of osteoclasts has a ruffled border that plays a key role in bone resorption. A proton – translocating ATPase pumps protons across a ruffled border into resorption area. This lowers the local pH to 4,0 and therefore facilitates the dissolution of hydroxyapatite from bone and creates the optimal pH for the activity of lysosomal hydrolases. Resorption of bone under the influence of osteoclasts is due to the increasing the *acid phosphatase* and other lysosomal enzyme activities. The products of bone resorption are taken up into the cytoplasm of osteoclast, probably digested further, and transferred into capillaries.

Many factors are involved in the regulation of bone metabolism. *Growth hormone* or, more likely, IGF-1, promotes growth of long bones at the epiphysial plates in growing children and appositional or acral growth in adults. *Parathyroid hormone (PTH)* increases the rate of dissolution of bone, including both organic and inorganic phases, which moves Ca^{2+} into extracellular fluid. But PTH influences the octeoclasts indirectly through osteoblasts. *Corticosteroids* inhibit osteoblasts. *Calcitonin, estrogens* (by inhibiting IL-6 production), *TGFβ, IFNα, PGE_2* inhibit osteoclasts. *Calcitriol* supports the calcium concentration in extracellular fluid which is optimal to mineralization. It has also direct effect on bone: 24.25-$(OH)_2$-D_3 promotes normal bone mineralization and synthesis of hydroxyapatite; 1.25-$(OH)_2$-D_3 regulates resorption process and low concentration of calcium ions facilitates the Ca mobilization from bone.

Metabolism in cartilage. Cartilage is an avascular tissue and obtains most its nutrients from synovial fluid. It exhibits slow but continuous turnover. Supporting metabolic equilibrium in cartilage matrix is associated with intensive function of *chondrocytes.* Low metabolic activity of cartilage tissue, especially in joint cartilage, is the consequence of very small amount of cells in the unit of cartilage volume. But level of chondrocyte metabolism at calculation per one cell corresponds to metabolism of other tissue cells. In joint cartilages of adult human

half of ***proteoglycan molecules*** are ***annually renovated***. Process begins from degradation of core proteins under the action of proteinases which occur in cartilage tissue mainly in latent state. Then glycosidases degrade GAGs.

But ***10 years*** are necessary for ***renovation* of *half of collagen molecules***. This synthesis provides the reparation of insignificant damages of cartilages tissue, which occurs during life. The most part of collagen molecules of cartilage matrix is kept during all ontogenesis.

Chondrocytes participate both in anabolic and catabolic processes. The degradation of major molecules of cartilage matrix (type II collagen and proteoglycans) is believed to include two phases. Initially macromolecules are cleft to big fragments. Then these fragments are degraded in cells by lysosomal hydrolytic enzymes.

Metabolic activity of chondrocytes is influenced by different factors. *Glucocorticoids* suppress biosynthesis activity of chondrocytes. Testosteron stimulates growth of cartilages. *Estrogens* inhibit biosynthesis DNA in GAGs by chondrocytes. IL-1 and TNFα appear to stimulate the production of proteases which can degrade collagen and other proteins found in cartilages.TNFβ and IGF-1 generally exert an anabolic influence on cartilage.

Chondrocytes undergo the action of hormones on the ground of the most intensive influence of matrix. Matrix can modify the effect of hormones. Mechanic factors also play regulatory role. That is very important for joint cartilages because their mechanic function is main. Prolonged joint movement limit leads to decreasing cartilage mass and diminishing proteoglycan concentration in matrix.

20.4 Disorders of Connective Tissue

Inherited disturbances of connective tissue. A number of genetic diseases result from abnormalities in the synthesis of collagens.

Osteogenesis imperfect is characterized by abnormal fragility of bones. Over 90% of patients with osteogenesis imperfect have mutations in the COL1A1 and COL1A2 genes. Other mutations affect RNA splicing. In general, these mutations result in decreased expression of collagen or in structurally abnormal chains that

assemble into abnormal fibrils.

Ehlers – Danlos syndrome comprises a group of inherited disorders whose principal clinical features are hyperextensibility of the skin, abnormal tissue fragility, increased joint mobility. This provides the participation of patients with Ehlers – Danlos syndrome in circus attraction as “gutta-percha boy” or “woman-snake”. At least 11types of Ehlers-Danlos syndrome have been recognized, most of which reflect a variety of lesions in synthesis of collagen.

Type IV is the most serious because of its tendency for spontaneous rupture of arteries or the bowel, reflecting abnormalities in type III collagen. Type VI is due to a deficiency of *lysyl hydroxylase*. It is characterized by marked joint hypermobility and a tendency to ocular rupture. Type VIIC Ehlers – Danlos syndrome is caused by a deficiency of *procollagen N – proteinase.* This leads to the formation of abnormal irregular collagen fibrils and is manifested by marked joint hypermobility and soft skin.

Alport’s syndrome refers to a number of genetic disorders (both X – linked and autosomal) affecting the structure of type IV collagen fibers, the major collagen found in the basement membranes of the renal glomeruli. The presenting sign is hematuria. Patients may eventually develop end-stage renal disease.

Menke’s disease (“kinky” or “steely” hair disease) is a disorder of copper metabolism. It is X – linked, affects only male infants, involves the nervous system, connective tissue, and vasculature, and is usually fatal in infancy. Copper is cofactor *lysine oxidase.* This is accompanied by disturbance of normal fibril formation.

Marfan’s syndrome is due to mutations in the gene for fibrillin. It is relatively freguent inherited disease affecting connective tissue. It affects the eyes (causing dislocation of the lens), the skeletal system. Most patients are tall, exhibit long digits (arachnodactyly) and hyperextensibility of the joints.

Mucopolysaccharidoses are the group of inherited disorders due to deficiencies of enzymes that degrade GAGs. This leads to accumulation of GAGs and to development of clinical symptoms. They are manifested by severe disorders

of connective tissue of many internal organs, by pathology of bones and joints, by mental retardation. Mucopolysaccharidoses belong to class of lysosomal diseases of accumulation. This group includes 10 nozologic units.

Table 20.2 Mucopolysaccharidoses

Name	Alternative designation	Enzyme defect	Urinary metabolites
Hurler, Scheie, Hurler – Scheie	MPS I	α-L-Iduronidase	Dermatan sulfate, heparan sulfate
Hunter	MPS II	Iduronate sulfatase	Dermatan sulfate, heparan sulfate
Sanfilippo A	MPS IIIA	Heparan sulfate N-sulfatase (sulfamidase)	Heparan sulfate
Sanfilippo B	MPS IIIB	α-N-Acetylglucosaminidase,	Heparan sulfate
Sanfilippo C	MPS IIIC	Acetyltransferase	Heparan sulfate
Morquio A	MPS IVA	Galactose 6-sulfatase	Keratan sulfate, C6-SO_4
Morquio B	MPS IFB	β-Galactosidase	Keratan sulfate
Maroteaux - Lamy	MPS IV	N-Acetylgalactosamine 4-sulfate (arylsulfatase B)	Dermatan sulfate
Sly	MPS VII	β-Glucuronidase	Dermatan sulfate, heparan sulfate, C4- and C6-SO_4

Specific laboratory investigations to help in their diagnosis are urine testing

for the presence of increased amount of GAGs and assay of suspected enzymes in white cells, fibroblasts, or sometimes in serum. In certain cases, a tissue biopsy is performed. Prenatal diagnosis can be made using amniotic cells or chorionic villus biopsy.

Acquired diseases due to disturbances of conective tissue metabolism include: dystrophic pathologies of bone tissue (osteoarthroses); disorders of synchronic processes of joint cartilage cell biodegradation and formation; inflammatory diseases; traumatic diseases; tumors of bone tissue, which can be divided into the primary tumors and methastases; diffuse diseases of connective tissue (synonym collagenoses).

Diffuse diseases of connective tissue: rheumatism, rheumatoid arthritis, lupus erythematosis, diffuse scleroderma, periarteritis nodosa. The inflammation of different organs and tissues, from the first connective tissue, development of autoimmunic processes, excessive formation of fibroid tissue are characteristic for diffuse diseases of connective tissue.

Feature of pathogenesis of practically all connective tissue diffuse diseases is the development of organospecific autoimmunity, which is manifested by hyperproduction of autoandibodies to components of nucleus and cytoplasm of cells (antibodies to DNA, RNA, antinuclear factor etc.), to immunoglobulins (rheumatoid factors), to anticlotting system components (lupous anticoagulant) etc. Autoimmunity development is due to disturbance of intercellular interaction of immune competent cells, particularly to the decrease of T-supressor function and the increase of B-lymphocyte activity. Collagenoses have similar clinical symptoms, especially in early stage of disease. They include: fever, arthritis or polyarthritis, myositis or myalgia, sometimes recidivating serositis, different lesion of internal organs (including pathologic changes of kidneys) generalized vasculitis, lymphadenopathy.

Practically all diseases of this group are accompanied by autoimmune syndrome, such as thyroiditis of Hashimoto, thrombocytopenic purpura etc.

Rheumatoidic arthritis is one of the most spread forms of collagenoses. Systemic lesion of connective tissue with joints deformation and disturbance of their functions occur. The inflammation results in hyperplasia of synovium, local joint erosion and finally to destruction of all the joint surfaces. In active phase of disease all the indices charactreristic for acute inflammation increase: level of fibrinogen, C-reactive protein in the blood. The acute increase of sialic acids and hexoseamines levels in blood serum and synovium, sialic acid and hydroxyproline contents in urine are observed.

The most progressive analysis is the determination of antibodies to cyclic citrulline peptide.

Traumatic diseases. Fractures are most spread of them.

Diseases of inflammatory origin. Osteomyelitis, arthritis, acute condition of arthrosis, festering wound belong to this group.

Dystrophic pathologies of bone tissue (osteoporosis, osteoarthrosis, rickets).

Dysplastic diseases. Paget's disease (deforming osteodystrophy) is due to local or plural increased metabolism of bone tissue. Biochemical changes in blood and urine express both osteoclastic and osteoblastic activities of bone tissue. They depend on both intencivity of resorption and renovation processes in rebuilding centre and amount of these centers.

Bone tissue tumors may be the primary (osteoma, the primary osteosarcoma, fibrosarcoma, chondrosarcoma, Eving's tumor, osteoid osteoma) or methastases (mainly, in consequence of development of bronchogenic carcinoma, tumors of mammal gland and prostate). It should be noted that the capacity of malignant tumor development depends on connective tissue state. Cancer can not be developed in organism which has connective tissue with normal reactivity.

Tests for Self-control

1. Which substance is the reliable marker of hyaline cartilage chondrogenic differentiation?

A. Collagen II type
B. Collagen I type
C. Collagen V type
D. Collagen IX type
E. Collagen XII type

2. The mucopolysaccharidoses signs (dwarfism, hypertrichosis, coarse features, hearing loss) are observed in the child. Which biochemical investigations are necessary to put the final differential diagnosis of mucopolysaccharidoses type?

A. Examination of corresponding GAGs level in urine
B. Examination of corresponding GAGs concentration in blood
C. Examination of corresponding enzymes in blood serum
D. Examination of corresponding enzymes in leukocytes
E. Examination of corresponding enzymes in urine

3. The early signs of rickets have been examined in the child. Which blood index from below mentioned is the evidence of vitamin D deficiency.

A. The increase of Ca level
B. The decrease of Ca level
C. The increase of 25-(OH)-D_3 level
D. The decrease of 25-(OH)-D_3 level
E. The increase of alkaline phosphatase activity

4. The increased extensibility and elasticity of skin, abnormal joint mobility provide the participation of the patients with Ehlers-Danlos syndrome in circus attraction as “gutta percha boys” and “women-snake”. Which biomolecules hereditary disturbances are observed under this disease?

A. Glycosaminoglycans
B. Collagen
C. Elastin
D. Gangliosides
E. Glycogen.

5. A child is diagnosed with an X-linked recessive mucopolysaccharidosis that causes heparan sulfate and dermatan sulfate deposition in his bones. Which enzyme deficiency leads to development of this disease?

A. α-L-iduronidase
B. L-iduronosulfate sulfatase
C. N-acetylgalactosamine-6-sulfatase
D. Amylo-1,6-glucosidase
E. Arylsulfatase A

6. A five-year old boy was normal at birth, but by the age 18 months he developed characteristic of short stature, mental retardation, limited movements

and coarse facial features. He was diagnosed with L-iduronidase deficiency. Which one of the following diseases does he have?

A. Hurler’s disease
B. Hunter’s disease
C. Sanfilippo A disease
D. Morquio’s disease
E. Marteaux-Lamy disease

7. Patient, 36-year old, suffers of collagenosis. Which metabolite increased amount is more possible to be found in the urine?

A. Indican
B. Hydroxyproline
C. Creatinine
D. Urea
E. Urobilinogen

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