FACULTY OF MEDICINE

Zuzana Országhová, Ingrid Žitňanová et al.

TEXTBOOK OF MEDICAL CHEMISTRY

2018
COMENIUS UNIVERSITY IN BRATISLAVA

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Reviewers:

Prof. MUDr. Ladislav Turecký, CSc.
Prof. RNDr. Peter Račay, PhD.

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PREFACE

Chemistry and biochemistry at medical faculties are integral parts of basic theoretic knowledge of contemporary graduate.

Educational book "Textbook of Medical Chemistry" is destined to students of study branches General Medicine and Dentistry for the course "Medical Chemistry". It is structured into 13 chapters concerning several topics: general and physical chemistry, organic chemistry, chemistry of natural substances (static biochemistry), enzymology and oxidative stress. Individual chapters provide comprehensible explanation of the fundamentals of physico-chemical, chemical and biochemical relationships applying in living systems as well as the knowledge of the structure of biologically important molecules (simple molecules and macromolecules) related to their properties and biological function.

The theoretical knowledge of this subject, which student obtains in the lectures, is verified in laboratory practices and is expanded in seminaries.

Our selection of topics makes the text especially appropriate for medical students and managing of problems gives the students satisfactory ground for effective study of biochemistry and other related study subjects (clinical biochemistry, immunology, pharmacology, physiology, pathophysiology a.o.) in the sense of comprehension of relative continuity and interdependence of biochemical processes or their function respectively.

We expect this study literature to serve as a basic study material for mentioned medical study branches for preparation to exam from "Medical Chemistry".

The authors thank their former colleagues Prof. Ing. Ľ.Bergendi, DrSc., Prof. RNDr. J.Čársky, CSc., RNDr. M. Hrnčiarová, CSc. and Ing. A. Liptáková, CSc. for their lifelong work on creating the study literature for future doctors that helped us to write this textbook.

Authors

Bratislava, 2018

1. Structure Of The Atom. Chemical Bond As a Part Of Biological Systems.

1.1. Classification of matter, its kinds and states

Matter is anything that occupies space and has mass, and there are three kinds of matter called **mixtures**, **elements** and **compounds** (Fig. 1.1.).

Matter is **mixture of pure substances**. Homogeneous mixtures are composed from one phase, for example, true solutions. Heterogeneous mixtures are composed minimally of two phases, such as suspensions, emulsions. Pure substances are with a definite, fixed chemical composition. A pure substance is either an **element** or a **compound**. Elements are the building blocks of compounds. Elements are substances that cannot be broken down into anything simpler, yet are stable enough to obtain, store (sometimes under special conditions), and use in experiments. Familiar examples are iron, copper, silver and gold. Compounds are invariably made from two or more elements. Water is a typical compound.

An atom is the smallest particle of an element that shows the chemical properties of element. **A molecule** is the smallest individual unit of a pure substance exhibiting chemical properties of a substance. **Elements** consist of one kind of atoms, compounds are built of more types of atoms. **Molecules** may consist of different numbers of atoms.

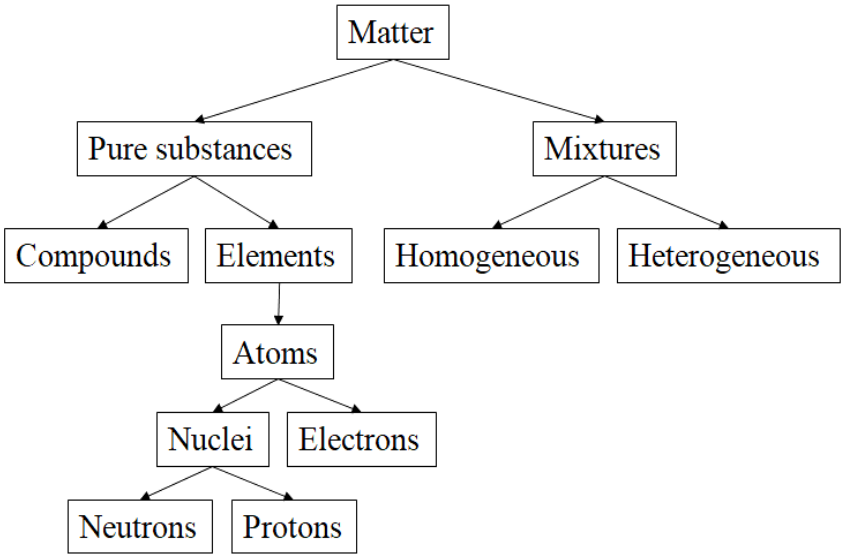


Fig. 1.1. Organization of matter

1.2. A simple view of atomic structure

An atom is the smallest representative particle of element. Each element has a unique kind of atoms that differs from the atoms of all other elements. The chemical behavior of an atom is directly related to its atomic structure. The fundamental particles of which atoms are composed are the proton, electron and neutron.

	relative mass	**relative charge**
proton	1	+1
neutron	1	0
electron	1/1836	-1

An atom consists of a very small, extremely dense, and positively charged **nucleus** around which negatively charged **electrons** of relatively very low masses are moving (Rutherford, 1911).

The nucleus is at the center of the atom and contains the protons and neutrons. Protons and neutrons are collectively known as **nucleons.** Virtually all the mass of the atom is concentrated in the nucleus, because the electrons weigh so little.

1.2.1. Protons and neutrons

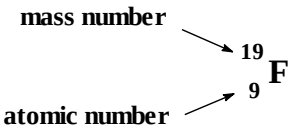
Number of protons = ATOMIC NUMBER (*Z*) of the atom

The atomic number is also given the more descriptive name of **proton number.**

Number of protons + Number of neutrons = MASS NUMBER (*A*) of the atom

The mass number is also called the **nucleon number.**

This information can be given simply in the form:



How many protons and neutrons has this atom got?

The atomic number counts the number of protons (9); the mass number counts protons + neutrons (19). If there are 9 protons, there must be 10 neutrons for the total to add up to 19.

The atomic number is tied to the position of the element in the Periodic Table (Tab.1.3.) and therefore the number of protons defines what sort of element you are talking about. So if an atom has 8 protons (atomic number = 8), it must be oxygen. If an atom has 12 protons (atomic number = 12), it must be magnesium.

Similarly, every chlorine atom (atomic number = 17) has 17 protons; every uranium atom (atomic number = 92) has 92 protons.

1.2.2. Isotopes

Atoms with the same atomic as well as mass numbers are termed **nuclides**. For example nuclide of fluorine mentioned above with atomic number Z = 9 and mass number A = 19 consists of atoms each of which contains 9 protons and 10 neutrons in nucleus.

Atoms of certain element can differ in the mass number, i.e. in the number of neutrons. Atoms which have **the same atomic number** but **different mass numbers** are called **isotopes.** They all have the same number of protons, but the number of neutrons varies. For example, the following nuclides constitute the isotopes of carbon: carbon-12 (^{12}C), carbon-13 (^{13}C), and carbon-14 (^{14}C)

	protons	neutrons	mass number
carbon-12	6	6	12
carbon-13	6	7	13
carbon-14	6	8	14

The fact that they have varying numbers of neutrons makes **no difference** whatsoever **to the chemical reactions of the carbon**.

Some of the elements occur naturally with only one nuclide, e.g. ^{19}F or phosphorus ^{31}P. Other elements have two, three (^{1}H, ^{2}H, ^{3}H; ^{16}O, ^{17}O, ^{18}O) or more naturally occurring isotopes. Even though an element has isotopes, all of the atoms of that element have the same chemical behavior and chemical properties. The existence of isotopes is reflected in the average mass of an atom of an element: The relative atomic masses (weights) listed in periodic table are the **average relative masses** of an atom of elements determined by considering the contribution of each natural isotope. Most naturally occurring nuclides are stable and retain their structure indefinitely.

However, some nuclides spontaneously decay over time – they are radioactive. **Radioactivity** depends on the instability of nuclei which decay by giving off nuclear particles (α or β accompanied by γ radiation in some cases) and forming **new nuclei** with different atomic numbers.

1.2.3. Electrons

Working out the number of electrons

Atoms are electrically neutral, and the positiveness of the protons is balanced by the negativeness of the electrons. It follows that in a neutral atom: **number of electrons = number of protons**

So, if an oxygen atom (atomic number = 8) has 8 protons, it must also have 8 electrons; if a chlorine atom (atomic number = 17) has 17 protons, it must also have 17 electrons.

Atomic orbitals and electron configuration of atoms

To understand the behaviour of electrons in atoms and molecules it requires the use of quantum mechanics (also called wave mechanic). According to this theory, every elementary particle behaves simultaneously as a particle or a wave. The basic equation of quantum mechanics is Schrödinger equation. The solution of the equation gives not the exact position of the electron, but the probability of finding the electron in a specific region around the nucleus. The most probable region in space at which electron is found is known as an **orbital**.

According to quantum mechanics, three quantum numbers are required to describe atomic orbitals. These numbers are derived from the mathematical solution of the Schrödinger equation for **the hydrogen atom**. Fourth quantum number that describes the behaviour of a specific electron – spin quantum number – completes the description of electrons in atoms.

The principal quantum number (*n*) determines the energy of an orbital and has an integral values 1, 2, 3…. With an increasing *n*, there is an increase in electron energy and in the size of orbitals (the "distance" of electrons from the nucleus increases).

The angular momentum quantum number (*l*) is related to the shape of atomic orbitals. The values of *l* depend on the value of the principal quantum number, *n*. For a given value of *n*, *l* has possible values from 0 to (*n*-1). If $n = 1$, there is only one possible value of *l:* $l = 0$. If $n = 2$, there are two values of *l:* $l = 0$ and $l = 1$. If $n = 3$, there are three values of *l*, given by 0, 1 and 2, etc. The value of *l* is generally designated by the letters **s**, **p**, **d**, **f**. Thus if $\boldsymbol{l = 0}$, we have an **s** orbital; if $\boldsymbol{l = 1}$, we have a **p** orbital; if $\boldsymbol{l = 2}$, we have a **d** orbital and so on. This system arises from early spectral studies and is summarized in Table 1.1. A collection of orbitals with the same value of *n* is frequently called a **shell**. One or more orbitals with the same *n* and *l* values are called a **subshell**.

The magnetic quantum number (m_l) describes the orientation of the orbital in space and has integral values from $-l$ to $+l$ including zero. For a certain value of *l*, there are $2l+1$ values of m_l. The number of m_l values indicates the number of orbitals in a subshell with particular *l* value. For example, the orbital where $n = 3$ and $l = 1$ is symbolized as 3p. There are three 3p orbitals (because there are three values of m_l, given by -1, 0, 1) which have different orientations in space. The orbital where $n = 3$ and $l = 2$ is symbolized as 3d. There are five 3d orbitals (because there are five values of m_l, given by -2, -1, 0, 1, 2). The shape of the orbitals and their orientation in space is schematically drawn in Fig. 1.2.

The electron spin quantum number (m_s) has the value of +1/2 or -1/2. These values correspond to the two possible spinning motions of the electron. Whereas an atomic orbital is defined by a unique set of three quantum numbers, an electron in an atomic orbital is defined by a unique set of four quantum numbers: *n*, *l*, m_l and m_s. As there are only two values of m_s, an orbital can by occupied only by two electrons which are spin–paired; one electron has a value of $m_s = +1/2$ and the other $m_s = -1/2$.

Tab.1.1. Quantum numbers for the first four levels of orbitals in the hydrogen atom

n	*l*	Name of orbital	m_l	Number of orbitals	Maximal number of electrons
1	0	1s	0	1 (1s)	2
2	0	2s	0	1 (2s)	8
	1	2p	-1, 0, 1	3 ($2p_x$, $2p_y$, $2p_z$)	
3	0	3s	0	1 (3s)	18
	1	3p	-1, 0, 1	3 ($3p_x$, $3p_y$, $3p_z$)	
	2	3d	-2, -1, 0, 1, 2	5 ($3d_{xy}$, $3d_{xz}$, $3d_{yz}$, $3d_{x^2-y^2}$, $3d_{z^2}$,)	
4	0	4s	0	1 (4s)	32
	1	4p	-1, 0, 1	3 ($4p_x$, $4p_y$, $4p_z$)	
	2	4d	-2, -1, 0, 1, 2	5 ($4d_{xy}$, $4d_{xz}$, $4d_{yz}$, $4d_{x^2-y^2}$, $4d_{z^2}$,)	
	3	4f	-3, -2, -1, 0, 1, 2, 3	7 4f	

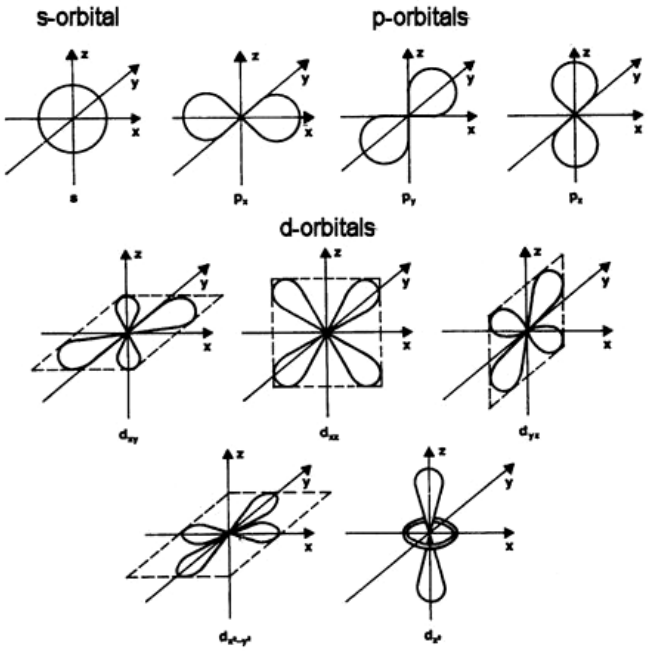


Fig. 1.2. Shapes of orbitals s, p and d

Size of orbitals

For a given atom, a series of orbitals with different values of *n* but the same values of *l* and m_l (for example 1s, 2s, 3s....) differ in their relative size (spatial extent). The larger the value of *n*, the larger the orbital, although this relationship is not linear. An increase in size also corresponds to an orbital being more diffuse. Fig. 1.3. shows us the hydrogen 1s, 2s and 3s orbitals. The hydrogen atom is a particularly simple system because it contains only one electron. Hydrogen's single electron can occupy any of its atomic orbitals. However, in the lowest energy state, the **ground state**, the electron resides in the **1s** orbital. If energy is put into the atom, the electron can be transferred to a higher-energy orbital, producing an **excited state**.

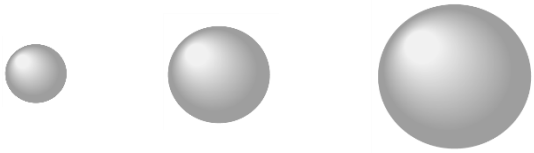


Fig. 1.3. Schematic representation of the hydrogen 1s, 2s and 3s orbitals

The situation is different for multi-electron atoms. To understand electronic behaviour in multi-electron atom, we must know the **electron configuration** of the atom.

Electron configuration of atoms

The electron configuration of an atom tell us how the electrons are distributed among the various atomic orbitals (it describes the electronic structure of an atom). A knowledge of the electron configurations helps to understand and predict the properties of the elements.

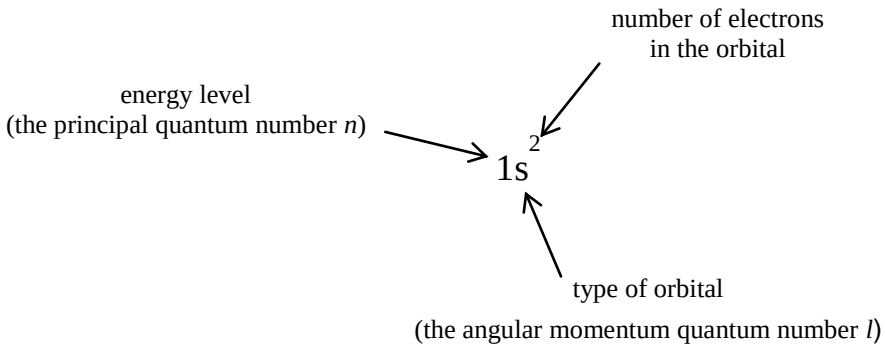
The general rules that are used to figure out the electron configuration of atoms are following:

1. In neutral atoms the number of electrons equals the number of protons in atomic nuclei.
2. The **building-up principle**. The building-up principle states that electrons occupy orbitals in the order of increasing energy. Orbital energy depends on the atomic number. The following sequence is approximately true (exact only for hydrogen atom) for the relative energies of orbitals in neutral atoms, lowest energy first:

   1s, 2s, 2p, 3s, 3p, 4s, 3d, 4p, 5s, 4d, 5p, 6s, 4f, 5d, 6p, 7s, 5f, 6d, 7p
3. **Pauli exclusion principle.** In a given atom no two electrons can have the same set of four quantum numbers (n, l, m_l, and m_s). Since electrons in the same orbital have the same values of n, l and m_l, this postulate says that they must have different values of m_s. Then, since only two values of m_s are allowed, an orbital can hold only two electrons, and they must have opposite spins. This principle will have an important consequences as we use the atomic model to account for the electron arrangements of the atoms in the periodic table.
4. The rule of maximum multiplicity **- Hund's rule**. Hund's rule states that the lowest energy configuration for an atom is the one having the maximum number of unpaired electrons allowed by the Pauli principle in a particular set of degenerate orbitals (orbitals that have the same energy). It means, that electrons may not be spin-paired in an orbital until each orbital in the set contains one electron; electrons singly occupying orbitals in a degenerate set have parallel spins, i.e. they have the same values of m_s. See electron configuration of carbon, below.

According to these rules, the ground-state electron configuration of an atom can be found by putting electrons in orbitals, starting with that of lowest energy and moving progressively to higher energy.

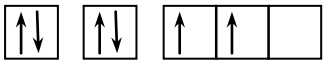
For example, helium has two electrons ($Z = 2$), hence the electron configuration of He is $1s^2$:



The electron configuration can also be represented by an **orbital diagram** that shows the spin of the electrons:

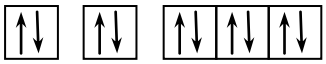
He 
1s

Carbon has six electrons ($Z = 6$). Two electrons occupy the 1s orbital, two occupy the 2s orbital, and two occupy 2p orbitals. Since there are three 2p orbitals with the same energy, electrons will occupy separate 2p orbitals (Hund´s rule):

C: $1s^2\ 2s^2\ 2p^2$ or 
1s 2s 2p

Note that the unpaired electrons in the 2p orbitals are shown with parallel spins. Orbitals that are not occupied by electrons are referred to as **vacant** orbitals.

In neon the 2p orbitals are completely filled:

Ne: $1s^2\ 2s^2\ 2p^6$ or 
1s 2s 2p

For sodium ($Z = 11$), the first ten electrons occupy the 1*s*, 2*s*, and 2*p* orbitals, and the eleventh electron must occupy the first orbital with $n = 3$, the 3s orbital. The electron configuration for sodium is $1s^2 2s^2 2p^6 3s^1$. To avoid writing the inner-level electrons, this configuration can be abbreviated as $[Ne]3s^1$, where [Ne] represents the electron configuration of neon, $1s^2 2s^2 2p^6$.

The chemical properties of elements depend chiefly on the electron configuration, especially on the configuration of **valence electrons**. Valence electrons are electrons in the outermost electron shell (**valence shell**) of the atom. For example, the valence electrons of the carbon and neon atoms are electrons in the 2s and 2p orbitals and sodium atom has one valence electron in 3s orbital. The valence electrons are very important, because they are involved in the formation of **chemical bonds**. Elements in a given group of the Periodic table have the same number of valence electrons and therefore have similar chemical properties. The electron configuration of some elements with emphasis on the valence orbitals and valence electrons is in Table 1.2.

Tab. 1.2. Electron configuration of some elements (valence orbitals and electrons are signed by bold type)

Element	Outermost electron shell	Element	Outermost electron shell	Element	Outermost electron shell
$_{1}$**H**	$\mathbf{1s^1}$	$_{7}$**N**	$1s^2\mathbf{2s^2 2p^3}$	$_{19}$**K**	$1s^2 2s^2 2p^6 3s^2 3p^6\mathbf{4s^1}$
$_{2}$**He**	$\mathbf{1s^2}$	$_{8}$**O**	$1s^2\mathbf{2s^2 2p^4}$	$_{20}$**Ca**	$1s^2 2s^2 2p^6 3s^2 3p^6\mathbf{4s^2}$
$_{3}$**Li**	$1s^2\mathbf{2s^1}$	$_{11}$**Na**	$1s^2 2s^2 2p^6\mathbf{3s^1}$	$_{26}$**Fe**	$1s^2 2s^2 2p^6 3s^2 3p^6\mathbf{3d^6 4s^2}$
$_{4}$**Be**	$1s^2\mathbf{2s^2}$	$_{15}$**P**	$1s^2 2s^2 2p^6\mathbf{3s^2 3p^3}$	$_{29}$**Cu**	$1s^2 2s^2 2p^6 3s^2 3p^6\mathbf{3d^{10} 4s^1}$
$_{5}$**B**	$1s^2\mathbf{2s^2 2p^1}$	$_{16}$**S**	$1s^2 2s^2 2p^6\mathbf{3s^2 3p^4}$	$_{30}$**Zn**	$1s^2 2s^2 2p^6 3s^2 3p^6\mathbf{3d^{10} 4s^2}$
$_{6}$**C**	$1s^2\mathbf{2s^2 2p^2}$	$_{17}$**Cl**	$1s^2 2s^2 2p^6\mathbf{3s^2 3p^5}$	$_{34}$**Se**	$1s^2 2s^2 2p^6 3s^2 3p^6 3d^{10}\mathbf{4s^2 4p^4}$

1.2.4. Periodicity in properties of the elements

The Periodic Law: The properties of elements are periodic functions of their atomic numbers. **The Periodic Table** is a two-dimensional tabular arrangement portraying the elements in terms of similarities and differences in **chemical properties**. In the table, the elements are arranged in rows in increasing order of atomic number, running from left to right across the table (Tab.1.3.).

The rows are called **periods** and are normally identified with **Arabic numerals** (*i.e.*, Period 3).

The columns are termed **groups.** There are two systems of group numbers**;** one using **Arabic numerals** (1, 2, 3) and the other two using **Roman numerals** (I, II, III). The Roman numeral names were used at first and are the traditional names; the Arabic numeral names are newer names that the International Union of Pure and Applied

Chemistry (IUPAC) decided to use as well. The IUPAC names were meant to replace the older Roman numeral systems as they used the same names to mean different things, which was confusing. Under an international naming convention, the groups are numbered numerically from 1 to 18 from the leftmost column (the alkali metals) to the rightmost column (noble gases). In each group, the outermost electron shell contains the same number of electrons for each member.

The block of elements between Groups 3 and 12 are called **transition metals**. Transition metals have from one to ten electrons in the **d** orbitals. These transition elements have very similar chemical properties.

Elements 58 to 71 are the **lanthanides** or **rare Earth** elements. Lanthanides are elements in which the **4f** orbitals are being filled. Elements 90 to 92 (to 103 if the elements that have been created artificially are counted) are termed the **actinide** elements. Actinides are elements in which the **5f** orbitals are being filled.

The elements in Group 17 are collectively termed **halogens.** Atoms of these non-metals contain seven electrons in their outer electron shell. All form univalent anions, e.g., Cl^-. They are oxidizing agents, decreasing in oxidizing power (and chemical reactivity) with increasing period, so that fluorine is the most reactive and iodine the least.

The elements in Group 18 are collectively termed **noble or inert gases**. Their electron configurations are very stable as the outer shell of electrons is complete. They gain or loose electrons only in extreme conditions and so do not normally enter into chemical reactions. They exist as monoatomic gases.

Tab. 1.3. Periodic table of elements

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
	I A	II A	III B	IV B	V B	VI B	VII B	VIII B			I B	II B	III A	IV A	V A	VI A	VII A	VIII A
1	$_{1}$**H**																	$_{2}$**He**
2	$_{3}$**Li**	$_{4}$**Be**											$_{5}$**B**	$_{6}$**C**	$_{7}$**N**	$_{8}$**O**	$_{9}$**F**	$_{10}$**Ne**
3	$_{11}$**Na**	$_{12}$**Mg**											$_{13}$**Al**	$_{14}$**Si**	$_{15}$**P**	$_{16}$**S**	$_{17}$**Cl**	$_{18}$**Ar**
4	$_{19}$**K**	$_{20}$**Ca**	$_{21}$**Sc**	$_{22}$**Ti**	$_{23}$**V**	$_{24}$**Cr**	$_{25}$**Mn**	$_{26}$**Fe**	$_{27}$**Co**	$_{28}$**Ni**	$_{29}$**Cu**	$_{30}$**Zn**	$_{31}$**Ga**	$_{32}$**Ge**	$_{33}$**As**	$_{34}$**Se**	$_{35}$**Br**	$_{36}$**Kr**
5	$_{37}$**Rb**	$_{38}$**Sr**	$_{39}$**Y**	$_{40}$**Zr**	$_{41}$**Nb**	$_{42}$**Mo**	$_{43}$**Tc**	$_{44}$**Ru**	$_{45}$**Rh**	$_{46}$**Pd**	$_{47}$**Ag**	$_{48}$**Cd**	$_{49}$**In**	$_{50}$**Sn**	$_{51}$**Sb**	$_{52}$**Te**	$_{53}$**I**	$_{54}$**Xe**
6	$_{55}$**Cs**	$_{56}$**Ba**	$_{57}$**La**	$_{72}$**Hf**	$_{73}$**Ta**	$_{74}$**W**	$_{75}$**Re**	$_{76}$**Os**	$_{77}$**Ir**	$_{78}$**Pt**	$_{79}$**Au**	$_{80}$**Hg**	$_{81}$**Tl**	$_{82}$**Pb**	$_{83}$**Bi**	$_{84}$**Po**	$_{85}$**At**	$_{86}$**Rn**
7	$_{87}$**Fr**	$_{88}$**Ra**	$_{89}$**Ac**	$_{104}$**Rf**	$_{105}$**Db**	$_{106}$**Sg**	$_{107}$**Bh**	$_{108}$**Hs**	$_{109}$**Mt**	$_{110}$**Ds**	$_{111}$**Rg**	$_{112}$**Cn**						
			*	$_{58}$**Ce**	$_{59}$**Pr**	$_{60}$**Nd**	$_{61}$**Pm**	$_{62}$**Sm**	$_{63}$**Eu**	$_{64}$**Gd**	$_{65}$**Tb**	$_{66}$**Dy**	$_{67}$**Ho**	$_{68}$**Er**	$_{69}$**Tm**	$_{70}$**Yb**	$_{71}$**Lu**	
			**	$_{90}$**Th**	$_{91}$**Pa**	$_{92}$**U**	$_{93}$**Np**	$_{94}$**Pu**	$_{95}$**Am**	$_{96}$**Cm**	$_{97}$**Bk**	$_{98}$**Cf**	$_{99}$**Es**	$_{100}$**Fm**	$_{101}$**Md**	$_{102}$**No**	$_{103}$**Lr**	

**** Lanthanides***

***** Actinides***

1.2.5. The electron structures of atoms in individual periods

The first period

Hydrogen has its only electron in the 1s orbital - **$1s^1$**, and at helium the first level is completely full - **$1s^2$.**

The second period

Lithium's electron goes into the 2s orbital because that has a lower energy than the 2p orbitals. Lithium has an electron structure of $1s^2$**$2s^1$.** Beryllium adds a second electron to the same level - $1s^2$**$2s^2$.**

Now the 2p levels start to fill. These levels all have the same energy, and so the electrons go in singly at first.

B $1s^2 2s^2 \mathbf{2p_x^1}$

C $1s^2 2s^2 2p_x^1 \mathbf{2p_y^1}$

N $1s^2 2s^2 2p_x^1 2p_y^1 \mathbf{2p_z^1}$

The next electrons to go in will have to pair up with those already there.

O $1s^2 2s^2 \mathbf{2p_x^2} 2p_y^1 2p_z^1$

F $1s^2 2s^2 2p_x^2 \mathbf{2p_y^2} 2p_z^1$

Ne $1s^2 2s^2 2p_x^2 2p_y^2 \mathbf{2p_z^2}$

The third period

At neon, all the second level orbitals are full, and so after this we have to start the third period with sodium. The pattern of filling is now exactly the same as in the previous period, except that everything is now happening at the 3-level.

For example:

		short version
Mg	$1s^2 2s^2 2p^6 3s^2$	$[Ne]3s^2$
S	$1s^2 2s^2 2p^6 3s^2 3p_x^2 3p_y^1 3p_z^1$	$[Ne]3s^2 3p_x^2 3p_y^1 3p_z^1$
Ar	$1s^2 2s^2 2p^6 3s^2 3p_x^2 3p_y^2 3p_z^2$	$[Ne]3s^2 3p_x^2 3p_y^2 3p_z^2$

The beginning of the fourth period

At this point the 3-level orbitals are not all full - the 3d levels have not been used yet. But if you refer back to the energies of the orbitals, you will see that the next lowest energy orbital is the 4s - so that fills next.

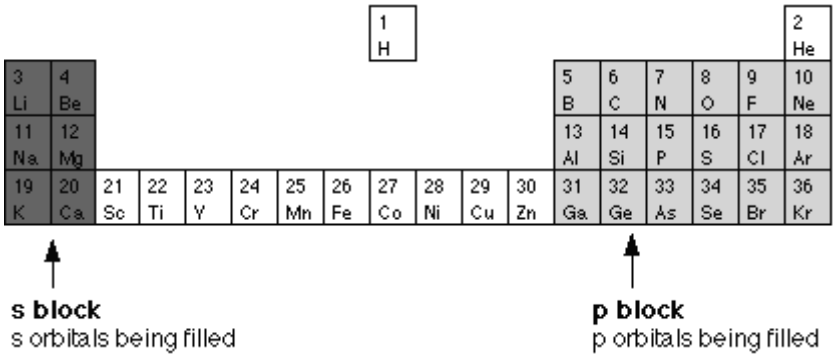
K $1s^2 2s^2 2p^6 3s^2 3p^6 \mathbf{4s^1}$

Ca $1s^2 2s^2 2p^6 3s^2 3p^6 \mathbf{4s^2}$

There is strong evidence for this in the similarities in the chemistry of elements like sodium ($1s^2 2s^2 2p^6 \mathbf{3s^1}$) and potassium ($1s^2 2s^2 2p^6 3s^2 3p^6 \mathbf{4s^1}$)

The outer electron governs their properties and that electron is in the same sort of orbital in both of the elements. That wouldn't be true if the outer electron in potassium was $3d^1$.

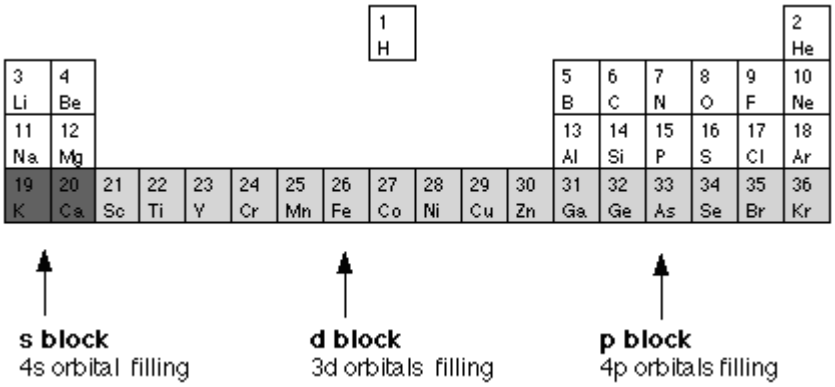
s- and p-block elements



The elements in group 1 of the Periodic Table all have an outer electron structure of ns^1 (where n is a number between 2 and 7). All group 2 elements have an outer electron structure of ns^2. Elements in groups 1 and 2 are described as s-block elements.

Elements from group 13 across to the noble gases all have their outer electrons in p orbitals. These are then described as p-block elements.

d-block elements



The **4s orbital has a lower energy than the 3d orbitals** and **so fills first**. Once the 3d orbitals have filled up, the next electrons go into **the 4p orbitals** as you would expect. d-block elements are elements in which the last electron to be added to the atom is in a **d orbital**.

d electrons are almost always described as, for example, d^5 or d^8 - and not written as separate orbitals. Remember that there are five d orbitals, and that the electrons will occupy them singly as far as possible. Up to 5 electrons will occupy orbitals on their own. After that they will have to pair up.

d^5 means 

d^8 means 

All the 3-level orbitals are written together, even though the 3d electrons are added to the atom after the 4s.

Sc $1s^2 2s^2 2p^6 3s^2 3p^6 \mathbf{3d^1 4s^2}$

Ti $1s^2 2s^2 2p^6 3s^2 3p^6 \mathbf{3d^2 4s^2}$

V $1s^2 2s^2 2p^6 3s^2 3p^6 \mathbf{3d^3 4s^2}$

Cr $1s^2 2s^2 2p^6 3s^2 3p^6 \mathbf{3d^5 4s^1}$

Chromium breaks the sequence. In chromium, the electrons in the 3d and 4s orbitals rearrange so that there is one electron in each orbital. It would be convenient if the sequence was tidy - but it is not!

Mn $1s^2 2s^2 2p^6 3s^2 3p^6 \mathbf{3d^5 4s^2}$ (back to being tidy again)

Fe $1s^2 2s^2 2p^6 3s^2 3p^6 \mathbf{3d^6 4s^2}$

Co $1s^2 2s^2 2p^6 3s^2 3p^6 \mathbf{3d^7 4s^2}$

Ni $1s^2 2s^2 2p^6 3s^2 3p^6 \mathbf{3d^8 4s^2}$

Cu $1s^2 2s^2 2p^6 3s^2 3p^6 \mathbf{3d^{10} 4s^1}$

Zn $1s^2 2s^2 2p^6 3s^2 3p^6 \mathbf{3d^{10} 4s^2}$

And at zinc the process of filling the d orbitals is complete.

The d-block elements are called the **transition metals** and the f-block elements (**lanthanides and actinides**) are called the **inner transition elements** or the rare earths elements.

1.2.6. First ionization energy

The first ionization energy is the energy required **to remove** the most loosely held **electron** from one mole of gaseous atoms to produce 1 mole of gaseous ions (**cation**) each with a charge of 1^+.

This is more easily seen in symbol terms.

$$X_{(g)} \longrightarrow X^+_{(g)} + e^-$$

It is the energy needed to carry out this change per mole of X (Tab.1.4). There is a general decrease of the first ionization energy of elements from top to bottom within a given group and increase from left to right within a given row of the periodic table. **Metals** have usually few outer-level electrons and incline to give positively charged ions (cations) by losing the electrons. Therefore, they are said to be **electropositive elements**, their values of electronegativity are low.

Tab. 1.4. The values of ionization energy for some elements (kJ/mol)

$H \rightarrow H^+$	**$Li \rightarrow Li^+$**	**$Na \rightarrow Na^+$**	**$K \rightarrow K^+$**	**$Rb \rightarrow Rb^+$**
1312.5	520.4	496.1	419.1	401.5
$Be \rightarrow Be^{2+}$	**$Cu \rightarrow Cu^{2+}$**	**$Ag \rightarrow Ag^+$**	**$Au \rightarrow Au^+$**	**$Zn \rightarrow Zn^{2+}$**
2646.1	2683.8	724.3	896.0	2625.2

1.2.7. First electron affinity

Ionization energies are always concerned to the formation of positive ions. Electron affinities are the negative ion equivalent, and their use is almost always confined to elements in groups 16 and 17 of the Periodic Table.

The first electron affinity is the energy released when each atom of 1 mol of gas acquires an electron to form 1 mol of gaseous 1^- ions.

This is more easily seen in symbol terms.

$$X_{(g)} + e^- \longrightarrow X^-_{(g)}$$

It is the energy released (per mol of X) when this change happens. First electron affinities have negative values. For example, the first electron affinity of chlorine is -349 kJ mol^{-1}. By convention, the negative sign shows a release of energy.

The first electron affinities of the group 7 elements:

F -328 kJ mol^{-1}

Cl -349 kJ mol^{-1}

Br -324 kJ mol^{-1}

I -295 kJ mol^{-1}

1.2.8. Second electron affinity

The second electron affinity is the energy required to add an electron to each ion in 1 mole of gaseous 1^- ions to produce 1 mole of gaseous 2^- ions. This is more easily seen in symbol terms.

$$X^-_{(g)} + e^- \longrightarrow X^{2-}_{(g)}$$

It is the energy needed to carry out this change per mole of X^-.

Why is energy needed to do this?

You are forcing an electron into an already negative ion.

$O_{(g)} + e^- \longrightarrow O^-_{(g)}$ $\quad 1^{st}$ EA = -142 kJ.mol^{-1}

$O^-_{(g)} + e^- \longrightarrow O^{2-}_{(g)}$ $\quad 2^{nd}$ EA = +844 kJ.mol^{-1}

The positive sign shows that you have to put in energy to perform this change. The second electron affinity of oxygen is particularly high because the electron is being forced into a small, very electron-dense space.

1.2.9. Oxidation states

Oxidation state (also called **oxidation number)** is a measure of the degree of oxidation of an atom in a substance. The oxidation numbers of the atoms in a covalent compound are defined as the imaginary charges the atoms would have if the shared electrons were divided equally between identical atoms bonded to each other or, for different atoms, were all assigned to the atom in each bond that has higher electronegativity. The oxidation number is represented by a Roman numeral. For ionic compounds containing monatomic ions, the oxidation number of the ion is equal to the net charge on the ion, for example K^+, Ca^{2+}, Fe^{3+} have oxidation numbers I, II and III respectively.

Here are the rules which allow the assignment of oxidation numbers in most compounds:

1. The oxidation number of a **free element** (uncombined element) is zero. Thus Na, Be, O_2 has the same oxidation number: **0**.
2. The oxidation number of **hydrogen** is **I**, except when it is bonded to metals in binary compounds (that is, compounds containing two elements). For example in LiH, CaH_2 its oxidation number is **-I**.
3. The oxidation number of **oxygen** in most compounds is **–II,** in hydrogen peroxide (H_2O_2) its oxidation number is **-I**.
4. **Fluorine** has an oxidation number of **-I** in all compounds. Other halogens have negative oxidation numbers when they occur as halide ions in their compounds (e.g. NaCl, KBr). When combined with oxygen, for example in oxoacids ($HClO_4$), they have positive oxidation number.
5. **In a neutral molecule, the sum of the oxidation numbers of all atoms must be zero**. **In a polyatomic ion, the sum of oxidation numbers of all atoms in the ion must be equal to the net charge of the ion**. For example in the sulfate anion SO_4^{2-}, the oxidation number of S is VI and that of O is -II. Thus the sum of the oxidation numbers is 6 + 4.(-2) = -2, which is equal to the net charge of the ion.

An element is said to be oxidized if its oxidation number increases in a reaction; if the oxidation number of the element decreases in a reaction, it is said to be reduced. The higher the oxidation state of a given atom, the greater is its degree of oxidation; the lower the oxidation state, the greater is its degree of reduction.

Fig. 1.4. shows some of the oxidation states found in compounds of the transition-metal elements.

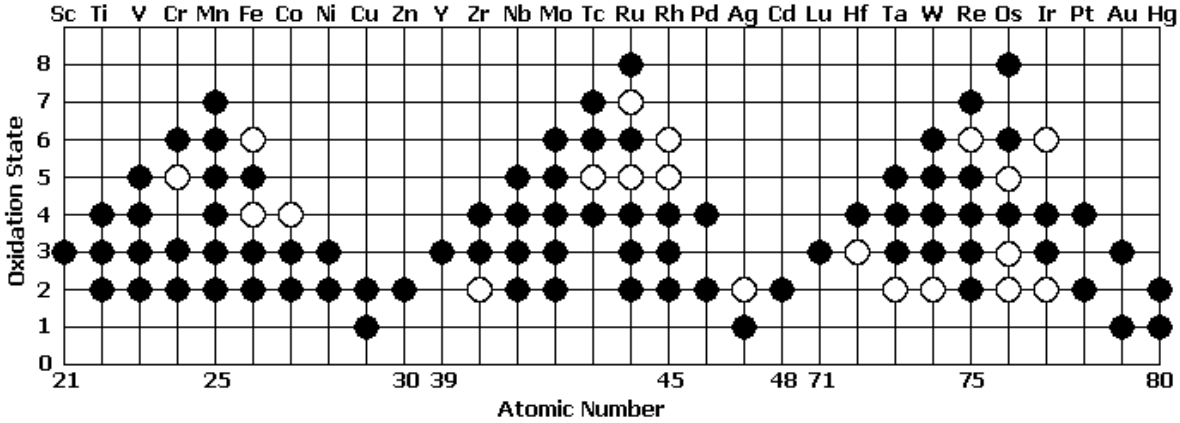


Fig. 1.4. The oxidation states found in compounds of the transition-metal elements.
A solid circle represents a common oxidation state, and a ring represents a less common (less energetically favourable) oxidation state.

1.3. Chemical bonds

The atoms of a compound are held together by chemical bonds formed by the interaction of electrons from each atom. The chemical bond means a net force of attraction that holds atomic nuclei within compounds near each other. Atoms are disposed to those chemical reactions that give them more stable, preferentially closed configurations of outer-shell electrons (see the "octet rule").

There are two ways in which atoms interact through their outer-shell electrons to attain the stable configuration:

1. Formation of molecules by mutual possession or **sharing a pair of electrons** which acts as a bonding force to link the atoms, **the covalent bond** (Fig. 1.5.)
2. Formation of oppositely charged ions by the transferring some valence electrons; the ions are attracted through electrostatic forces, **the ionic bond** (Fig. 1.5.)

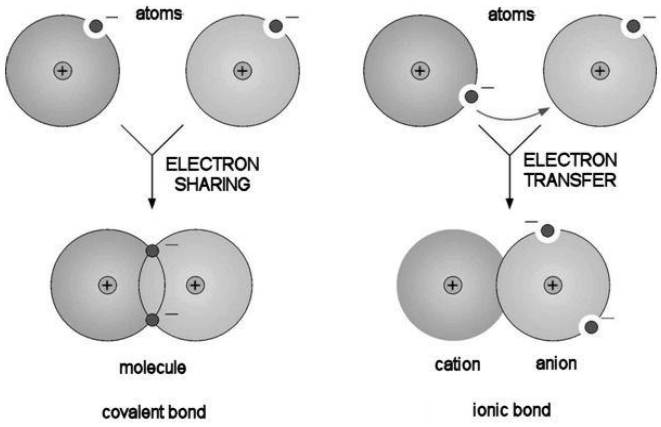


Fig. 1.5. Comparison of covalent and ionic bond

1.3.1. Covalent bond

When nonmetals or metalloids form compounds with other nonmetals, the atoms attain a stable configuration by **sharing** one or more **electron pairs** between the atoms (Fig. 1.5.).

1.3.1.1. Single covalent bond

One shared pair means one covalent bond. It is drawn as one line connecting the atoms and represents an overlap of two half-filled atomic orbitals which have turned into a bonding molecular orbital occupied by two electrons of opposite spins- by a shared bonding pair. A covalent bond which result from an end-to-end overlap of half filled **s** or **p orbitals**, with cylindrical symmetry around the bond axis, is called a **σ bond** (Fig. 1.6.a). A bond formed by coupling of atoms of the same element is a **nonpolar bond**, the electron pair is shared equally. The formation of diatomic molecules such as hydrogen or chlorine lead to a single covalent bond (**σ bond).**

A covalent bond between nonmetals with **different electronegativity** values is **called a polar covalent** bond, because of an unequal sharing of the bonding electron pair. If the difference in electronegativity is smaller than 0.4, the covalent bond is still classified as **nonpolar**.

Definition of electronegativity

Electronegativity is a measure of the tendency of an atom to attract a bonding pair of electrons. The Pauling scale is the most commonly used. Fluorine (the most electronegative element) is assigned a value of 4.0, and values range down to cesium and francium which are the least electronegative at 0.7.

What happens if two atoms of equal electronegativity bond together?

If the atoms are equally electronegative, both have the same tendency to attract the bonding pair of electrons, and so it will be found *on average* half way between the two atoms. To get a bond like this, A and B would usually have to be the same atom. You will find this sort of bond in, for example, H_2 or Cl_2 molecules.

What happens if B is slightly more electronegative than A?

B will attract the electron pair rather more than A does.

That means that the B end of the bond has more than its fair share of electron density and so becomes slightly negative. At the same time, the A end (rather short of electrons) becomes slightly positive. Symbol δ is used to indicate a fractional charge; δ^+ means partially positive charge and δ^- partially negative charge.

Definition of polar bonds

The polar bond is a covalent bond in which there is a separation of charge between one end and the other - in other words in which one end is slightly positive and the other slightly negative. Examples include most covalent bonds. The hydrogen-chlorine bond in HCl or the hydrogen-oxygen bonds in water are typical.

What happens if B is a lot more electronegative than A?

In this case, the electron pair is dragged right over to B's end of the bond. To all intents and purposes, A has lost control of its electron, and B has complete control over both electrons. Ions have been formed.

A "spectrum" of bonds

The implication of all this is that there is no clear-cut division between covalent and ionic bonds. In a pure covalent bond, the electrons are held on average exactly half way between the atoms. In a polar bond, the electrons have been dragged slightly towards one end.

How far does this dragging have to go before the bond counts as ionic? There is no real answer to that. You normally think of sodium chloride as being a typically ionic solid, but even here the sodium has not *completely* lost control of its electron. Because of the properties of sodium chloride, however, we tend to count it as if it were purely ionic.

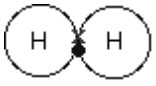
The absolute value of the difference in electronegativity of two bonded atoms tells the degree of polarity in their bond. Although dividing points between bond types are not sharply defined, the following rule is helpful in distinguishing between them:

-
- No electronegativity difference or a small electronegativity difference (less than about 0.4) between two atoms leads to a **non-polar covalent** bond (H-H, O-O, C-H).
- An electronegativity difference from about 0.4 to 1.7 leads to a **polar covalent** bond (H-Cl, H-O)
- An electronegativity difference greater than about 1.7.leads to **an ionic** bond (NaCl, KF).

Some simple covalent molecules

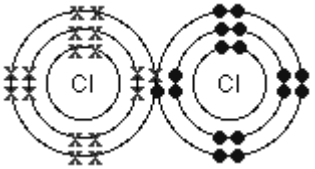
Hydrogen

Hydrogen atoms need two electrons in their outer level to reach the noble gas structure of helium. Once again, the covalent bond holds the two atoms together as the pair of electrons is attracted to both nuclei.



Chlorine

For example, two chlorine atoms could both achieve stable structures by sharing their single unpaired electron as in the diagram.

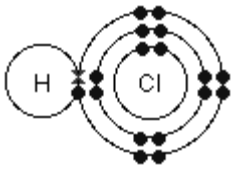


The fact that one chlorine has been drawn with electrons marked as crosses and the other as dots is simply to show where all the electrons come from. In reality there is no difference between them.

The two chlorine atoms are said to be joined by a covalent bond. The reason that the two chlorine atoms stick together is that the shared pair of electrons is attracted to the nucleus of both chlorine atoms.

Hydrogen chloride

The hydrogen has a helium structure, and the chlorine an argon structure.



Shapes of simple molecules and ions

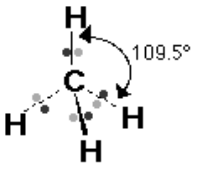
One of the most important features of covalent bonds is their **strong directionality**. It is this property which determines the specific three-dimensional structure of the molecule.

The electron pair repulsion theory

The shape of a molecule or ion is governed by the arrangement of the electron pairs around the central atom. All you need to do is to work out how many electron pairs there are at the bonding level, and then arrange them to produce the minimum amount of repulsion between them. You have to include both bonding pairs and lone pairs.

Four electron pairs around the central atom

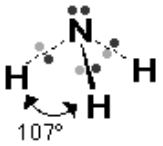
There are lots of examples of this. The simplest is ***methane, CH_4***. Carbon is in group 4, and so has 4 outer electrons. It is forming 4 bonds to hydrogen atoms, adding another 4 electrons - 8 altogether, in 4 pairs. Because it is forming 4 bonds, these must all be bonding pairs. Four electron pairs arrange themselves in space in what is called a ***tetrahedral*** arrangement. A tetrahedron is a regular triangularly-based pyramid. The carbon atom would be at the centre and the hydrogen atoms at the four corners. All the bond angles are 109.5°.



Other examples with four electron pairs around the central atom

Ammonia, NH_3

Nitrogen is in group 5 and so has 5 outer electrons. Each of the 3 hydrogen atoms is adding another electron to the nitrogen's outer level, making a total of 8 electrons in 4 pairs. Because the nitrogen is only forming 3 bonds, one of the pairs must be a lone pair (triangular pyramid). The electron pairs arrange themselves in a tetrahedral fashion as in methane.



In this case, an additional factor comes into play. Lone pairs are in orbitals that are shorter and rounder than the orbitals that the bonding pairs occupy. Because of this, there is more repulsion between a lone pair and a bonding pair than there is between two bonding pairs. That forces the bonding pairs together slightly - reducing the bond angle from 109.5° to 107°.

Remember this:

Greatest repulsion **lone pair - lone pair**

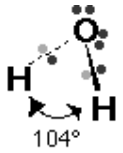
lone pair - bond pair

Least repulsion **bond pair - bond pair**

Although the electron pair arrangement is tetrahedral, when you describe the shape, you only take notice of the atoms. Ammonia is pyramidal - like a pyramid with the three hydrogen atoms at the base and the nitrogen at the top.

Water, H_2O

Following the same logic as before, you will find that the oxygen has four pairs of electrons, two of which are lone pairs. These will again take up a tetrahedral arrangement. This time the bond angle closes slightly more to 104°, because of the repulsion of the two lone pairs. The shape isn't described as tetrahedral, because we only "see" the oxygen and the hydrogen atoms - not the lone pairs. Water is described as ***bent*** or ***V-shaped.***



1.3.1.2. Multiple covalent bond

Multiple covalent bonds arise between two σ-bonded atoms when their non-hybridized and half-filled p or d-orbitals situated in one plane overlap side-to side. The resulting second or third covalent bond is called a **π bond**, the bonding electron pair of which lies half above and half below the bond axis. A double covalent bond (e.g. CH_2=CH_2) consists of one σ bond and one π bond. A molecule of N_2 has a triple covalent bond (N≡N) composed from one σ bond and two π bonds (Fig. 1.6.).

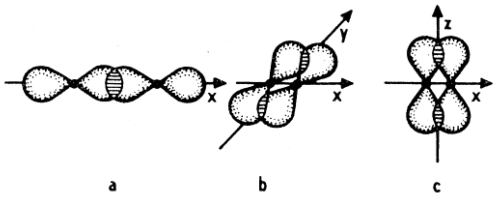
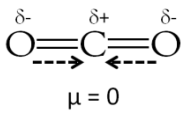


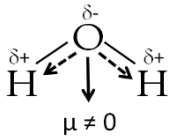
Fig. 1.6. Schema of bonds in molecule of nitrogen: a) σ bonds, b) c) π bonds

1.3.1.3. Dipole moment

Polarity is an important molecular property. The polarity of a molecule is measured by its **dipole moment.** Dipole moment is equal to the product of the charges (q) and the distance between them (d); $\mu = q.d$. For diatomic molecules such as H─F and H─Cl that have only one bond, when the bond is polar so is the molecule. Whether polyatomic molecules are polar in an overall sense depends **not just on the presence of polar bonds** but also **on the geometry of the molecule**. It is possible for the polarities of individual bonds to cancel out each other. A good approximation is to assign a dipole moment (which is a vector, shown as an arrow pointing from the negative charge to the positive charge) to each bond, and then obtain the total dipole moment by carrying out a vector sum of the bond dipoles. In linear molecule CO_2, for example, each carbon-oxygen bond has a dipole. However, because the dipoles are equal in magnitude and point in opposite directions, the total molecular dipole moment vanishes:



That is the reason, why in highly symmetrical molecules such as BF_3 (trigonal planar symmetry) or CF_4 (tetrahedral symmetry) the net dipole moment is zero even though the individual bonds may be strongly polar and such molecules are **nonpolar**. On the other hand, **water molecule is polar.** Because of the lone pairs on oxygen, the structure of H_2O is bent (see description of its geometry above) and the vectors representing the dipole moment of each O─H bond do not cancel each other out:



In a similar way, NH_3 molecule is also polar. Dipole moments are an important source of intermolecular forces.

1.3.2. Coordinate bond

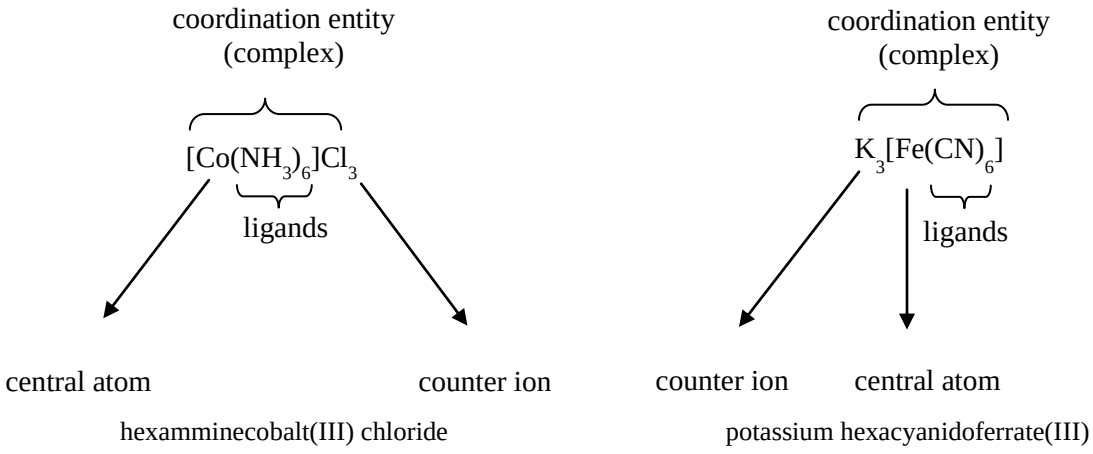
Coordinate bond (also sometimes called a dative bond) is a special case of bond where the electrons for sharing are supplied by one atom – **donor** atom. It means that a **coordinate bond** is distinguished by the **ligand** donor atom donating both electrons of a lone electron pair (nonbonding electron pair) to an empty orbital on the **central atom** to form the bond. The **ligand** is an ion or molecule having at least one donor atom with a lone electron pair, for example F^-, O^{2-}, OH^-, CN^-, H_2O, NH_3. The **central atom** is the electron pair acceptor (possesses vacant orbitals) and is conventionally metal, for example Cu, Co, Fe, Mn. Metal ions may exist and form coordinate bonds in a number of oxidation states. A **coordination entity** (or **complex**) is an ion or neutral molecule that is composed of a central atom to which is attached a surrounding array of ligands. The formula of the entire complex, whether charged or not, is enclosed in square brackets.

Examples:

1. $[Co(NH_3)_6]^{3+}$
2. $[Fe(CN)_6]^{3-}$
3. $[CuCl_2(H_2O)_2]$
4. $[Ni(CO)_4]$

In these complexes, the central atoms are cobalt in the oxidation state of III, iron in the oxidation state of III, copper in the oxidation state of II and nickel in the oxidation state of 0, respectively and ligands attached to them are either neutral molecules NH_3, H_2O and CO or anions $CN^-$and Cl^-.

The **coordination compound** is any neutral compound that contains a coordination entity, for example $[Co(NH_3)_6]Cl_3$ or $K_3[Fe(CN)_6]$:



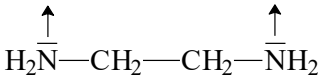
The number of σ-bonds between ligands and the central atom (number of donor atoms directly bound to the central atom) is called the **coordination number**. For example, the coordination numbers of Co^{3+} in coordination compound $[Co(NH_3)_6]Cl_3$ and of Fe^{3+} in $K_3[Fe(CN)_6]$ are 6. The most common coordination numbers are 6 and 4.

Ligands such as F^-, O^{2-}, OH^-, CN^-, H_2O, NH_3 are classical examples of **monodentate** ligands, it means that these ligands have only one donor atom able to form a bond with the same central atom. Many ligands offer more than one donor atom capable of binding **to the same central atom**, such ligands are called a **polydentate (multidentate)** ligands or **chelate** ligands. The number of such ligating atoms in a single chelating ligand is indicated by the adjectives **bidentate**, **tridentate**, **tetradentate**, **pentadentate**, etc. It means, when two donor

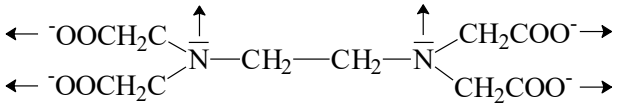
atoms of a ligand are bound to the same central atom, it is a **bidentate** ligand; three bound donor atoms yields a **tridentate**, and four bound is called a **tetradentate** ligand.

The cyclic structures formed when more than one donor atom from the same ligand is bound to the central atom are called **chelate rings** and the process of coordination of these donor atoms is called **chelation.** The complexes thus created are called **chelates**. Chelate ligands forms more stable complexes than do monodentate ligands. **Usually, the higher the denticity, the more stable is the complex formed**.

Common example of bidentate ligand is ethylenediamine (en) and chelation leads to a five-membered chelate ring. Ethylenediaminetetraacetic acid (EDTA), a hexadentate ligand, is an example of a multidentate ligand that has six donor atoms with a lone electron pairs that can be used to bond to a central atom:



ethylenediamine (en)



ethylenediaminetetraacetate ($EDTA^{4-}$)

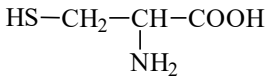
In medical practice, chelate ligands (**chelating agents**), are widely used for direct treatment of metal poisoning because they are capable of binding to toxic metal ions to form complex structures which are easily excreted from the body removing toxic metal ions from intracellular or extracellular spaces (see Chapter 2.4.3.). Chelating agents are also employed as extracting agents in industrial and laboratory separation of metals and as metal-ion buffers and indicators in analytical chemistry. Many commercial dyes and a number of biological substances, including chlorophyll and hemoglobin, are chelate compounds.

1.3.2.1. Biological ligands for metal ions

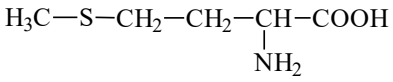
Within the biological context, metals are present mainly in oxidized form as ionized metal centers, which are surrounded by electron pair donating ligands. The three most important classes of biologically important ligands are: **proteins** with amino acid side chains that can be used for coordination, specially biosynthesized **macrocyclic chelate ligands** and **nucleic acids** with nucleobases as potentially coordinating components.

Proteins as ligands

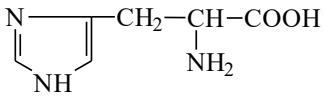
Proteins, including enzymes, consist of α amino acids, which are connected via peptide bonds, –C(O) –N(H) –. The functional groups in the side chains of amino acids residues, such as –COOH, $–NH_2$, –OH and –SH are particularly well suited for metal coordination. The common donor atoms in proteins are sulfur atom of cysteines, nitrogen atoms of histidines and oxygen atoms of glutamates, aspartates and tyrosinates. Another potential donor atoms (though less common) for metal ion coordination are sulfur atoms of methionines, nitrogen atoms of arginines and lysines and oxygen atoms of serines.



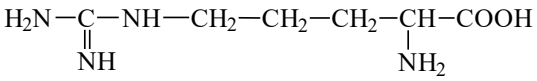
cysteine



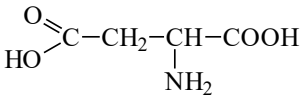
methionine



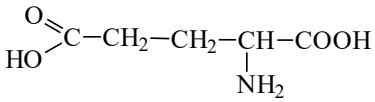
histidine



arginine



aspartic acid



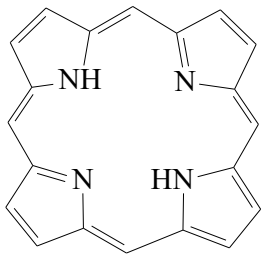
glutamic acid

Polypeptide chain of proteins thus acts as a huge chelate ligand, employing metal-binding amino acid residues. This is often the way how metals are bound in **metalloproteins** (see Chapter 9.3.3), one important branch of which is the **metalloenzymes**.

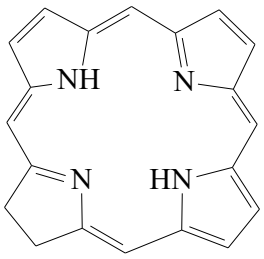
For example important antioxidant enzyme Cu/Zn-superoxide dismutase (Cu/Zn-SOD), contains copper and zinc in the active site. Copper is coordinated by four nitrogen atoms from four histidine residues. One of them acts as a bridge between the copper and the zinc ion. One oxygen from aspartate and two nitrogen atoms from two other histidine residues complete the coordination of zinc atom.

Tetrapyrrole ligands

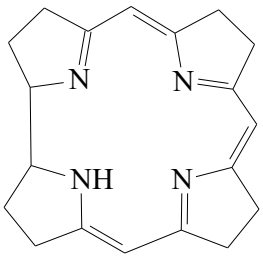
The tetrapyrroles are fully or partially unsaturated tetradentate macrocyclic ligands which can tightly bind metal ions. A pyrrole ring in a molecule is a five-atom ring where four of the ring atoms are carbon and one is nitrogen. In cyclic tetrapyrroles, lone electron pairs on nitrogen atoms can bind a metal ion such as iron, magnesium or cobalt. The resulting complexes are among the most common and best known bioinorganic compounds: **hemoproteins, chlorophylls,** and the **cobalamins**. The **heme** prosthetic group, which consist of an iron center and a substituted porphyrin ligand is found in **metalloproteins** hemoglobin, myoglobin, cytochromes and peroxidases. Chlorophylls contain Mg^{2+} as the central atom and chlorin as a ligand, cobalamins (the coenzymatically active forms of vitamin B_{12}) contain cobalt as the central atom and corrin as a ligand.



porphyrin
(or porphin in unsubstituted form)



chlorin



corrin

Nucleobases, nucleotides and nucleic acids as ligands

Suitable ligands for metal ions are also information-carrying nucleic acids and oligo- and polynucleotides. The formation, replication and cleavage of nucleic acid polymers (RNA, DNA) as well as their structural integrity require the presence of metal ions (e.g. Mg^{2+}). Coordination sites are negatively charged phosphate/carbohydrate

backbone and heterocyclic nucleobases with nitrogen or oxygen donor atoms. Formation of coordinate bond between metals and nucleic acids is important also from clinical point of view. For example

metal-nucleic acid interactions are the basis for application of cisplatin and its derivatives as anticancer chemotherapeutic agents (see Chapter 2.5).

1.3.3. Ionic bond

The ionic bond is the mutual electrostatic force of attraction between oppositely charged ions in an ionic compound. **Electrons are transferred from one atom to another** resulting in the formation of **positive ions - cations** and **negative ions** – **anions** (Fig.1.7.). Ionic compounds are made up of combinations of oppositely charged ions occurring in a crystal lattice in the definite ratio that ensures that all of the electrical charges on the assembled ions cancel out so that the substance is electrically neutral. The electrostatic attractions between the positive and negative ions hold the compound together.

Simple (binary) ionic compounds can originate in chemical reactions between two elements, the electronegativity values of which **differ more than 1.7** (usually s-block metals with oxygen, sulphur and halogens, respectively). In those reactions, the complete transfer of one or more valence electrons occurs from the metal atom to the nonmetal. Both atoms gain the configuration of the nearest noble gas by forming monoatomic ions, **without any sharing of electron bonding pairs.** The ionic bond is sometime referred to as **the extremely polarized type** of covalent bond.

Consider the formation of the ionic compound sodium chloride from sodium and chlorine. The electron configuration of sodium is $1s^2 2s^2 2p^6 3s^1$, or [Ne] $3s^1$, and that of chlorine is $1s^2 2s^2 2p^6 3s^2 3p^5$, or [Ne] $3s^2 3p^5$. The electronegativity of chlorine is much greater than that of sodium. Because of this large difference, electron will be transferred from sodium to chlorine to form **chloride anions** and **sodium cations** in the compound. Thus, both ions gain electron configuration of the nearest noble gas: chlorine needs one electron to fill its 3s and 3p valence orbitals and to achieve the configuration of argon ($1s^2 2s^2 2p^6 3s^2 3p^6$). On the other side, by losing one electron, sodium can achieve the configuration of neon ($1s^2 2s^2 2p^6$). One electron is therefore transferred:

$$\mathbf{Na}\ [Ne]3s^1 + \mathbf{Cl}\ [Ne]3s^2 3p^5 \rightarrow \mathbf{Na^+}\ [Ne] + \mathbf{Cl^-}\ [Ar]$$

To predict the formula of the ionic compound, we simply recognize that chemical compounds are always electrically neutral – they have the same quantities of positive and negative charges. In this case we have equal numbers of Na^+ and Cl^- ions, and the formula of the compound is NaCl.

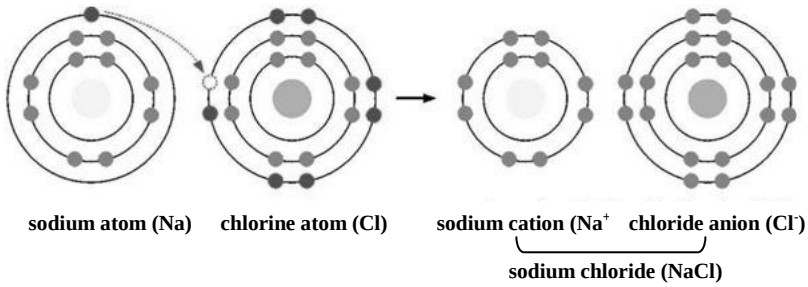
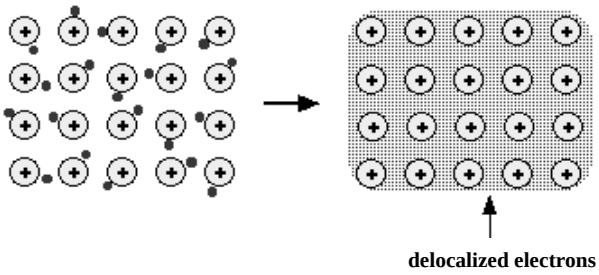


Fig. 1.7. Origin of ionic bond in molecule of NaCl

1.3.4. Metallic bond

Metals exist predominantly in the solid state. Atoms of metals are bonded in a closed-packed lattice. However, they are not held rigidly by covalent bonds or by surrounding array of anions. A model of the metallic state is suggested in which the metal atoms exist as cations and electrons resulting from the ionization of atoms are distributed throughout the lattice so as to overcome the mutual repulsion of the cations and to set up the net attraction forces between electrons and cations. With such a model, the binding electrons belong to the crystal of a metal as a whole (an **electron "gas"),** they can move throughout the lattice at continuous bands of energy levels, thus providing conducting electricity. **The electrons** can move in metal freely within molecular orbitals, and so each electron becomes detached from its parent atom. The electrons are said also to be ***delocalized.*** The

metal is held together by **the strong forces of attraction** between **the positive nuclei** and **the delocalized electrons**. This is sometimes described as "an array of positive ions in a sea of electrons":



The free movement of delocalized electrons is responsible for high electrical and thermal conductivities of metals.

1.3.5. Intermolecular forces

In addition to chemical bonding between atoms or ions, there is another type of attractive forces that exists between molecules (or atoms), known as intermolecular forces. Though this forces are generally much weaker than intramolecular forces (e.g. ionic or covalent bonds), they play very important role in biological systems, because they have a very significant influence on the three-dimensional structures of proteins, nucleic acids, polysaccharides and membrane lipids.

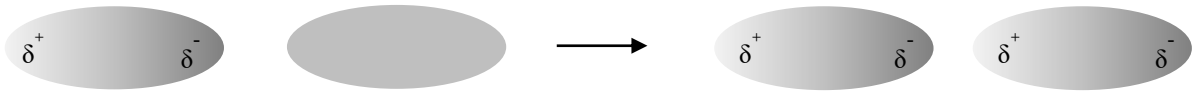
1.3.5.1. Van der Waals forces

Van der Waals forces includes: **dipole–dipole, dipole-induced dipole** and **London** (instantaneous **dipole-induced dipole)** forces. Van der Waals forces are electrostatic, involving attractions between positive and negative species, it means between polar molecules with permanent dipoles or nonpolar molecules (or atoms) with instantaneous induced dipoles.

1. Dipole-dipole forces are forces that act between polar molecules - between the partially positive end of one polar molecule and the partially negative end of another polar molecule. It means, they occur between molecules that possess **permanent** dipole moments. The larger the dipole moments, the greater the force. These forces occur between the polar molecules such as HCl, HBr or H_2S.

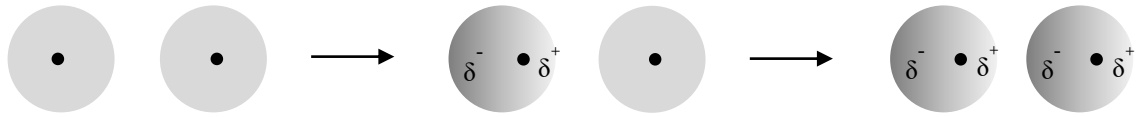
dipole **dipole**

2. Dipole-induced dipole forces are the attractive interactions between a polar molecules with permanent dipole and the induced dipoles. If we place a polar molecule near a nonpolar molecule (or atom), the electron distribution of the nonpolar molecule (or atom) is distorted by the force exerted by the polar molecule. The resulting dipole in the molecule is said to be an **induced dipole**, because the separation of positive and negative charges in the nonpolar molecule or atom is due to the proximity of the polar molecule. The ease with which a dipole moment can be induced depends not only on the strength of the permanent dipole but also on the polarizability of the neutral molecule. **Polarizability** is the ease with which the electron distribution in the neutral molecule (or atom) can be distorted. Generally, the larger the number of electrons and more diffuse the electron cloud in the molecule (or atom), the greater its polarizability. By diffuse cloud we mean an electron cloud that is spread over an appreciable volume, so that the electrons are not held tightly by nucleus.



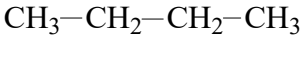
polar molecule **nonpolar molecule** **dipole** **induced dipole**

3. London forces, also called **dispersion** forces, are attractive forces between nonpolar molecules (or atoms), due to their mutual polarizability. Polarizability give us the clue to why gases containing nonpolar molecules or atoms (for example He or N_2) are able to condense. In a helium atom the electrons are moving at some distance from the nucleus. The atoms are nonpolar and so possess **no permanent dipole moment**. If we could freeze the motion of the electrons at any given instant, both electrons could be on one side of the nucleus. At just that instant, the atom has an **instantaneous** dipole moment. The motions of electrons in one atom influence the motions of electrons in its neighbours. The instantaneous dipole on one atom can induce an instantaneous dipole on an adjacent atom, causing the atoms to be attracted to each other. At very low temperatures, this attraction is enough to hold the atoms together, causing helium gas to condense.

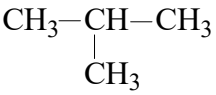


two helium atoms, no polarization | **instantaneous dipole** | **instantaneous dipole** | **induced dipole**

Dispersion forces usually increase with molar mass, because molecules with larger molar mass tend to have more electrons and dispersion forces increase in strength with the number of electrons (molecules have higher polarizability). For example, the melting and boiling points of the straight-chain alkanes increase with the number of carbon atoms, and thus with molar mass. This is a consequence of the increasing strength of dispersion forces between heavier molecules. Methane, ethane, propane and butane are all gases at room temperature, but the hydrocarbons that follow them in the alkane series are liquids and alkanes beyond about $C_{17}H_{36}$ are solids. Molecular shape also influences the magnitudes of dispersion forces. For example, the hydrocarbon molecules butane and 2-methylpropane both have a molecular formula C_4H_{10}, but the atoms are arranged differently. In butane the carbon atoms are arranged in a single chain, but 2-methylpropane is a shorter chain with a branch. Intermolecular attraction is greater for butane because the molecules can come in contact over the entire length of the longer butane molecules. Less contact is possible between the shorter and branched 2-methylpropane molecules.



butane



2-methylpropane

The London dispersion forces are also components of the forces between polar molecules.

1.3.5.2. Hydrogen bond

The hydrogen bond is a special type of dipole-dipole interaction. It is the attractive force between the hydrogen attached to an electronegative atom of one molecule and another electronegative atom of the different (intermolecular hydrogen bond) or the same molecule (intramolecular hydrogen bond). Usually the electronegative atom is oxygen, nitrogen or fluorine, which has at least one lone electron pair. A typical hydrogen bond may be depicted as:

$$X{-}H + Y{-}Z \rightarrow X{-}H\text{.....}Y{-}Z$$

The dots denote the hydrogen bond and X and Y are F, O or N. Because of their high electronegativity, they have a partial negative charge (δ-). The hydrogen then has the partial positive charge (δ+). In some cases X and Y are the same. It is best considered as an electrostatic interaction, heightened by the small size of hydrogen, which permits proximity of the interacting dipoles or charges. The hydrogen bond is generally stronger than ordinary dipole-dipole and dispersion forces, but weaker than true covalent and ionic bonds. Early evidence for hydrogen bonding came from the study of boiling points of compounds. Normally, the boiling points of a series of similar compounds containing elements in the same group increase with increasing molar mass. But, as Figure 1.8. shows, some exceptions were noticed for the hydrogen compounds of Groups 15, 16 and 17 elements. In

each of these series, the lightest compound (NH_3, H_2O, HF) has the highest boiling point, contrary to the expectations based on molar mass. It is because molecules held together by hydrogen bonding are more difficult to separate. It takes a lot more kinetic energy in an increased temperature to break the hydrogen bonds to free NH_3, H_2O, HF molecules as the gas.

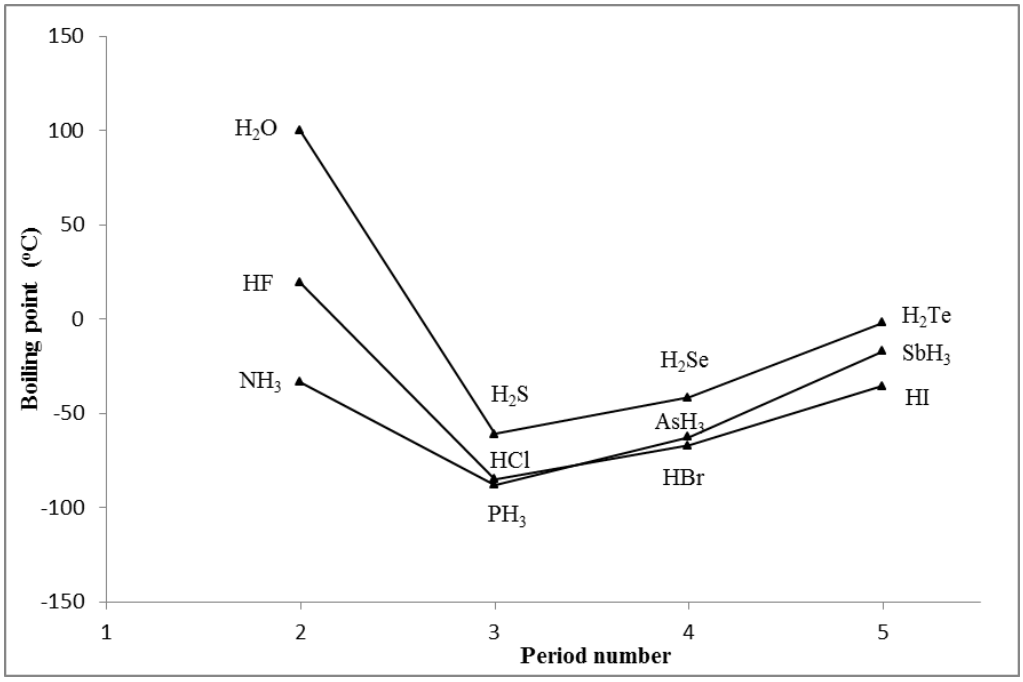


Figure 1.8. Boiling points versus formula masses for the binary, nonmetal hydrides of the elements in groups 15, 16 and 17.

The Figure 1.9. shows the hydrogen bonds between water molecules. Water is the most abundant substance in living systems with many important functions, see Chapter 2.3.1. Water has a high **specific heat capacity**, it means that water is able to absorb a high amount of heat before increasing in temperature, allowing humans to maintain body temperature **(thermoregulation)**. The reason for this is that to raise the temperature of water, many intermolecular hydrogen bonds must be broken. Thus, water can absorb a substantial amount of heat while its temperature rises only slightly.

Just as it takes a lot of heat to increase the temperature of liquid water, it also takes an unusual amount of heat to vaporize water, because hydrogen bonds must be broken in order for molecules to fly off as gas, it means that water has also a high **heat of vaporization.** In many living organisms, including in humans, the evaporation of sweat, which is 90 percent water, allows the organism to cool so that a consistent, healthy body temperature is maintained.

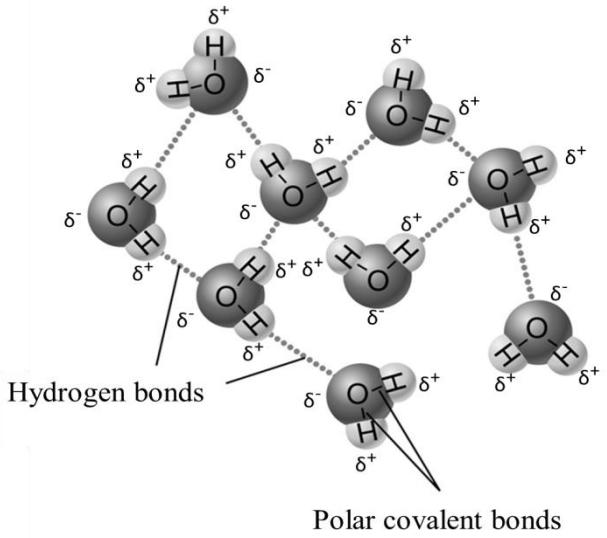


Figure 1.9. Schematic representation of hydrogen bonds in water

The hydrogen bonds may be **intermolecular** or **intramolecular**. The hydrogen bonds between NH_3, H_2O, HF molecules are **intermolecular** hydrogen bonds, it means they occur between separate molecules in a substance. **Intramolecular** hydrogen bonds are those which exist within one single molecule. This occurs when two functional groups of a molecule can form hydrogen bonds with each other. Intramolecular hydrogen bonds do not influence the physical properties of substances such as the boiling and melting points.

The hydrogen bond is a powerful force in determining the structures and properties of many compounds and is very important in biological systems:

The hydrogen bonds

- hold the two complementary strands of DNA together, see Chapter 10.4.2.
- hold polypeptide chain together in secondary structures (as α-helix and β-sheet conformation)
- contribute to the stabilization of tertiary structures of proteins, see Chapter 9.3.1.
- help enzymes bind to their substrate, see Chapter 12

Hydrogen bonds are also important in stabilizing the antibody-antigen interaction and play an important role in the transfer of genetic information (e.g. binding of transcription factors to DNA or to each other).

1.3.5.3. Hydrophobic interactions

Hydrophobic interactions describe the relations between water and a low water-soluble molecules. Water is a polar solvent (see Chapter 1.3.1.3). It readily dissolves most biomolecules, which are generally charged or polar compounds; compounds that dissolve easily in water are **hydrophilic**. In contrast, nonpolar solvents such as chloroform and benzene are poor solvents for polar biomolecules but easily dissolve those that are **hydrophobic** - nonpolar molecules such as lipids and waxes. In aqueous solutions, nonpolar molecules (or nonpolar region of the molecules) tend to cluster together to avoid contact with water. The forces that hold nonpolar molecules or nonpolar regions of the molecules together are called **hydrophobic interactions.** Many biomolecules are amphipathic; proteins, certain vitamins and the sterols and phospholipids of membranes all have polar and nonpolar surface regions. Structures composed of these molecules are stabilized by hydrophobic interactions among the nonpolar regions. Hydrophobic interactions among lipids and between lipids and proteins, are the most important determinants of structure in biological membranes. Hydrophobic interactions between nonpolar amino acids stabilize the three-dimensional structures of proteins.

Hydrophobic interactions also play an important role in the binding of the substrate to the enzyme and in antigen-antibody interactions.

Control questions:

1. Can the atoms with the following electron configuration be changed to a stable ions? If they can, what would be the electrical charge on the ion?
   a) $1s^2 2s^2 2p^5$
   b) $1s^2 2s^2 2p^6 3s^2 3p^6 4s^1$
2. What is the oxidation number of iron in $Fe_2(SO_4)_3$ and in $FeSO_3$?
3. What are the oxidation numbers of carbon and hydrogen in methane and what is meant when single bonds between carbon and hydrogen is called a sigma bond?
4. Explain the formation of a covalent bond.
5. Suppose that atoms X and Y form diatomic molecule, and that atom X is less electronegative than atom Y. Is the bond X─Y polar? If so, where are the δ^+ and the δ^- charges located?
6. Select the polar compound/compounds:
   a) CO_2
   b) NH_3
   c) H_2O
   d) CCl_4
7. What are an ionic compounds? How is electrical neutrality maintained in an ionic compound?
8. Compare and contrast the properties of molecular compounds and ionic compounds. Which elements are most likely to form ionic compounds?
9. Which of the following compounds are likely to be ionic? Which are likely to be molecular?
   $SiCl_4$, LiF, C_2H_4, glucose, $CaCl_2$, KCl, H_2O, SO_2, urea
10. Explain the terms coordinate bond, coordination compound and coordination number.
11. Why NH_3 and H_2O molecules can act as ligands in coordination compounds?
12. Ligands in complexes can be:
    a) halide ions
    b) OH^- ions
    c) corrin
    d) Cr^{3+} ions
13. Indicate the oxidation number of central atom and its coordination number in $K_3[Co(CN)_6]$ and $[Cu(NH_3)_4]SO_3$.
14. Explain what is chelate and which compounds can be chelate ligands. Write two examples of chelate ligands.
15. Why water plays an important role in human body thermoregulation?
16. Explain the principle of hydrophobic interactions.

2. CLASSIFICATION OF ELEMENTS. IMPORTANCE OF ELEMENTS IN THE ORGANISM. TOXIC ELEMENTS.

If we look at the periodic table we can find around 25 elements that are required by most, if not all, biological systems. Six elements, carbon, hydrogen, nitrogen, oxygen, phosphorus and sulfur provide the building blocks for major cellular components including proteins, nucleic acids, lipids, polysaccharides and metabolites. But life cannot survive with only these principal elements. It is now clear that around 20 additional elements are essential for most species to function. Conduction of nerve impulses, hydrolysis and formation of adenosine triphosphate (ATP), regulation of gene expression, control of cellular processes and signaling and catalysis of many key reactions of metabolism require elements such as calcium, magnesium, potassium, iron, zinc and many others. All elements that play an important role in the physiological context are **biogenic elements.**

2.1. Classification of biogenic elements

Biogenic elements are building blocks of living organisms; they take part in the metabolism and play an important biological role.

According to their abundance in organism biogenic elements can be classified into **macroelements** and **microelements.**

Macroelements include 11 elements in total. They form up to 99 % of any organism, and can be further subdivided into:

a) a group of stable **primary** elements (elements found in all of Earth's living systems, often in relatively large quantities, form up 2 – 60 % of total organism weight). These elements are **O, C, H** and **N.**

b) a group of stable **secondary** elements (elements found in living systems in relatively small quantities, form up 0.05 – 2 % of total organism weight). These elements are **Na, K, Ca, Mg, P, S** and **Cl**.

Microelements (or **trace elements**), which are present in organism in amounts less than 0.05 %.

Microelements can be subdivided into:

a) a group of metals: **Fe, Cu, Zn, Mn, Co, Ni, Mo, V, W**

b) a group of semi-metals (or metalloids) and nonmetals: **B, Si, F, I, Se**

Trace elements play an important role in enzymatic activities, where they can be a part of the cofactor. Enzymes that need a trace metal element for their activity are called metalloenzymes.

Some of biogenic elements are essential for all living form (bacteria, plants, animals) other differs in various biological species. For example **V** is essential for some nitrogen fixing bacteria as part of enzyme *nitrogenase*. *Bromoperoxidase* and *iodoperoxidase* enzymes in marine algae, microscopic fungi and lichens also require vanadium for proper functioning, but its essentiality for humans has not been proven yet.

First four elements, **O, C, H, N**, are present in a large amount because they are building blocks of "bio-mass"; together with phosphorus and sulfur provide the building blocks for major cellular components including proteins, nucleic acids, lipids, polysaccharides and metabolites. The values for **O** and **H** also reflect the high content of water in human body. Calcium is the most abundant metallic element and its main quantitative use is the stabilization of the endoskeleton. Three transition metals, **Fe, Zn** and **Cu** are needed in significant amounts and trace quantities of many other transition elements are required to maintain proper physical functioning. Nonnegligible quantities of obviously nonessential elements such as **Br**, **Al**, **Sr**, **Ba** or **Li** and toxic elements such as **As, Pb, Cd** or **Hg** are also present in the body. All these elements are probably incorporated due to a chemical similarity (indicated by →) with important essential elements ($Li^+ \rightarrow Na^+$, K^+; Sr^{2+}, $Ba^{2+} \rightarrow Ca^{2+}$; $Br^- \rightarrow Cl^-$; $Al^{3+} \rightarrow Fe^{3+}$; Cd^{2+}, $Pb^{2+} \rightarrow Zn^{2+}$).

Criteria of including the chemical element to biogenic elements are based on many years research of individual pathways and mechanisms. The classification has not been finished yet and it can be changed based on new information. As our knowledge of the chemistry of living systems increases, other elements might be added to the list of essential elements.

2.2. Biological functions of elements

Inorganic elements are involved in all life processes. Main biological functions of the elements are following:

a) **Structural function.** The formation of hard structures in the form of exo- or endoskeleton (bones, teeth) via biomineralization. This function is represented mainly by **Ca**, **Mg** (as divalent cations) and **P, O, C, Si, S, F** as parts of anions, e.g. PO_4^{3-}, CO_3^{2-}.

b) **Charge carriers.** Simple atomic ions are superbly suited as charge carriers for very fast information transfer. Electrical impulses in nerves, triggering of muscle contraction are initiated with the fastest possible effect by sudden fluxes of simple inorganic ions (**K^+, Na^+, Ca^{2+}**)

c) **Metabolism** (synthesis and degradation of organic compounds). Many biochemical processes are catalyzed by enzymes that require trace element as cofactor (**Zn, Ni, Mn, Fe**).

d) **Electron transfer.** Transition metals with multiple oxidation states facilitate the transfer of electrons which is essential for energy transfer in organisms. Biological ligands can stabilize metals in unusual oxidation states and fine tune redox potentials (**$Fe^{II}/Fe^{III}/Fe^{IV}$, Cu^{I}/Cu^{II}, $Mn^{II}/Mn^{III}/Mn^{IV}$, $Mo^{IV}/Mo^{V}/Mo^{VI}$**)

e) **Activators of small molecules**. Transition metals allow organisms to carry out energetically and mechanistically difficult reactions under physiological conditions, e.g.:
   - transport and storage of O_2: **Fe, Cu**
   - fixation of N_2: **Mo, Fe, V** (conversion to ammonia)
   - reduction of CO_2: **Ni, Fe** (reduction to methane)

f) **Various specific functions:**
- organometallic reactivity: **Co** (in vitamin B_{12}, see Chapter 11.2.7)
- hormonal action: **I** (triiodothyronine and thyroxine produced by the thyroid gland)

2.3. Importance of elements in the organism

2.3.1. Non-metallic elements

Hydrogen

Hydrogen is the most abundant chemical element in the universe and the third most abundant element in the human organism (with 10 % contents). Hydrogen is present in all organic compounds and in water. Unique, non-covalent interactions between electronegative atom and a hydrogen atom covalently bonded to another electronegative atom in the same or another molecule (e.g. O, N), **hydrogen bonds**, are very common in living organisms and play an important role in many chemical processes. Hydrogen bonds are responsible for unique properties of water (high boiling point, high heat of vaporization and specific heat capacity). They hold complementary strands of DNA together, and are responsible for determining the three-dimensional structure of folded proteins including enzymes and antibodies.

Hydrogen plays a particularly **important role in acid-base chemistry**, in which many reactions involve the exchange of **protons** (H^+) between soluble molecules. **Hydrogen transfer** from organic compounds to oxygen (formation of H_2O) is the principle of **biological oxidations**, in the course of which organism gains energy in the form of **adenosine triphosphate (ATP)**.

Oxygen

Oxygen is the most abundant element of the Earth; it occurs in elemental state as dioxygen **O_2** and ozone **O_3**, or combined in innumerable compounds (oxides, silicates, carbonates, etc.). Oxygen is also a constituent of water and of nearly all biological molecules; it is a key component of proteins and carbohydrates, for example. Atmospheric O_2 (about 21 vol. %) comes almost entirely from photosynthesis by green plants. Photosynthetic cells absorb light energy and use it to drive electrons from water to carbon dioxide, forming products such as glucose, releasing O_2 into the atmosphere:

$$n\ CO_2 + n\ H_2O \longrightarrow C_nH_{2n}O_n + n\ O_2$$

In human organism, oxygen is the biogenic macroelement that represents 65 % of the body weight. O_2 is transported from the lungs to the peripheral tissues by the iron-containing protein hemoglobin. In cells, oxygen occurs together with carbon and hydrogen in most organic compounds that have either **structural** or **metabolic functions.** O_2 is essential for **cellular respiration** in all aerobic organisms. It serves as an **electron acceptor**: in

the inner mitochondrial membrane electrons derived from different fuels of the body flow through the cascade of redox reactions (**respiratory chain**) to oxygen, reducing it to water. Simultaneously there is created proton gradient which is driving force for generation of ATP .

Oxygen participates in metabolic reactions in the body and during these reactions can create **reactive oxygen species**, such as **singlet oxygen, hydrogen peroxide, hydroxyl radicals** and **superoxide anion radical** (see Chapter 13).

Oxygen therapy is widely used in the management of a number of chronic and acute health conditions. Oxygen therapy is useful in the treatment of **pneumonia, emphysema, some heart disturbances** and another diseases. The therapeutic use of oxygen under pressure is known as **hyperbaric oxygen therapy**. Hyperbaric oxygen therapy in hyperbaric chamber may be used to treat carbon monoxide poisoning, burns, radiation injury or decompression sickness.

Ozone (O_3)

Ozone, **O_3**, is the less stable triatomic allotrope of oxygen. It is a blue, diamagnetic gas with a characteristic pungent odour. Ozone is a trace constituent of the upper layers of the atmosphere, where it plays an important role as an **absorber of UV radiation** and is formed by electrostatic discharge in the presence of molecular oxygen. Ozone is very toxic for human organism, **irritates** airways and can be **neurotoxic**. Ground-level ozone is an air pollutant with harmful effects on the respiratory systems of animals.

Ozone is powerful oxidizing agent and at low concentrations it is used for drinking water sterilization and for air purification. Small ozone doses help in the **treatment of surface caries**, promotes **wound healing** and **epithelization**.

Compounds of oxygen and hydrogen

Water (H_2O)

Water is the most abundant substance in living systems, making up 70 % or more of the weight of most organisms. Its unique physical properties, which include the ability to solvate a wide range of organic and inorganic molecules, derive from water's dipolar structure and exceptional capacity for hydrogen bonds formation. These properties affect the structure and function of biomolecules.

The main functions of water in the organism can be summarized to following points:

1. Water is a **universal solvent** and **transport medium** of inorganic and organic compounds (both, low and high molecular weight).
2. It is **a structural component** of biological macromolecules (hydration water).
3. It is **an activator** of certain chemical reactions.
4. Water takes part essentially in organism **thermoregulation**.
5. Water is also central to **acid-base balance**. As a fundamental factor it secures stability of internal environment of cells and organisms. Internal environment has a stabilizing, buffering capacity.
6. Water is **substrate** or **product** of many biochemical reactions (photosynthesis, cellular respiration).

Hydrogen peroxide (H_2O_2)

Hydrogen peroxide, when pure, is almost colourless (very pale blue) liquid, less volatile than water and somewhat more dense and viscous. Mixtures of H_2O_2 and organic or other readily oxidized materials are dangerously explosive. In H_2O_2 the oxidation state of oxygen is -I, intermediate between the values for O_2 and H_2O, and can act either as an oxidizing or a reducing agent. It spontaneously decomposes to water and oxygen, a reaction that is accelerated in the presence of peroxidases, mainly *catalase*, which are ubiquitous in human blood and tissues. Organisms naturally produce hydrogen peroxide as a by-product of oxidative metabolism. As H_2O_2 belongs to **reactive oxygen species** and plays an important role in **Fenton reaction** it must be quickly converted into other, less dangerous substances. Therefore organisms have evolved enzymes to prevent oxidative damage to cells and tissues caused by hydrogen peroxide and other reactive oxygen species, (see Chapter 13.2.).

H_2O_2 demonstrates broad-spectrum efficacy against viruses, bacteria, yeasts and bacterial spores. Diluted solution of H_2O_2 (3 %) is used medically for cleaning wounds, removing dead tissue, or as an oral debriding agent.

Carbon

Carbon occurs both as the free element (**graphite, diamond**) and in combined form, mainly as the carbonates of Ca, Mg and other electropositive elements. It also occurs as CO_2, a minor but crucially important constituent of the atmosphere. In human organism, carbon is **the biogenic macroelement** that represents 18 % of the body weight. Together with hydrogen, oxygen and nitrogen make up more than 99 % of the mass of most cells and provide the building blocks of proteins, nucleic acids, lipids, saccharides and metabolites. Carbon can form single bonds with hydrogen atoms, and both single and double bonds with oxygen atoms. Of greatest significance in biology is the ability of carbon atoms to form very stable carbon–carbon single bonds. Each carbon atom can form single bonds with up to four other carbon atoms. Covalently linked carbon atoms in biomolecules can form linear chains, branched chains, and cyclic structures. To these carbon skeletons are added groups of other atoms, called **functional groups**, which confer specific chemical properties on the molecule. It seems likely that the bonding versatility of carbon was a major factor in the selection of carbon compounds for the molecular machinery of cells during the origin and evolution of living organisms. No other chemical element can form molecules of such widely different sizes and shapes or with such a variety of functional groups. Among a lot of inorganic compounds containing carbon an important role plays **bicarbonate buffer system** (HCO_3^-/H_2CO_3). It is one of the major buffering systems used to maintain the pH of mammalian blood. A small amount of insoluble calcium and magnesium carbonates, $\mathbf{CaCO_3}$ and $\mathbf{MgCO_3}$, can be found in bones.

Carbon dioxide, $\mathbf{CO_2}$ is one of the end products of cellular respiration. In the blood most of the CO_2 produced in metabolism is transported as **hydrogencarbonate** (or **bicarbonate**) **ion** HCO_3^- and 15-20 % of the CO_2 is carried as **carbamate** bound to the N-terminal amino groups of hemoglobin, forming **carbaminohemoglobin**. A small portion of carbon dioxide, about 5 %, remains unchanged and is transported dissolved in blood.

Carbon monoxide, CO is colourless and odourless gas and is highly **toxic to aerobic organisms**. It is produced by the incomplete burning of various fuels, including coal, wood, charcoal, oil, kerosene, propane, and natural gas. Its toxicity follows from the binding of CO at a very low concentration to hemoglobin to the same site as oxygen (200 times stronger than oxygen). This product is called **carboxyhemoglobin** (HbCO) and is ineffective for oxygen delivery. The initial symptoms of low to moderate CO poisoning are similar to the flu (but without the fever). They include fatigue, shortness of breath, nausea and dizziness. High-level CO poisoning results in progressively more severe symptoms, including mental confusion, vomiting, loss of muscular coordination loss of consciousness and ultimately death. Carbon monoxide poisoning is treated with 100 % oxygen at high pressure (hyperbaric oxygen therapy), which facilitates the dissociation of CO from the hemoglobin.

On the contrary, the controlled *in vivo* release of CO appears to have several important physiological effects. Cells and tissues produce small amounts of CO as an elimination product of cellular metabolism, largely during the degradation of heme to biliverdin catalyzed by microsomal *heme oxygenase*. CO functions as a signaling molecule in the neuronal system, involving the regulation of neurotransmitters and as a vasodilatory agent.

Nitrogen

Nitrogen, in the form of nitrogen gas $\mathbf{N_2}$ is the most abundant element in the Earth's atmosphere (78 % by volume). It is an essential macroelement for humans; the human body contains about 3 % of nitrogen, which is present in amino acids, nucleotides, hormones, and many other biologically important compounds. However N_2 is unavailable for direct use by most organisms because there is a triple bond between the two nitrogen atoms with bond energy 945 kJ/mol, making the molecule almost inert. Only some prokaryotes, such as free bacteria of the *Azotobacter* strain or *Rhizobium* bacteria that live symbiotically on the roots of some plants, are capable of converting ("**fixing**") extremely stable atmospheric nitrogen (N_2) into ammonia:

$$N_2 + 10\ H^+ + 8\ e^- + 16\ ATP \longrightarrow 2\ NH_4^+ + 16\ ADP + 16\ P_i + H_2$$

To catalyze this reaction nitrogen-fixing bacteria utilize the enzyme ***nitrogenase***. Other bacteria, the nitrifying bacteria, oxidize ammonia to nitrites and nitrates. Plants can generally use either ammonia or nitrate as their sole source of nitrogen to produce the biologically important nitrogen molecules, but vertebrates must obtain nitrogen in the form of amino acids or other organic compounds.

In nitrogen metabolism an important role plays **nitric oxide (NO), a natural free radical molecule**. NO is synthesized from L-arginine by the enzyme *nitric oxide synthase*. NO is the endothelium-derived relaxing factor, which causes **vasodilation by relaxing vascular smooth muscle** and, therefore, lowers blood pressure. NO also acts as **a neurotransmitter**, **prevents platelet aggregation** and plays an essential role in macrophage function. NO-induced relaxation of cardiac muscle is the same response brought about by nitroglycerin tablets and other nitrovasodilators taken to relieve angina pectoris, the pain caused by contraction of a heart deprived of O_2 because of blocked coronary arteries. Nitric oxide has a very short half-life in tissues (3–10 seconds) because it

reacts with oxygen and superoxide, and then is converted into **nitrates** and **nitrites** including peroxynitrite ($O{=}NOO^-$), **a reactive nitrogen species.** Nitrovasodilators produce long-lasting relaxation of cardiac muscle because they break down over several hours, yielding a steady stream of NO. Nitrates are toxic in higher concentrations; they can for example oxidize hemoglobin to methemoglobin, which is ineffective for oxygen delivery. From nitrite and nitrate salts, **nitrous acid** (HNO_2) can be formed. Nitrous acid is a potent accelerator of the deamination of nucleobases. It deaminates cytosine (to uracil), adenine (to hypoxanthine) and guanine (to xanthine).

Phosphorous

Phosphorus is an important element in all known forms of life. In the form of the phosphate **(PO_4^{3-})** it provides stable backbone of **DNA and RNA**. It is present in many other biologically important compounds, such as **phospholipids** in cell membranes, **phosphoproteins** (phosphate group usually regulates protein function) and high energy phosphate esters, for example a fundamental energy source **ATP**. As a component of these important biological substances, phosphorus plays a central role in **energy** and **cell metabolism**.

Phosphorus is constituent of **hydroxyapatite $Ca_5(PO_4)_3(OH)$** and **fluorapatite $Ca_5(PO_4)_3F$**, mineral components of **bones** and **teeth**. Fluorapatite is more resistant to acid attack than hydroxyapatite is. For this reason, toothpaste contains a source of fluoride anions (e.g. sodium fluoride, sodium monofluorophosphate), which allows exchange of fluoride anions for hydroxide anions in hydroxyapatite in the teeth. Too much fluoride results in dental fluorosis and/or skeletal fluorosis.

Phosphate buffer system (**$H_2PO_4^-/HPO_4^{2-}$**) is one of the main biological buffer systems which regulates the acid-base balance of animal body fluids.

Sulfur

Sulfur represents about 0.2 % of our total body weight, similar to potassium. The body (adult, 70 kg) contains approximately 140 grams of sulfur bound in many important biomolecules. Sulfur is present in proteins as a part of amino acids **methionine,** and **cysteine**. Sulfur is also present in **coenzyme A**, **α-lipoic acid**, and two B vitamins, **thiamine** and **biotin** (see Chapter 11.2. and 12.2.). Sulfur is part of other important molecules (e.g. mucopolysaccharides **heparin**, **chondroitin sulfate** and **keratan sulfate)**. **Glutathione (GSH)**, an important sulfur-containing tripeptide (γ-glutamyl-cysteinyl-glycine) belongs to low-molecular weight antioxidants and it is readily oxidized by reactive oxygen/nitrogen species to glutathione disulfide **(GSSG)**. GSH/GSSG is the major redox couple that determines the antioxidative capacity of cells. Disulfide bonds (S-S bonds) formed between cysteine residues in peptide chains are very important for protein structure and stability. For example, disulfide bonds are largely responsible for the mechanical strength and insolubility of the protein **keratin**, found in outer skin, hair and feathers.

Fluorine

Fluorine occurs in organism as the negatively charged ion, fluoride **(F^-)**. Fluoride anions are absorbed in the stomach and small intestine. Once in the bloodstream they rapidly enter mineralized tissue (bones and developing teeth). At usual intake levels, fluoride anions do not accumulate in soft tissues. Fluorides are used in trace amounts in toothpastes and other medical preparations for the **prevention of dental caries**. F^- small radius allows it to either displace the larger hydroxide anion (OH^-) in the predominant mineral in bones and teeth, **hydroxyapatite**, forming **fluorapatite**, or to increase crystal density by entering spaces within the hydroxyapatite crystal. Moreover, fluorides have been found **to inhibit bacterial enzymes**, resulting in reduced acid production by cariogenic bacteria.

Ingestion of excess fluoride ions can cause **fluorosis** which affects the teeth and bones. Fluorosis manifests itself through discoloration of the teeth, deformation of the skeleton, renal failure and muscle weakness. Moreover excess of F^- ions can cause inhibition of numerous enzymes by binding of F^- to the metal center or due formation of insoluble CaF_2 with Ca^{2+} ions. (Calcium ions are important for many physiological processes, see **calcium** role).

Chlorine

Chlorine as chloride **(Cl^-)** is an essential macroelement being found mainly in extracellular fluids. Along with hydrogencarbonate HCO_3^- it is the most important free anion. Chloride is essential for the **regulation of osmotic**

pressure and **acid-base balance**. It is the chief anion of the gastric juice where it is accompanied by the hydrogen ions thus participating in the **maintenance** of the gastric juice **pH**.

In the body, the concentration of chloride ions is closely regulated. Any significant decrease or increase may have harmful or fatal consequences. **Hypochloremia**, excessive depletion of chloride ions through losses of certain gastrointestinal fluids (e.g. vomiting), by heavy sweating or by kidney disorders may lead to alkalosis due to an excess of hydrogencarbonate, since the inadequate level of chloride is partially compensated for or replaced by hydrogencarbonate. High levels of Cl^- in the blood, **hyperchloremia** result in acidosis.

Chlorine is powerful bleaching, oxidizing and disinfecting agent. It is very effective for the deactivation of pathogenic microorganisms and is one of the most commonly used disinfectants for water disinfection.

Iodine

The heaviest stable halogen was already recognized as an essential element for higher organisms in the middle of the 19th century. Iodine is present in thyroid gland in the form of polyiodinated small organic compounds: the **thyroid hormones thyroxine** (tetraiodothyronine) and even more active **triiodothyronine**. The thyroid gland traps iodine from the blood and incorporates it into thyroid hormones that are stored and released into the circulation when needed. Thyroid hormones regulate a number of physiological processes, including **growth**, **development** and the control of **energy metabolism** and associated processes. Thyroid hormones also affect **cardiovascular, central nervous** and **reproductive systems**.

Symptoms of reduced thyroid activity (**hypothyroidism**) include fatigue, weight gain and cold intolerance. One of the most devastating effects of maternal iodine deficiency is **congenital hypothyroidism**, which can result in irreversible mental retardation. Low thyroid activity due to iodine deficiency may be compensated by excessive growth of the organ (goiter) with an increased tendency for tumor formation; it can be counteracted by supplementing iodide preparations (iodinated drinking water or cooking salt). Due to the extreme localization of iodine in the human body, tumors of the thyroid can be successfully diagnosed and treated using the radioactive isotopes ^{131}**I** and ^{123}**I**. Excessive amounts of the thyroid hormones (**hyperthyroidism**) cause nervousness, heart palpitations, weight loss and heat intolerance.

Selenium

Selenium is the trace element that is essential in small amounts, but like all essential elements, it is toxic at high levels. This nonmetal is chemically related to sulfur and is found in **selenocystein**e selenium analogue of sulfur-containing amino acid cysteine. Humans and animals require selenium for the function of a number of selenium-dependent enzymes. It is an essential component of enzyme selenium-dependent ***glutathione peroxidase*** (**GPx**). **GPx** is an antioxidative enzyme that reduces potentially damaging reactive oxygen species, such as hydrogen peroxide and lipid hydroperoxides, to harmless products like water and alcohols by coupling their reduction with the oxidation of **glutathione**. Selenium also takes part in **thyroxine** conversion to **triiodothyronine** in thyroid hormone biosynthesis. Most of the biologically more active triiodothyronine in the circulation and inside cells is created by the removal of one iodine atom from thyroxine in a reaction catalyzed by selenium-dependent enzyme - ***iodothyronine deiodinase***. Thus, selenium is an essential element for normal development, growth and metabolism.

Selenium deficiency is rare; selenium deficiency symptoms can occur on extremely selenium-poor soil. Selenium deficiency may contribute synergistically with iodine deficiency to the development of **goiter** and **hypothyroidism** and can also lead to liver necrosis and an increased susceptibility for liver cancer. Characteristic symptoms of **selenium overload** are the loss of hair and disorders of the central nervous system.

2.3.2. Metallic elements

Sodium and **potassium**

Sodium and potassium are biogenic macroelements. While $\mathbf{Na^+}$ is an important **extracellular** cation, $\mathbf{K^+}$ is typical **intracellular** cation; in most mammalian cells, 98 % of K^+ is intracellular, for Na^+ the situation is the reverse. This concentration differential ensures a number of major biological processes, such as cellular **osmotic balance, transport** through the cell membranes and **transmission of nerve impulses** both within the brain and from the brain to other parts of the body.

Absorption of **sodium** cations in the small intestine plays an important role in the absorption of chloride anions, amino acids, glucose and water. The latter is important for maintaining water balance in the body; thus the concentration of Na^+ influences extracellular fluid volume, including blood volume. In general, sodium retention

results in water retention and sodium loss results in water loss. Disorders of sodium balance – **hyponatremia** and **hypernatremia** – are the most common electrolytic disturbances seen in clinical medicine. **Hyponatremia** may result from increased fluid retention or increased sodium loss (vomiting, excessive sweating, the use of some diuretics). Symptoms of hyponatremia include headache, nausea, vomiting, muscle cramps, fatigue, disorientation and fainting. If sodium levels fall rapidly, seizure, coma and brain damage can occur. A high plasma sodium concentrations, **hypernatremia** is much less common than hyponatremia and rarely caused by excessive sodium intake (e.g. from the ingestion of large amounts of seawater or intravenous infusion of concentrated saline solution). Hypernatremia generally develop from excess water loss, frequently accompanied by an impaired thirst mechanism or lack of access to water. Symptoms of hypernatremia in the presence of excess fluid loss may include dizziness or fainting, low blood pressure, and diminished urine production.

Potassium is crucial to heart function and plays a key role in skeletal and smooth muscle contraction. **Hypokalemia** is most commonly a result of excessive loss of potassium (e.g. from prolonged vomiting, the use of some diuretics). Severe hypokalemia may result in an abnormal heart rhythm (cardiac arrhythmia) that can be fatal. Elevated serum potassium concentrations are referred to as **hyperkalemia**. Hyperkalemia occurs when potassium intake exceeds the capacity of the kidneys to eliminate it. Hyperkalemia may also result from a shift of intracellular potassium into the circulation, which may occur with the rupture of red blood cells (hemolysis) or tissue damage (e.g. trauma or severe burns). The most serious complication of hyperkalemia is the development of an abnormal heart rhythm, which can lead to cardiac arrest.
The major role in regulating the balance of electrolytes and water play **mineralocorticoids**. They act especially on kidneys, where they facilitate reabsorption of sodium ions and water from urine and excretion of potassium ions.

Magnesium

Mg^{2+} is the fourth most abundant cation in the body and the second most prevalent intracellular cation. Nearly 99 % of the total body magnesium is located in **bones** or in the **intracellular space**. Magnesium is a critical cation in numerous intracellular processes. Mg^{2+} is an essential factor for biological **phosphate transfer reactions**, such as phosphorylations by kinases and dephosphorylations by phosphatases. Mg^{2+} is required for the activity of over 300 enzymes which play an important role in **nucleic acid metabolism**, **glycolysis** (of the ten enzymes involved in the glycolytic pathway, five are Mg^{2+} dependent), **oxidative phosphorylation** and in many other processes.

Further important roles are its Ca^{2+}- analogous **function in bones and teeth**, **stabilization of the cell membranes** and **conduction of nerve impulses** and **muscle contraction**. Magnesium deficiency (**hypomagnesemia**) in healthy individuals who are consuming a balanced diet is quite rare because magnesium is abundant in both plant and animal foods and because the kidneys are able to limit urinary excretion of magnesium when intake is low. Effects of Mg^{2+} deficiency are reduced mental and physical performances due to insufficient energy production via phosphate transfer and due to the inhibition of protein metabolism and neurological, cardiovascular and metabolic disorders. Acute magnesium deficiency is treated with intravenous magnesium sulfate. Elevated serum levels of magnesium (**hypermagnesemia**) may result in a fall in blood pressure (hypotension). Some of the later effects of magnesium toxicity, such as lethargy, confusion, disturbances in normal cardiac rhythm and deterioration of kidney function, are related to severe hypotension. As hypermagnesemia progresses, muscle weakness and difficulty breathing may occur. Severe hypermagnesemia may result in cardiac arrest.

In plants, magnesium plays a key role in **photosynthesis** as Mg^{2+} is the central atom of the chlorophyll molecule. Activity of many key enzymes involved in photosynthetic carbon metabolism is greatly affected by small variations in Mg^{2+} concentration.

Calcium

Calcium is an essential macroelement in the human body. Over 99 % of the total body calcium is located in **bones** and **teeth** and the mineral component of bone consists mainly of **hydroxyapatite $Ca_5(PO_4)_3(OH)$** crystals. The other 1 % of the total body calcium exists outside of the skeleton, mainly in **extracellular space**. The extracellular concentration of Ca^{2+} is much higher (10^4 times) than that in the cytosol of the average mammalian cell (which is around 10^{-7} M). In order to preserve normal physiological function, calcium concentrations in the blood and fluid that surround cells are tightly controlled by **parathyroid hormone (PTH)**, **calcitriol** and **calcitonin**. A slight drop in blood calcium levels (e.g. in the case of inadequate calcium intake) is sensed by the **parathyroid glands**, resulting in their increased secretion of **PTH**. Elevations in PTH rapidly decrease urinary excretion of calcium but increase urinary excretion of phosphorus and stimulate bone

resorption, resulting in the release of bone mineral (calcium and phosphate) - actions that restore serum calcium concentrations. PTH also stimulates conversion of **vitamin D** to its active form - **calcitriol**, which increases the uptake of calcium from the small intestine (see Chapter 11.1.2.). The third hormone - **calcitonin** stimulates deposition of calcium into bones, which decreases its blood concentration.

In addition to its **structural function** (bones, teeth), Ca^{2+} ion plays central role in many fundamental physiological processes:

1. it affects **neuromuscular excitability** of muscles (together with the ions K^+, Na^+, Mg^{2+}) and it takes part in **muscle contraction**,
2. it takes part in regulation of **glycogenolysis** in liver and in regulation of **gluconeogenesis** in kidneys and liver,
3. it decreases cell membrane and capillary wall permeability, what results in its **anti-inflammatory**, **anti-exudative** and **antiallergic effect**,
4. it is necessary for **blood coagulation**,
5. it influences **secretion of insulin** into the circulation and secretion of digestion enzymes into small intestine.

A low blood calcium level (**hypocalcemia**) usually implies abnormal parathyroid function since the skeleton provides a large reserve of calcium for maintaining normal blood levels, especially in the case of low dietary calcium intake. Other causes of low blood calcium concentrations include chronic kidney failure and vitamin D deficiency. Hypocalcemia is characterized by tetany, cramps, altered mental status, seizures, muscle spasms and hypotension. Chronically low calcium intakes in growing individuals may contribute to accelerated bone loss and ultimately to the development of **osteoporosis** (osteoporosis is a multifactorial disorder and nutrition is only one factor contributing to its development and progression).

High level of serum calcium (**hypercalcemia**) indicates primary hyperparathyroidism or malignancy. Hypercalcemia can be also associated with the consumption of large quantities of calcium supplements in combination with antacids. Mild hypercalcemia may be without symptoms or may result in loss of appetite, nausea, vomiting, abdominal pain, fatigue and hypertension. More severe hypercalcemia may result in confusion, delirium and coma.

Iron

Iron, element 26 in the periodic table, is the second most abundant metal (after aluminium) and the fourth most abundant element of the earth's crust. Its position in the middle of the elements of the first transition series implies that iron has the possibility of various oxidation states (from −II to VI), the principal ones being **II** and **III**.

The average amount of iron in the human body (adult, 70 kg) is 4 - 5 g and iron is thus the most abundant transition metal in our organism. About 70 % of this amount is used for oxygen transport and storage (**hemoglobin**, **myoglobin**), almost 30 % is present in the storage forms, mostly as **ferritin,** and about 1 % is bound to the transport protein **transferrin** and to various iron-dependent enzymes.

The daily absorption rate of iron supplied by food amounts to 1 - 2 mg. Iron enters the body by absorption, primarily in the duodenum through the action of enterocytes. In the blood iron binds to **transferrin**, which delivers iron to the blood-forming cells in the bone marrow for the hemoglobin synthesis or to the liver and muscle cells, where it can be utilized in the biosynthesis of iron-containing enzymes or myoglobin. Unused iron is stored mainly in **ferritin**. **Hemosiderin** is another iron storage form found in organisms, particularly during iron overload (it is assumed that this insoluble species is formed via lysosomal decomposition of ferritin). Storage and transport proteins **ferritin**, **hemosiderin** and **transferrin** are non-heme iron proteins and bind an iron in its ferric ($\mathbf{Fe^{3+}}$) state.

Hemoglobin and **myoglobin** are hemoproteins, it means that they contain **heme** prosthetic group, which consists of a complex organic ring structure, protoporphyrin, to which an iron atom in its ferrous ($\mathbf{Fe^{2+}}$) state is bound. **Hemoglobin** is found exclusively in red blood cells, where its main function is to transport oxygen (O_2) from the lungs to the capillaries of the tissues. Furthermore it can transport H^+ and CO_2 from the tissues to the lungs. **Myoglobin**, present in heart and skeletal muscle, functions both as a reservoir for oxygen and as an oxygen carrier that increases the rate of transport of oxygen within the muscle cells.

Other important hemoproteins are ***catalase*** and ***cytochrome c oxidase***. ***Catalase*** is an enzyme that protects the cells against the toxic action of hydrogen peroxide by catalyzing its decomposition into molecular oxygen and water (see Chapter 13.2.1.). ***Cytochrome c oxidase*** plays a crucial role in aerobic life being the terminal oxidase

of the mitochondrial electron transfer chain that catalyzes reduction of molecular oxygen to water. Besides iron cytochrome c oxidase contains also **copper** as the other metal in the active site.

Iron uptake, transport and storage are highly regulated; proteins function as endogenous multidentate chelating ligands that tightly bound iron in the chelate structures. An excess of free iron (or more accurately, loosely bound) is dangerous for any organism as highly reactive hydroxyl radicals can be generated in the presence of superoxide anion radical or hydrogen peroxide, see **Haber-Weiss** and **Fenton reaction**, Chapter 13.1.1.

Iron deficiency in organism causes iron-deficiency **anemia**. This type of anemia results from a discrepancy between iron availability and the amount required for production of red blood cells. The main causes of iron deficiency are the poor availability of iron in the diet, malabsorption and chronic blood loss. Treatment for iron-deficiency anemia will depend on its cause and severity. Treatment includes dietary changes, supplements, surgery and severe iron-deficiency anemia may require a blood transfusion.

Iron overload due to gene defects (thalassemia, hereditary hemochromatosis), as well as an excessive iron intake can cause severe pathological symptoms. **Hereditary hemochromatosis** is a genetic disorder that increases the amount of iron that the body absorbs from the gastrointestinal tract. Iron is then deposited in various organs, mainly in the liver, but also in the pancreas, heart, endocrine glands and joints and causes cirrhosis, liver cancer, diabetes, cardiomyopathy and arthritis. Treatment of iron overload includes therapeutic phlebotomy and iron chelation therapy (chelating agent **desferrioxamine**).

Copper

Copper is the trace element and is essential for the function of most living organisms. Copper relevant oxidation states are **I** and **II**, but bivalent copper Cu(II) represents the most stable oxidation state of copper. The total amount of copper in the human body (adult, 70 kg) is relatively small, about 150 mg. Copper absorbed from the small intestine is transported in the blood bound predominantly to serum albumin. Copper taken up by the liver may be stored in hepatocytes (predominantly bound to metallothionein), secreted into plasma, or excreted in bile. About 80 % of the copper circulating in the blood is an integral part of protein **ceruloplasmin**. In addition to **transporting** copper, **ceruloplasmin** has the **ferrooxidase activity** - the ability to catalyze the oxidation of Fe^{2+} to Fe^{3+}, thus influencing the iron metabolism (Fe^{3+} is taken up by transferrin which delivers iron to the cells where it can be utilized in the biosynthesis of iron-containing enzymes). **Ceruloplasmin** may also function as an **antioxidant**. As free copper and iron ions are powerful catalysts of free radical formation, ceruloplasmin can prevent oxidative damage to biomolecules by:

1. binding of copper ions (both Cu^{+} and Cu^{2+})
2. oxidation of Fe^{2+} to Fe^{3+} (ferroxidase activity) which prevents free ferrous ions (Fe^{2+}) from participating in harmful free radical generating reactions
3. scavenging of superoxide anion radical ($O_2^{\bullet -}$)

Important copper-containing enzymes are ***cytochrome c oxidase*** (see section Iron) and ***Cu/Zn-superoxide dismutase*** (***Cu/Zn-SOD***). ***Cu/Zn-SOD*** catalyzes the disproportionation (dismutation) of toxic superoxide anion radical $O_2^{\bullet -}$ to molecular oxygen and hydrogen peroxide (see Chapter 13.2.1.). The hydrogen peroxide formed in this process can further disproportionate to yield O_2 and H_2O in reaction catalyzed by the iron-containing enzyme *catalase*.

Copper deficiency is rare among healthy people and the most common cause of copper deficiency are Crohn's disease, celiac disease, gastric surgery due to malabsorption or high dietary zinc intakes. As copper plays an important role in iron metabolism, lack of copper leads to iron-deficiency **anemia**. Copper deficiency may also lead to decreased number of white blood cells, osteoporosis and some neurologic diseases, because copper is required for a number of enzymes which are necessary for energy production, formation of bones, connective tissues (collagen, keratin), neurotransmitter biosynthesis and many others.

Genetic disorder causing copper deficiency is called **Menke's disease**. The resulting symptoms in infants include severe disturbances in mental and physical development; an effective therapy must rely on intravenously administered copper compounds.

Another copper-related genetic disorder is **Wilson**'**s disease**. Wilson's disease causes **excessive copper accumulation** in the liver and brain, leading to liver failure, progressive neurological disorders, or psychiatric illness. A therapy of this disease, and of acute copper poisoning, requires the administration of Cu-specific chelate ligands such as **D-penicillamine**. This ligand contains both **S** (thiolate) and **N** (amine) donor atoms, in order to guarantee specificity for copper and a hydrophilic carboxylic functional group, which makes the resulting complex excretable.

Excess of copper is toxic; similarly to iron, copper ions are prone to participate in the formation of reactive oxygen species.

Zinc

With approximately 2 g per 70 kg body weight, zinc is the second most abundant transition element in the human organism, following iron. Under physiological conditions, this element occurs only in the oxidation state **II** and thus, in contrast to iron and copper, zinc is not redox active. The daily zinc requirement has been estimated at between 3 mg (small children) and 25 mg (pregnant women). A relatively large tolerance exists for higher doses before symptoms of zinc poisoning become manifest. Zinc is absorbed in the small intestine and in the blood stream is transported predominantly by albumin. Zinc plays an essential role in cell membrane integrity, it helps manage insulin action and blood glucose concentration and has an essential role in the development and maintenance of the body's immune system. Zinc is also required for bone and teeth mineralization, normal taste and wound healing.

Zinc is a cofactor of several hundred enzymes (e.g. ***Cu/Zn-SOD, alcohol dehydrogenase***, ***carbonic anhydrase***, ***carboxypeptidase***), which catalyze **the metabolic conversion** or **degradation** of proteins, nucleic acids, lipids, porphyrin precursors and other important bioorganic compounds (*synthases, polymerases, ligases, transferases and hydrolases).* Other functions lie in **structural stabilization** of proteins and peptides conformation of (e.g. role of zinc in insulin, *Cu/Zn-SOD*) and of transcription-regulating factors.

It is thus not surprising that **zinc deficiency** can lead to severe pathological effects. Symptoms of zinc deficiency include growth disorders, reduced sense of taste and a lack of appetite, enhanced disposition for inflammations and an impairment of the immune system and of the reproduction, delayed wound healing and dermatitis. They are usually a consequence of impaired zinc absorption rather than of undersupply.

Zinc is essential for many processes; however, **excess** of zinc is toxic. This type of toxicity occurs from selfsupplementation or prolonged use of oral zinc supplements for medicinal purposes. Excess of zinc may also affect copper status with symptoms of neutropenia and anemia, impaired immune function and lower plasma HDL concentrations.

Cobalt

Cobalt is a relatively rare element of the earth's crust, which is essential to mammals as an **integral part of vitamin B_{12}**. Its most common oxidation numbers are **II** and **III**. The human body (adult, 70 kg) contains approximately 1 mg of cobalt, 85 % of which is in the form of vitamin B_{12}. Cobalt is readily absorbed in the small intestine, but the retained cobalt serves no physiological function since human tissues cannot synthesize vitamin B_{12} in the intestine and vitamin B_{12} must be obtained from the diet. Most of the consumed cobalt is excreted in the urine with very little being retained, mainly in the liver and kidneys. Though for cobalt only one specific compound is known (vitamin B_{12}), recent studies show that cobalt may be linked with iodine in the formation of thyroid hormones. **Function** of **vitamin B_{12}** and its **deficiency symptoms** are described in detail in Chapter 11.2.7.

A **cobalt deficiency** as such has never been produced in humans. Signs and symptoms that are sometimes attributed to cobalt deficiency are actually due to lack of **vitamin B_{12}**.

The **toxicity** of cobalt is relatively low compared to many other metals, however it is toxic and carcinogenic at **higher concentrations**. Effects on lungs, including asthma and pneumonia have been found in workers who breathed high levels of cobalt in the air. Workers exposed to cobalt-containing hard metal compounds exhibited a significantly higher risk of developing lung cancer. Cobalt-mediated free radical generation contributes to the toxicity and carcinogenicity of cobalt.

Some radioactive isotopes of cobalt, such as **^{57}Co** or **^{60}Co**, are used in treating patients in nuclear medicine and in research, for example to study defects in vitamin B_{12} absorption. By injecting a patient with vitamin B_{12}, labeled with radioactive cobalt, the physician can study the path of the vitamin through the body and discover any irregularities.

Manganese

Manganese is an essential trace element in all forms of life. Manganese has access to three oxidation states of relevance to biology: **II, III** and **IV**. Absorption of manganese from the gastrointestinal tract is related inversely to the level of calcium, phosphorus and iron in the diet. The main excretion route is through the bile. Manganese is a cofactor and an activator of many enzymes involved in the metabolism of proteins, lipids and carbohydrates

and in the detoxification of reactive oxygen species. Manganese is an essential cofactor of enzymes called ***glycosyltransferases***; these enzymes are required for the synthesis of proteoglycans that are needed for the formation of healthy cartilage and bone. Manganese is also a cofactor in enzymes such as ***arginase, catalase*** and ***superoxide dismutase* (*Mn-SOD*)**. ***Arginase*** is involved in the conversion of the L-arginine to form L-ornithine and urea. In mammals, hepatic **arginase** is the terminal enzyme of the urea cycle, which represents the major end product of nitrogen metabolism. ***Mn-SOD*** is present in eukaryotic mitochondria and also in many bacteria. This enzyme catalyzes the two-step dismutation of superoxide anion radical. Mechanism is similar to that of *Cu/Zn-SOD* but manganese atom oscillates between trivalent and divalent oxidation states, according to the following equation:

$$\text{Enzyme-Mn}^{III} + O_2^{\bullet -} \longrightarrow \text{Enzyme-Mn}^{II} + O_2$$

$$\text{Enzyme-Mn}^{II} + O_2^{\bullet -} \xrightarrow{2H^+} \text{Enzyme-Mn}^{III} + H_2O_2$$

Manganese deficiency is rare, because this element is nutritionally essential only in small amounts. Low levels of this element in the body can result in skeletal abnormalities, impaired reproductive function and growth, and altered carbohydrate and lipid metabolism.

Manganese toxicity upon **overexposure** is associated with inhalation in occupational settings (mining, alloy production, welding) and may result in neurobehavioral and neurological effects, with symptoms similar to those of Parkinson's disease, including tremors, postural instability and rigidity.

Molybdenum

Molybdenum is the only element from the second period of transition metals (4d) that is essential for most living organisms. While it is relatively rare in the earth's crust, molybdenum is the most abundant transition metal in seawater in its most stable hexavalent (**VI**) form as molybdate MoO_4^{2-}, at pH 7. This ion shows a close resemblance to the biologically important sulfur-transporting sulfate ion, SO_4^{2-}. The physiologically relevant oxidation states lie between **IV** and **VI**.

In humans, molybdenum is an essential element in several enzymes, including ***xanthine oxidase***, ***sulfite oxidase*** and ***aldehyde oxidase***. ***Xanthine oxidase*** inserts hydroxyl groups into derivatives of the purine bases adenine and guanine, finally converting xanthine to uric acid, the final product of purine degradation, which is excreted in the urine. ***Sulfite oxidase*** catalyzes the oxidation of sulfite to sulfate, a reaction that is necessary for the metabolism of sulfur-containing amino acids and ***aldehyde oxidase*** participate in the alcohol metabolism, it catalyzes oxidation of aldehydes into carboxylic acid. ***Xanthine oxidase*** and ***aldehyde oxidase*** also play a role in the metabolism of drugs and toxic substances. Molybdenum is also present within tooth enamel and may prevent its decay.

Molybdenum deficiency is rare because amount of molybdenum required is relatively small. Low levels of this element can result in low serum uric acid, neurological problems and mental retardation. A deficiency in molybdenum has also been linked to tooth decay and cavities. Molybdenum is copper **antagonist** and excessive dietary intake of molybdenum induces a secondary copper deficiency. **Molybdenum overload** causes anemia, high levels of serum uric acid, bone and joint deformities and impaired reproductive function.

Chromium

Under physiological conditions, the typical oxidation states of chromium are **III** and **VI**, the latter as **chromate**, $\mathbf{CrO_4^{2-}}$, at pH 7. Chromium carcinogenicity was first identified over a century ago and **Cr(VI)** compounds were amongst the earliest chemicals to be classified as carcinogens.

However trivalent chromium, **Cr(III)**, is generally believed to be an essential trace element and to have a role in maintaining proper carbohydrate and lipid metabolism, probably by enhancing insulin signaling. This stimulation of insulin activation occurs without changing the concentration of insulin. However, chromium deficiency is difficult to achieve. After entering the body from an exogenous source, **Cr(III)** binds to transferrin, an iron-transporting protein in the plasma. Trivalent chromium is the most stable form found in living organisms, but is not as bioavailable as its hexavalent counterpart (**VI**). **Cr(VI)** (as chromate, $\mathbf{CrO_4^{2-}}$) is strong oxidizing agent and can oxidatively damage DNA and proteins. $\mathbf{CrO_4^{2-}}$ can actively enter the cells through channels for the transfer of the isoelectric and isostructural anions, such as SO_4^{2-} and HPO_4^{2-} channels and reach the cell nucleus unless it is rapidly reduced. Inside the cell there are three major reducers of **Cr(VI)**: ascorbic acid, glutathione and cysteine. If the level of the reducing agents is low compared with the amount of **Cr(VI)**, then

more labile and more strongly oxidizing **Cr(V)** and **Cr(IV)** intermediates are formed. These intermediates produce free radicals that can directly attack the DNA and effect bond cleavage, crosslinking and, as a consequence, faulty gene expression.

Vanadium

Vanadium may be beneficial and possibly essential in humans, but certainly essential for some marine organisms and mushrooms. Vanadium is a constituent of enzymes ***haloperoxidases*** as well as ***nitrogenases*** in some nitrogen-fixing organisms (*Azotobacter*). The usual oxidation states of the vanadium are **III**, **IV** and **V**.

Though its role as a micronutrient in humans has not yet been well defined, particular interest has been given to the study of the therapeutic applications of vanadium compounds. It has been shown that these compounds are implicated in many biological processes as **inhibitors of cancerous tumor growth** or **insulin-mimetics** drugs. **Insulin-mimetic** (insulin-like) activity encompasses the ability to lower elevated blood glucose levels in vivo. Vanadium stimulates glucose uptake and subsequent oxidation in glucose-metabolizing cells as well as glycogen synthesis *in vitro*. In addition, it promotes the inhibition of glycolysis and gluconeogenesis, and inhibits lipolysis (activates lipogenesis).

Nickel

The most common oxidation state of nickel in biosystems is **II**. Nickel is often only one of several components of complex enzymes, which may otherwise contain several coenzymes as well as additional inorganic elements.

Nickel-dependent enzymes are for example ***urease, CO dehydrogenase, Ni-superoxide dismutase* (*Ni-SOD*)** and are found in bacteria and some eukaryotes. ***Urease*** catalyzes the hydrolysis of **urea**, forming ammonia and carbon dioxide. Urea is a very stable molecule, which normally hydrolyzes very slowly and the catalytic activity of the enzyme increases the rate of hydrolysis by a factor of about 10^{14}. Many methanogenic bacteria contain enzyme ***CO dehydrogenase*** which catalyzes the oxidation of CO to CO_2. This reaction is enzymatically reversible and may therefore serve as an alternative method of CO_2 fixation (assimilation) by photosynthetic bacteria. ***Ni-SOD*** catalyzes disproportionation of superoxide anion radical $O_2^{\bullet -}$ to molecular oxygen and hydrogen peroxide.

Essentiality of nickel in humans has not been proven. Nickel can enter body via inhalation, ingestion and dermal absorption. The amount of nickel absorbed by the gastrointestinal tract depends on the type of nickel species in the food, the content and the absorptive capacity. Normally, only 1-2 % of ingested nickel is absorbed. The daily intake of nickel has been estimated to be in the range 35 - 300 μg per day. Nickel is toxic at higher concentrations. Workers exposed to nickel-containing compounds exhibited increased risk of respiratory tract and nasal cancers. Nickel is also one of the most common causes of **allergic contact dermatitis**.

2.4. Toxic elements

2.4.1. Toxicity of metal ions in biological systems

The previous chapters have demonstrated that many elements (in the form of their chemical compounds) are essential for life and their deficiency results in impairment of biological functions. However, even such essential substances will be poisonous if present in excess (Paracelsus principle "The dose makes the poison"). With regard to the toxicity, we distinguish the group of elements for which **exclusively negative effects** have been found to date. This group includes metals such as **mercury, lead, cadmium, beryllium, thallium** and **arsenic** (semi-metal).

The toxicity of metal ions is based on:

1. **Mutual substitution** of essential metal in the active site of metalloenzymes by toxic metal with similar but not identical chemical characteristics: $Zn^{2+} \leftrightarrow Pb^{2+}$ or Cd^{2+}, $Ca^{2+} \leftrightarrow Pb^{2+}$ or Cd^{2+}, $K^{+} \leftrightarrow Tl^{+}$.
2. **Binding** to the functional groups of proteins, nucleic acids, lipids, e.g. -SH, -NH-, -OH, -COOH (acidic protons may be substituted by metal cations). It affects enzyme activity, transport processes across the cell membranes and can cause changes in genetic information.
3. **Oxidative damage** to important macromolecules. Metal-mediated formation of reactive oxygen and nitrogen species causes various modifications of DNA bases, enhanced lipid peroxidation and changes in biological functions of proteins.

The toxic effect of metal ions depends on:

1. **the dose**
2. **physicochemical properties** (phase state, solubility, oxidation state, chemical form)
3. **duration and route of exposure** (inhalation, ingestion, skin penetration and in case of teratogenic and embryotoxic substances through placenta)
4. **organism** (the age, gender, genetics and nutritional status of exposed individuals)

2.4.2. Exposure, absorption, transport, distribution and excretion

Air, food and water are **the major exposure media** for humans. In urban areas and in the vicinity of industries inhaled air is often a major, direct exposure route. Air is also especially important for occupational exposures. Metals and their compounds occur naturally in food and drinking water, because they are intrinsic components of the earth's crust and biota. Contaminated air may pollute soil and water secondarily, resulting in contaminated crops and vegetables.

The physicochemical properties of metals in exposure media play an important role in determining the extent of **absorption** and **distribution** of metal in organism. One of the major factors influencing the availability and absorption of metals is **solubility** of metal compounds in water and lipids. **The chemical form** in which the metal occurs is also of great importance, because absorption can be entirely different for different compounds of the same metal. This is particularly so when **inorganic** and **organometallic compounds** of the same metal are compared. Organometallic compounds are compounds having bonds between metal atom and carbon atom of an organyl group, e.g. CH_3-, CH_3CH_2-. Generally organometallic compounds because of their lipophilic character tend to pass membranes, including the very discriminating blood-brain barrier, much easier than inorganic metal compounds and usually are more toxic. For example **methylmercury** (CH_3-Hg^+) is almost completely absorbed (approximately 90-100 % absorption), whereas inorganic Hg(II) salts are absorbed to an extent 10 % or less.

The **transport** and **distribution** of metals depend on the form in which the metal occurs in the blood, which is the main transporting medium in the body. The lymph may also constitute an important route for transport of metals, for example, from the lung into the blood circulation. The toxic metal ions can penetrate the cell membranes by the same transport mechanism, as the chemically related essential metal ions. For example the structure of arsenate and chromate oxyanions mimic those for endogenous anions phosphate and sulfate respectively, and are transported across the cell membranes the same way as these endogenous anions.

The **biological half-life** is used to predict the accumulation of a metal in the body as a whole or in individual organs. It defines the time it takes for the body to excrete half of a certain accumulated amount. Several of the toxic metals have a long biological half-life and tend to accumulate in the body. For cadmium, the biological half-life in man is estimated to be 10-30 years. For arsenic and chromium it is only hours or days.

Effects of different metals on specific target organs are presented in Table 2.1.

Various routes exist by which a metal may be **excreted** from the body. The most important excretory pathways are the **gastrointestinal** and **renal** ones.

Concentration of the toxic metal is frequently determined in **blood**, **urine** and **hair**. For example **blood levels**, particularly red cell levels, of **methylmercury** are valuable for assessing the concentration of methylmercury in CNS, as well as in the body as a whole. Urinary levels cannot be used, because the excretion of methylmercury in urine is extremely small, the main excretion route being the bile. In contrast, measuring the **urinary** concentration of **As** is useful in assessing **recent** exposure to As, because most arsenic that is absorbed from the lungs or from the gastrointestinal tract is excreted in the urine within 1-2 days. Biomonitoring for **As** in **hair** and nails is particularly useful in evaluating **chronic** exposures to As.

Tab. 2.1. Effects of different metals on specific target organs.

Heavy metal	Target organs
Lead	long bones, brain, liver, kidney, placenta
Arsenic	CNS, skin, hair
Cadmium	kidney, liver
Mercury	brain, liver, kidney, immune system
Chromium	lungs, liver, kidney, genitals, skin
Beryllium	lungs, skeleton, skin
Thallium	degenerative effect to all cells

2.4.3. Treatment of metal poisoning

Organisms have developed various detoxification strategies in order to remove unwanted inorganic substances, e.g. transformation from toxic to less toxic states ($Hg^{2+} \leftrightarrow Hg^{0}$). Certain proteins can bind toxic ions up to a certain storage capacity and thus remove them from the circulation. Special membranes can hinder the passing of highly charged ions into brain, fetus or cell nucleus.

In the **treatment** of metal poisoning, prevention of further absorption (removal from exposure, minimizing absorption from the gastrointestinal tract), elimination of absorbed poison (diuresis, dialysis, **chelation therapy**) and general supportive therapy is a priority.

Chelation therapy is indicated in the treatment of metal poisoning, as well as in the treatment of metal-storage diseases (Wilson's disease) and in blood transfusion overload (iron overload due to thalassemias). **Chelating agent** is a **multidentate ligand** that can form a chelate with a metal atom (see Chapter 1.3.2.). The ligand should possess electron donor groups showing a high affinity for the metal to be removed, thus releasing it from complexes with proteins or other endogenous ligands in a form which it can be readily excreted.

Basic principles that define efficiency of chelating agent are:

1. high affinity of chelating agent to toxic metal
2. low toxicity of chelating agent and of formed complex under the physiological pH
3. minimal interaction with biomolecules in organism
4. permeation to tissues where metal is deposited
5. minimal chelating agent metabolism
6. fast elimination of created complex from organism

Treatment is the most effective if the chelating agent is given while the metal is still in the circulation or in the extracellular fluid compartment, because once intracellular, the metal is less accessible.

The most important chelating agents:

1. Polyaminopolycarboxylic acids (used as an antidote for lead intoxication) have a low toxicity as their salts with Na^{+}, Ca^{2+} and Zn^{2+} (e.g. **$CaNa_2EDTA$**, calcium disodium salt of ethylenediaminetetraacetic acid).
2. Compounds containing -SH ligands, e.g. **dimercaprol** (2,3-dimercapto-1-propanol), **DMPS** (2,3-dimercapto-1-propanesulfonic acid) and **DMSA** (2,3-dimercaptosuccinic acid). These dithiol compounds successfully compete with protein sulfhydryl groups and are effective chelating agents mainly for Pb^{2+}, Hg^{2+}, As^{3+}, Sb^{3+} intoxication.
3. **D-penicillamine** used in case of Hg^{2+}, Cu^{2+}, Zn^{2+}, Pb^{2+} poisoning.
4. Crown ethers used selectively in the case of Cd^{2+}, Hg^{2+}, Pb^{2+} poisoning.
5. **Desferrioxamine** (Desferal) in the case of iron and aluminium poisoning. It is indicated for example in the treatment of transfusion-associated iron overload.

2.4.4. Toxicity of individual metals

Mercury

Mercury was used for centuries both as a medicine and a poison and is currently used for many commercial purposes. Mercury is the only metal which is liquid at room temperature. There are three main forms of mercury: **elemental mercury** and **inorganic** and **organic compounds** (methyl-, ethylmercury).

All forms induce toxic effects, the extent of the effects depends on the form of Hg at the time of exposure, the duration of exposure and the route of exposure. The toxicity of mercury compounds is based on the strong affinity of this metal for thiol ligands (containing **–SH** groups), such as cysteine. Mercury compounds affect tertiary and quaternary structure of proteins and significantly influence their activity. Toxic mercury compounds are rapidly distributed in the body to those parts, which feature the highest affinity towards this heavy metal; mercury is mainly found in the **liver**, **kidney** and **nervous system**, especially the **brain**. The **biological half-life** depends on the form of Hg and vary from a few days to month.

Humans are exposed to all forms of mercury through different pathways. **Elemental, metallic mercury (Hg^{0})** is poorly absorbed from the gastrointestinal tract, but is very volatile and mercury vapour is efficiently absorbed from the lungs. Elemental mercury is highly soluble in lipids; a characteristic that facilitates its diffusion across the alveoli into the circulation, as well as its distribution throughout the lipophilic compartments of the body including passage across the **blood-brain** barrier into the **central nervous system** and across the **placenta**,

where it lodges in the fetal brain. Mercury vapour has however a limited survival time; it is rapidly oxidized to Hg(II) in tissue and then reacts in the same way as mercuric mercury.

For **inorganic mercury** salts common routes of exposure are the gastrointestinal tract and skin. Especially toxic are soluble **mercuric Hg(II)** salts (e.g. **$HgCl_2$)**. Mercuric mercury does not readily cross the blood-brain barrier or the placental barrier; however it does accumulate in the placenta, fetal tissues and amniotic fluid. The critical organs are the **kidneys** and the **intestinal tract**.

An especially toxic form is represented by **organometallic compounds**, particularly by the **methylmercury** (H_3C-Hg^+). Organometallic compounds usually originate from biological sources, mainly fresh- or saltwater fish and the dominant route of exposure is through the ingestion. They are absorbed more completely from the gastrointestinal tract than inorganic salts because they are more lipid soluble. As they have high lipid solubility they are distributed throughout the body, accumulating mainly in the **brain**, kidney and liver. Organometallic compounds also cross the placental barrier attributing to **neurological** symptoms and **teratogenic** effects.

Acute exposure caused by inhaled elemental mercury causes **pulmonary symptoms** (shortness of breath, chest pain) and **effects on CNS** (lethargy, confusion, tremor). Acute exposure to inorganic mercury results in **gastrointestinal effects** (metallic taste, gastric and abdominal pain, necrosis of the intestinal mucosa) and **renal effects. Chronic exposure** induces toxic effects in **nervous** (increased excitability, memory loss, depression), **gastrointestinal** (diarrhea), **cardiovascular** (hypertension, tachycardia) and **renal** (polyuria, failure) systems. All forms of mercury are **toxic to the fetus**, but methylmercury most readily passes through the placenta and maternal exposure can lead to spontaneous abortion or retardation. For treating mercury poisoning thiol-containing chelating agents such as **dimercaprol, DMSA, DMPS** and **penicillamine** are used. In severe cases of toxicity when renal function has declined hemodialysis is used.

Lead

Historically, lead is the "oldest" recognized toxic metal and it is also the one which has been most extensively spread into the environment by humans. In contrast to mercury and cadmium, it is not particularly rare in the earth's crust. After absorption lead is transported by blood; within blood most of lead is present in the erythrocytes. From blood the absorbed lead is distributed to other organs. Lead accumulates mainly in **teeth** and in the **skeleton**, among the soft tissues, the **liver** and the **kidney** attain the highest concentrations of this metal. Lead is excreted from the body mainly through the urine and feces. The **biological half-life** depends strongly on the location in the body. In blood and soft tissues the half-life is about **1 month**. The major part of incorporated lead is stored in the bone tissue due to the similar properties of **Pb^{2+}** and **Ca^{2+}**. The **half-life** there may reach **30 years** or more, with effects on the development of degenerative processes such as **osteoporosis**.

Lead toxicity arises not only due to its ability to substitute other bivalent cations like **Ca^{2+}** or **Zn^{2+}** but also due to its great affinity to **-SH** groups of proteins, affecting various fundamental biological processes of the body.

Very toxic lead compounds are **organometallic** compounds, e.g. tetraethyl lead, which was used as fuel additive. Organometallic compounds may cause severe disorders of the **central** and **peripheral nervous system** (e.g. cramps, paralysis, loss of coordination). The toxicity of organometallic cations results from the permeability of membranes, including the very discriminating **blood-brain** barrier for such lipophilic species. Furthermore, lead passes the placenta and may cause effects on the nervous system of the fetus. Poisoning with **inorganic** lead compounds on the other hand primarily causes **gastrointestinal** (colics) and **hematological** symptoms such as **anemia** due to inhibition of heme synthesis - even relatively low concentrations of lead inhibit Zn^{2+} dependent enzyme which catalyzes an essential step in porphyrin and thus in heme biosynthesis. Further toxic effects of lead poisoning include **reproductive disorders** such as sterility and miscarriages. Heavy lead exposure may cause renal dysfunction and chronic renal failure. **Acute lead poisoning** causes classic symptoms, including abdominal and muscle pains, nausea and vomiting, seizures. The most commonly used chelating agents for the **treatment** of lead poisoning are combination of **$CaNa_2EDTA$** with **dimercaprol**.

Cadmium

Cadmium is a cumulative, very toxic metal. In its ionic form, **Cd^{2+}** shows great chemical similarity with two biologically very important metal ions: the lighter homologue **Zn^{2+}** and **Ca^{2+}**, which comes very close in size.

Cd^{2+} can displace this cations and interfere with their physiological action. Cadmium, similar to lead, has a great affinity to **-SH** groups of proteins, affecting their activity but cadmium is generally regarded to be far more toxic than lead.

Humans normally absorb cadmium into the body either by ingestion or inhalation. The absorption of cadmium compounds through the skin is negligible. Cadmium can be incorporated through food, with the liver and kidney

of slaughter animals and wild mushrooms being particularly rich in this element. Important route of exposure for the general population is smoking tobacco. A low intake of iron, zinc and calcium increases the degree of its absorption. After absorption from the lungs or the gastrointestinal tract, cadmium is transported through the blood to other parts of the body. Cadmium in blood is found mainly in the blood cells. Cadmium is distributed to many of the organs, the greatest amounts of Cd^{2+} were found in the **liver**, **kidneys** and **muscles** with particularly long **biological half-life**, **10-30 years**. In contrast to many other heavy metals and toxic elements, cadmium **does not** easily pass into the **central nervous system** or the **fetus**, because, in its ionized form and under physiological conditions, it cannot be bioalkylated to form stable, membrane-penetrating organometallic compounds. Cadmium **accumulates** in the body and only small portion of the Cd^{2+} absorbed is excreted. The daily excretion takes place through the feces and urine.

The critical organ in cadmium poisoning is the kidney. **Chronic exposure** leads to **renal tubular dysfunction**. Chronic cadmium poisoning may also lead to the **disturbance of calcium metabolism**, **osteoporosis** and **osteomalacia**. Extremely painful deformations of the skeleton have been observed and characterized in Japan in the middle of 20^{th} century and named as "itai-itai disease". Long term inhalation causes **pulmonary disorders** (e.g. chronic obstructive lung disease). **Acute exposure** because of inhalation leads to dryness of the nose and throat, headaches, chest pains and lung edema. Acute gastrointestinal symptoms are nausea, vomiting and abdominal pain. Cadmium is classified as a **human carcinogen**.

There is no specific **treatment** for cadmium poisoning. Because of the long half-life of cadmium in the kidneys and in the liver, which are the critical organs, primary prevention is essential. When there are signs of osteomalacia, large doses of vitamin D are given.

Arsenic

Arsenic is a semi-metallic element found in soils, groundwater, surface water, air and some foods. Arsenic in the environment occurs in both organic and inorganic compounds in its trivalent **(III)** or pentavalent **(V)** state. Trivalent arsenic compounds are generally more toxic than pentavalent arsenic compounds. **In organic**, especially methylated form, arsenic compounds are **less toxic** and are quite common, particularly in marine organisms such certain fish and crustaceans. After absorption by the lungs or the gastrointestinal tract, arsenic is transported by the blood to other parts of the body. The highest levels of arsenic in humans are found in **hair**, **nails** and **skin**. The main route of excretion is through the **kidneys**, only a low percent is excreted in feces. Arsenic has a relatively short **biological half-life**, most arsenic is excreted in the urine **within 1-2 days**.

Arsenic exerts its toxicity by inhibiting around 200 enzymes **involved in cellular energy pathways** and **DNA synthesis and repair**.

The toxicity of **As(III)** occurs mainly through the interaction of trivalent arsenic with sulfhydryl groups **(-SH)** in proteins. Arsenic binding to a specific protein could alter the conformation of the protein resulting in loss of its function. For example As(III) binds with two -SH groups of the enzyme complex *pyruvate dehydrogenase* which converts the end product of glycolysis - pyruvate to acetyl coenzyme A, which then enter the citric acid cycle (Krebs cycle). Inhibition of the *pyruvate dehydrogenase* complex can block the citric acid cycle and ultimately **decrease the production of ATP**, resulting in cell damage and death.

As(V) in the form of inorganic arsenate ($HAsO_4^{2-}$) is a molecular analogue of phosphate (HPO_4^{2-}) and thus can compete for phosphate anion transporters and replace phosphate in some biochemical reactions. For example, arsenate can substitute phosphate in the formation of ATP (forming ADP-arsenate) and other phosphate intermediates involved in glucose metabolism, which could slow down the normal metabolism of glucose, and interrupt the production of energy.

Acute arsenic exposure results in **gastrointestinal effects** (e.g. nausea, vomiting, thirst, abdominal pain) and **neurological effects** (e.g. headaches, dizziness) on the central and peripheral nervous system.

Chronic exposure is associated with adverse health effects on several systems of the body, including **dermal** (hyperpigmentation, **skin cancer), nervous, renal, hepatic, hematological** and **cardiovascular** systems. Arsenic is classified as a **human carcinogen** and ingested or inhaled arsenic is involved in the development of several cancers (skin, lungs, bladder, kidneys and liver).

Effective chelating agents for the **treatment** of arsenic poisoning are **dimercaprol, DMPS** and **DMSA**.

Thallium

Thallium forms a stable, highly toxic monovalent cation $\mathbf{Tl^+}$ under the physiological conditions. Tl^{3+}, the other stable oxidation state is easily reduced to Tl^+. Absorption of thallium compounds is rapid after inhalation, skin

contact and is nearly complete after digestion. After absorption, thallium compounds are widely distributed in the body, the highest concentration being accumulated initially in the kidneys. Excretion occurs through both urine and feces. Excretion also occurs through hair, which in unexposed subjects has been shown to contain the highest concentration of thallium in any tissue. Thallium has the **biological half-life** from 3 to 8 days.

Thallium compounds are extremely toxic. The majority of thallium toxic properties are due to its chemical similarity to potassium: the **ionic radii of Tl^+** and **K^+** are quite similar and Tl^+ can penetrate membranes via potassium ion channels and pumps to reach nearly all regions of an organism and cause disorders. Enzymes requiring the presence of K^+ for their activity, such as *Na^+/K^+-ATPase* or pyruvate kinase, an important enzyme of glycolysis, are inhibited by thallium. Thallium also crosses the placental barrier. **Acute poisoning** is characterized by **gastroenteritis** with nausea, vomiting and abdominal pain within hours of absorption. Involvement of the **nervous system** then becomes apparent after a few days, with peripheral neuropathy, mental confusion or delirium and convulsions, with respiratory and circulatory involvement followed by death. After an interval of 1-3 weeks, **alopecia** (loss of hair) develops. **Neurologic symptoms** (paresthesias) predominate with **chronic exposure** and tend to progress, even despite decreasing blood thallium levels.

For the **treatment** of thallium poisoning chelation therapy is not recommended as the thallium complexes formed are highly lipophilic and may increase thallium levels in the brain.

Suitable countermeasures following thallium poisoning include dialysis and high supplementation with K^+ in combination with administration of large quantities of mixed-valent iron cyanide complexes such as **Prussian blue**, simplified as $KFe[Fe(CN)_6]$ in colloidal form. Prussian blue particles are able to function as nontoxic cation exchangers; they do not release significant amounts of cyanide but can bind monocations such as Tl^+ and Cs^+ instead of K^+ and thus remove them from the organism.

Beryllium

Beryllium is a strategic and critical material for many industries. As a result of the increasing industrial use of beryllium, occupational exposure to the metal may be an important issue. Exposures to beryllium are much more hazardous by the inhalation route than by the ingestion route. Beryllium and its compounds are poorly absorbed from the gastrointestinal tract. Inhalation exposure to beryllium compounds results in long-term storage of beryllium in lung tissue and may lead to **chronic beryllium disease** (berylliosis) and **lung cancer**.

Once incorporated, beryllium is excreted only very slowly from organisms and is deposited in the skeleton. As a lighter homologue of magnesium, divalent beryllium can reach the cell nucleus, where it exerts **mutagenic** as well as **carcinogenic** effects. Beryllium interacts also with the **immune system**, its compounds being allergenic contact toxins. A possible chelation therapy of beryllium poisoning is not feasible, since Be^{2+} specific O-containing ligands are also strongly binding such biologically important metal ions as Mg^{2+} and Fe^{3+}.

2.5. Application of inorganic compounds in medicine

Inorganic or metal-containing medicinal compounds may contain either chemical elements essential to life forms, for example iron salts used in the treatment of anemia, or nonessential/toxic elements that carry out specific medicinal purposes, for example platinum-containing compounds as antitumor agents or technetium and gadolinium complexes as medical diagnostic tools, thus playing a great number and variety of roles in modern medicine both as **therapeutic** and as **diagnostic** agents.

In the present, preventive medicine, such as early recognition and treatment of cancer or coronary artery diseases, plays a very important role. A major tool in prevention is based on the use of non-invasive techniques, such as **magnetic resonance imaging (MRI)** and **computed tomography (CT)** or techniques that are used in nuclear medicine, such as **scintigraphy** and **single photon emission computed tomography (SPECT)**. Nuclear medicine imaging involves the administration of a radiolabelled molecules called a **radiopharmaceuticals**.

X-ray and MRI contrast agents

The use of **X-ray radiation** (discovered by Wilhelm C. Röntgen in 1895) to visualize the internal anatomic structures of a patient without surgery is still one of the most important diagnostic tools in modern medicine. As a rule, materials of high density or high atomic number (*Z*) tend to better absorb X-rays. While the large contrast between electron-dense bones and the surrounding more permeable soft tissues allows high-resolution imaging, the native contrast between the different soft tissues is so small, that unenhanced x-ray imaging cannot differentiate between them. In order to achieve better X-ray absorption than observed for biological tissues, elements of higher atomic number are incorporated into the contrast agent molecule. **Contrast agents** are a class

of pharmaceuticals that, when administered to a patient, **enter** and **pass through anatomic regions** of interest to provide **transient contrast enhancement,** and are **completely excreted** afterwards without being metabolized. In the form of suspension, relatively insoluble **$BaSO_4$** is widely used for gastrointestinal tract imaging. Water-soluble **iodinated** aromatic contrast agents are used for X-ray computed tomography (CT) of the head and the body for the evaluation of neoplastic and non-neoplastic lesions, for angiography throughout the cardiovascular system and many others.

Among the existing imaging techniques, **MRI** stands out thanks to the excellent spatial resolution and the outstanding capacity of differentiating soft tissues. This technique offers exquisite anatomical images of soft tissues based on resonance detection of protons largely in water or lipids. The magnetic resonance image can be enhanced by the administration of suitable MRI contrast agents. Contrary to contrast agents used in X-ray computed tomography, MRI contrast agents are not directly visualized in the image. Only their effects are observed as they alter water protons relaxation times and consequently the intensity of the signal. Metal ions most suitable for this application are those having the higher number of unpaired electrons, especially **Gd (III)** (seven unpaired electrons). Its administration as a free ion is strongly toxic even at low doses. For this reason, it is necessary to use ligands that form very **stable chelates** with Gd (III), for example polyaminocarboxylic acids. Gd (III) chelates in clinical use are for example Dotarem® (lesions in the blood-brain barrier), Eovist® (imaging of the liver).

Radiopharmaceuticals

Radiopharmaceuticals are compounds that contain radioactive atoms (radionuclides or radioisotopes) within their structure, for example complexes containing various radioactive isotopes of gallium, cobalt, technetium, rhenium and rhodium, that are widely exploited to deliver radiation to the targeted sites.

They play an important role in non-invasive diagnosis and therapy of various diseases such as cancer or neurodegeneration. In the case of the diagnostic imaging agents the goal is to use a radioisotope that emits radiation allowing good image of the target organ, but has only little effect on surrounding tissue. This is achieved with **γ** emitting isotopes. **α-** or **β-** emitting radionuclides are applied for therapeutic purposes; if the nuclides also emit **γ** radiation, they can simultaneously image the distribution of the therapeutic agent. They deposit the decay energy of the particle within a short range, which will lead to very high local doses accompanied by corresponding biological damage, necrosis, or apoptosis of the targeted cells, so they are widely used for the treatment of cancers. The physical half-life of the radionuclide should be long enough to allow administration and sufficient accumulation in target tissue in the patient. On the other hand, the radioactive decay time should be short enough to minimize the radiation dose to the patient. An ideal radiopharmaceutical is one that rapidly localizes **specifically in the target organ** causing **minimum damage to normal tissues**, **remains in it for the duration of study**, and is **quickly eliminated** from the body.

The most common isotopes for radiotherapy and radiodiagnostics are:

1. **^{131}I**, mainly in the form of iodide, with exclusive selectivity for the thyroid gland
2. **^{67}Ga**, which is used in localizing inflammatory processes or cancer cells
3. **^{99m}Tc**, in a wide variety of complexes for the imaging of various organs

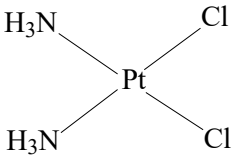
^{99m}Tc is the most widely used radionuclide in diagnostic medicine. It emits γ ray; this emission is sufficiently energetic to allow visualization of sites deep within the human body. ^{99m}Tc labelled compounds are used to image heart and kidney functions, spread of cancer to bone and is indicated for detecting coronary artery disease by localizing myocardial ischemia and infarction. The **in vivo behaviour** of the ^{99m}Tc radiopharmaceutical depends on the **ligands** that surround the metal and **charge** and **lipophilicity of the complex**. For example, negatively charged compounds tend to clear through the kidneys, many positively charged complexes accumulate in the heart and an overall neutral complex is required for crossing the blood–brain barrier.

Radioisotopes such as **^{67}Cu, ^{90}Y, ^{188}Re** and **^{212}Bi** (preferably α and β emitters) are used in radioimmunotherapy; they can be selectively incorporated into tumor tissue, allowing very specific tumor cell killing.

Boron neutron capture therapy: Stable inorganic isotopes such as ^{10}B, ^{97}Ru and^{157}Gd can have an indirect radiotherapeutic effect. Boron neutron capture therapy is a binary form of treatment of cancer in which a compound containing isotope **^{10}B** is selectively delivered to tumor tissue prior to irradiation by thermal neutrons. Upon irradiation, interaction of a ^{10}B atom with these thermal neutrons produces an **α** particles, a high-energy ^{7}Li ions and a low-energy **γ**-ray. This therapy is used especially for the treatment of glioma (tumors originating from the brain or spinal cord) and cutaneous melanoma.

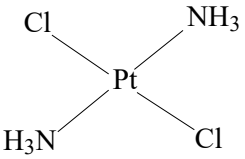
Anticancer therapeutic agents

The Pt(II) complex **cisplatin**, $[Pt(NH_3)_2Cl_2]$, is one of the most widely used anticancer drugs, particularly for the treatment of testicular and ovarian carcinoma and against bladder, cervical and lung tumors and tumors in the head/neck.



cis-diamminedichloridoplatinum(II)

cisplatin



trans-diamminedichloridoplatinum(II)

Cisplatin reacts with DNA in the cell nucleus, where the concentration of chloride ions is markedly lower than in extracellular fluids. In media containing low concentrations of chloride cisplatin loses its chloride ligands to form positively charged mono- and diaaqua species: $[Pt(NH_3)_2Cl(H_2O)]^+$ and $[Pt(NH_3)_2(H_2O)_2]^{2+}$. Water ligands are in turn replaced by nucleobases (main donor atom is N7 nitrogen atom of guanine) from DNA strands and form cross-link adducts. Such adducts leads to marked conformational alterations in DNA structure and are able to inhibit DNA and RNA synthesis and thereby induce cell death. The most common side effects of a cisplatin therapy include kidney and gastrointestinal problems, which may be attributed to the inhibition of enzymes through coordination of platinum to sulfhydryl groups in proteins. Similar platinum-containing antitumor drugs in clinical use are **carboplatin** and **oxaliplatin** with broader spectrum of antitumor activity or improved safety.

Several **Pd(II)**, **Ru(II)**, **Rh (I)**, **Rh(II)**, **Rh(III)** complexes have been prepared and are currently investigated for their anti-tumor properties on the basis of their similar properties as compared to cisplatin.

Inorganic compounds in non-cancer therapy

Lithium

Lithium is clinically used in the treatment of **manic depression** (or **bipolar disorder)**. Lithium salts, usually **Li_2CO_3** are administered orally in several controlled doses per day. Difficulties in the therapy result from the toxicity of lithium at higher concentrations.

Magnesium

Magnesium-based drugs have a great number of potential uses, including the treatment of **eclampsia**, **arrythmias**, **stroke** and **myocardial infarction**. Mg^{2+} ion plays a physiological role in processes pertinent to ischemia; it is complexed with ATP and is an important cofactor in cellular energy metabolism and protein synthesis. Magnesium hydroxide together with aluminium hydroxide (Maalox®) act as **antacids** by neutralizing stomach acid. Magnesium citrate and magnesium sulfate are used as **laxatives**. This products are used for the short-term of relief of **constipation**, and to cleanse the bowels prior to certain procedures, such as a colonoscopy, usually with other products. In the intestinal lumen the poorly absorbable magnesium ions exert an osmotic effect and cause water to be retained in the intestinal lumen. This increases the fluidity of the intraluminal contents and results in a laxative action. Magnesium is an attractive therapeutic agent because it is inexpensive, widely available and intravenous and intramuscular administration yields predictable serum concentrations.

Gold

The monovalent form of gold **Au(I)** has therapeutic importance in the treatment of **rheumatoid arthritis** (RA). Most of the Au compounds used as antirheumatic agents contain thiolate ligands RS^-, that stabilize oxidation state I. Among the first gold compounds used for the treatment of RA are Solganol® and Aureotan®, containing thioglucose as a ligand. A disadvantage of this gold drugs is that they are administered to patient via intramuscular injection and gold is accumulated in organs such as kidneys resulting in nephrotoxicity, which leads to leakage of protein and blood into the urine. A relatively new gold-based drug that finds wide use is Auranofin® which can be administered orally and the concentration of gold in kidneys is considerably less.

Bismuth

Bismuth compounds were first used in the seventeenth century as a treatment for syphilis and other bacterial infections. **Bi(III)** compounds containing hydroxycarboxylate ligands (salicylate, citrate) have applications as remedies for **gastrointestinal disorders**. The preparations Pepto-Bismol® and De-Nol® are used for gastric and duodenal ulcer therapy and to treat non-ulcer dyspepsia and have antimicrobial activity against *Helicobacter pylori.*

Sodium nitroprusside

Sodium nitroprusside, **$Na_2[Fe(CN)_5(NO)]$**, is a nitrosyl complex, long in clinical use. It is administered in case of suddenly elevated blood pressure (e.g. during surgery) or after myocardial events. After infusion of the drug, the desired reduction of the blood pressure begins within 1-2 minutes. The reason for the therapeutic effect is, that the complex releases **NO**, which leads to the **relaxation of the smooth muscles of the blood vessels**. Alternatively, nitroglycerine can be administered in case of angina pectoris.

Some other elements with therapeutic and diagnostic effects are listed in Table 2.2.

Tab. 2.2. Some examples of inorganic elements and compounds with medicinal purposes

Element	Some medically-relevant uses
Calcium	$CaCO_3$: antacid, $CaSO_4 \cdot 1/2\ H_2O$: plaster ingredient, $Ca(lactate)_2$: dietary supplement
Aluminium	$Al(OH)_3$: adjuvant in vaccines, antacid
Gallium	$Ga(NO_3)_3$: treatment of cancer-related hypercalcemia
Iron	$FeSO_4$, complex organic Fe(II) salts (succinate, fumarate): for iron deficiency
Cobalt	vitamin B_{12}: treatment of pernicious anemia
Zinc	$Zn(gluconate)_2$: dietary supplement, ZnO: skin ointment
Copper	$Cu(histidine)_2$: Menke's disease treatment
Vanadium	insulin mimetics, V(IV) and V(V) complexes in clinical use for DM type 2
Silver	$AgNO_3$: cauterizing agent, histology: silver staining
Helium	^3He for MRI
Cesium	^{129}Cs: (γ-particle emitter) for myocardial imaging
Radium	^{223}Ra (α-particle emitter): for treatment of skeletal metastases

Control questions:

1. Except of biogenic elements, nonessential and toxic elements are also present in the body. Explain why.
2. Name the biological functions of biogenic elements (generally). Why Zn is not involved in electron transfer, while metals like Fe, Cu, Mn and Mo are?
3. What is the biological function of nitric oxide? Can this molecule be toxic?
4. What are hemoproteins, name 3 examples and their functions.
5. Why is carbon monoxide toxic?
6. Can iron be toxic? If yes, give the reasons.
7. Ceruloplasmin has a ferrooxidase activity. Explain what it means and the biological importance of this activity.
8. What are the functions of Zn-containing proteins?
9. Co is bound in complex with corrin in vitamin B_{12}. What type of ligand corrin is? Is the formed complex stable?
10. Write the reaction which is catalyzed by Mn-SOD.
11. Name three main mechanisms of action of toxic metals, i.e. what is the metal toxicity based on.
12. What is chelation therapy based on? Characterize chelating agents.
13. What are organometallic compounds? What is the base of their toxicity?
14. Why can be $BaSO_4$ used for gastrointestinal tract imaging?
15. What are radiopharmaceuticals and what is their application? Name some examples.

3. DISPERSED SYSTEMS

3.1. The types of dispersed systems

Dispersed system is formed by dispersing of a substance in a solvent. The sizes of the particles intimately mixed with a solvent determine some physical properties of the mixtures (Tab. 3.1.). Mixtures are either homogeneous or heterogeneous. **Homogeneous** mixtures are those in which the tiniest samples are everywhere identical in composition and properties. **Heterogeneous** mixtures are any mixtures that are not homogeneous (e.g. mixture of water and ice). A homogeneous part of a heterogeneous mixture is called a phase, that is physically separated from its other parts. There are two kinds of relatively stable homogeneous mixtures: **solution** and **colloidal dispersion**. A third kind, the **suspension**, is unstable, being homogeneous only when constantly stirred. The suspension is on the borderline between homogeneous and heterogeneous (e.g. the **blood**, while moving, is a suspension, besides being a solution and a colloidal dispersion). The kinds of homogeneous mixtures differ fundamentally in the sizes of the particles involved, and the differences in the size can alone cause interesting and important changes in properties. See Tab. 3.1. for a summary of the chief properties of solutions, colloidal dispersions, and suspensions.

According to the size of dispersed particles systems are:

1. **True solutions** – are homogeneous mixtures in which the particles of both the solvent and the solutes have the size of atoms, or ordinary ions and molecules, in the range of **0.1 to 1 nm** (1 nm = 10^{-9} cm)**.**
2. **Colloidal dispersions** – are homogeneous mixtures consisting of very large clusters of ions or molecules, or macromolecular compounds in which the dispersed particles have the diameter in the range of **1 nm to 1000 nm**.
3. **Crude dispersions** – **suspensions** – on the borderline between homogeneous and heterogeneous mixtures, in which the dispersed or suspended particles have the diameter over **1000 nm.**

Tab. 3.1. Properties of dispersed systems

Dispersion	Analytical (true solutions)	Colloidal	Crude
Particles size	< 1 nm (0.1 – 1 nm) all particles are of atoms, ions or small molecules	1 – 1000 nm particles are large clusters of atoms, ions, or small molecules, or very large ions or molecules	over 1000 nm
Particles movement	no Brownian motion	Brownian motion	slow Brownian motion
Particles filterability	cannot be separated by filtration	cannot be separated using filter paper, can be separated using semipermeable membrane	can be separated by filtration,particles are trapped by filter paper
Diffusion	rapid	slow	no diffusion
Osmotic pressure	high	small	no osmotic pressure
Visibility	cannot be seen with electron-microscope	can be seen with a high power microscope	may be seen with a low power microscope, or by eyes
Sedimentation	most stable to gravity	less stable to gravity, they sediment in the strong centrifugal field	unstable to gravity , they separate under the influence of gravity
Aging	are stable to aging	less stable to aging (with time they get old)	unstable to aging
Optical properties	transparent no Tyndall effect	often translucent or opaque, may be transparent Tyndall effect	cannot be transparent

The **types** of colloidal and crude dispersed systems, according to a character of dispersed particles and dispersing medium are presented in Tab. 3.2.

Tab. 3.2. The types of colloidal and crude-dispersed systems

	Dispersed particles		
Dispersing medium	**Gas**	**Liquid**	**Solid**
gas	- -	**liquid aerosol** (mist, fog, clouds, certain air pollutants)	**smoke** (dust in smog)
liquid	**foam** (whipped cream, suds)	**emulsion** (milk, mayonnaise)	**suspension** (blood, sand in water) **hydrosol** (blood plasma, starch in water) **gels (are sols that adopt a semisolid form)** fruit jellies, gelatin)
solid	**solid foam** (pumice, marshmallow)	**solid emulsion** (butter, cheese)	**solid sol** (pearls, opals, alloys)

3.2. True solutions

3.2.1. Characteristics

Solutions are **homogeneous systems** (solid, liquid or gaseous), which consist of at least two components – a **dissolved** substance – a **solute** (one or more) and a **solvent** (the component present in the greatest amount), in variable proportion, in which the particles of both solutes and solvent have sizes of atoms, or ordinary ions and molecules, in the range of 0.1 to 1 nm.

Solutes may be liquids, gases, or solids.

Gas systems are usually mixtures (e.g. air is a mixture of 78 % nitrogen, 21 % oxygen, 1 % inert gases and 0.03 % carbon dioxide). Solid homogeneous systems are e.g. alloys.

We usually think of solutions as **liquids**. A liquid solvent can dissolve a **solid** substance (e.g. solution of sugar in water), a **liquid** substance (e.g. vinegar – acetic acid in water) or a **gas** substance (e.g. carbonated beverages – carbon dioxide in water).

Solubility depends **mainly** on the **features of dissolved substance**, **solvent** and **temperature.** Solubility is commonly reported as the weight of a substance per 100 g of saturated solution (the solution with the maximum quantity of the solute that can dissolve in a given quantity of solvent), at 25 °C. Dissolution of substances is a complex physicochemical process, in which intermolecular and intramolecular bonds of dissolving substances and forces among solvent molecules interrupt, and new associations between the solvent molecules and molecules or ions of solute arise.

Various factors affect solubility. Molecular structure determines **the polarity of a molecule**. **Solvents** are **polar** (water) and **nonpolar**. **Polar solutes** (electrolytes and nonelectrolytes) with polar groups in a molecule dissolve well in **polar solvents,** while **nonpolar solutes** dissolve in **nonpolar solvents.** The ratio of **polar –hydrophilic** to **nonpolar – hydrophobic** (or lipophilic) parts of molecules of organic substances determines their solubility. For example, solubility of carboxylic acids in polar solvents decreases with the extension of carbon chain. **Cholesterol** does not dissolve in water (neither in water medium of blood), because the entire cholesterol molecule is hydrocarbon–like. It has only one polar group (–OH), and this represents too small portion of the molecule to make cholesterol sufficiently polar to dissolve either in water or in water medium of blood.

The most important **polar solvent** is **water,** the compound necessary for life. In water medium the reactions in living organisms proceed. Total water is about 60 % of body mass (in adults). The total volume of body fluids in the average 70 kg person is about 40 liters. The fluid is divided into two major compartments and moves

between them: **intracellular** fluid **–** approximately 25 l held within the body´s cells and **extracellular** fluid – approximately 15 liters present outside the cells. Extracellular fluids are for example *blood plasma*, *interstitial fluid* (the fluid between cells), *lymph* (the interstitial fluid that is transported from the spaces between the cells back to the blood circulatory system), *cerebrospinal fluid* (surrounding the brain and spinal cord), *fluid in gastrointestinal tract*, *urine* and variety of other fluids.

Intake and elimination of water must be in equilibrium (balance) in organism. From the viewpoint of water homeostasis, the total volume of water is not as important as its distribution among the three compartments: intravascular, interstitial, and intracellular system. Continuous water exchange proceeds among the three systems, however, the water volume in these spaces remains constant in a certain range. The main regulatory factors include osmotic, hydrostatic and oncotic pressure (kidney being the central regulation organ).
The covalent bonds in water molecule are polar because oxygen atom is more electronegative than atom of hydrogen. Water does act like an **electrical** molecular **dipole** (Fig. 3.1.), and therefore it is an excellent polar solvent. By a molecular dipole, we mean a molecule that has a positive and the negative end. Two dipoles brought close enough will attract or repel one another. The positive end of one molecule attracts the negative end of another.

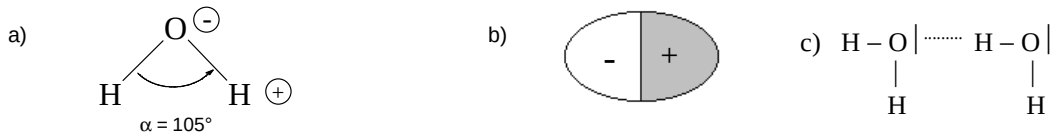


Fig. 3.1. Schematic arrangement of structure: a) water molecule, b) electrical dipole, c) hydrogen bond

Polar particles of substances and ions in water are surrounded by water molecules which negative ends point toward the positively charged ions.

Pressure has little effect on the solubilities of liquids or solids, but increased pressure increases the solubility of gases. An increase in temperature decreases the solubility of a gas, but the effect of temperature on the solubility of a solid varies with the identity of a solid.

True solutions are:

1. **ionic** – solution of **electrolytes**, in which ions are present, formed by electrolytic dissociation of ionic compounds or by ionization of polar covalent compounds. An **electrolyte** is a chemical compound that dissociates into ions and hence is capable of transporting an electric charge - i.e. an electrolyte conductor; unlike metals the flow of charge is not a flow of electrons, but is movement of ions.

When ionic compounds dissolve in water, the associated ions in the solid separate because water reduces the strong electrostatic forces between them. The polar water molecules surround individual ions at the surface of crystal and penetrate between them, reducing the strong inter-ionic forces that bind ions together and letting them move off into solution as **hydrated ions** (Fig. 3.2.). This process is called **electrolytic dissociation:**

$$NaCl \xrightarrow{H_2O} Na^+ + |\underline{\underline{Cl}}|^-$$

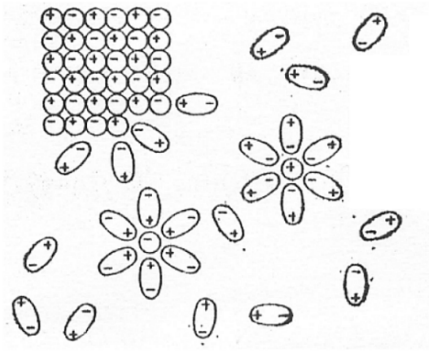


Fig. 3.2. Dissolution of ionic compounds in a polar solvent

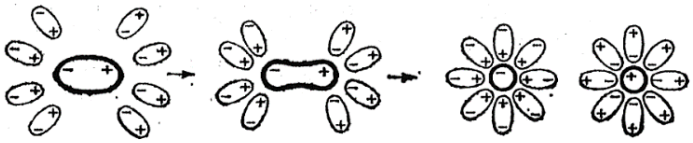
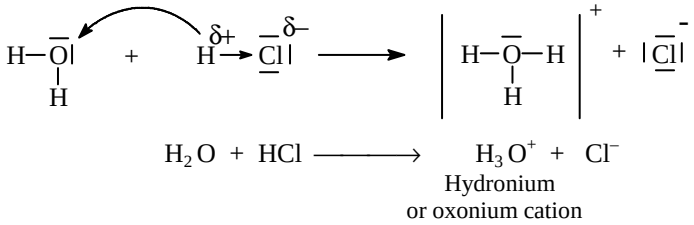


Fig. 3.3. Scheme of ionization of a polar covalent compound in a polar solvent

2. **molecular** – solutions of nonelectrolytes, contain molecules of compounds in a solution (e.g. water solution of glucose or urea).

When polar covalent compounds dissolve (Fig. 3.3.) in a polar solvent, a polar covalent bond is heterolytically cleaved by water molecules and ions are formed. This is called **ionization**:



3.2.2. Ways of expressing solution composition

Concentration is a measure of the **amount of solute** dissolved in a given **amount of solution**. The amount of solute can be expressed either in **mass units** (such as grams) or as **amount of substance** (in moles).

Ways of expressing concentration are present in Tab. 3.3. Solution composition can be described in terms of amount of **substance concentration (molarity), molal concentration (molality), weight (or mass) concentration**, **weight fraction**, **weight percentage**, **volume fraction and volume percentage.**

Amount of substance (n) is a basic SI quantity, having a basic unit of 1 mole (symbol mol). This is such an amount of a substance, which contains a number of specific particles (atoms, molecules, ions, radicals or electrons) equal to the number of atoms in 0.012 kg of ^{12}C carbon.

Amount of substance concentration means the amount of substance of the dissolved solute (here denoted as the solute B) divided by the solution volume. **The symbol** of amount of substance concentration is **c** (e.g. c(NaCl), c_{NaCl} or **[NaCl]**. The basic unit of the amount of substance concentration (c) is mol/l.

Weight (mass) concentration is used when the molecular weight (M_r) of a substance is unknown.

Weight (w %, or % w/w) **or volume percentage** (v %, or % v/v) is sometimes used in practice. These units express the number of weight or volume units of the solute B present in 100 weight or volume units of the solution. They are obtained by multiplying mass fraction (w_B) or volume fraction (v_B) by 100:

$$w\,\% = w_B \,.\, 100 \qquad \text{or} \qquad v\,\% = v_B \,.\, 100$$

Tab. 3.3. Quantities and units of concentration

Quantity	Symbol	Definition	Unit
Amount of substance concentration (older name - molar concentration, molarity)	$c_B = \frac{n_B}{V_{solution}}$	Amount of substance of the dissolved compound B divided by the solution volume	$mol.m^{-3}$ $mol.dm^{-3} = mol.l^{-1}$
Molal concentration (older name - molality)	$m = \frac{n_B}{m_{solvent}}$	Amount of substance of the dissolved compound B divided by the solvent weight	$mol.kg^{-1}$ water
Weight (or mass) concentration	$\rho_B = \frac{m_B}{V}$	Weight of the dissolved compound B divided by the solution volume	$kg.l^{-1}$ ($g.l^{-1}$)
Weight fraction (or mass fraction) --- **Weight percentage**	$w_B = \frac{m_B}{m_{solution}}$ --- $w\% = (m_B/m).100$	Weight of the dissolved compound B divided by the solution weight --- Weight of the dissolved compound B in 100 weight units of a solution	g/g (kg/kg)
Volume fraction --- **Volume percentage**	$v_B = \frac{V_B}{V_{solution}}$ --- $v\% = (V_B/V).100$	Volume of the dissolved compound B divided by the solution volume --- Volume of the dissolved compound B in 100 volume units of a solution	ml/ml (l/l) –

3.2.2.1. Calculations of solution composition and solution preparation – practical examples

1. **How many grams of NaCl (M_r=58,44) is needed to prepare 1000 ml of NaCl solution with concentration c = 0.15 $mol.l^{-1}$?**

   $m = c \times V \times M_r = 0.15 \times 1 \times 58.44 = 8.766$ g NaCl

2. **Prepare 500 g 0.9 % (w/w) of NaCl solution.**

   W % = m(B)/m(R) .100

   m (B)= w% × m(R)/100 = 0.9×500 /100 = 4.5 g NaCl

3. **Mass concentration of albumin (M_r=69000) in blood plasma is 38 $g.l^{-1}$. What is amount of substance concentration of albumin?**
   $c=n/V= (m/M_r)/V)=m/(M_r \times V)=38/(69000 \times 1)= 0.55 \times 10^{-3}$ $mol.l^{-1}$

4. **How many grams of glucose (M_r=180) is needed to prepare 10 ml of glucose solution with concentration c= 0.5 $mol.l^{-1}$?**
   $m = c \times V \times M_r = 0.5 \times 0.01 \times 180 = 0.9$ g glucose

5. **The volume of 2 ml of a glucose solution with concentration 5 $mmol.l^{-1}$ was diluted with 6 ml of water. Calculate concentration of resulting solution.**
   $c_1V_1 = c_2V_2$
   $c_2=c_1V_1/V_2 = 5 \times 2/(2+6) = 5 \times 2/8 = 1.25$ $mmol.l^{-1} = 1.25 \times 10^{-3}$ $mol.l^{-1}$

6. **Calculate the concentration of glucose solution, which was prepared by mixing the volume of 15 ml glucose solution with concentration 5 $mmol.l^{-1}$ with 25 ml of glucose solution with concentration 4 $mmol.l^{-1}$?**
   $c_1V_1 + c_2V_2 = c_3V_3$
   $c_3 = (c_1V_1 + c_2V_2)/V_3 = (5.15+4.25)/40 = 4.375$ $mmol.l^{-1}$

3.2.3. Properties of true solutions

Colligative properties are those properties of solutions, that do not depend on the structure and the chemical composition of a solute, but depend only on the number of solute particles (molecules or ions formed by dissociation) present in a solution. Colligative properties of solutions include:

- **freezing point depression in a solution**
- **boiling point elevation in a solution**
- **osmotic pressure**

Because a nonvolatile solute lowers the freezing point and raises the boiling point, it extends the liquid state of the solvent. The vapor pressure of a solution containing a nonvolatile solute is always less than the vapor pressure of the pure solvent, since the presence of the solute **decreases the number of solvent molecules** per unit volume and thus proportionately lowers the escaping tendency of the solvent molecules. **Raoult's law** states that the vapor pressure of a solution is directly proportional to the mole fraction of the solvent. Raoult's law holds for ideal solution.

The processes based on colligative properties are **diffusion**, **osmosis** and **dialysis**.

Diffusion is a process of a spontaneous movement of particles of a dissolved compound from a region of higher concentration to a region of lower concentration, to distribute themselves uniformly. Diffusion happens very rapidly in gases, but much more slowly in liquids. In liquids, diffusing molecules encounter and collide with a large number of solvent molecules, and such collisions impede their progress. The rate of diffusion depends on differences in concentration within a solution: the greater concentration difference, the greater the rate of diffusion. Diffusion takes place at transport of gas and nutrients between cells of the body and other environment constitutes. Low-molecular weight compounds, such as water, urea, nitrogen, oxygen, carbon dioxide are transported through biological membranes by diffusion.

Osmosis and dialysis (migration of solvent – osmosis, or migration of solution – dialysis through semipermeable membranes) also depend only on the number of solute particles (molecules or ions).

Osmosis is a phenomenon, which arises at the boundary between a solution and a solvent (or two solutions with different concentrations of the same compound) that are separated by a semipermeable osmotic membrane (membrane that is permeable only to solvent molecules, but not to molecules of the solute). During osmosis, there is always a net flow of solvent from the more dilute area to the compartment in which the solution is more concentrated. By permeation of the solvent into the solution, excess pressure is created. *The pressure needed to prevent the net flow of solvent from the diluted solution to the concentrated one is called* **osmotic pressure**. Osmotic pressure is measured by **osmometer** (Fig. 3.4.).

Dialysis is process similar to osmosis, with the following difference: during dialysis not only water molecules can permeate through the membranes, but also molecules of the solute (with exception of macromolecules, e.g. protein molecules). Pores of **dialyzing membranes** are larger than those of osmotic membranes. Dialysis takes place in living organisms. Cell membranes have properties of dialyzing membranes, but their permeability is highly selective. Selective migration of molecules or ions through cell membranes is an important mechanism for entry of nutrients into and exit of waste products from the cells.

Blood dialysis (hemodialysis)

Hemodialysis, removal of waste metabolic products (such as urea or creatinine) or toxins, is performed in kidney. If the kidneys do not work efficiently, these wastes built up in the blood and threaten the life of the patient. The artificial kidney is one remedy for **hemodialysis**. The bloodstream is diverted from the body and pumped through a long, coiled tube that serves as the dialyzing membrane (Fig. 3.5.) A solution called the **dialysate** circulates outside of the tube. The dialysate is prepared not only to be isotonic with blood, but also to have the same concentrations of all the essential substances that should be left in the blood.

In the case of the same concentrations, the rate at which such solutes migrate out of the blood equals the rate at which they return. The dialysate is kept very low in the concentration of the wastes, so the rate at which they leave the blood is greater than the rate at which they can get back in. In this manner, hemodialysis slowly removes the wastes from the blood.

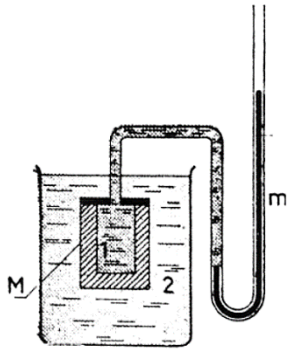


Fig. 3.4. Osmosis. Measure of osmotic pressure
(M-semipermeable membrane, m-mercury manometer, 1-solution, 2-solvent)

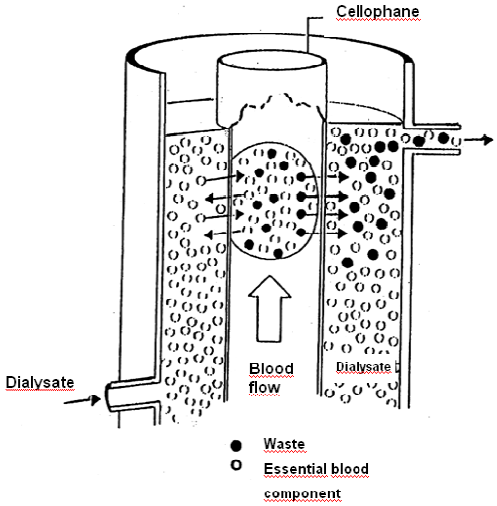


Fig. 3.5. Hemodialysis
(M – semipermeable membrane)

3.2.3.1. Osmotic pressure

Osmotic pressure - π of diluted solutions depends on the number of solute particles and can be expressed as the relationship:

$$\pi = i.c.RT$$

where: i – number of solute particles in solution ; c - amount of substance concentration of solute in solution; R – gas constant (8.314 $J.K^{-1}.mol^{-1}$); T – temperature in Kelvin K (0 °C = 273.15 K);

Osmotic pressure is directly proportional to the concentration of all particles of solutes in a solution. Osmotically active are all compounds in a solution, **electrolytes, organic compounds and macromolecular compounds** (e.g. **proteins**). Each particle, that arises in the solution by dissociation contributes to the osmotic pressure, so the amount of substance concentration of the solution is multiplied by the number of particles in the solution, **i** (for compounds that are not dissociated in the solution, i = 1). It means the osmotic pressure of solutions with the same amount of substance concentration depends just on the number of particles, it means electrolytes have higher osmotic pressure than non-electrolytes.

Osmotic pressure is determined by **amount of substance concentration of all osmotically active particles of the solution** (ions, molecules, or macromolecules), which is called **osmolarity - c_{osm}** (expressing concentration of osmotically active particles of compounds dissolved in the volume of one liter of a solution) or **osmolality** (related to one kg of solvent - water):

c_{osm} = i.c **- osmolarity - [$mol.l^{-1}$]** **older units:[$Osmol.l^{-1}$]**
- osmolality - [$mol.kg^{-1}$ H_2O] **[$Osmol.kg^{-1}$] H_2O**

For example, osmolarity of :
a) NaCl solution with concentration c = 0.15 $mol.l^{-1}$ is c_{osm} = 2× 0.15 = 0.3 $mol.l^{-1}$,
b) $MgCl_2$ solution with concentration c = 0.15 $mol.l^{-1}$ is c_{osm} = 3× 0.15 = 0.45 $mol.l^{-1}$,
c) glucose solution with concentration c = 0.15 $mol.l^{-1}$ is c_{osm} = 1× 0.15 = 0.15 $mol.l^{-1}$.

The osmotically effective concentration (osmolarity) of blood serum can be calculated from the relationship for osmotic pressure, and from the known blood osmotic pressure of around 780 kPa:

$$c_{osm} = \frac{\pi \text{ (blood)}}{RT} = \frac{780 \text{ kPa}}{8.3 \text{ J.K}^{-1}.\text{mol}^{-1} \cdot 310 \text{ K}} = 0.3 \text{ mol.l}^{-1}$$

Under physiological conditions, osmolarity of the internal environment, $c_{osm} = 285 \pm 10$ **mmol.l^{-1}.**

This osmolarity value is maintained at a constant level, thus protecting cells from excessive volume changes.

Maintaining a constant osmolarity is provided by regulatory systems of the organism, and it is performed mainly with the help of water and ions transfer between different compartments (extra- and intracellular) and by the action of kidney (osmo- and volume receptors).

Two solutions of **equal osmolarity** are called **isotonic** or **isoosmotic** solutions. When we compare two solutions with different osmolarities, the solution with lower osmolarity (or osmotic pressure) is called **hypotonic** and the solution with higher osmolarity is called **hypertonic** solution. A solution of **0.15 mol.l^{-1} NaCl** corresponds to the osmotic pressure of body fluids (blood), therefore it is called **isotonic** (**isoosmotic) saline solution** or **physiological saline solution**. Physiological solutions are solutions, which composition, pH value and buffering properties resemble blood plasma (Tab. 3.4.). In some medical situations, body fluids need replacement or nutrients have to be given by intravenous drip. The osmolarity of the solution being added should match that of the fluid inside. Any solution to be added in any large quantity into the bloodstream has to be isotonic.

The osmotic condition of living cells separated by semipermeable membranes results from balance between the rate of water entering the cell and the rate of water leaving the cell. The red blood cells (erythrocytes) circulate in the bloodstream, and their membranes behave as semipermeable membranes. Within each red blood cell there is an aqueous fluid with dissolved and colloidally dispersed substances (see Fig. 3.6.a). Although the colloidal particles are too large to dialyze, they contribute to colloid osmotic pressure. They help to determine the direction of dialysis through the membranes. The relations between a cell's inner and outer concentrations lead to three different osmotic conditions: hypotonic, hypertonic, and isotonic, illustrated in Fig. 3.6. When red cells are placed in pure water or in hypotonic solution, the fluid inside the red cell is more concentrated than the surrounding liquid. Dialysis now occurs to bring fluid into the red cell. Enough fluid moves in to make the red cell burst open. The rupturing of red cells is called **hemolysis** (Fig. 3.6.b). Red cells hemolyze if placed in hypotonic environment. Red cells undergo crenation, if placed in a hypertonic environment (Fig. 3.6.c).

Determination of erythrocyte hemolysis has a diagnostic importance.

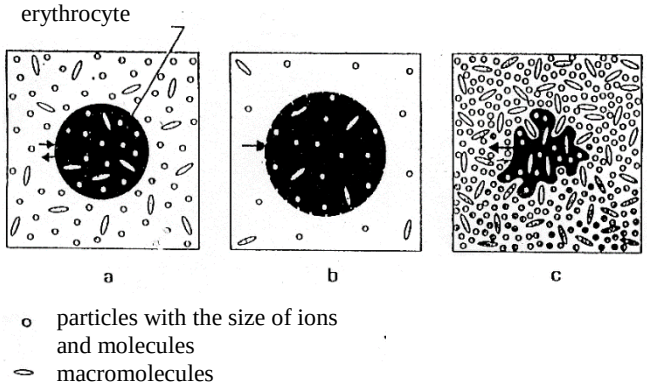


Fig. 3.6. Red blood cells in: isotonic (a), hypotonic (b) and hypertonic (c) environment

Tab. 3.4. Examples of physiological, isoosmotic (isotonic) solutions in biology

Solution composition (g.l^{-1})	**Physiological solutions**		
	Ringer´s Lock´s (g.l^{-1})	**Ringer´s Tyrode´s (g.l^{-1})**	**(mmol.l^{-1})**
NaCl	9.0	8.0	137.9
KCl	0.42	0.2	2.7
$CaCl_2$	0.24	0.2	1.8
$NaHCO_3$	0.2	0.1	1.19
$MgCl_2$	-	0.1	1.0
Na_2HPO_4	-	0.05	0.23
Glucose	1.0	1.0	5.5

All dissolved solutes contribute to overall osmotic pressure. The intracellular fluid, the interstitial fluid, and blood all contain a variety of dissolved solutes and colloid particles. All these fluids contain relatively high levels of electrolytes, e.g. charged particles such as sodium, potassium, chloride, and bicarbonate ions (Tab. 3.5.). The electrolyte (ions) makeup of the extracellular fluids (plasma and interstitial fluid) are very similar. But, ions are distributed unevenly between the intracellular and extracellular compartments. Osmolarity of the two compartments is, however, equal and both compartments are electroneutral. The osmotic pressures due to these electrolytes are balanced.

Compared to the electrolyte concentrations, the colloidal protein concentration is very low. Yet, there is a difference in the protein concentration among blood plasma, interstitial fluid and intracellular fluids. The difference in the protein concentration gives **blood plasma** a **higher osmotic pressure** than that exhibited by the **interstitial fluid**. **Colloid osmotic** pressure is termed **oncotic** pressure (see subchapter 3.2.3.5.). The oncotic pressure is the property of colloid solutions.

Tab. 3.5. Electrolyte content of body fluids

Solute	Osmotically active concentration – osmolarity (mmol.l^{-1}) of main solutes in the body fluids (average values)		
	Extracellular fluids		Intracellular fluids
	Blood plasma	Interstitial fluids	
Na^+	144	137	10
K^+	5	4.7	141
Ca^{2+}	2.5	2.4	0
Mg^{2+}	1.5	1.,4	31
Cl^{-1}	107	112.7	4
HCO_3^-	27	28,3	10
HPO_4^{2-} , $H_2PO_4^-$	2	2	11
SO_4^{2-}	0.5	0.5	1
proteinate	1.2	0.2	4
other solutes	13	13	90.2
total osmolarity	303.7	302.2	302.2

3.2.3.2. Ionic strength

The ionic strength of a solution is a measure of electrolyte concentration and is calculated by:

$$\mathbf{I = \frac{1}{2} \sum c_i z_i^2}$$

where c_i is the molar concentration of a particular ion and z is the charge on the ion. The sum is taken over all ions in the solutions. Due to the square of z_i multivalent ions contribute strongly to the ionic strength.

For a 1:1 electrolyte such as **sodium chloride**, where each ion (cation or anion) is singly - charged, the ionic strength is equal to the concentration.

For the electrolyte **$MgSO_4$**, however, each ion is doubly-charged, leading to an ionic strength that is four times higher than an equivalent concentration of sodium chloride:

$I = \frac{1}{2} (c(+2)^2 + c(-2)^2) = 4c$

3.2.3.3. Importance of the ionic strength

A number of biochemically important events (for example: **protein solubility** and **rates of enzyme action**) vary with the ionic strength of a solution.
The solubility of proteins is strongly dependent on the salt concentration (ionic strength) of the medium. Proteins are usually poorly soluble in pure water. Their solubility increases as the ionic strength increases, because more and more of the well-hydrated inorganic ions are bound to protein´s surface, preventing aggregation of the molecules (**salting in**). At very high ionic strengths, the salts withdraws the hydrate water from the proteins and thus leads to the aggregation and precipitation of the molecules (**salting out**). For this reason, adding salts such

as ammonium sulphate $(NH_4)_2SO_4$ makes it possible to separate proteins from a mixture according to their degree of solubility (fractionation).

3.2.3.4. Calculations of osmolarity and ionic strength – practical examples

1. **Calculate the ionic strength of $FeSO_4$ with concentration 0.02 mol.l^{-1}.**
   $I = ½ (1 \times 0.02 \times (+2)^2 + 1 \times 0.02 \times (-2)^2) = 0.08$ mol.l^{-1}

2. **Calculate osmolarity and the ionic strength of K_2SO_4 with concentration 0.1 mol.l^{-1}.**
   $c_{osm} = 3 \times c = 0.3$ mol.l^{-1}
   $I = ½ (2 \times 0.1 \times (+1)^2 + 1 \times 0.1 \times (-2)^2) = 0.3$ mol.l^{-1}

3. **Calculate osmolarity and the ionic strength of a solution containing 0.02 mol.l^{-1} NaCl and 0.02 mol.l^{-1} $BaSO_4$.**
   $c_{osm} = 2 \times 0.02 + 2 \times 0.02 = 0.08$ mol.l^{-1}
   $I = ½ (1 \times 0.02 \times (+1)^2 + 1 \times 0.02 \times (-1)^2 + 1 \times 0.02 \times (+2)^2 + 1 \times 0.02 \times (-2)^2) = 0.10$ mol.l^{-1}

4. **Calculate concentration c of K_3PO_4 solution which is isoosmotic with physiological solution.**
   c_{osm} (K_3PO_4) = c_{osm} (NaCl) = 0.30 mol.l^{-1}
   $4 \times c = 0.30$ mol.l^{-1}
   $c = 0.075$ mol.l^{-1}

5. **Calculate concentration c of $CaCl_2$ solution which is isoosmotic with K_3PO_4 solution with concentration 5 mmol.l^{-1}.**
   c_{osm} ($CaCl_2$) = c_{osm} (K_3PO_4)
   $3 \times c = 4 \times 5$ mmol.l^{-1} = 20 mmol.l^{-1}
   $c = 6.66$ mmol.l^{-1}

3.2.3.5. Colloid osmotic pressure

Colloid osmotic pressure is termed **oncotic** pressure. It is represented (caused) by the blood components (proteins) which can not pass the membrane to reach the same concentration on both sides of capillary membranes. Although blood oncotic pressure represents only 1 % of the total blood osmotic pressure, it is still of a great importance. Small ions and molecules can be dialyzed in both directions between the blood and the interstitial compartment. Large protein molecules do not have this ability, therefore their presence produces excess osmotic pressure of blood relative to the interstitial fluid.

Due to the higher blood colloid osmotic pressure, interstitial fluid tends to dialyze into circulation (into blood) to maintain the equilibrium of osmotic pressures. Blood pressure at the arterial side of the capillary network is sufficient to prevent this dialysis. Water and dissolved substances leave the blood for interstitial fluid instead. Substances are exchanged. Nutrients are transferred into cells and cell waste products are removed. At the venous side of capillaries, blood pressure is too low to prevent natural diffusion of the fluid back to circulation. The fluid contains the waste products. These mutual relationships are shown in Fig. 3.7.

The important function of oncotic pressure is to **maintain water within capillaries**. If capillaries become more permeable for proteins (in certain abrupt impairments, e.g. during surgical procedures or extensive burns), proteins migrate from blood. Proteins loss results in the loss of blood oncotic pressure that has been assisting in return of fluids from tissues back to blood. As a result, the total blood volume decreases rapidly, what drastically reduces the ability of blood to transfer oxygen and to eliminate carbon dioxide (CO_2). Decrease of blood volume associated with insufficient brain oxygen supply leads to shock.

Decreased blood protein concentration (hypoproteinemia) is observed also under other conditions as well, e.g. during kidney diseases and starvation. Although it is a slow process, it can lead to a decrease of colloid osmotic pressure as well. Liquid is accumulated in interstitial compartments, resulting in **edema** development. Edema occurs at certain stages of starvation, when organism starts to metabolize circulating proteins, due to their absence in the diet. The same occurs when oncotic pressure in tissues is increased (during inflammation), water is transferred to tissues and causes edema.

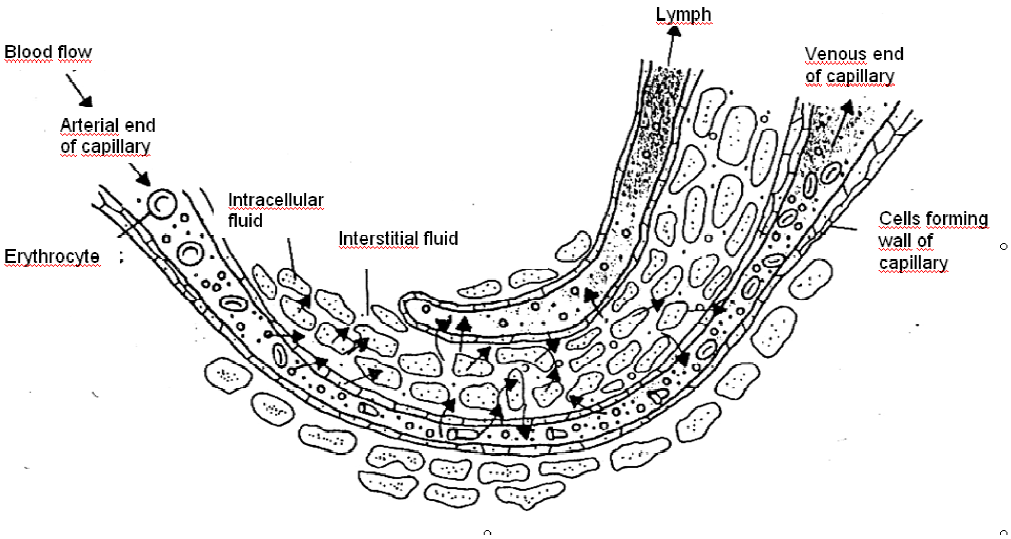


Fig. 3.7. Exchange of compounds between blood and tissues

3.3. Colloidal solutions

3.3.1. Properties of colloidal dispersions

Colloids are defined not by the kind of matter they contain but by the size of the particles involved. A **colloidal dispersion** is a mixture in which the dispersed particles have diameters in the range of 1 nm to 1000 nm and consist of very large clusters of ions or molecules.

High–molecular weight (**macromolecular) compounds (e.g. proteins, polysaccharides**), in the process of dissolution spontaneously form colloidal solutions. Low–molecular weight compounds may form colloidal solutions as a consequence of clustering of molecules into aggregates – **micelles** (e.g. soap solutions).

In organism there are many substances in colloidal state. Almost all reactions proceed in a colloid environment. Colloidal state allows formation of the large surface needed for many chemical reactions. Colloids are rich in water which is also necessary for the reactions. All proteins in the organism are in a colloidal state.If a native protein loses water, it loses also the colloidal character and its vital functions are diminished. Properties of dispersed systems are presented in Tab. 3.1.

Colloidal particles may be separated from analytical particles by the dialysis. Colloidal dispersion properties are determined by the character of a bond between dispersed particles and a dispersion medium. Blood carries many substances in colloidal dispersions, including a variety of proteins.

When colloidal dispersions are in a fluid state, the dispersed particles, although large, are not large enough to be trapped by the ordinary filter paper during filtration. They are large enough to reflect and scatter light. Light scattering by a colloidal dispersion is called the **Tyndall effect**, after the British scientist John Tyndall. The effect is responsible for the milky, partly obscuring character of smog, or the way sunlight sometimes seems to stream through a forest canopy.

When a colloidal dispersion is fluid, the large colloidally dispersed particles eventually settle down due to gravity. The settling process can take time ranging from a few seconds to many decades, depending on the system. One of the factors that keeps the particles dispersed is their constant bumping by molecules of the solvent. Evidence for this bumping can be seen by looking at the colloidal system under a good microscope. You can't actually see the colloidal particles, but you can see the light scintillations caused as they move erratically and unevenly about. This motion of colloidal particles is called the **Brownian motion**.

Emulsions are colloidal dispersions of two liquids. An emulsion may be prepared by shaking together two immiscible liquids. Emulsions usually are not stable (e.g. the oil soon separates from the aqueous layer). An emulsion can be stabilized by a third component called **emulsifying agent**.

Biologically important emulsifying agents are salts of **bile acids**. They are important for digestion and resorption of nutrients. By the time dietary lipids (fats) have passed through the stomach and entered the small intestine, it

has been separated into liquid droplets suspended in an aqueous medium. In the intestine, emulsification is accomplished by the action of biologically surface-active compounds - salts of bile acids. Their presence at the oil–water boundary combined with the mechanical action of the intestine emulsifies the lipid droplets, greatly increasing their total surface area. The increase in the surface area of lipid droplets results in a similar increase in the rate of their digestion.

Dispersed colloidal particles are usually electrically charged. In colloidal particles there are forces which prevent their aggregation, expansion, as well as sedimentation. These forces result from the electric charge of colloidal particles. As particles contain the charge they can move in the direct electric field (electrophoresis). Electric charge on the surface of particles is formed by dissociation of functional groups of molecules. If a particle contains acidic –COOH group, anion $–COO^-$ is formed, if it contains basic $–NH_2$ group, cation NH_3^+ is formed. In case the particles are amphoteric (they contain both acidic and basic groups in their molecules), according to the pH of environment zwitterion (dipolar ion), anion or cation will be formed.

Colloidal systems demonstrate **colloidal osmotic – oncotic pressure** (see subchapter 3.2.3.5.) and the **zeta potential**.

3.3.2. Classification of colloidal systems

Types of colloidal and crude-dispersed systems according to the state of dispersed particles and dispersed medium are illustrated in Tab. 3.2.

Classification of colloidal dispersions according to their properties:

1. **lyophilic (hydrophilic) – sols,**
2. **lyophobic (hydrophobic) – sols,**
3. **micelles – association colloids.**

3.3.2.1. Lyophilic colloids

Lyophilic colloids are formed in spontaneous dissolving of macromolecular substances. They are mainly formed of high molecular weight organic compounds. The particles of lyophilic colloids are stabilized in a solution (prevention of aggregation) by solvation (hydration) shell, i.e. oriented solvent molecules (Fig. 3.8.). The loss of hydration shell after an excess of a neutral salt (electrolyte) is added into the solution results in salting out (precipitation) of particles from the solution. The living cells represent solutions of lyophilic colloids (as well as crude dispersions).

Under the certain conditions, the dispersed phase in a colloidal system **coagulates** so that the whole mass, including the liquid, set to an extremely viscous body known as the **gel**. Because the formation of a gel is accompanied by taking up water or some other solvents, the gel is said to be hydrated or solvated. Apparently, the fibers of the dispersed substance form a complex three–dimensional network, the interstices being filled with a medium or a dilute solution of a dispersed phase (Fig. 3.9).

Globular proteins, usually form colloidal solutions of normal viscosity – **sols.** Sols that adopt a semisolid, semirigid form are gels. From macromolecular compounds, gels arise by swelling in a solvent – acceptation of water molecules by solid polymers.

Gels exhibiting high viscosity are formed from fibrous proteins (gelatin from collagen, polysaccharide gels – dextran, sephadex). Gels are elastic and keep the stable shape.

Gels represent the most important colloidal systems in nature, give to living cells elasticity and flexibility, allow electrolyte diffusion, exert high surface for metabolic reactions to rapidly proceed.

The cellular wall of the living cells is colloidal, and within the cell there is a gel. Gels undergo aging**,** particles coagulate, gel volume diminishes and water is displaced.

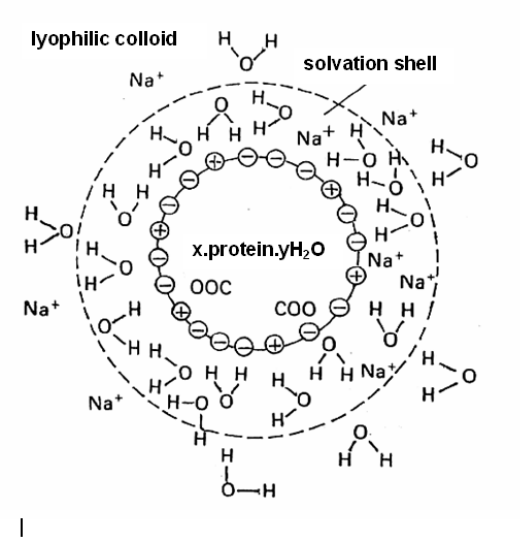


Fig. 3.8. Lyophilic colloid particle

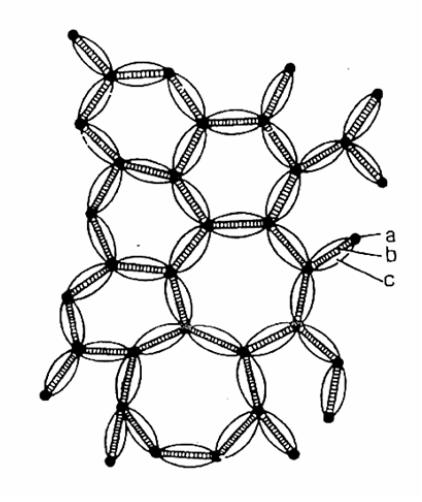


Fig. 3.9.Structure of gel (a – hydrophobic part, b – hydrophilic, c – hydration shell)

3.3.2.2. Lyophobic colloids

Lyophobic colloids are made by aggregation of low molecular weight substances in a solution. They are formed of clusters of inorganic molecules, e.g. As_2S_3 and their electric charge keeps them in the solution. The charge loss or its decrease results in the coagulation (formation of aggregates). Addition of an electrolyte results in irreversible separation of colloidal particles from the solution. This thickening causes formation of crude dispersions, which are visible in electron microscope.

3.3.2.3. Association colloids – micelles

Association colloids – micelles, are formed by dissolving the low-molecular **amphipathic compounds**. Amphipathic compound is a compound with both hydrophilic – polar, and hydrophobic (lyophilic) – nonpolar groups in its molecule. The molecules of amphipathic compounds, when mixed in the right proportion of water, spontaneously become grouped into colloidal particles – micelles (Fig. 3.10.). A micelle is a globular aggregation, in which **t**he **hydrophobic tails** gather together and the **hydrophilic heads** have maximum exposure to the aqueous medium. Soaps and detergents are examples of amphipathic compounds.
Amphipathic compounds **form the basic architecture of animal cell membranes. Cell membranes are made of phospholipids and proteins. Phospholipids are amphipathic substances. They form micelles of extended shape, that would produce two rows of molecules or a lipid bilayer arrangement.**
In the lipid bilayer of cell membranes, hydrophobic groups become positioned within the membrane and the hydrophilic heads face the water at each surface of the bilayer **(see also Fig. 8.9. in subchapter 8.3.)**

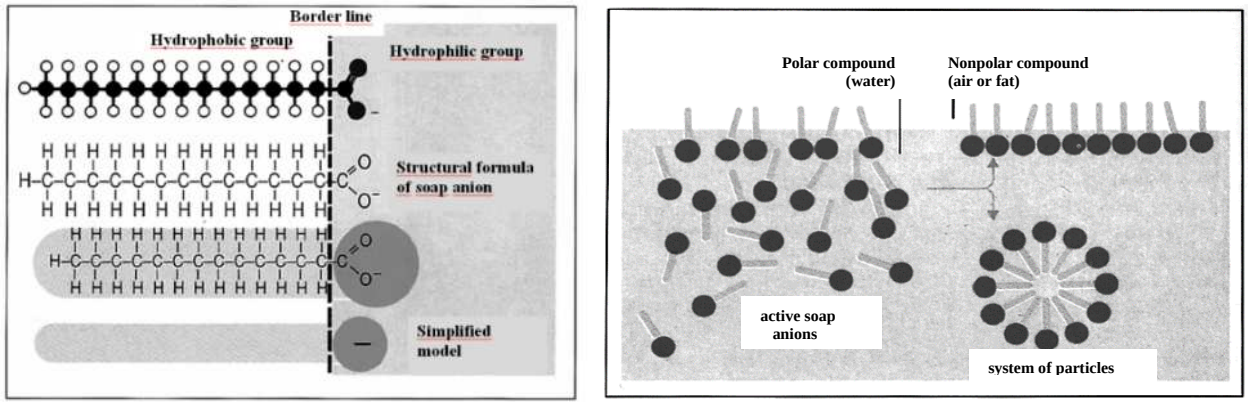


Fig. 3.10. Structure of soap anions (left) and a micelle (right) in water

The long–chain carboxylic acids are insoluble in water, but their salts (sodium, potassium) – soaps are almost completely miscible with water. However, they do not dissolve as we might expect, that is, as individual ions. When soap is shaken with water it forms a **colloidal dispersion** – not a true solution. These soap solutions contain **aggregates of soap molecules** called **micelles** (Fig. 3.10.). Soap micelles are usually spherical clusters of carboxylate ions that are dispersed throughout the aqueous phase. So, the polar, or **hydrophilic** ends of the molecule on the surface of the micelle that is presented to the water and the nonpolar, or **hydrophobic** (**lipophilic**), carbon chains are directed toward the center of the micelle. The outer part of each micelle is negatively charged, and the positive sodium ions congregate near the micelles (the sodium salts are scattered throughout the aqueous phase as individual solvated ions).

The nonpolar alkyl chains of the soap remain in a nonpolar environment – in the interior of the micelle. The polar carboxylate groups are exposed to a polar environment – that of the aqueous phase. Because the surface of micelles is negatively charged, individual micelles repel each other and remain dispersed throughout the aqueous phase.

Soaps serve their function as dirt removers in a similar way. Most dirt particles (for example on the skin) become surrounded by a layer of an oil or fat. Water molecules alone are unable to disperse these greasy globules because they are unable to penetrate the oily layer and separate the individual particles from each other or from the surface to which they are stuck. Soap solutions, however, are able to separate the individual particles because hydrocarbon chains can dissolve in the oily layer (Fig. 3.11.). As this happens each individual particle develops an outer layer of carboxylate ions and presents the aqueous phase with a much more compatible exterior – a polar surface. The individual globules now repel other and thus, become dispersed throughout the aqueous phase, shortly thereafter they make their way down the drain. Unusually low surface tension is a soap property, which gives a soap solution more wetting power than plain water has. A combination of the emulsifying power and the surface action of soap solutions enables them to detach dirt, grease, and oil particles from the surface being cleaned and to emulsify them so that they can be washed away.

Synthetic detergents function in the same way as soaps, they have long nonpolar alkane chains with polar groups at the end. The polar groups of most synthetic detergents are sodium sulfonates or sodium sulfates.

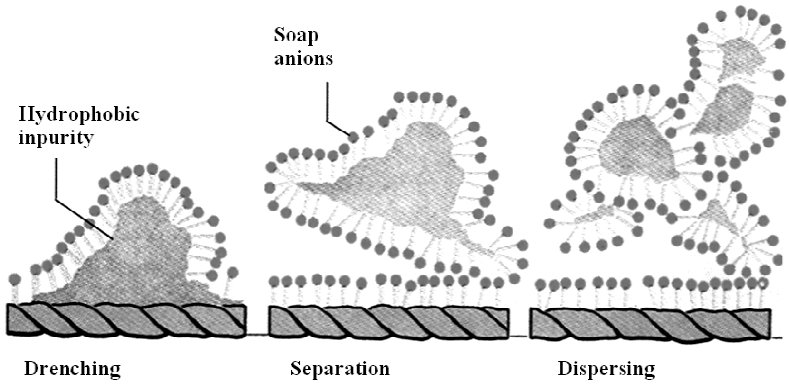


Fig. 3.11. Surface activity of soap anions, micelle

3.3.3. Biological importance of colloids and colloidal systems

- all living organisms are composed of highly structured colloidal systems
- protoplasm is a viscous (semi-fluid, jelly-like) substance. It is surrounded by the cell wall.
- every cell has an internal colloidal system arranged in patterns to create specific functions
- blood, lymph, proteins, mucus, cytosol, nucleus, cell membranes are biocolloids
- high dispersity of colloidal particles play a role in adsorption processes
- the changes of colloidal osmotic state of cytosol influence distribution of water between the cell and its environment, which may lead to edema formation

Control questions

1. What is the definition of true solutions?
2. What is the definition of diffusion?
3. What is the definition of osmosis?
4. What is the definition of dialysis and practical use of hemodialysis in medicine?
5. What is the definition of semi-permeable membrane?
6. Explain the difference between true and colloid solutions.
7. What is the definition of lyophobic colloids?
8. What is the definition of lyophilic colloids?
9. What is the definition of colloidal dispersions?
10. What is the definition of suspensions?
11. What is the Tyndall effect?
12. What are the colligative properties of solutions?
13. What is the definition of osmotic pressure?
14. What is the definition of oncotic pressure?
15. Explain the hemolysis of erythrocytes.
16. What is the definition of emulsions? Give an example of biological important emulsifying agent.
17. What are the emulsifying agents and what is their role in making emulsions?
18. What are sols?
19. What are gels?
20. What are the association colloids-micelles?
21. What is the definition of amphipathic compounds?
22. Explain the conditions of edema development.
23. Explain the biological importance of colloids.

4. BASIC CHEMICAL PROCESSES. pH. ACIDS AND BASES. BUFFERS

A reaction means a change. Chemical reactions involve rearrangements in the electron layers of atoms resulting in a conversion of reactants to products. During this process following parameters can be evaluated:

1. The rate of a reaction (reaction kinetics).
2. State of equilibrium and its properties (chemical equilibrium).
3. Changes in energy (energetics of chemical reactions).

Quantitative and qualitative aspects of a chemical process can be described by a chemical equation. This equation must meet the principle of mass conservation and electroneutrality. This principle implies that mass can neither be created nor destroyed, although it may be rearranged in space. Thus, during any chemical reaction in an isolated system, the total mass of the reactants or starting materials must be equal to the mass of the products.

Chemical reactions can be classified in many ways. In living systems there are four particularly important types of chemical processes:

1. Acid-base reactions (proton transfer between acid and base).
2. Oxidation-reduction reactions (electron transfer between reacting substances).
3. Displacement reactions (precipitation reactions – formation of volatile products).
4. Reactions of complex formation or decomposition (formation or cleavage of coordination bond).

4.1. Reaction rates (chemical kinetics)

Chemical reactivity is controlled by two broad factors: thermodynamics and kinetics (Fig.4.1.).

Thermodynamics considers these questions: which state is more stable? Reactants or products? Should this reaction occur? Kinetics- the subject of this chapter- considers the question: what controls the rate of a reaction?

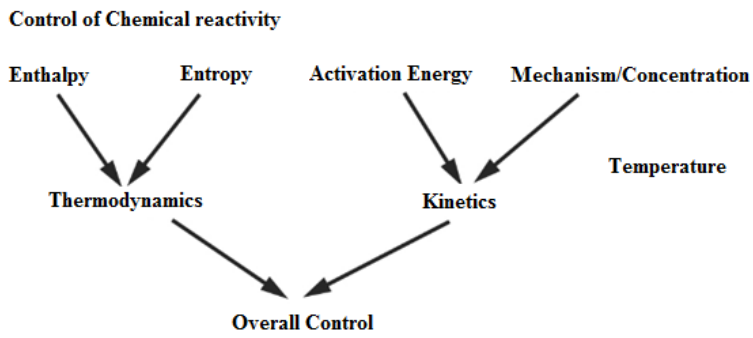


Fig. 4.1. Chemical reactivity and its control

In order for a reaction to occur, it must be both thermodynamically and kinetically favored.

Kinetic control of a reaction arises from the manner in which the reaction takes place- its mechanism, the energy barrier required to be overcome- the activation energy and many other factors such as the concentration of reactants, pressure, temperature, and enzyme activity, can impact the rate of a reaction.

The rate of a chemical reaction is a measure of how quickly reactants are converted into products. Chemical reactions occur due to collisions of molecules, atoms or ions involved in the reaction. Upon collision, some bonds are broken and others formed. The collisions must provide a minimum of energy (the *activation energy*) to start a chemical reaction.

The interval required for a chemical change or reaction to occur is called the reaction time. Every reaction has its own unique reaction time and a reaction rate.

The reaction rate (v) **is defined as a change of concentration of a reactant (or formed products) per time unit.**

$$\text{Rate} = \frac{\text{change in reactant concentration}}{\text{time interval}}; \qquad v = \frac{dc}{dt}$$

The reaction reaches equilibrium when its rate is constant. General definition of concentration effect on rate of chemical reactions is specified in **Guldberg-Waage law** or **rate equation (the Law of Mass Action):** the rate of a chemical reaction is proportional to the concentrations of the reacting substances. Consider a typical chemical reaction::

$$A + B \rightarrow C$$

The rate (v) of the reaction is:

$$v = k\,[A]\,[B] \qquad (\text{or: } v = k\,c_A\,c_B)$$

where [A] and [B] are the molar concentrations of a reactant A and B ($mol.l^{-1}$), and k is the rate constant.

Rate constant *k* is a main kinetic feature (characteristics) of a certain reaction. It gives the rate of reaction in which reacting substances are in unit concentrations. It depends on the properties of reacting substances and temperature. The higher k value, the faster the reaction proceeds. The rate constant is related to the activation energy by a relationship known as the **Arrhenius equation**:

$$k = A \times e^{-Ea / RT}$$

where: R is the gas constant (8.314 JK^{-1} mol^{-1}), T is the temperature on the Kelvin scale, E_a is the activation energy in joules per mole, and A is a constant called the frequency factor, which is related to the frequency of collisions and the orientation of the reacting molecules.

In a chemical reaction the concentration of reactants and products change with time (Fig. 4.2.). The concentration of reactant A and reactant B decreases, and the concentration of product C increases. The rate is positive for the appearance of a product and for the disappearance of reactants. When the concentrations of products and reactants no longer change with the time, a chemical reaction has reached equilibrium.

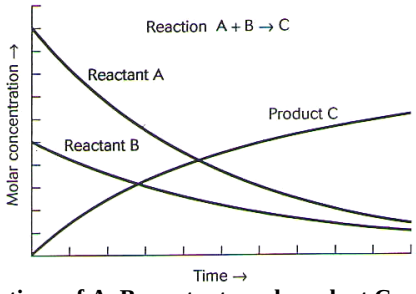


Fig. 4.2. Concentrations of A, B reactants and product C as a function of time

A reaction can be also characterized by the half -time of the reaction. **The half-time** of a reaction (t½) is the time required to consume the half amount of original concentration of the reactant. As example, the half-time of reaction is used for expression of radioactive decomposition (breakdown) or for elimination/chemical degradation of xenobiotics (toxicants, drugs) from organism.

4.1.1. Temperature

The rates of chemical reactions increase with a rise in temperature. Some reaction rates are very sensitive to temperature changes, whereas other are only slightly affected. The reason is that all reactions have an "activation barrier," i.e. an energy level that must be overcome before the start of reactant conversion to products. Therefore, temperature and activation energy are important factors in the control reaction rate. The lower the activation energy, the higher the reaction rate, and vice versa. The reason is because, molecules are constantly in motion and collide with one another in solution, and any collision such as this can create a chemical reaction between

them. If the energy released from the collision is higher than that of the activation energy required for the creation of the intermediate, the chemical reaction will take place. If no, the chemical reaction will not start. But the high activation energy can be overcome with higher temperatures. A general rule that can be used is that on the average a 10 ºC rise in temperature doubles the reaction rate. Temperature and activation energy effects are part of the rate constant, k, as described by the Arrhenius equation.

In living organisms, chemical reactions are dramatically affected by changes in temperature. Since these reactions run under enzyme actions, the effect of temperature on the reaction rate is limited by enzyme denaturation at the higher temperature, as stated later. In general, temperature rising results in an increased metabolic rate. On the contrary, temperature lowering slows down metabolism of an organism. The technique of the body temperature lowering (hypothermia) in patients undergoing a surgery can be used to prevent deterioration of vital tissues. As example, hypothermia is often used when blood circulation is interrupted under heart surgery. When surgery is completed and circulation resumed, the patient body temperature is increased gradually.

4.1.2. Catalysts

A **catalyst** is a substance that increases a reaction rate when it is present in the reaction mixture. Catalysts serve one of two functions: making reactions faster or making them more "selective." In either case, the catalyst provides an alternative mechanistic pathway for the reaction, which changes the activation energy and changes the rate. Catalysts are usually required only in small amounts and their amount is the same (unchanged before and after the reaction.

Basic features/characteristics of a catalyst:

1. It lowers the activation energy of a reaction.
2. It does so by becoming an active participant in the chemical process, but it emerges unchanged.
3. It does not alter the results of a reaction; it changes only the speed at which the reaction takes place.
4. Any catalyst for the forward reaction of a chemical equilibrium also acts as a catalyst for the reverse reaction.

Catalysts in animals and plants allow these organisms to carry out reactions at rates sufficient for the organism to survive. Catalysts in organisms are called **enzymes** (see subchapter 12.).

4.2. Oxidation-reduction reactions

Oxidation-reduction (redox) reactions take place in the world at every moment. In fact, they are directly related to the origin of life. For instance, oxidation of nutrients forms energy and enables human beings, animals, and plants to thrive.

An oxidation-reduction (redox) reaction is a type of chemical reaction that involves a transfer of electrons between two species. Oxidation is loss of electrons (or hydrogen), reduction is gain of electrons (hydrogen).

An oxidizing agent (oxidant) is substance which oxidizes something else. A reducing agent (reductant) reduces something else.

In a redox reactions, the number of electrons lost by the **reductant** must be equal to the number of electrons gained by the **oxidant** (Tab. 4.1.). To decide when a redox reaction has taken place, we assign a number to an atom that defines its **oxidation state**, called its **oxidation number**. If that number undergoes a change as a result of a chemical reaction, the reaction is a redox reaction. Furthermore, the changes in oxidation number of reductant and oxidant are used to balance redox reaction equations.

Tab. 4.1. Three different definitions of oxidation and reduction

Oxidation		Reduction
Gain oxygen	← O	Lose oxygen
Lose hydrogen	→ H	Gain hydrogen
Lose electrons	→ e^-	Gain electrons

Oxidation and reduction go hand in hand. You cannot have one without the other. When one substance is oxidized, another must be reduced. In reaction:

$$Cu + Cl_2 \longrightarrow CuCl_2$$

Copper is oxidized and chlorine is reduced. Chlorine is called the **oxidizing agent**, that is, the agent that brings about the oxidation of another substance. Copper is the **reducing agent**; it supplies the electrons that cause chlorine to be reduced. Because reduction and oxidation are always paired, the term **redox** is often used to describe these reactions.

Oxidation numbers are numerical values assigned to elements in compounds according to the following rules:

1. The oxidation number of free elements is zero (0).
2. In compounds, Group IA and Group IIA elements are assigned oxidation numbers of +1 and +2, respectively.
3. In most compounds, hydrogen has an oxidation number +1.
4. In most compounds, oxygen has an oxidation number −2 (in peroxides is −1).
5. For compounds, the algebraic sum of all the oxidation numbers must be 0.
6. For ions, the algebraic sum of all the oxidation numbers must equal the charge on the ion.

The special case of oxidation-reduction reaction is **dismutation (disproportionation)**. It is the reaction in which one compound containing certain element in the "middle" oxidation number (e.g. 0) changes to two products. In one of them the element has a lower oxidation number (e.g. −1) than in the initial compound and in the other product the element has a higher oxidation number (e.g. +1). One element is simultaneously reduced and oxidized to form two different products. For example:

$$Cl_2^{(0)} + H_2O \longrightarrow HCl^{(-1)} + HOCl^{(+1)}, \quad \text{or} \quad 2\,O_2^{\cdot -} + 2\,H^+ \xrightarrow{SOD} O_2 + H_2O_2$$

where SOD is enzyme superoxide dismutase (see subchapter 13.2.1.).
Autooxidation is oxidation process running under action of air oxygen on compound at normal or mildly increased temperature.

4.2.1. Biological oxidation-reduction reaction

Although oxidation and reduction must occur together, it is convenient when describing electron transfer to consider the two halves of an oxidation-reduction reaction separately. For example oxidation of ferrous ion by cupric ion:

$$Fe^{2+} + Cu^{2+} \longleftrightarrow Fe^{3+} + Cu^{+}$$

This reaction can be described in terms of two half-reactions:

$$(1)\quad Fe^{2+} \longleftrightarrow Fe^{3+}$$
$$(2)\quad Cu^{2+} \longleftrightarrow Cu^{+}$$

The electron-donating ion, reducing agent or reductant is Fe^{2+}; the electron-accepting ion, oxidizing agent or oxidant is Cu^{2+}. A given agent, such as an iron cation existing in the ferrous (Fe^{2+}) or ferric (Fe^{3+}) state, functions as a conjugate reductant-oxidant pair (redox pair). In redox reactions we can write equation:

$$\text{electron donor} \longleftrightarrow e^- + \text{electron acceptor}$$

In the reversible half-reaction (1) together Fe^{2+} and Fe^{3+} constitute a **conjugate redox pair**.

The electron transfers in the oxidation-reduction reaction of organic compounds are not fundamentally different from those of inorganic species. In biological systems, oxidation is often synonymous with **dehydrogenation**, and many enzymes that catalyze oxidation reactions are **dehydrogenases**.

Electrons are transferred from one molecule (electron donor) to another (electron acceptor) in one of four different ways:

1. Directly as *electrons*. For example, the Fe^{2+} / Fe^{3+} redox pair can transfer an electron to the Cu^+ / Cu^{2+} redox pair:

$$Fe^{2+} + Cu^{2+} \longleftrightarrow Fe^{3+} + Cu^{+}$$

2. As *hydrogen atoms*. A hydrogen atoms consist of a proton (H^+) and a single electron (e^-). In this case we can write the general equation: $AH_2 \leftrightarrow A + 2\ e^- + 2\ H^+$, where AH_2 is the hydrogen/electron donor. AH_2 and A together constitute a conjugate redox pair (A / AH_2), which can reduce another compound B (or redox pair, B / BH_2) by transfer of hydrogen atoms:

$$AH_2 + B \longleftrightarrow A + BH_2$$

3. As a *hydride ion* ($:H^-$), which consists of two electrons and one proton. This occurs in the case of NAD-linked dehydrogenases (described in subchapter 12.2.1.).

4. Through direct *combination with oxygen*. In this case, oxygen combines with an organic reductant and is covalently incorporated in the product, as in the oxidation of a hydrocarbon to an alcohol:

$$R{-}CH_3 + \tfrac{1}{2}\,O_2 \longleftrightarrow R{-}CH_2OH$$

The hydrocarbon is the electron donor and the oxygen atom is the electron acceptor.

4.2.2. Reduction potentials

When two conjugate redox pairs are together in solution, electron transfer from the electron donor of one pair to the electron acceptor of the other may occur spontaneously. The tendency for a reaction depends on the relative affinity of the electron acceptor of each redox pair for electrons. **The standard reduction potential (E^0)** is the tendency for a chemical species to be reduced, and is measured in volts at standard conditions. Electrochemists have chosen as a standard of reference the half-reaction:

$$H^+ + e^- \longleftrightarrow \tfrac{1}{2}\,H_2$$

The electrode at which this half-reaction occurs is arbitrarily assigned a standard reduction potential of 0.00 V. When this hydrogen electrode is connected through an external circuit to another half-cell in which an oxidized species and its corresponding reduced species are present at standard concentration (each solute at 1 $mol.l^{-1}$, each gas at 101.3 kPa, or 1 atm), electrons tend to flow through the external circuit from the half-cell of lower standard reduction potential to the half-cell of higher standard reduction potential. By convention, the half-cell with the stronger tendency to acquire electrons is assigned a positive value of E°.

The reduction potential of a half-cell depends not only on the chemical species present but also on their activities, approximated by their concentrations. W. Nernst (Nobel price in year 1920) derived an equation that relates standard reduction potential (E°) to the reduction potential (E) at any concentration of oxidized and reduced species in the cell:

$$E = E^\circ + \frac{RT}{nF} \ln \frac{[\text{electron acceptor}]}{[\text{electron donor}]}$$

where R is gas constant (8.314 $JK^{-1}mol^{-1}$), T is temperature (Kelvin degree), n is the number of electrons transferred per molecule, and F is the Faraday constant ($9.68 \cdot 10^4$ $Cmol^{-1}$). At 298 K (25 °C), this expression reduces to:

$$E = E^\circ - \frac{0.026}{n} \ln \frac{[\text{electron acceptor}]}{[\text{electron donor}]}$$

Many half-reactions of interest to biochemists involve protons. As in the definition of $\Delta G'^\circ$(transformed standard Gibs free energy), biochemists define the standard state for oxidation-reduction reactions as pH 7 and express reduction potential as $\Delta E'^\circ$, the standard reduction potential at pH 7. The standard reduction potentials given in Table 4.2. are values of E'° and are therefore only valid for systems at neutral pH. Each value represents the potential difference when the conjugate redox pair, at 1 $mol.l^{-1}$ concentrations and pH 7, is connected with the standard (pH 0) hydrogen electrode.

Tab. 4.2. Standard reduction potentials of some biologically important half-reactions, at 25 °C and pH 7

Half-reaction	E°′ (V)
$2\ H^+ + 2\ e^- \rightarrow H_2$	− 0.420
$NAD^+ + H^+ + 2\ e^- \rightarrow NADH$	− 0.320
Lipoic acid + $2\ H^+ + 2\ e^- \rightarrow$ dihydrolipoic acid	− 0.290
$S + 2\ H^+ + 2\ e^- \rightarrow H_2S$	− 0.243
Glutathione + $2\ H^+ + 2\ e^- \rightarrow$ 2 red. glutathione	−0.230
Riboflavin + $2\ H^+ + 2\ e^- \rightarrow$ dihydroriboflavin	− 0,208
Pyruvate + $2\ H^+ + 2\ e^- \rightarrow$ lactate	− 0.185
Fumarate + $2\ H^+ + 2\ e^- \rightarrow$ succinate	0.031
Cytochrome b (Fe^{3+}) + $e^- \rightarrow$ cyt b (Fe^{2+})	+ 0.075
Ubiquinone + $2\ H^+ + 2\ e^- \rightarrow$ ubihydroquinone	+ 0.100
Cytochrome c (Fe^{3+}) + $e^- \rightarrow$ cyt c (Fe^{2+})	+ 0.254
Cytochrome a (Fe^{3+}) + $e^- \rightarrow$ cyt a (Fe^{2+})	+ 0.290
$O_2 + 2\ H^+ + 2\ e^- \rightarrow H_2O_2$	+ 0.295
$Fe^{3+} + e^- \rightarrow Fe^{2+}$	+ 0.774
$O_2 + 4\ H^+ + 4\ e^- \rightarrow 2\ H_2O$	+ 0.815

Oxidation-reduction potentials are of great *importance for medicine*. Enzyme systems and substrates form **biological series of potentials** in cells, in which each system is theoretically able to reduce the system located below in the series and to oxidize the system located above. The oxidation-reduction potentials of biological redox systems allow to determine the direction and sequence of oxidation-reduction reactions in biological systems. The sequential character of enzymatic reactions in the "respiratory chain" rule out a sharp change of two interacting systems allowing a gradual release of energy during biological oxidation.
Standard reduction potentials can be used to calculate the free-energy change (see also Chapter 5.2.6, eq. 5.11):

$$\Delta G = nF\,\Delta E, \qquad \text{or} \qquad \Delta G'^{\circ} = nF\,\Delta E'^{\circ}$$

4.3. Chemical equilibrium

4.3.1. Electrolytes

All aqueous fluids of the living systems – plants or animals – contain dissolved molecules and ions. Blood, for example, contains sodium and chloride ions at low concentrations, several other ions at even smaller concentrations, as well as molecules of glucose and other molecular compounds. To understand these fluids at their molecular level, therefore, it requires a study of the chemical properties of ions.

Solid ionic compounds do not have electrical conductivity because the ions are not free to move. Electrical conductivity requires the movement of charged particles. In the solid state, electrostatic forces hold the ions together in a crystal lattice structure, which is, in short, a 3D interconnected ion network. In a liquid, the ionic compound dissociates into its respective ions. Ions can move and conduct electricity. This flow of ions is an electric current. Charge-motion is required for there to be electric current. For this reason, ionic compounds are called **electrolytes**. Water and compounds such as glucose, ethanol, gasoline, which when dissolved in water do not dissociate to ions and conduct electricity, are called **nonelectrolytes.**

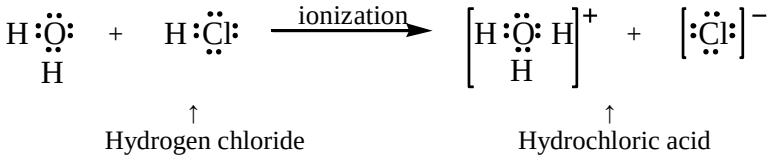
When an electrolyte (ionic compound) dissolves in water, we say that **dissociation** occurs because the oppositely charged ions separate from each other and form ions. Certain chemical systems except electrically neutral molecules contain the particles with electric charge. While dissolving, many substances spontaneously fall into electrically charged particles, which were named **ions** by Faraday. Ions can have positive or negative charge. Ions with a positive charge are **cations**, and with a negative charge are **anions**.

As the molecules have the total charge zero, in solution there is the same number of positive charges on cations and negative charges on anions (electroneutrality law). According to the number of ions into which electrolytes fall, these can be divided into *binary* electrolytes (2 ions, e.g. KCl), *ternary* electrolytes (3 ions, e.g. $ZnCl_2$) and *quaternary* electrolytes (4 ions, e.g. $FeCl_3$).

Free ions can be formed for example during the dissolving of ionic crystals, which create the structure of most salts. Attractive forces between the ions in crystal grate are weakened by solvent, because ions are coated with

molecular dipoles of the solvent. This interaction is called **solvation**, in case of water solutions **hydration of ions**.

Apart from dissociation, many molecular compounds (non-ionic, polar covalent compounds) can also generate ions in water by a different way – in the process of ionization. **Ionization** is the formation of ions by a chemical reaction of a molecular compound with the solvent (interaction between the molecules of a dissolved compound and molecules of a polar solvent). Hydrogen chloride, for example, undergoes ionization as it dissolves in water. We can describe this reaction as follows:



Pure hydrogen chloride, either as a gas or a liquid, contains no ions. Yet when HCl (g) dissolves in water, essentially 100 % of its molecules react with water to give hydronium and chloride ions. This solution tis called *hydrochloric acid*.

The passage of electricity through a solution with dissolved ions is called **electrolysis.** Electricity in metals is a flow of electrons. The atoms of metal elements are characterized by the presence of valence electrons - electrons in the outer shell of an atom that are free to move about. Because valence electrons are free to move they can travel through the lattice that forms the physical structure of a metal. These "free electrons" allow metals to conduct an electric current. Based on this property of metals, **electrodes** are manufactured. An electrode is used to take an electric current to or from a source of power, a piece of equipment, or a living body. Electrodes are used to provide current through nonmetal objects to alter them in numerous ways and to measure conductivity for numerous purposes. Examples include electrodes for medical purposes (EEG, ECG, defibrillator), for electrophysiology techniques in biomedical research, for chemical analysis using electrochemical methods (measurement of pH) etc. The negative electrode is called the **cathode** and it attracts positively charged ions - cations

The negative ions or anions are naturally attracted to the **anode**, the positive electrode.

Molten ionic compounds are also electrolytes. For electrolysis to happen, ions must be mobile. When ions are immobilized in the solid state, no electrolysis occurs. If the solid, ionic compound is heated until it melts, however, than the ions become mobile, and molten salts conduct electricity. Thus the term *electrolyte* can refer either to a solution of ions or to the pure, solid ionic compound.

Electrolytes are not equally good at enabling the flow of electricity. A **strong electrolyte is** a substance which is fully dissociated in water solutions (for example all soluble salts, KOH, HCl). A **weak electrolyte** is a substance that generates ions in water (solvent) only to a small percentage of its molar concentration. Typical examples are aqueous ammonia, acetic acid.

We can summarize the relationships we have just studied as follows. Be sure to notice the emphasis on percentage ionization as the feature dominating these definitions:

Strong electrolyte	- One that is strongly dissociated or ionized in water (solvent) – a high percent ionization
Weak electrolyte	- One that is weakly ionized in water (solvent) – a low percent ionization
Nonelectrolyte	- One that does not dissociate or ionize in water (solvent) – essentially zero percent ionization

4.3.2. Equilibrium in chemical reactions

When reactants first come together - before any products have been formed - their rate of reaction is determined in part by their initial concentrations. As the reaction products accumulate, the concentration of each reactant decreases and so does the reaction rate. However, chemical reactions do not proceed in one direction only. As a reaction occurs, product molecules are formed and they begin to participate in the reverse reaction, which re-forms the reactants.. Thus, the forward and backward reactions can occur at the same time. After some time, the

rates of the forward and reverse reactions become equal, so that the concentrations of reactants and products stop changing.

When the ***rate of product formation is equal to the rate of reactant formation, an equilibrium is established:***

$$\text{reactants} \underset{\textit{reverse reaction}}{\overset{\textit{forward}}{\rightleftharpoons}} \text{products}$$

Let's consider a general equation

$$a\text{A} + b\text{B} \longleftrightarrow c\text{C} + d\text{D}$$

If reactants A and B are in equilibrium with products C and D. the **equilibrium** for the system is defined by:

$$K_{eq} = \frac{[\text{C}]^c\,[\text{D}]^d}{[\text{A}]^a\,[\text{B}]^b} \qquad (4.1)$$

where brackets indicate equilibrium concentrations of reactants and products; *a*, *b*, *c*, *d* represent the stoichiometric coefficients that balance the equation..

The equilibrium constant (K_{eq}) is proportional to the ratio of products and reactants concentrations. This ratio is a fixed value that is independent of the rate at which the reaction proceeds. The K_{eq} depends on the nature of the reactants and products, the temperature, and the pressure (particularly in reactions involving gases). In the presence of an enzyme or other catalyst, the reaction rate may increase, but the final ratio of product to reactant will always be the same. Because a catalyst accelerates the rates of the forward and reverse reactions by the same factor, it does not change the value of the rate constants of both reactions. Thus, under standard physical conditions (25 °C and 1 atm pressure, for biological systems), the K_{eq} is always the same for a given reaction, whether or not a catalyst is present. Notice, the K_{eq} tells nothing about how fast a reaction proceeds. The equilibrium can however change with temperature. The direction of change depends on whether the reaction is exo- or endothermic. If the reaction is exothermic in the forward direction – evolves heat – the equilibrium constant decreases with increasing the temperature. For reactions that are endothermic in the forward direction, the equilibrium constant increases with increasing temperature. The size of $\boldsymbol{K_{eq}}$ indicates the position of equilibrium.

The size of the equilibrium constant tells us about the yield of the reaction. The value of K_{eq} is small, less than 1, when the denominator in the equilibrium law is larger than the numerator. The denominator carries the reactant concentrations, so a larger denominator means that reactants' concentrations are greater than those of the products. A small value of K_{eq} means that the reactants are favored at equilibrium.

Conversely, a value of K_{eq} greater than 1 means that the products are favored, because their molarities appear in the numerator in Equation 4.1. We can summarize the relationships of K_{eq} to position of equilibria as follows:

$K_{eq} < 1$,	reactants are favored at equilibrium
$K_{eq} > 1$,	products are favored at equilibrium

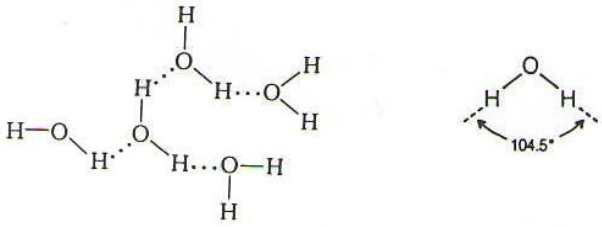
K_{eq} is a constant in the midst of other changes. Two Norwegian scientists Guldberg and Waage were the first to realize this important fact about chemical equilibria. No matter how we try to change the concentrations of individual species in the equilibrium and thereby make the equilibrium shift, the value of K_{eq} remains constant. The concentration of reactants does not necessarily equal the concentration of products at equilibrium. These relative concentrations depend on the particular system considered. What is necessarily true at equilibrium is that each time some reactants changes to a product, an equal amount of product changes back to a reactant. Because molecules are still reacting, even though their concentrations do not change, we say that equilibrium is a **dynamic** situation (dynamic equilibrium). Therefore, an equilibrium may be disturbed. A disturbance to an equilibrium is called a **stress**. The system in chemical equilibrium can be temporarily displaced from equilibrium with changing of conditions: concentration, temperature, (and pressure of gas). A French chemist, H. L. Le

Chatelier, summarized the effect of these variations. His rule is called **Le Chatelier's principle**: **If a stress (such as a change in concentration, pressure, or temperature) is applied to a system in equilibrium, the equilibrium shifts in a way that tends to undo the effect of the stress** (the system adjusts in such a way as to minimize the stress).

4.3.3. Water, its properties and functions

Roughly 60 % of the adult body mass is water Its content varies in the course of life and the individual parts of a body contain different amounts of water (Tab. 4.3.). Generally, the water content is higher in younger organisms than in older ones, both, from the phylogenetic and ontogenetic viewpoints.

Water is distributed between intracellular and extracellular compartments of the body. The movement of water depends on the concentration of solutes (or **osmolality**) of each compartment. Because water is a dipolar molecule with an uneven distribution of electrons between the hydrogen and oxygen atoms, it forms **hydrogen bonds** (see subchapter 1.3.5.2.) with other molecules and acts as **a solvent**. A hydrogen bond is a weak noncovalent interaction between the hydrogen of one molecule and the more electronegative atom of an acceptor molecule.



Based on this property, polar organic and inorganic salts can be readily dissolved in water. Compounds that dissolve easily in water are **hydrophilic** (Greek, "water-loving"). In contrast, nonpolar solvents such as chloroform and benzene are poor solvents for polar biomolecules but easily dissolve those that are **hydrophobic -** nonpolar molecules such as lipids and waxes.

The structure of water also allowed it to resist temperature changes and therefore water is an important agent in **thermal regulation** of the body. Water is also an important **transport medium** because many of the compounds produced in the body or originated from food are dissolved in water (hormones, minerals, vitamins etc.) and transported to other parts of body.

Many of the compounds dissolved in water also contain chemical groups that act as acids and bases, releasing or accepting hydrogen ions. The hydrogen ion content and the amount of body water are controlled to maintain body **homeostasis -** a constant environment for the cells.

Water itself dissociates to a slight extent (autoionization), generating hydrogen ions (protons, H^+) and hydroxide ions (OH^-). Pure water contains not only H_2O, but also equal concentrations of hydrated protons, called ***hydronium ions*** (H_3O^+).

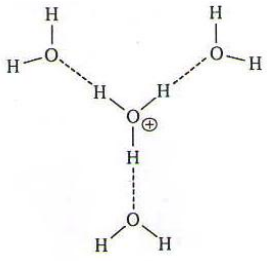
Tab. 4. 3. Water content in some organisms

Organism	Water content (%)	Organism	Water content (%)
Human embryo	93	Bacteria	
Newborn	72	Escherichia coli	75 – 80
Adult man		Green vegetables	
− whole body	60 – 62	− average	70 − 85
− skeleton	20 − 27	− leaves	70 − 92
− brain	70	− wood	30 − 60
− muscles	76	− dry seeds	5 − 20
− blood	80	Fungi	35 − 90

These molecular species are formed by the **autoionization** of water:

$$H_2O + H_2O \longleftrightarrow H_3O^+ + OH^- \qquad (4.2)$$

The formula for the hydrated proton is written as $\mathbf{H_3O^+}$, but since each hydrated proton actually interacts with three water molecules, $H_9O_4^+$ is a more accurate formula. For convenience, however, the hydrated proton is usually represented as H_3O^+, or even as H^+, since it is understood that the hydrogen ion in water is always hydrated:



The autoionization of water can be analyzed quantitatively. At the equilibrium the equilibrium constant (K_{eq}) for the ionization of water (4.2) is given by:

$$K_{eq} = \frac{[H_3O^+][OH^-]}{[H_2O]^2} \qquad (4.3)$$

where $[H_3O^+]$ is the concentration of hydronium ions, $[OH^-]$ is the concentration of hydroxide ions and $[H_2O]$ is the concentration of water.

Since one liter of water has a mass of 1000 g, and since the gram molecular weight of water is 18 $g.mol^{-1}$, water has a concentration of 1000 g/18 $g.mol^{-1}$, or 55.6 $mol.l^{-1}$. The equation (4.3) thus collapses to:

$$55.6^2 \,.\, K_{eq} = [H_3O^+][OH^-] \qquad (4.4)$$

The equilibrium constant for the autoionization of water, as measured by electrical conductivity studies, is $3.24 .\times 10^{-18}$ (at 25 °C). Substituting this value in equation (4.4) yields:

$$[H_3O^+][OH^-] = 1\times10^{-14}\ mol^2.l^{-2} \qquad (4.5)$$

The quantity 1×10^{-14} ($mol^2.l^{-2}$) in equation (4.5) is called the **ionic product of water** and is designated $\boldsymbol{K_w}$. Therefore:

$$K_w = [H_3O^+][OH^-] = 1\times10^{-14}\ mol^2.l^{-2} \qquad (4.6)$$

The only way to change K_w is to change the temperature. We can obtain two pieces of information from the expression for the ionic product of water. First, very little water exists in the form of its ionic products; that is, K_w is very small. Second, given that the product of hydronium ion and hydroxide ion concentrations is a constant, we know that, when the concentration of H_3O^+ in water increases, the concentration of OH^- must decrease, and vice versa. Thus three situations can exist for the relative concentrations of hydronium and hydroxide ions in water:

- in ***neutral*** solution, $[H_3O^+] = [OH^-] = 10^{-7}\ mol.l^{-1}$
- in ***acidic*** solutions, $[H_3O^+] > [OH^-]$
- in ***basic*** solutions, $[H_3O^+] < [OH^-]$

Because of $K_w = 1\times10^{-14}\ mol^2.l^{-2}$, we can calculate the value of one of the two concentration terms $[H_3O^+]$ or $[OH^-]$, if we know the other.

The autoionization of water and its participation in acid-base reactions are fundamental to the progress of many biochemical reactions or functions of many biomolecules such as proteins and nucleic acids.

4.3.4. The pH scale

Many biochemical reactions and processes depend on the concentration of hydrogen ions, even though these silent partners often do not appear explicitly in the process. The transport of oxygen in the blood, the biochemical reactions catalyzed by enzymes, and the generation of metabolic energy during respiration and photosynthesis are several of the many biochemical phenomena that depend upon hydrogen ion concentration. The range over which $[H_3O^+]$ varies is enormous, from 0.1 $mol.l^{-1}$ in the stomach to less than 10^{-7} $mol.l^{-1}$ in the cytosol of a cell. Because it is difficult to plot such a wide range of values on graphs, it is convenient to use a logarithmic scale to define the quantity called **pH**, where:

$$pH = -\log [H_3O^+] \tag{4.7}$$

Hydroxide ion concentration can be similarly expressed in terms of **pOH**:

$$pOH = -\log [OH^-] \tag{4.8}$$

The relationship between pH and pOH is:

$$pH + pOH = 14 \tag{4.9}$$

Thus, a neutral solution has a pH value of 7.0. **Acidic solutions have pH values smaller than 7.0, whereas basic solutions have pH values greater than 7.0.** Since the scale of pH is logarithmic, a change in pH of one unit means that the hydronium ion concentration has changed by a power of 10 (Tab. 4.4.):

Tab. 4.4. The pH and pOH scales and their relation to H_3O^+ and OH^- concentration

$[H_3O^+]$ ($mol.l^{-1}$)	1	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	**10^{-7}**	10^{-8}	10^{-9}	10^{-10}	10^{-11}	10^{-12}	10^{-13}	10^{-14}
pH	0	1	2	3	4	5	6	**7**	8	9	10	11	12	13	14
$[OH^-]$ ($mol.l^{-1}$)	10^{-14}	10^{-13}	10^{-12}	10^{-11}	10^{-10}	10^{-9}	10^{-8}	**10^{-7}**	10^{-6}	10^{-5}	10^{-4}	10^{-1}	10^{-2}	10^{-1}	1
pOH	14	13	12	11	10	9	8	**7**	6	5	4	3	2	1	0

Most of body cells and fluids have pH value different from 7. pH values of some cells and body fluids are given in Tab. 4.5.

Tab. 4.5. pH values of some cells and body fluids

Body cells	**pH**	**Body fluids**	**pH**
Erythrocytes	7.00	Blood	7.36 – 7.44
Platelets	7.28	Gastric juice	1.00 – 2.00
Osteoblasts	8.50	Duodenal juice	6.50 – 7.60
Prostatic cells	4.50	Urine	5.00 – 8.00
Sceletal muscle cells	6.90	Bile	6.20 – 8.50
		Saliva	7.00 – 7.50
		Pancreatic juice	7.50 – 8.80

4.4. Acids and bases. Acid-base equilibria

During metabolism, the body produces a number of acids that increase the hydrogen ion concentration of the blood or other body fluids and tend to lower the pH. However, the pH of the blood is normally maintened between 7.36-7.44, and intracellular pH at approximately 6.9-7.4. To understand how this acid-base balance is kept, we must understand the basic rules of acid and base behaviour in a solution.

Acid and *base* can be defined in several ways. According to the Arrhenius theory of acids and bases, an acid is a compound that increases the concentration of H^+ ions that are present when added to water. These H^+ ions form the hydronium ion (H_3O^+) when they combine with water molecules. Arrhenius bases are substances which produce hydroxide ions in solution. This theory successfully describes how acids and bases react with each other to make water and salts. However, it does not explain why some substances that do not contain hydroxide ions, can make basic solutions in water. The Brønsted-Lowry theory of acids and bases addresses this problem. According to this theory, ***acids*** are compounds that ***donate a hydrogen ion (H^+)*** to a solution, and ***bases*** are compounds that ***accept hydrogen ions***. A compound that acts as both a Brønsted-Lowry acid and base together is called **amphoteric,** for example H_2O, HSO_4^-, $H_2PO_4^-$, or amino acids. When a Brønsted acid dissociates, it increases the concentration of hydrogen ions in the solution [H^+]; conversely, Brønsted bases dissociate by taking a proton from the solvent (water) to generate [OH^-]. Based on this definition also molecules or ions (positive as well as negative) can act as acids and bases.

Because there is a proton exchange in these reactions, acids and bases are termed as **protolytes** and these reactions are called as **protolytic reactions**, . In protolytic reactions an acid reacts with a base; *a proton donor reacts with a proton acceptor*.

When an acid and base react and transfer a proton, another base and acid are produced. *The* **acid** *that loses a proton* forms a **conjugate base** of the acid. Every acid has a conjugate base, and vice-versa and every base has a conjugate acid. The acids and bases, whose formulas differ by just **a proton** (H^+), are called **conjugate acid – base pairs:**

Acid	⟷	**Proton**	+	**Base**
HCl	⟷	H^+	+	Cl^-
H_2SO_4	⟷	H^+	+	HSO_4^-
HSO_4^-	⟷	H^+	+	SO_4^{2-}
NH_4^+	⟷	H^+	+	NH_3
H_2O	⟷	H^+	+	OH^-
H_3O^+	⟷	H^+	+	H_2O

In order for an acid to act as a proton donor, a base (proton acceptor) must be present to receive the proton. An acid does not form its conjugate base unless a second base is present to accept the proton. When the second base accepts the proton, it forms its conjugate acid, the second acid. When hydrogen chloride , HCl, reacts with anhydrous ammonia, NH_3, forming ammonium ions, NH_4^+, and chloride ions, Cl^-, hydrogen chloride ($acid_1$) gives up a proton forming chloride ion, its conjugate base ($base_1$). Ammonia act as the proton acceptor and therefore is a base ($base_2$). The proton combines with ammonia to give its conjugate acid, the ammonium ion ($acid_2$). The equations for this and several other acid-base reactions are as follows:

$Acid_1$	+	**$Base_2$**	⟷	**$Acid_2$**	+	**$Base_1$**
HCl	+	NH_3	⟷	NH_4^+	+	Cl^-
HNO_3	+	F^-	⟷	HF	+	NO_3^-
NH_4^+	+	S^{2-}	⟷	HS^-	+	NH_3

In aqueous solutions of acids *the proton is donated to water* and the **hydronium ion** is formed. For example, when gaseous hydrogen chloride is dissolved in water, virtually every HCl molecule transfers its proton to water, and a solution of hydronium ions and chloride ions results:

$$HCl + H_2O \longrightarrow H_3O^+ + Cl^-$$

The transfer of a proton from an acid to water is called **ionization**. The proton in the hydronium ion is attached to oxygen by a nonbonded pair of electrons on oxygen. The terms proton, hydrogen ion, or hydronium ion are often used interchangeably in describing aqueous acid solutions.

Acids that can give up one proton are *monoprotic*, as hydrochloric acid, nitric acid, perchloric acid ($HClO_4$). *Diprotic* acid can transfer two protons to water or to a base, as in case of sulfuric acid. Transfer of a proton

yields in hydrogen sulfate ion HSO_4^-; transfer of the second proton yields the sulfate ion SO_4^{2-}. Triprotic acids can transfer three protons to water or a base. Phosphoric acid is *triprotic* because it may transfer three protons to water and form $H_2PO_4^-$, HPO_4^{2-} and PO_4^{3-}.

All Brønsted acids produce hydronium ions in solution but in different amounts.

According to the degree of ionization, acids and bases can be divided to strong and weak acids and bases. ***Strong acids*** belong to the group of strong electrolytes. They are almost completely dissociated in aqueous solutions. The resulting anions do not react with water, they are not able to bind H^+. Anions of strong acids are weak conjugate bases and they do not participate in acid-base reactions. Hydrochloric, hydrobromic and hydroiodic acid belong to binary strong acids. Oxoacids involve mainly sulphuric acid, nitric acid, chloric, and perchloric acid.

Strong acids

HCl	**HBr**	**H_2SO_4**	**HNO_3**	**$HClO_3$**	**$HClO_4$**	**CF_3COOH**	**CCl_3COOH**	**R-SO_3H**	**R-O-SO_3H**

Strong bases belong to the group of strong electrolytes. They include metal hydroxides of the first and second main subgroup and tetraalkylammonium hydroxides.
They completely dissociate to ions in water. The ions do not enter acid-base reactions. In clinical biochemistry, the cations of strong bases are often called "strong" cations (e.g. Na^+, K^+, $Ca^{2+,}$ Mg^{2+}).

Strong bases

NaOH	**KOH**	**$Ca(OH)_2$**	**$Mg(OH)_2$**	**$Ba(OH)_2$**

Weak acids belong to weak electrolytes. They react only partly with water, that is, only a small percentage of the acid and base molecules ionize in water to form hydronium and hydroxide ions, respectively.

The weak inorganic acids include hydrocyanic acid (HCN), nitrous acid (HNO_2), hydrofluoric acid (HF), and carbonic acid (H_2CO_3). Organic compounds containing the carboxyl group (−COOH) are the weak acids.

To consider weak acids and weak bases in quantitative terms, we must consider them to be systems in chemical equilibrium, characterized by equilibrium constants. For an acid with the general formula HA (e.g. acetic acid), the equilibrium constant for ionization is obtained from the equation for ionization:

$$\underset{\text{Weak acid}}{HA} + H_2O \longleftrightarrow H_3O^+ + \underset{\text{Conjugate base}}{A^-}$$

The anion of a weak acid (A^-) is a strong conjugate base; it participates in acid-base reactions (see hydrolysis). The weaker the acid, the higher affinity of anions to H^+ and thus the stronger the conjugate base is.
The equilibrium constant expression for its ionization is:

$$K_{eq} = \frac{[H_3O^+][A^-]}{[HA][H_2O]}$$

The concentration of water is 55.6 $mol.l^{-1}$ and is so large compared to that of the other components of the equilibrium that its value changes very little when the acid HA is added. Therefore, it is included in the **acid ionization constant, K_a:**

$$\boldsymbol{K_a} = K_{eq}[H_2O] = \frac{[H_3O^+][A^-]}{[HA]} \qquad (4.10)$$

Similarly, ***weak bases*** are weak electrolytes. Ammonia (NH_3) is an example of a weak base. Many organic compounds containing nitrogen are also weak bases. These organic bases can be considered to be derivates of ammonia, with other groups substituting for one or more of the hydrogen atoms of ammonia. Examples of organic bases are methylamine (CH_3NH_2), pyridine (C_5H_5N), and others. Weak bases, like ammonia or the bicarbonate ion, react incompletely with water, usually to a small percentage, to make some OH^-.

An equilibrium is established in which the unchanged base is favored. For a base with the general formula B (e.g. NH_3), the equilibrium constant for ionization is obtained from the equation for ionization:

$$\underset{\text{Weak base}}{B} + H_2O \longleftrightarrow \underset{\text{Conjugate acid}}{BH^+} + OH^-$$

The **base ionization constant** for this equilibrium, $\boldsymbol{K_b}$, is defined by the following equation:

$$\boldsymbol{K_b} = K_{eq}[H_2O] = \frac{[BH^+][OH^-]}{[B]} \qquad (4.11)$$

The values of K_a and K_b are indicative of the relative amounts of products and reactants at equilibrium. The larger the value of K, the larger is the percentage of ionization at the same concentration. The smaller the values of the ionization constant, the smaller the amount of product relative to reactant, or in terms of ionization, the smaller the degree or extent of ionization. In other words, the value of this constant provides us with a quantitative measure of just how "weak" a weak acid or weak base is. **The smaller the ionization constant, the weaker the acid or base.**

For the same reason that the pH concept was devised, analogous pK_a and pK_b expressions, based on K_a and K_b, have been defined. The pK_a is the negative logarithm of K_a, and the pK_b is the negative logarithm of K_b:

$$pK_a = -\log K_a\,; \qquad pK_b = -\log K_b \qquad (4.12)$$

The relationship between the pK_a and pK_b values for an acid and its conjugate base:

$$pK_a + pK_b = 14.00 \quad (25\ ^\circ C) \qquad (4.13)$$

Table 4.6. lists various weak acids and weak bases along with their ionization constants K and pK values at 25 °C. Note that, reading down each list, as ***the ionization constants become smaller, the corresponding pK values become larger.***

Tab. 4.6. Values of K and pK for some weak acids and weak bases

Acid			Base		
Name (formula) of acid	$\boldsymbol{K_a}$	**p$\boldsymbol{K_a}$**	**Name (formula) of base**	$\boldsymbol{K_b}$	**p$\boldsymbol{K_b}$**
Citric acid	7.1×10^{-4}	3.15	Dimethylamine	5.81×10^{-4}	3.24
Ascorbic acid	7.9×10^{-5}	4.10	Methylamine	4.59×10^{-4}	3.34
Acetic acid	1.8×10^{-5}	4.74	NH_3	1.75×10^{-5}	4.70
Carbonic acid	4.5×10^{-7}	6.35	HPO_4^{2-}	1.60×10^{-7}	6.80
$H_2PO_4^-$	6.3×10^{-8}	7.20	HCO_3^-	2.20×10^{-8}	7.65
NH_4^+	5.7×10^{-10}	9.24	Cocaine	3.90×10^{-9}	8.41
HCO_3^-	5.6×10^{-11}	10.25	Acetate ion	5.60×10^{-10}	9.26
HPO_4^{2-}	4.5×10^{-13}	12.35	Aniline	4.17×10^{-10}	9.38

Calculation of pH:

strong acid,	$pH = -\log [H_3O^+]$	
strong base,	$pOH = -\log [OH^-]$	$pH = 14 - pOH$
weak acid,	$pH = \frac{1}{2}\, pK_a - \frac{1}{2} \log c_a$	
weak base:	$pOH = \frac{1}{2}\, pK_b - \frac{1}{2} \log c_b$	$pH = 14 - \frac{1}{2}\, pK_b + \frac{1}{2} \log c_b$

Where: K is ionization constant of an acid or base and c is concentration ($mol.l^{-1}$); $pK = -\log K$
In all calculations it has to be distinguished between strong or weak acid (base).

Strong acids and bases are completely dissociated in the solution. From total concentration of an acid or hydroxide, we can directly derive the concentration of H^+ or OH^- ions, respectively. In monoprotic acids the

concentration $[H^+]$ is equal to the concentration of acid (c_a). Similarly, in monobasic hydroxides the concentration $[OH^-]$ equals to the concentration of hydroxide (c_b). Then we can calculate the pH of a strong acid (base) solution from the expressions:

$pH = -\log [H_3O^+]$ $\quad pOH = -\log [OH^-]$ $\quad pH + pOH = 14$
$pH = -\log c_a$ $\quad pOH = -\log c_b$

In polyprotic acids (polybasic hydroxides) we have to consider the number of H^+ or OH^- ions liberated by the dissociation of one mole of a substance.

pH calculation examples for strong acids and bases.

1. What is the *pH* of sulfuric acid solution (c = 0.02 mol.l^{-1})?

$H_2SO_4 \rightarrow \mathbf{2}H^+ + SO_4^{2-}$
$c(H^+) = \mathbf{2}\ c\ (H_2SO_4) = 2 \times 0.02$ mol.l^{-1}
$pH = -\log c\ (H^+) = -\log (0.04) = \mathbf{1.4}$

Sulfuric acid solution has pH = 1,4.

2. What is the amount of substance concentration of HCl at *pH* = 2.7?

$HCl \rightarrow H^+ + Cl^-$
$c\ (H^+) = 10^{-pH} = 10^{-2.7} = 2.0 \times 10^{-3}$ mol.l^{-1}
$c(HCl) = c(H^+) = \mathbf{2.0 \times 10^{-3}\ mol.l^{-1}}$

Amount of substance concentration of HCl is 2 mmol.l^{-1}.

3. What is the pH of KOH solution (c= 0.02 mol.l^{-1})?

$c\ (OH^-) = c(KOH) = 0.02$ mol.l^{-1}
$pOH = -\log c(OH^-) = -\log (0.02) = 1.7$
$pH = 14 - pOH = 14 - 1.7 = \mathbf{12.3}$

KOH solution (c = 0.02 mol.l^{-1}) has pH = 12.3.

4. Solution of $Ba(OH)_2$ has pH = 12.5. What is the amount of substance concentration of this hydroxide?

$Ba(OH)_2 \rightarrow Ba^{2+} + 2\ OH^-$
$pOH = 14 - pH = 14 - 12.5 = 1.5$
$c(OH^-) = 10^{-pOH} = 10^{-1,5} = 3{,}2 \times 10^{-2}$ mol.l^{-1}
$c\ (Ba(OH)_2) = \frac{1}{2}\ c(OH^-) = \mathbf{1.6 \times 10^{-2}\ mol.l^{-1}}$

Amount of substance concentration of this solution is 16 mmol.l^{-1}.
Weak acids and bases dissociate only partially in solutions.
We can calculate the pH of a **weak acid** solution from the expressions:

$\mathbf{pH = \frac{1}{2}\ (pK_a - \log c_a)}$ $\qquad \mathbf{pK_a = -\log K_a}$

$\mathbf{pH = \frac{1}{2}\ pK_a - \frac{1}{2}\log c_a}$

Similarly, expressions for calculating pH of a weak base:

$\mathbf{pOH = \frac{1}{2}\ (pK_b - \log c_b)}$ **resp.** $\mathbf{pH = 14 - \frac{1}{2}\ pK_b + \frac{1}{2}\log c_b}$,

where **pK = – log K** of certain type of acid (A) or base(B) and **c** is the amount of substance concentration of acid or base.

$pK_a + pK_b = 14$

pH calculation examples for weak acids and bases

1. What is the pH formic acid solution (c = 0.032 $mmol.l^{-1}$)? $pK_a = 3.75$

$pH = ½ (pK_a – \log c_a) = ½ (3.75 – \log 0.032)$
pH = **2.62**

Formic acid solution has pH = 2.62.

2. Calculate pH of ammonia solution (c = 64 $mmol.l^{-1}$) . $pK_b = 4.75$

$pH = 14 – ½ (pK_b - \log c_b)$
$pH = 14 – ½ (4.75 - \log 0.064)$
pH = **11.03**

pH of ammonia solution is 11.03.

4.4.1 Hydrolysis of salts

Salt is a compound formed by neutralization reaction between an acid and a base . They generally ionize in water furnishing cations and anions. The cations or anions formed during ionization of salts either exist as hydrated ions in aqueous solutions or interact with water to regenerate the acids and bases. The process of interaction between cations or anions of salts and water is known as hydrolysis of salts (Tab.4.7).
Salts formed by the neutralization of **strong acid** and **strong base** are neutral (neutral salts) as the bonds in the salt solution will not break apart. They generally get **hydrated** but do not hydrolyze (e.g. NaCl). Salts formed by the neutralization of **weak acid** and **strong base** are basic (basic salts, e.g. CH_3COONa), while salts formed by the neutralization of **strong acid** and **weak base** are acidic (acidic salts. e.g. NH_4Cl).
The process of hydrolysis starts with dissociation of a salt when dissolved in water and after that dissociated ions can be hydrolyzed. Salts are divides into 4 groups:

A . Salts of weak acids and strong hydroxides (e.g. CH_3COONa, KCN, Na_2CO_3, $NaHCO_3$, KNO_2)

These salts dissociate to cation and anion in an aqueous solution, e.g. $CH_3COONa \rightarrow Na^+ + CH_3COO^-$. The cation Na^+ comes from the strong hydroxide (NaOH), does not react with water, however it is hydrated and is found in a hydrated form in the solution. The anion CH_3COO^-comes from the weak acid (CH_3COOH) and is the subject of hydrolysis, i.e. it reacts with water to form acetic acid until equilibrium is established:

$$CH_3COO^- + H_2O \longleftrightarrow CH_3COOH + OH^-$$

This results in an increase in concentration of OH^- ions which makes the solution alkaline. pH of the solution is greater than7.

B. Salts of weak bases and strong acids (e.g. NH_4Cl, $(NH_4)_2SO_4$, NH_4NO_3)

$$NH_4Cl \longleftrightarrow NH_4^+ + Cl^-$$

Anion of this salt (Cl^-) comes from the strong acid (HCl), it does not react with water, and is found in the hydrated form in the solution. Cation of this salt is the subject of hydrolysis:

$$NH_4^+ + H_2O \longleftrightarrow NH_3 + H_3O^+$$

The final solution is acidic in nature because of formation of H_3O^+ ions in the course of hydrolysis of the ammonium cation. pH of such solutions is less than 7.

C. Salts of weak acids and weak bases (e.g. CH_3COONH_4, NH_4NO_2, $CuNO_2$)

After a salt dissociation in water, the ions undergo independently hydrolysis, and both hydroxide and oxonium ions are formed. The pH of the final solution can be acidic, basic or neutral depending on the nature of acids and bases involved. If the acid HA is stronger than the base B, then $pK_a<pK_b$ and pH < 7, the solution has acidic reaction. And, if the base is stronger, then $pK_a>pK_b$ and pH > 7, the solution has basic reaction. If the dissociation constants of both components are nearly equal ($pK_a \approx pK_b$), pH of hydrolyzed salt solution remains neutral.

D. Salts of strong acids and strong bases (e.g. NaCl, Na_2SO_4)

The salts of strong acids and strong bases are completely dissociated in aqueous solutions. Cations and anions do not hydrolyze but they are found in hydrated forms in solutions and pH of these solutions remains neutral.

Tab. 4.7 The hydrolysis of salts

The origin of the salt	Hydrolysis occurs	Hydrolyzing ion	pH of a solution
Strong acid + strong base	no	none	**7**
Strong acid + weak base	yes	cation	**< 7**
Weak acid + strong base	yes	anion	**> 7**
Weak acid + weak base	yes	cation + anion	**≈ 7**

4.5. Buffers

Many acids are formed in the course of metabolic processes in our bodies. Our stomach produces hydrochloric acid. Our muscles produce lactic acid. When starch and glucose are metabolized, the pyruvic acid is formed. Carbon dioxide from respiration produces carbonic acid in the blood. The physiological pH in human blood is kept in the narrow range of pH about 7.4 (7.4 ± 0.04). A slight change outside of this range can be devastating to cells and the entire body. Therefore the body must have several defense mechanisms to protect the acid-base homeostasis of the body. There are three important mechanisms the body uses to regulate pH. The first is the system of chemical buffers, the second line of defense is the respiratory system, and last, is the urinary system. These three mechanisms work together to keep body pH within that narrow range.

The first line of defense against pH changes is comprised by **chemical buffers** in the body. A buffer keeps the pH of a solution constant by taking up protons that are released during reactions, or by releasing protons when they are consumed by reactions. The observation that partially neutralized solutions of weak acids or bases are resistant to changes in pH when small amounts of strong acids or bases are added led to the concept of the "buffer". Chemically, a buffer solution usually contains approximately equal concentrations

a/ ***of a weak acid and salt of its conjugate base***,

b/ ***of a weak base and salt of its conjugate acid***.

How does a buffer work? Consider a buffer solution consisting of weak acetic acid (CH_3COOH) and sodium acetate (CH_3COONa). Addition of sodium acetate to acetic acid solution inhibits its ionization. If a strong acid is added to this solution, the hydronium ions produced by the added acid donate protons to the acetate ion in solution:

$$\boxed{CH_3COOH + CH_3COO^- + Na^+} + \mathbf{H}^+ + Cl^- \longleftrightarrow 2\,CH_3COO\mathbf{H} + Na^+ + Cl^-$$

$$CH_3COO^- + H_3O^+ \longleftrightarrow CH_3COOH + H_2O$$

Although the reaction is reversible to a slight extent, most of the protons remain attached to the acetic acid product. Remember, acetic acid is a *weak* acid, and it cannot transfer the proton back to the water molecule efficiently. Since most of the protons are tied up in this way, the pH of the solution changes very little.
When a strong base is added, the hydroxide ions react with hydronium ions already in the buffer because of the presence of acetic acid:

$$\boxed{CH_3COOH + CH_3COO^- + Na^+} + Na^+ + \mathbf{OH^-} \longleftrightarrow 2\ CH_3COO^- + 2\ Na^+ + \mathbf{H_2O}$$

$$H_3O^+ + OH^- \longleftrightarrow 2\ H_2O$$

Acetic acid is a *weak* acid, but it is an acid, and that means that it produces some hydronium ions in solution. The added hydroxide ions are tied up. Some of the hydronium ions originally present in the buffer are used up in this process. They are immediately replaced by further ionization of the acetic acid in the buffer:

$$CH_3COOH + H_2O \longleftrightarrow CH_3COO^- + H_3O^+$$

The concentration of hydronium ions returns to approximately the original value, and the pH is only slightly changed.
The pH of a buffer solution remains constant when a limited amount of strong acid or base are added to it. A solution's ability to resist pH changes when an acid or base is added to it is called its **buffer capacity**. *Buffer capacity* is defined as ***the quantity of strong acid (base) that has to be added to one liter of a buffer solution to change its pH value by one unit***. For optimal buffering action to take place, the concentrations of conjugate acid and conjugate base in the buffer system must be approximately equal and whose pH equals p*K* value of the acid or base. The larger the difference between pH and p*K* of the buffer system, the smaller its capacity to accept or donate a proton. The greater the concentrations of conjugate acid and base, the greater the capacity of the buffer to absorb changes in hydronium or hydroxide ion concentrations.

4.5.1. Quantitative aspects of buffer systems

The pH values of buffered solutions can be calculated from ionization constants and buffer concentrations. The buffer is made by dissolving a weak acid [HA] and its sodium salt, [NaA] in water.
Recall Equation 4.10, which defines K_a for a weak acid, HA:

$$K_a = \frac{[H_3O^+][A^-]}{[HA]}$$

Because we want to know $[H_3O^+]$ and then pH, let us rearrange this equation to give us an expression for $[H_3O^+]$:

$$[H_3O^+] = K_a \times \frac{[HA]}{[A^-]} \qquad (4.14)$$

We can convert Equation 4.14 into a form that includes pH instead of $[H_3O^+]$. If we take the logarithm of both sides of Equation 4.14, and then multiply every resulting term by – 1, we get:

$$-\log [H_3O^+] = -\log K_a - \log \frac{[HA]}{[A^-]}$$

We can recognize expression for pH and pK_a in this, so we can write:

$$\text{pH} = pK_a + \log \frac{[A^-]}{[HA]} = pK_a + \log \frac{[salt]}{[acid]} \qquad (4.15)$$

Equation 4.15 is the **Henderson-Hasselbalch equation** and is used for calculation of pH of buffers composed of a ***weak acid and its conjugate base (salt).***

Calculation of pH of buffer solution consisting *of a **weak base and salt of its conjugate acid**:*

$$pH = 14 - pK_b + \log \frac{[base]}{[salt]} \qquad (4.16)$$

Calculation example:
Calculate pH of acetate buffer, which contain in 1000 ml of solution 0.2 mol CH_3COOH and 0.5 mol CH_3COONa. K_a (CH_3COOH) = 1.8 x 10^{-5}.

$$pH = pK_a + \log \frac{[salt]}{[acid]}$$

pH = 4.75 + log (0.5/0.2) = **5.138**

4.5.2. Buffers in biological systems

Almost every biological process is pH dependent; a small change in pH produces a large change in the rate of the process. This is true not only for the many reactions in which the H^+ ion is a direct participant, but also for those in which there is no apparent role for H^+ ions. The enzymes that catalyze cellular reactions, and many of the molecules on which they act, contain ionizable groups with characteristic pK_a values. The protonated amino and carboxyl groups of amino acids and the phosphate groups of nucleotides, for example, function as weak acids; their ionic state depends on the pH of the surrounding medium. Ionic interactions are among the forces that stabilize a protein molecule and allow an enzyme to recognize and bind its substrate.

Cell and organisms maintain a specific and constant cytosolic pH, keeping biomolecules in their optimal ionic state, usually near pH 7. In multicellular organisms, the pH of extracellular fluids is also tightly regulated (Tab. 4.5.). The constancy of pH is achieved primarily by biological buffers. Four buffer systems of body fluids take part in stabilizing pH of body. These systems act in concert. In addition, lung and kidney play a role in the process of maintaining acid-base balance.

The main buffer systems in the organism are:
- Bicarbonate buffer system (H_2CO_3 / HCO_3^-).
- Phosphate buffer system ($H_2PO_4^-$ / HPO_4^{2-}).
- Proteinate buffer system (protein / proteinate).
- Oxyhemoglobin / hemoglobin.

Bicarbonate buffer system

The buffering action of blood depends upon the equilibrium of carbonic acid, bicarbonate, and carbon dioxide. Carbon dioxide is both dissolved in blood, CO_2 (aq), and present in the air spaces of the lungs, CO_2 (g). Carbon dioxide produced by cells as an end product of metabolism diffuses into the erythrocytes of the blood. There, it is converted into bicarbonate ion in a reaction catalyzed by the enzyme *carbonic anhydrase*. This process can be represented by a two-step process:

$$CO_2\,(aq) + H_2O \longleftrightarrow H_2CO_3$$

$$H_2CO_3 + H_2O \longleftrightarrow H_3O^+ + HCO_3^-$$

Furthermore, CO_2 (aq), is also in equilibrium with CO_2(g):

$$CO_2\,(g) + H_2O \longleftrightarrow CO_2\,(aq)$$

The Henderson-Hasselbalch equation must be slightly modified so that is uses the formula of carbon dioxide, not the formula of the associated weak acid, H_2CO_3. We cannot employ the usual symbol, pK_a, because we are not working with a system at 25 °C but at 37 °C. Moreover, we're not working with H_2CO_3 for which pK_a = 6.35. To handle these different conditions, we use what is called an *apparent acid ionization constant*, symbolized as *K*', and a corresponding apparent p*K*'. The accepted value of p*K*' for the bicarbonate buffer under body conditions is 6.1, so we can write:

$$pH = 6.1 + \log \frac{[HCO_3^-]}{[CO_2\,(aq)]}$$

Under normal pH conditions in human arterial blood, $[HCO_3^-]$ = 24 mmol.l^{-1}, and $[CO_2]$ = 1.2 mmol.l^{-1}. The pH of human arterial blood is then calculated by putting the values into the equation. The pH is 7.4.
If the pH of blood falls because of a metabolic process that produces excess hydrogen ions, the concentration of H_2CO_3 decreases due to formation CO_2 (aq), that enters the gas phase. Thus, the increase in proton concentration is compensated by an increase in pressure of CO_2 that is eliminated by the lungs from the body. On the other hand, if the pH of the blood rises, the pH is rapidly restored because atmospheric CO_2 (g) converts to CO_2 (aq) and then to H_2CO_3 in the capillaries of the lungs. Whether there is an increase or decrease of the pH, the reservoir of CO_2 (g) restores the equilibrium of the blood buffer. Since this reservoir is large and can be changed quickly by altering the breathing rate, the pH of the blood does not change under the physiological conditions (Fig. 4.3.). Hydrogen carbonate buffer system provides approximately 53 % of blood buffer capacity.

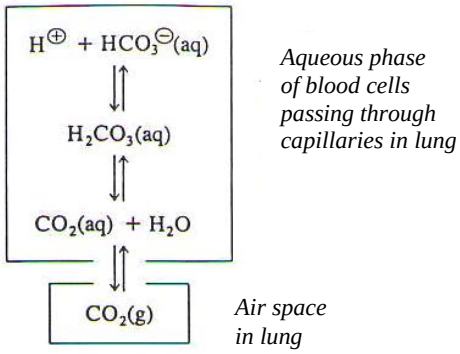
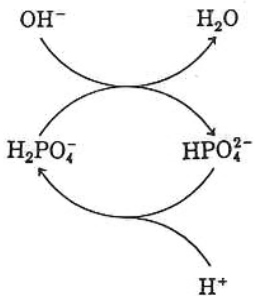


Fig. 4.3. pH regulation of the blood

Phosphate buffer system
This buffer system acts in cytoplasm of cells and is an ideal urinary buffer. It consists of a weak acid $H_2PO_4^-$ as a donor and a conjugate base HPO_4^{2-} as a proton acceptor:

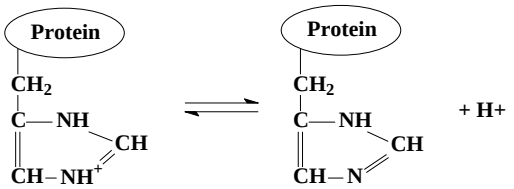


The phosphate buffer system is maximally effective at the pH close to its pK_a of 6.86 and thus tends to resist pH changes in the range between about 5.9 and 7.9. It is therefore an effective buffer in biological fluids; in mammals, for example, most cytoplasmic compartments have a pH in the range of 6.9 to 7.4. The formation of an acid is more common in metabolism, and HPO_4^{2-} is the more important component of the buffer. The normal $[H_2PO_4^-]$ / $[HPO_4^{2-}]$ ratio in the cells is about 1: 4, and the buffer neutralizes acid more efficiently than base. The $H_2PO_4^-$ formed from the reaction of HPO_4^{2-} with acid is eliminated by excretion in the urine. This system has a relatively small share in maintaining blood pH – about 5 %.

Protein buffer system

Proteins act as a third type of blood buffer. Proteins are ampholytic substances. These molecules can act as proton acceptors and proton donators. Proteins provide for about 7 % of the total buffer capacity of blood.

Buffer function is carried out mainly by:
a) imidazole groups of histidine,
b) sulfhydryl groups of cysteine:

a) 

b) $R{-}SH \longleftrightarrow R{-}S^{-} + H^{+}$

Hemoglobin buffer system

The buffer capacity of the hemoglobin system is about 35 %. Hemoglobin as well as oxyhemoglobin (HbO_2) have character of weak acids with pK = 7.71 and 7.16. Based on the pK value HbO_2 is a stronger acid than hemoglobin. Ionization and binding of H^+ ions is carried out on imidazole group of histidine residues of globin part of the molecule. The changes in ionization of Hb are in correlation with formation and ionization of carbonic acid.

In **lungs** during oxygenation protons are released according to equation:

$$HHb + O_2 \rightarrow HHbO_2 \rightarrow HbO_2^{-} + H^{+}.$$

The released protons bind to HCO_3^- and H_2CO_3 is formed. Carbonic acid is cleaved to CO_2 and water with carbonic anhydrase and CO_2 is eliminated by pulmonary ventilation.

In the peripheral tissue cells, oxygen is released and the processes proceed according to the following equations:

$$HbO_2^{-} \longrightarrow Hb^{-} + O_2 \qquad \text{and} \qquad Hb^{-} + H^{+} \longrightarrow HHb$$

The feedback bond of proton to hemoglobin can also occur. The source of the proton is H_2CO_3 according to the following equation:

$$H_2O + CO_2 \longrightarrow H_2CO_3 \longrightarrow HCO_3^{-} + H^{+}$$

By this way blood and erythrocytes, which are moving from tissues to lungs and back, allow continual one-direction transport of CO_2 from tissues to lungs where it is eliminated to external environment. This transport is called **isohydric trasport** of CO_2 because it proceeds without change of pH.

In clinical practice the measurement of partial pressures of CO_2 (pCO_2) and O_2 (pO_2 is of a great importance in the evaluation of the acid-base balance and its imbalance. Similarly, the determination of the level of alkali in blood – so called base excess or deficit (BE) is of a great importance. The BE is defined as the amount of a strong acid that must be added to each liter of fully oxygenated blood to return the pH to 7.40 at a temperature of 37°C and a pCO_2 of 5.3 kPa. The predominant base contributing to base excess is bicarbonate. Thus, a deviation of serum bicarbonate from the reference range is ordinarily mirrored by a deviation in base excess. **BE represents a component of blood buffer capacity. It alters under the different physiological and pathological states and it serves as diagnostic tool.**

Acid-base balance in the blood exists when the pH of blood is in the range of 7.36 to 7.44. **Acidemia/alkalemia** is a decrease/increase in pH of **the blood.** It is a serious condition requiring prompt attention, because all the equilibria that involve H^+ in the oxygenation or the deoxygenation of blood are sensitive to pH. If the pH falls below 6.8 or rises above 7.8, life is not possible. The term **acidosis/alkalosis** means a pathological state caused by a disease that results in an abnormal increase/decrease in acid in **the body**.

In certain circumstances – for example, in the lung disease emphysema, pneumonia, poliomyelitis, heart failure, or when a person is under anesthesia, the ventilation rate may be too low, and CO_2 is not removed from the lungs

rapidly enough. Consequently, the bicarbonate buffer system will "back up", hydrogen ion will not be removed by reaction with HCO_3^-, and the blood pH will fall under 7.4. This pathological condition is called **respiratory acidosis**. An immediate treatment consists of intravenous bicarbonate infusion. Conversely, if CO_2 is removed from the lungs faster than it arrives, the blood pH will rise over 7.4. This state, called **respiratory alkalosis**, can result from hyperventilation, which may occur in extreme fevers or severe hysteria, under circumstances of great excitement (rapid breathing) and other pathological conditions. First aid for this condition is to have the patient breathe into a paper bag, an action that increases the CO_2 content of the lungs.

Metabolic acidosis is a lowering of pH as a result of a metabolic disorder, rather than as a result of a failure of the blood buffer system. For example, a large and serious decrease in pH can occur as a result of uncontrolled diabetes and as the result of some low carbohydrate – high protein diets. The blood pH may fall from the normal 7.4 to as low as 6.8. The increased H^+ concentration is due to the large amounts of acidic compounds produced in the liver. The bicarbonate buffer system attempts to compensate for the H^+ excess, producing excess of CO_2, which must be eliminated in the lungs. However, so much CO_2 is lost by ventilation that the absolute concentration of the buffer system decreases. The capacity of the buffer system is severely compromised and cannot reduce the metabolically produced excess of H^+. In such cases, buffer capacity can be temporarily restored by intravenous administration of sodium bicarbonate.

An increase in pH as a result of a metabolic disorder is called **metabolic alkalosis**. This condition can arise when excessive amounts of H^+ ion are lost, for example, during excessive vomiting. H^+ starts removing from the blood and the pH of the blood is rising. Intake of excessive amounts of antacids also will cause the blood pH to rise. Immediate treatment consists of intravenous administration of ammonium chloride

These simple disturbances in acid-base balance can also combine and result in *combined failures* of acid-base balance.

Control questions:

1. Calculate pH of a hydrochloric acid solution with concentration 0.0001 $mol.l^{-1}$.

2. Calculate pH of buffer solution which is prepared by mixing 15 ml of NaH_2PO_4 solution, c = 0.1 $mol.l^{-1}$ and 5 ml of NaOH solution, c = 0.1 $mol.l^{-1}$. pK = 7.2.

3. What is the pH of solution prepared by mixing:
   a) 200 ml 0.1 $mol.l^{-1}$of acetic acid with 100 ml 0.1 $mol.l^{-1}$of sodium acetate (pKa = 4.75)
   b) 100 ml 0.1 $mol.l^{-1}$of acetic acid with 100 ml 0.1 $mol.l^{-1}$of sodium acetate (pKa = 4.75)
   c) 100 ml 0.1 $mol.l^{-1}$of acetic acid with 200 ml 0.1 $mol.l^{-1}$of sodium acetate (pKa = 4.75)

4. What is the pH of solution which was prepared by mixing 10 ml of 20 $mmol.l^{-1}$ of acetic acid solution and:
   a) 10 ml of water
   b) 10 ml 10 $mmol.l^{-1}$NaOH

5. At which ratio is needed to mix the solution consisting of 0.1 $mol.l^{-1}$ of sodium monohydrogen phosphate solution and 0.1 $mol.l^{-1}$ sodium dihydrogen phosphate to obtain buffer with pH = 7.5? pKa = 7.2.

5. LIFE AND ENERGY

5.1. Organism and energy

Life is an energy demanding process. Living cells and organisms must perform work to stay alive and to reproduce themselves. The ability to harness energy and to channel it into biological work is a fundamental property of a living organism. All of the physiological processes, like muscle work, cell division, healing, thinking or the process of cell dying require energy. What energy is and how is acquired by organism? How does organism transform and utilize energy? The answers to these questions are given by **bioenergetics**, the field of science concerned with the energy involved in making and breaking of chemical bonds in the molecules found in biological organisms. It can also be defined as the study of energy relationships and energy transformations in living organisms.

A living organism as the thermodynamic system exists in a dynamic steady state and it is never at equilibrium with their surroundings. Maintaining this steady state requires the constant investment of energy. When the cell can not longer generate energy, it dies and begins to decay toward equilibrium with its surroundings. The dynamic steady state is ensured by the flow of energy and mass to the thermodynamic system.

In autotrophs (bacteria, plants) energy flow through the system is supplied principally by solar radiation. This solar energy is converted into the necessary useful work to maintain the plant in its complex, high-energy configuration by a process called photosynthesis. Mass, such as water and carbon dioxide, also flows through plants, providing necessary raw materials, but not energy. For heterotrophs such as animals, energy flow through the system is provided by eating high energy biomass, either plant or animal. The breaking down of this energy-rich biomass, and the subsequent oxidation of part of it (e.g., carbohydrates), provides a continuous source of energy as well as raw materials. Maintenance of the complex, high-energy condition associated with life is not possible apart from a continuous source of energy.

In biosphere, autotrophs and heterotrophs live together in a vast, interdependent cycle in which autotrophic organisms use atmospheric carbon dioxide to build their organic biomolecules, some of them generating oxygen from water in the process. Heterotrophs in turn use the organic products of autotrophs as nutrients and return carbon dioxide to the atmosphere. Some of the oxidation reactions that produce carbon dioxide also consume oxygen, converting it to water. Thus carbon, oxygen, and water are constantly cycled between the heterotrophic and autotrophic worlds, with solar energy as the driving force for this global process. All living organisms also require a source of nitrogen, which is necessary for the synthesis of amino acids, nucleotides, and other compounds. Thus, in addition to the global carbon and oxygen cycle, a nitrogen cycle also operates in the biosphere. These cycles of matter are driven by an enormous flow of energy into and through the biosphere, beginning with the capture of solar energy by photosynthetic organisms and use of this energy to generate energy-rich carbohydrates and other organic nutrients; these nutrients are then used as energy sources by heterotrophic organisms (Fig. 5.1).

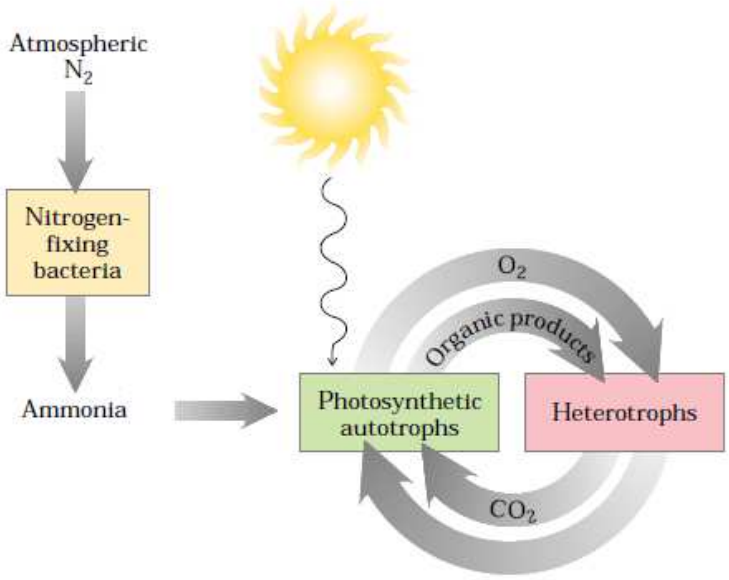


Fig. 5.1. The flow of energy and mass in the biosphere

However, a source of energy alone is not sufficient to explain the origin or maintenance of living systems. Think of this example. An automobile with an internal combustion engine, transmission, and drive chain provides the necessary mechanism for converting the energy in gasoline into comfortable transportation. The movement and transport would be impossible without such an „energy transducer". In a similar way, food would do little for a man whose stomach, intestines, liver, or pancreas were removed. Therefore, an organism must have a means of converting this energy into the necessary useful work to be able to build and maintain complex living processes.

This „energy transducer" is represented in an organism by the specific metabolic pathways, in which energy of substrates is converted to specific work. To illustrate, cells in the human are able to oxidize glucose in the metabolic pathways of glycolysis and oxidative phosphorylation. In these principal metabolic pathways energy captured in glucose is converted to adenosine triphosphate (ATP), the universal energy currency in all organisms. ATP supplies the energy for muscle contraction and this process involves the change of chemical energy to mechanical work of the muscle. Or, the high-energy phosphate bonds of ATP can also be used for biochemical work in the metabolic pathways which synthesize large molecules (e.g. DNA, proteins).

Energy transformation in the living system can be divided into three phases:

- **nutrient (fuel) oxidation** (carbohydrates, lipids, proteins) where enzymes that catalyze cellular oxidation capture released energy in electrons into just a few types of universal electron carriers (NADH, NADPH, $FADH_2$). This process results in the conservation of free energy released by substrate oxidation
- **energy transformation** to high-energy phosphate bonds of ATP. In this process electrons from reduced electron carriers are transferred by the mitochondrial electron transport chain to O_2. Energy of O_2 reduction is conserved in ATP generation in the process of oxidative phosphorylation (Fig. 5.2)

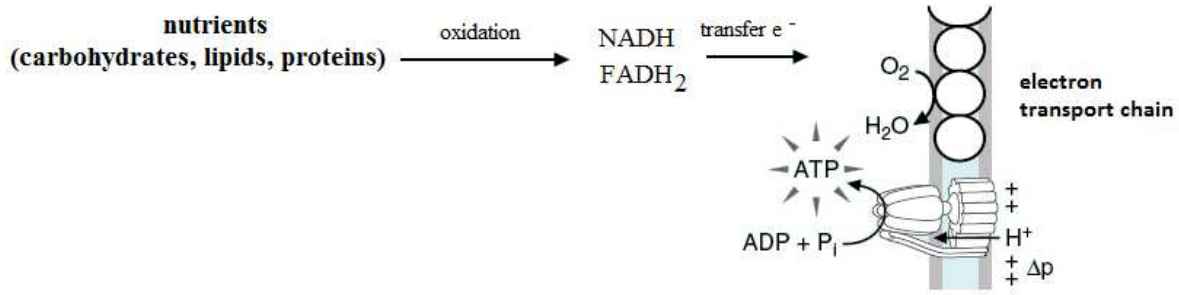


Fig. 5.2. Scheme of energy conversion of nutrients to energy of ATP

- **utilization** of energy of **energy rich** phosphate bonds in energy demanding cellular processes

The energy of ATP is employed to perform several types of the work:

- **mechanical work** where the energy of ATP hydrolysis results in conformational change of the protein with subsequent mechanical movement, e.g. muscle contraction
- **transport work (active transport across the membranes)** in which energy is used to transport molecules against a concentration gradient, e.g. Na^+ transport out of the cell by Na^+/K^+ pump
- **biochemical work** where energy of **energy rich** phosphate bonds is used in anabolic pathways for synthesis of large and complex biomolecules such as glycogen, proteins, DNA or for detoxification processes when toxic compounds are converted to nontoxic compounds that can be excreted (NH_4^+ conversion to urea in liver)

Some of the energy from fuel oxidation is converted into heat in the process of **thermogenesis**.

Fuel oxidation is regulated to maintain **ATP homeostasis.** Regardless of whether the level of cellular fuel utilization is high (increased ATP consumption) or low (decreased ATP consumption), the available ATP within the cell is maintained at a constant level by appropriate increases or decreases in the rate of fuel oxidation. Problems in ATP homeostasis and energy balance occur in some diseases such as obesity, myocardial infarction or hyperthyroidism.

Cell metabolism is highly coordinated cellular process and the remarkable ability of the organism is to deal with energy in super-efficient way. This efficacy is reflected in **coupling** of energy generating pathways (catabolic-degradative) to energy utilizing (anabolic-biosynthetic) pathways. So energy yielding processes can transfer energy directly to those requiring energy (Fig. 5.3)

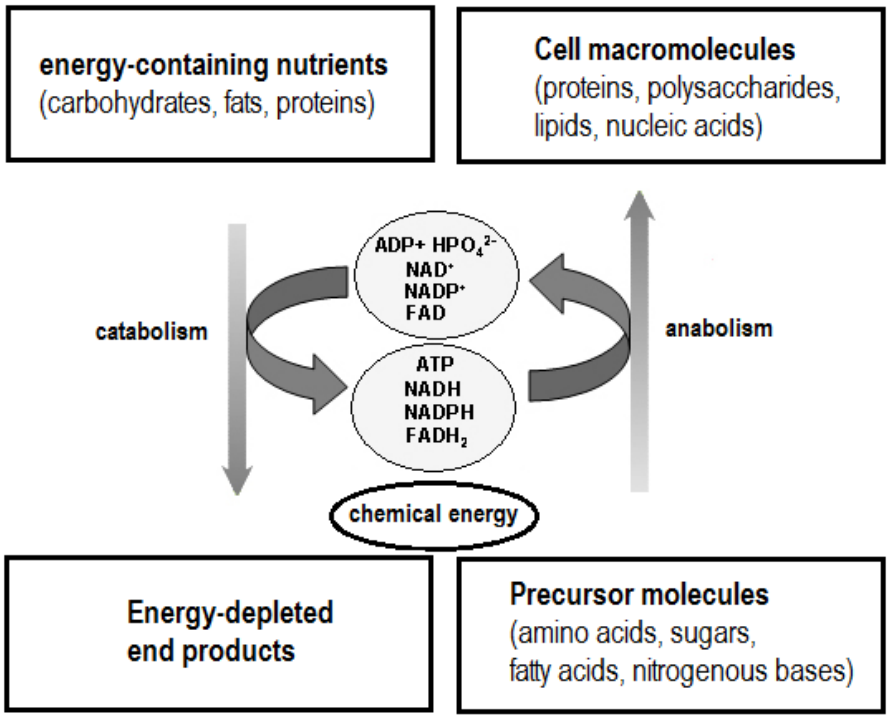


Fig.5.3. Energy relationships between catabolic and anabolic pathways
Catabolic pathways deliver chemical energy in the form of ATP, NADH, NADPH, and $FADH_2$. These energy carriers are used in anabolic pathways to convert small precursor molecules into cell macromolecules.

Since the flow and energy transformation follow the basic laws of physics, the utility of thermodynamic laws in biological systems is reviewed. The basic meaning is that the laws of thermodynamics can help to predict the feasibility of a physical process, its direction, to understand why molecules adopt their natural conformation or why move through cell membranes. But thermodynamics says nothing about the speed of processes (with speed of chemical processes deals chemical kinetics).

5.2. The basic law of thermodynamics

5.2.1. Organism as thermodynamic system

The molecules and ions contained within a living organism differ in kind and in concentration from those in the organism's surroundings. Although the characteristic composition of an organism changes little through time, the population of molecules within the organism is far from to be static. Small molecules, macromolecules and

supramolecular complexes are continuously synthesized and then broken down in chemical reactions that involve a constant flux of mass and energy through the system. The hemoglobin molecules carrying oxygen will be degraded and entirely replaced by new hemoglobin molecules. The glucose ingested with recent meal will be converted into carbon dioxide or fat and will be replaced with a fresh supply of glucose and the cycle will be repeated. Despite these transformations, the amounts of hemoglobin and glucose in the blood remain nearly constant because the rate of synthesis or intake of each just balances the rate of its breakdown, consumption, or conversion into some other product. The constancy of concentration is the result of a **dynamic steady state,** a steady state that is far from equilibrium. Maintaining this steady state requires the constant investment of energy. When the cell can no longer generate energy, it dies and begins to decay toward equilibrium with its surroundings. In this way the living organism is **an open thermodynamic system** which exchanges both energy and matter with its surroundings through its boundary. If the system exchanges energy but not matter with its surroundings, it is **a closed system.** If the system exchanges neither matter nor energy with its surroundings, it is said to be **isolated.**

5.2.2. The first law of thermodynamics

Many quantitative observations on the interconversion of different forms of energy led to the formulation of two fundamental laws of thermodynamics.

The first law of thermodynamics, which is the empirical law, is the principle of the conservation of energy. It states: *In any physical or chemical change, the total energy of a system, including its surroundings, remains constant.* It implies that within the universe energy may change form or it may be transported from one region to another, but it cannot be created or destroyed.

The equation that supports the first law of thermodynamics mathematically is:

$$\mathbf{\Delta U = Q + W} \tag{5.1}$$

where ΔU is the total change in internal energy of a system, Q is the heat exchanged between a system and its surroundings, and W is the work done on the system (W<0). If the work is made by the system (W>0), the equation 5.1 is

$$\mathbf{\Delta U = Q - W}$$

The internal energy of a system (U) encompasses many different kinds, including the kinetic energy associated with the motions of the atoms, the potential energy stored in the chemical bonds of the molecules or the gravitational energy of the system. It is nearly impossible to sum all of these contributions up to determine the absolute energy of the system. That is why we only worry about ΔU, the change in the energy of the system from the initial state to the terminal state. That means that ΔU is the state function. The internal energy would decrease if the system gives off heat or does work. Therefore, internal energy of a system increases when the heat increases (this would be done by adding heat into a system). The internal energy would also increase if work were done onto a system. Any work or heat that goes into or out of a system changes the internal energy. However, since energy is never created nor destroyed (the first law of thermodynamics), the change in internal energy always equals zero. If energy is lost by the system, then it is absorbed by the surroundings. If energy is absorbed into a system, then that energy was released by the surroundings. Basically, ΔU formulates the „net" energy change in the system, i.e. the difference in energy obtains from surroundings and energy released to surroundings (Fig. 5.4). Based on above, the well-known statement „*it is impossible to construct perpetual motion device*" is a popular formulation of the first law of thermodynamics.

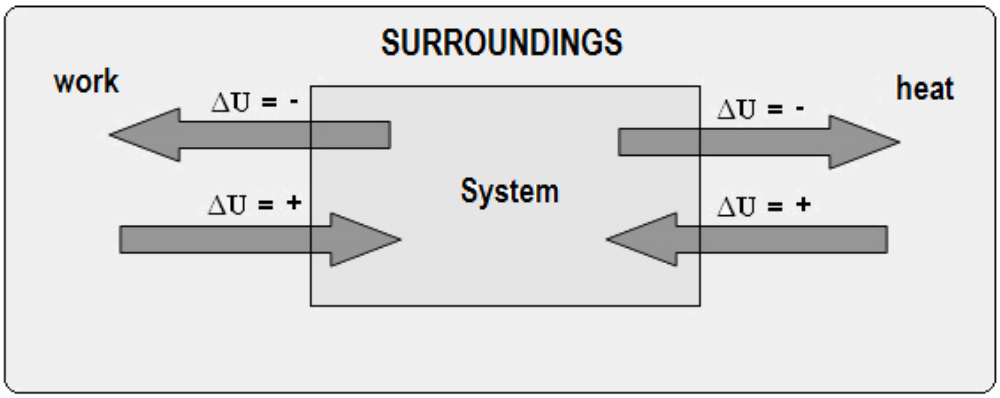


Fig. 5.4. Internal energy of a system, work and heat

Heat (Q) and **work (W)** are the forms of energy by means of the system changes its internal energy. Heat stands for the disordered form of energy, while work is the ordered form of energy.

Energy of the system is the state function, i.e. the energy of any system depends on the state of the system (initial and final) and not on the way, nature and pathways by which the system gets into the final state. For example, all glucose molecules contain the same amount of energy independent of biosynthetic pathways in which they were created. Heat and work are not the state quantities because they depend on the pathway by which the system has got from the initial to the final state.

According to the first law of thermodynamics, the energy in our consumed fuel can never be lost. Consumed fuel is either oxidized to meet the energy demands of the basal metabolic rate and exercise, or it is stored as fat. Thus, an intake of fuels in excess of those expended results in weight gain.

5.2.3. Hess's law

Hess's law states that *the heat evolved or absorbed in a chemical process is the same whether the process takes place in one or in several steps.* This is also known as the law of constant heat summation. Hess's law is saying that if you convert reactants A into products B, the overall enthalpy change (ΔH) will be exactly the same whether you do it in one step or two steps or however many steps (Fig.5.5)

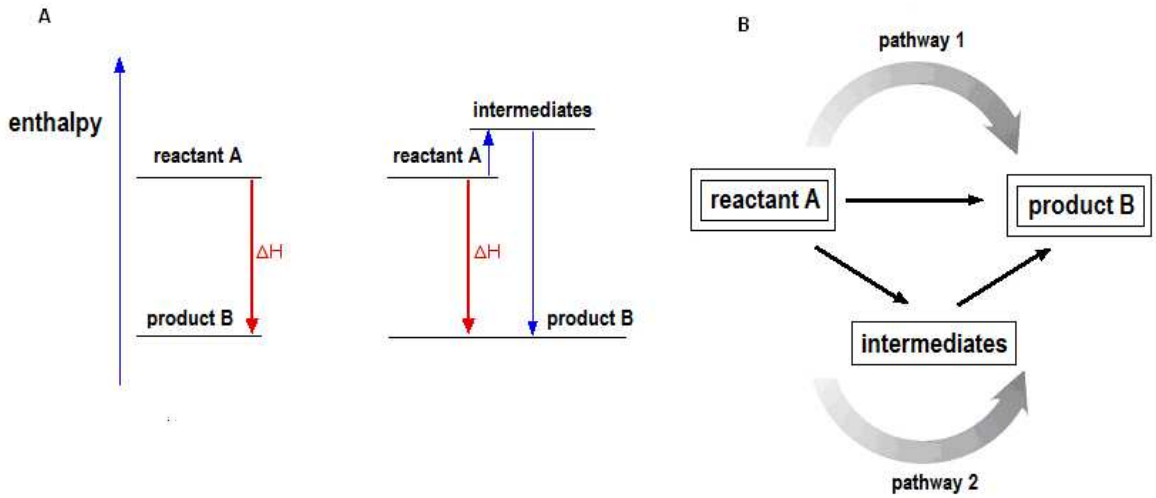


Fig.5.5. Graphical (A) and schematic (B) representation of enthalpy change in a chemical reaction which proceeds through intermediates or two steps

Enthalpy (H) is **the heat content** of a system and in a chemical reaction, the enthalpy of the reactants or of products is equal to their total bond energies.

The mathematical formulation of enthalpy is

$$\mathbf{H = U + pV} \tag{5.2}$$

where H is enthalpy of the system, U is the internal energy of the system, p is pressure and V is the volume of the system.

Compare now the definition of the total change in internal energy of a system (eq. 5.1) and the definition of enthalpy (eq. 5.2). Did you find out that they are very similar? What is the difference between them?

Enthalpy vs. internal energy of the thermodynamic system

In biochemical thermodynamics the enthalpy is also the state function and it is usually measured as heat transferred to or from the system under constant pressure (p). Because biological systems are generally held at constant pressure and volume ($\Delta V = 0$), the expression $pV = 0$ (it expresses the work) in the eq. 5.2. and

$$\mathbf{H = U} \tag{5.3}$$

Similar to the internal energy, enthalpy is not measured directly but as the change in enthalpy between the inital and the final state

$$\mathbf{\Delta H = \Delta U} \tag{5.4}$$

where ΔH is the change of enthalpy (J/mol), ΔU is the change of the internal energy.

That means, if the chemical reaction occurs under circumstances where the volume of the reaction is held constant, then the change in internal energy is equal to heat transferred in the reaction. So the difference between entropy and energy is insignificant in most biochemical systems.

When chemical reactions occur, the change of energy content of the system can be often detected as a temperature change, i.e. the system can release or absorb heat and the products have a different energy content than the original reactants. An **exothermic reaction** is one which gives out energy to the surroundings, usually in the form of heat energy. The products contain less energy than the reactants and ΔH is negative ($\Delta H<0$). If the products contain more energy than the reactants, heat is taken in or absorbed from the surroundings and the change is called an **endothermic reaction** ($\Delta H>0$).

The thermodynamic quantities are generally referred to standard conditions. The usual standard reference conditions are 298 K (25 °C and 1 atm/101 kPa), and other criteria may apply e.g. physical state of substance - gaseous, liquid, solid phase state, 1 molar solution etc. The values defined at this standard conditions are called **the standard values of thermodynamic functions** and they are indexed as ΔH^0, ΔU^0.

For example, the standard heat of water formation (atmospheric pressure, 25 °C) is ΔH = - 286 kJ/mol and this heat is released to surroundings and it is exothermic reaction

$$\tfrac{1}{2}\, H_2\,(g) + O_2\,(g) \rightarrow H_2O\,(l)$$

Thus, using Hess's law with standard heats of formation we can calculate heat of formation of any chemical reaction.

In summary, the relationship between the change in the internal energy of the system during a chemical reaction and the enthalpy of a reaction can be summarized as follows

1. enthalpy of a reaction or energy change of a reaction (ΔH) is an amount of energy or heat released ($\Delta H < 0$), or consumed ($\Delta H > 0$) in the reaction
2. the heat given off or absorbed when a reaction is run at constant volume or at constant pressure is equal to the change in the internal energy of the system
3. the difference between ΔU and ΔH for the system is small for reactions that involve only liquids and solids because there is little if any change in the volume of the system during the reaction. However, the

difference between ΔU and ΔH can be relatively large for reactions that involve gases, if there is a change in the number of moles of gas in the course of the reaction (Eq. 5.2)

5.2.4. The second law of thermodynamics

The second law of thermodynamics, which can be stated in several forms, is the law that formulates the spontaneity of processes. It describes the flow of energy in irreversible processes. It states: *the universe always tends toward increasing disorder: in all natural processes, the entropy of the universe increases.*

Many chemical and physical processes are reversible and yet tend to proceed in a direction in which they are said to be spontaneous. This raises a question: What makes a reaction spontaneous? What drives the reaction in one direction and not the other? The tendency of a spontaneous reaction to give off energy can't be the only driving force behind a chemical reaction. There is another factor that helps determine whether a reaction is spontaneous. This factor, known as **entropy (S)**, is a measure of the disorder of the system.

Entropy is the state function again and it defines the extent of randomness or disorder of the system. Any change in randomness of the system is expressed as the entropy change, ΔS, which by convention has a positive value when randomness increases.

The mathematical formula is

$$\Delta \mathbf{S} = \mathbf{Q/T} \quad \text{or} \quad \Delta \mathbf{S} = \Delta \mathbf{H/T} \tag{5.5}$$

where ΔS is entropy, Q is the flow of heat to or from the system, T is absolute temperature (K), ΔH is enthalpy and the total change of entropy is defined as

$$\Delta \mathbf{S}_{\mathbf{total}} = \Delta \mathbf{S}_{\mathbf{system}} + \Delta \mathbf{S}_{\mathbf{surroundings}} \tag{5.6}$$

The state with the maximal value of entropy is called **thermodynamic equilibrium**. The system in equilibrium is the most stable and has the highest entropy. In this meaning the death of an organism is thermodynamically the most stable and disordered state of the system, where the entropy of the system is equal to the entropy of surroundings.

Several factors can influence entropy, such as

- *volume change* of the system. When the volume is increased, more energy levels become available within which the system's energy can be dispersed
- *temperature change* - at higher temperatures, molecules have greater kinetic energy, making more energy levels accessible. This increases the number of energy levels within which the system's energy can be dispersed, causing entropy to increase
- *molecular complexity* - molecules can also exhibit rotational and vibration motions. Ordered forms (cyclic, spiral) restrict the motion of atoms and molecules and so their entropy is low. For example, under normal physiological conditions, polypeptides spontaneously fold into unique three-dimensional structures called native proteins. The functional or native state of a protein has very low entropy because its conformation is highly restricted. However, native proteins denature in the course of the time, their structures become disturbed and the entropy rises. That is why the organism degrades „old" proteins and synthesizes new ones.
- *increase in the number of particles in the system* – for example, passive ion transport through cell membranes increases the disorder of the system. To keep the entropy low, the organism must perform the work by pumping ions back through a cell membrane. This activity requires the input of energy which is obtained by ATP hydrolysis. So order is the result of work and energy investment.

These concepts may be summarized as follows:

- $\Delta S_{system} > 0$ it implies that the system becomes more disordered during the reaction and the reaction is *spontaneous* (however, this does not in any way describe how fast the reaction occurs). The spontaneous reaction is exothermic, the flow of heat is released into surrounding ($\Delta H<0$). $\Delta S_{system} > 0$ also in the case of some irreversible processes when there is no change in the surroundings. All spontaneous processes of fluid flows, heat flows and material flows are irreversible. As example, cells grow irreversibly, a drop of dye

expanding in water is also irreversible process because molecules diffuse and mix. $\Delta S_{system} > 0$ when heat flows from a hot to a cold object, and there is no change in the surrounding of the two objects, so $\Delta S_{total} > 0$.

- $\Delta S_{system} < 0$ it implies that the system becomes less disordered (more structured) during the reaction, the reaction requires energy to occur and the flow of heat is from surroundings to the system ($\Delta H>0$) (Tab. 5.1)

5.2.5. Entropy and order of biological systems

Living organisms consist of collections of molecules much more highly organized than the surrounding materials from which they are constructed. Life evolved in the process of evolution from the simple organized forms to the most complicated forms of life, including a human being. DNA, RNA and proteins are informational macromolecules where chemical energy is used to form the covalent bonds between the subunits. In addition, the cell must invest energy to order the subunits in their correct sequence. The investment of energy results in increased order in a population of molecules and decrease in entropy. But according to the second law of thermodynamics, the tendency in nature is toward ever-greater disorder in the universe. Does it mean that evolution violates the second law of thermodynamics? Surely not. How is it possible? The answer is hidden in **the transformation** of energy. A living organism is involved in a continuous and steady state process of taking up low-entropy food and energy from the environment. In the course of metabolic reactions, energy is used to form cell organized structure, i.e. order with a low level of entropy. However, a part of energy is released in the form of heat and waste. In doing so, surroundings becomes more disordered and its entropy is increased. Living organisms are able to decrease their own entropy by increasing the entropy of surroundings. Thus, **the local decrease in entropy as the result of formation more ordered structures is followed by increase in entropy of surroundings** and the second law of thermodynamics is not violated.

Another characteristic feature of the ordered structure of living organism is the **information content** of its macromolecules. Information can be measured in the terms of ordered arrangement of structure's subunits. For example, proteins and nucleic acids, in which the specific linear sequence of the subunits is highly ordered, are low in entropy and high in information content. Maintaining a state of high information content requires the input of energy. The ability of living organism **to couple energy, work and information** results in production of informational biomolecules. Doing so, the living system is functional and individual cells possess specific functions (muscle, nervous cells etc.). The functionality depends on some kind of complexity and the complexity is not the result of random processes. For example, it is extremely improbable that amino acids in a mixture would spontaneously condense into a single type of protein, with a unique sequence. What the difference is between order and complexity? Think about this example, consider crystals. They are very orderly, spatially periodic arrangements of atoms (or molecules) but they carry very little information. Nylon is another example of an orderly, periodic polymer (a polyamide) which carries little information. Nucleic acids and protein are aperiodic polymers, and this **aperiodicity** is what makes them able to carry much more information. By definition then, a periodic structure has order. An aperiodic structure has complexity. Living organisms are distinguished by their specified complexity. Crystals fail to qualify as living because they lack complexity; mixtures of random polymers fail to qualify because they lack specificity. Three sets of letter arrangements show nicely the difference between order and complexity in relation to information:

1. An ordered (periodic) and therefore specified arrangement:

   INFORMATION INFORMATION INFORMATION

2. A complex (aperiodic) unspecified arrangement:

   AGDCBFE GBCAFED ACEDFBG

3. A complex (aperiodic) specified arrangement:

   THIS SEQUENCE OF LETTERS CONTAINS INFORMATION!

Based on above, it can be concluded that informational macromolecules have a low degree of order but a high degree of specified complexity. This aspect according to recent research probably makes the basic difference between living organism and inanimate things.

Tab. 5.1 Relationship among the change of enthalpy, entropy and the spontaneity of a reaction

Change of enthalpy	Change of entropy	Is the reaction spontaneous?
Exothermic ($\Delta H < 0$)	increased disorder ($\Delta S > 0$)	Yes, $\Delta G < 0$
Endothermic ($\Delta H > 0$)	decreased disorder ($\Delta S < 0$)	No, $\Delta G > 0$

5.2.6. Gibbs free energy

According to the second law of thermodynamics, $\Delta S_{total} > 0$ (eq. 5.6.) for a spontaneous process. What we are usually concerned with and usually measure, however, are the properties of the system rather than those of the surroundings or those of the universe overall. Therefore, it is convenient to have a thermodynamic function that enables us to determine whether or not a process is spontaneous by considering the system alone. To express the spontaneity of a process more directly, we introduce another thermodynamic function called **the Gibbs free energy** (G), or simply free energy. Gibbs free energy expresses *the amount of energy capable of **doing work** during a reaction at constant temperature and pressure.* G has units of energy just as H and TS do. Like enthalpy and entropy, free energy is a state function. The change in free energy, ΔG, of a system for a process that occurs at constant temperature is

$$\mathbf{\Delta G = \Delta H - T\Delta S} \qquad (5.7)$$

where ΔG is the change of Gibbs free energy (J/mol), ΔH is the change of enthalpy, T absolute temperature (K), ΔS is the change of entropy.

Equation 5.7 enables us to predict the spontaneity of a process using the change in enthalpy, the change in entropy, and the absolute temperature. At constant temperature and pressure, for processes that are spontaneous as written (in the forward direction), ΔG is negative. The reaction is said to be **exergonic**. For example, biosynthesis of water

$$\mathbf{2H + \tfrac{1}{2}\, O_2 \rightarrow H_2O} \qquad \Delta G° = -237\ kJ.mol^{-1}$$

For processes that are not spontaneous as written but that are spontaneous in the reverse direction, ΔG is positive. The reaction is said to be **endergonic**. The examples of endergonic reactions in an organism are biosynthetic reactions which require the input of energy for synthesis of energy-rich products. When the system is at equilibrium, ΔG is zero.

- $\Delta G < 0$ The reaction is spontaneous in the forward direction (and nonspontaneous in the reverse direction)
- $\Delta G > 0$ The reaction is nonspontaneous in the forward direction (and spontaneous in the reverse direction).
- $\Delta G = 0$ The system is at equilibrium.

At this point is worth stressing the difference between an exothermic/exergonic reaction and an endothermic/endergonic reaction. You surely noticed that in eq. 5.1. are two functions: heat and work. The change of entropy is small (insignificant) in most of the classical chemical reactions and we consider only ΔH. Thus, an exothermic or endothermic reaction describes **the flow of heat energy**. In biochemical reactions the system is able to do a work and the change of entropy can be significant. Gibbs free energy considers **both the change of internal energy as well as the change of entropy of the system** and these reactions are called exergonic and endergonic.

The value of ΔG for a reaction can be influenced by initial concentration of substrates and products, temperature, pH and pressure. Similar to the rest of thermodynamic functions, ΔG is also confined to the standard conditions (25°C; 101.3 kPa). **The standard Gibbs free energy** ΔG^0 for a reaction refers to *the energy change for a reaction starting at 1 M substrate and product concentrations and proceeding to equilibrium.* It is the constant typical for every chemical reaction and its value can be find in the thermodynamic tables. Most biochemical reactions, however, occur in well-buffered aqueous solutions near pH 7. Both the pH and the concentration of water are essentially constant. Biochemists therefore define a different standard state, in which the concentration of H^+ is 10^{-7} M (pH 7), that of water is 55.5 M and Mg^{2+} (it binds to ATP) is commonly taken to be constant in solution at 1 mM. Physical constants based on this biochemical standard state are called **standard transformed**

constants and are written with a prime ($\Delta G'^0$) to distinguish them from the untransformed constants used by chemists and physicists (Tab. 5.2.) Although the difference between cellular conditions (pH 7; 37°C) and standard conditions is very small, the difference between cellular concentration of ATP, ADP and inorganic phosphate (P_i) is huge and greatly affects the availability of energy in the cell.

Tab. 5.2. Standard Gibbs free energy changes of some biochemical reactions

Type of reaction	**$\Delta G'^\circ$ (kJ/mol)**
Hydrolytic reactions	
ATP + H_2O → ADP + P_i	-30.5
ATP + H_2O → AMP + PP_i	-45.6
PP_i + H_2O → 2 P_i	-19.2
UDP-glucose + H_2O → UMP + glucose 1-phosphate	-43.0
Glucose 6-phosphate + H_2O → glucose + P_i	-13.8
Ethyl acetate + H_2O → ethanol + acetate	-19.6
Glutamine + H_2O → glutamate + NH_4^+	-14.2
Maltose + H_2O → 2 glucose	-15.5
Lactose + H_2O → glucose + galactose	-15.9
Elimination of water	
Malate → fumarate + H_2O	3.1
Rearrangement reactions	
Glucose 1-phosphate → glucose 6-phosphate	-7.3
Fructose 6-phosphate → glucose 6-phosphate	-1.7
Oxidation	
Glucose + $6O_2$ → $6CO_2$ + 6 H_2O	-2.84
Palmitate + 23 O_2 → $16CO_2$ + 16 H_2O	-9.77

The tendency of a chemical reaction to go to completion can be expressed as **an equilibrium constant (K_{eq})**. For the reaction

aA + bB → cC + dD

the equilibrium constant K_{eq}, is given by

$$K_{eq} = \frac{[C]^c[D]^d}{[A]^a[B]^b} \tag{5.8}$$

where [A], [B], [C], [D] are concentration of reactants (substrates) or products. Gibbs showed that ΔG for any chemical reaction is a function of the standard free-energy change, $\Delta G'^0$, a constant that is characteristic of each specific reaction, and a term that expresses the initial concentrations of reactants and products:

$$\Delta G = \Delta G'^0 + RT \ln \frac{[C]^c[D]^d}{[A]^a[B]^b} \tag{5.9}$$

In equilibrium, no driving force remains, no work can be done and $\Delta G = 0$. Then eq. 5.9 is transformed to

$$\Delta G'^\circ = - RT \ln K_{eq} \tag{5.10}$$

where R is the universal gas constant (8.31 J/molK), T absolute temperature (K).

Thus, $\Delta G'^{\circ}$ is simply a second way (besides K_{eq}) of expressing the driving force on a reaction. Because K_{eq} is experimentally measurable, we have a way of determining $\Delta G'^{\circ}$, the thermodynamic constant characteristic of each reaction. When $K_{eq} > > 1$, $\Delta G'^{\circ}$ is negative; opposite when $K_{eq} < < 1$, $\Delta G'^{\circ}$ positive (Tab. 5.3)

Tab. 5.3. Relationship among Gibbs free energy, ekvilibrium constant and spontaneity of reaction

K_{eq}	$\Delta G'^{\circ}$ (kJ/mol)	Spontaneity of reaction
10^{4}	-23	Spontaneous (→)
10^{2}	-11	Spontaneous (→)
$10^{0} = 1$	0	Equilibrium
10^{-2}	+11	Reversible (←)
10^{-4}	+23	Reversible (←)

Be warn the difference between ΔG and $\Delta G'^{\circ}$: $\Delta G'^{\circ}$ describes the change of Gibbs free energy under standard biochemical conditions, ΔG describes an actual free energy of a reaction taking place under actual cell conditions in the cell, i.e. actual concentrations of substrates, pH, temperature etc. The criterion of the spontaneity of a reaction is not the $\Delta G'^{\circ}$ but ΔG. Some reactions, despite their thermodynamically favorable state ($\Delta G<0$) do not run, in fact. The reason is the existence of the high initial energy barrier. These reactions require the initial input of energy called **activation energy** to overcome the activation barrier and proceed spontaneously. In cells **enzymes** are utilized as the tool for decrease of the activation barrier. The binding of an enzyme to a substrate is exergonic and the energy released by this binding reduces the activation energy for the reaction.

In living organisms an exergonic reaction can be coupled to an endergonic reaction to drive otherwise unfavorable reactions (Fig.5.6).

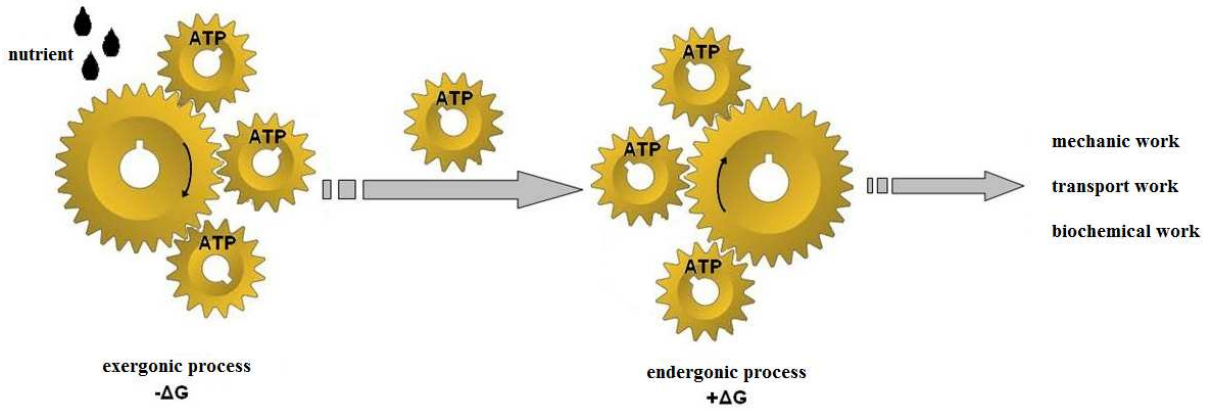


Fig. 5.6. Coupling of exergonic and endergonic reactions. ATP produced in an exergonic reaction is utilized in an endergonic reaction to run this energy unfavorable reaction

Coupling is based on these preconditions: the $\Delta G'^{\circ}$ values in a reaction sequence are **additive**, the pathway acquires an **overall negative $\Delta G'^{\circ}$** and there is **a common intermediate** in the reaction pathway. Shortly:

The first reaction	$A \rightarrow B$	$\Delta G_1'^{\circ}$
The second reaction	$B \rightarrow C$	$\Delta G_2'^{\circ}$
Sum:	$A \rightarrow C$	$\Delta G_1'^{\circ} + \Delta G_2'^{\circ}$

Oxidation and reduction reactions represent the major part of cell metabolism and they involve the transport of electrons. Each oxidation/reduction reaction makes or takes a fixed amount of energy ($\Delta G'^{\circ}$), which is directly proportional to the difference in reduction potentials of the oxidation/reduction pair ($\Delta E'^{\circ}$). **The reduction potential** of a compound, E°', is a measure in volts of the energy change when that compound accepts electrons (becomes reduced). $-E^{\circ}'$ is the energy change when the compound donates electrons (becomes oxidized). E°' can be considered an expression of the willingness of the compound to accept electrons. Some examples of reduction

potentials are shown in Table 5.4. Oxygen, which is the best electron acceptor, has the largest positive reduction potential + E'° (i.e., is the most willing to accept electrons and be reduced) and the transfer of electrons from all compounds to O_2 is energetically favorable and occurs with energy release. In an organism, the more negative the reduction potential of a compound, the greater is the energy available for ATP generation as universal energy currency in an organism when that compound passes its electrons to oxygen.

The direct relationship between the energy changes in oxidation/reduction reactions and ΔG'° is expressed by the equation

$$\mathbf{\Delta G'^\circ = -n\ F\ \Delta E'^\circ} \qquad (5.11)$$

where n is the number of electrons transferred, F is Faraday's constant (96 500 C/mol) and ΔE'° standard reduction potential.

Tab. 5.4. Reduction potentials of some oxidation/reduction reactions

Redox system	ΔE'° (V)
$½\ O_2 + 2H^+ + 2e^- \rightarrow H_2O$	0.82
2 cytochrome a-Fe^{3+} + $2e^- \rightarrow$ 2 cytochrome a-Fe^{2+}	0.29
2 cytochrome c-Fe^{3+} + $2e^- \rightarrow$ 2 cytochrome c-Fe^{2+}	0.25
Ubiquinone + $2H^+ + 2e^- \rightarrow$ ubiquinone-H_2	0.10
Fumarate + $2H^+ + 2e^- \rightarrow$ succinate	0.03
FAD + $2H^+ + 2e^- \rightarrow FADH_2$	- 0.18
Oxaloacetate + $2H^+ + 2e^- \rightarrow$ malate	- 0.10
Pyruvate + $2H^+ + 2e^- \rightarrow$ lactate	- 0.19
$NAD^+ + 2H^+ + 2e^- \rightarrow NADH + H^+$	- 0.32

Compounds are oxidized in the body in essentially three ways:

- the transfer of electrons from the compound as a hydrogen atom or a hydride ion
- the direct addition of oxygen from O_2
- the direct donation of electrons (e.g. $Fe^{2+} \rightarrow Fe^{3+}$)

Fuel oxidation in an organism involves the transfer of electrons to NAD^+ or FAD coenzymes which become reduced ($NADH+H^+$ or $FADH_2$). Electrons from reduced form of coenzymes are transferred to O_2 and the energy derived from reoxidation of coenzymes is available for the generation of ATP by oxidative phosphorylation. Which of the transfer is more favorable and provides more energy for ATP production? Find out it from the following calculation that utilizes the eq. (5.11) and Tab. 5.4.

The transfer of electrons from NADH donates electrons to O_2, then ΔE'° = + 0.32 (opposite of that shown in Tab. 5.4., the table shows values for accepting electrons) and ΔE'° = 0.82 for the transfer of electrons to O_2 . Then the ΔE'° for the net rection is calculated from the sum of the half-reactions

$$\Delta E'^\circ = E^\circ_{acceptor} + E^\circ_{donor}$$

$$\Delta E'^\circ = 0.82 + 0.32 = 1.14\ V$$

the number of transferred electrons is 2 (n=2)

$$\Delta G'^\circ = -n\ F\ \Delta E'^\circ = -2 \times 96{,}500 \times 1.14 = 220{,}019\ J/mol = \mathbf{-220\ kJ/mol}$$

The calculation for $FADH_2$

$$\Delta E'^\circ = 0.82 + 0.18 = 1.0\ V$$

the number of transferred electrons is 2

$$\Delta G'^\circ = -n\ F\ \Delta E'^\circ = -2 \times 96{,}500 \times 1.0 = 193{,}000\ J/mol = \mathbf{-193\ kJ/mol}$$

Based on the calculations can be concluded that the transfer of electrons from $NADH+ H^+$ to O_2 provides more energy for ATP synthesis than the transfer electrons form $FADH_2$.

5.2.7. Dependence of ΔG on reactant and product concentrations

One aspect of free energy changes contributing to the forward direction of anabolic pathways is the dependence of ΔG (eq. 5.9), the free energy change of a reaction, on the initial substrate and product concentrations. The dependence is of a great importance to regulation of metabolic pathways under physiological conditions and helps us to understand the diverted metabolic pathways under pathological conditions. Reactions in the cell with a positive ΔG'° can proceed in the forward direction if the concentration of substrate is raised to high enough levels, or if the concentration of product is decreased to very low levels. Product concentrations can be very low if, for example, the product is rapidly used in a subsequent energetically favorable reaction, or if the product diffuses or is transported away.

The effect of substrate and product concentration on ΔG and the direction of a reaction in the cell can be illustrated with conversion of glucose 6-phosphate (glu-6-P) to glucose 1-phosphate (glu-1-P) (Fig. 5.7). This reaction is the reaction in the pathway of glycogen synthesis and degradation. The reaction is catalyzed by the enzyme phosphoglucomutase and it is reversible.

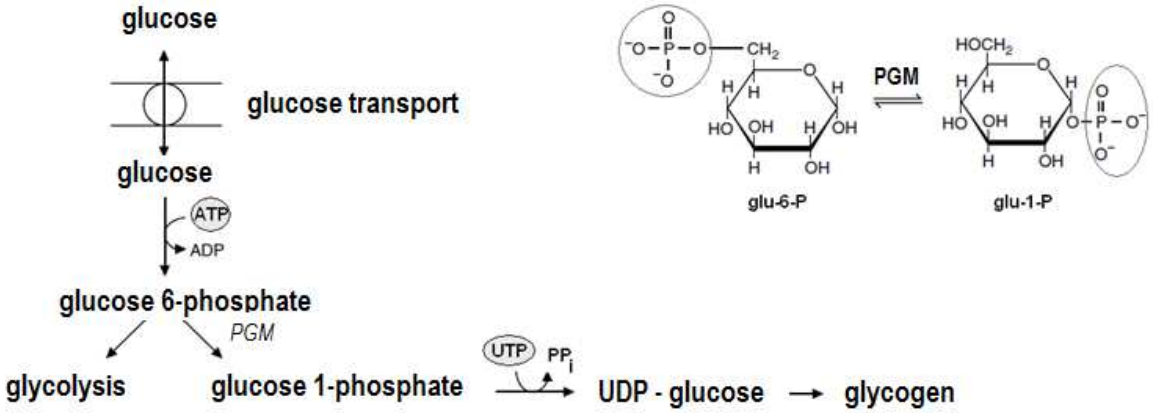


Fig. 5.7. Metabolic pathway of glycogen synthesis
ATP, UTP - energy rich compounds with high-energy bonds which donate energy for the reaction, PGM - phosphoglucomutase

Glu-1-P has a higher phosphate bond energy than glu-6-P because the phosphate is on the aldehyde carbon. The ΔG'° for the forward direction (glu-6-P → glu-1-P) is therefore positive (ΔG'° = +6.89 kJ/mol). Beginning at equimolar concentrations of both compounds, there is a net conversion of glu-1-P back to glu-6-P and at equilibrium the concentration of glu-6-P is higher than glu-1-P. The exact ratio is determined by ΔG'° for the reaction and can be calculated from the equation 5.10.

$$\Delta G'^{\circ} = - RT \ln K_{eq}$$

where K_{eq} is given by the ratio of the product concentration (P) to the substrate concentration (S)

$$K_{eq} = [\text{glu-1-P}] / [\text{glu-6-P}]$$

then

$$\Delta G'^{\circ} = -RT \ln ([\text{glu-1-P}]/[\text{glu-6-P}])$$

$$6.89 = -8.31 \times 10^{-3} \times 298 \times \ln ([\text{glu-1-P}]/[\text{glu-6-P}])$$

$$[\text{glu-1-P}]/[\text{glu-6-P}] = e^{-2.78}$$

$$[\text{glu-1-P}]/[\text{glu-6-P}] = \underline{\mathbf{0.062}}$$

so the ratio of [glu-1-P] to [glu-6-P] at equilibrium is 0.062.

However, if another reaction uses glu-1-P such that this ratio P/S will decrease to e.g. 0.032, then

$\Delta G = \Delta G'^{\circ} + RT \ln ([\text{glu-1-P}]/[\text{glu-6-P}])$

$\Delta G = 6.89 + 8.31 \times 10^{-3} \times 298 \times \ln 0.032$

$\Delta G =$ **- 1.63 kJ/mol (negative value!)**

Thus, a decrease in the ratio of product to substrate has converted the synthesis of glu-1-P from thermodynamically unfavorable to a thermodynamically favorable reaction that will proceed in forward direction (to glycogen synthesis) until equilibrium is reached.

The direction of this reaction is facilitated by the favorable energy balance of the overall pathway of glycogen synthesis from glucose. The glycogen synthesis incorporates reactions that expend high-energy bonds to compensate for the energy-requiring steps. Because the $\Delta G'^{\circ}$ values for a sequence of reactions are additive, the overall pathway becomes energetically favorable. The synthesis of glucose 6-phosphate is the first step in the utilization of glucose by many organisms:

glucose $+ P_i \rightarrow$ glucose 6-P $+ H_2O$ $\qquad \Delta G'^{\circ} = + 13.8$ kJ/mol

If the reaction were to proceed by addition of inorganic phosphate to glucose, glu-6-P synthesis would have a positive $\Delta G'^{\circ}$. However, in the organism this reaction is coupled to cleavage of the high-energy ATP bond through a phosphoryl transfer reaction

$ATP + H_2O \rightarrow ADP + P_i$ $\qquad \Delta G'^{\circ} = - 30.5$ kJ/mol

The energy released by ATP hydrolysis is used for the glu-6-P synthesis. The energy balance of glucose phosphorylation is calculated from the sum of the two reactions

glucose $+ P_i \rightarrow$ glucose 6-P $+ H_2O$

$ATP + H_2O \rightarrow ADP + P_i$

---

the sum: glucose + ATP → glu-6-P + ADP

$\Delta G = \Delta G'^{\circ}_1 + \Delta G'^{\circ}_2$

$\Delta G = +13.8 + (-30.5) =$ **- 16.71 kJ/mol**

The overall reaction is exergonic. In the next step, there is the conversion of glu-6-P to glu-1-P. The energy favorable direction of the reaction is obtained by using glu-1-P at the next reaction sequence (see calculation above). At the next step the cleavage of UTP and PP_i provide the energy (32.2 kJ/mol) for the generation of the activated intermediate UDP-glucose. In the last step of the glycogen synthesis the high-energy bonds of UDP-glucose (-13.8 kJ/mol) are used to bind glucosyl unit to the existing chain of glycogen (Fig. 5.7). Because free-energy changes are additive and the sequence of reactions share a common intermediate, the overall metabolic pathway is thermodynamically favorable and results in glycogen synthesis.

5.3. Energy rich compounds

Many biochemical pathways form activated intermediates containing high-energy bonds to facilitate biochemical work. The high-energy bonds, called **energy rich bonds** (marked as ~) are not a special kind of chemical bonds. They are covalent bonds with the high bond energy which is released during a bond cleavage. Energy rich bonds are defined by the ΔG for ATP hydrolysis ($\Delta G'^{0} = -30.5$kJ/mol). Any bond that can be hydrolyzed with the release of approximately as much, or more, energy than ATP is called a high-energy bond or a **energy rich** bond. The meaning of generation of the **energy rich** compounds in an organism is to store energy and facilitate the energy transfer to perform most of the work required in the cell.

5.3.1. ATP, phosphorylation and their meaning in organism

ATP plays a special role in an organism because links catabolism and anabolism reactions. Cells obtain free energy in a chemical form by the catabolism of nutrient molecules, and they use that energy to make ATP from ADP and P_i. ATP then donates some of its chemical energy to endergonic processes such as the biosynthesis of macromolecules, the transport of substances across membranes against concentration gradients, and mechanical motion. Through donation of energy ATP participates in the reactions, with the final result that ATP is converted to ADP and Pi or, in some reactions, to AMP and 2 P_i. This cycle is called **the ATP-ADP cycle**.

ATP (adenosine triphosphate) is made up of three main components, the base adenine, a phosphate chain made of three phosphate groups and a ribose sugar backbone (Fig. 5.8). It is a nucleotide that contains a large amount of chemical energy stored in two high-energy (energy rich) phosphate bonds. When these bonds are hydrolyzed, energy is released (– 30.5 kJ/mol) because the products of the reaction are more stable than the reactants. The instability of the phosphoanhydride bonds arises from their negatively charged phosphate groups. The hydrolytic cleavage of the terminal phosphoanhydride bond in ATP separates one of the three negatively charged phosphates and thus relieves some of the electrostatic repulsion in ATP. ADP has fewer negative charges to repeal each other and P_i is more stable because the electrons of the oxygen double bond are shared by all the oxygen atoms (resonance structure).

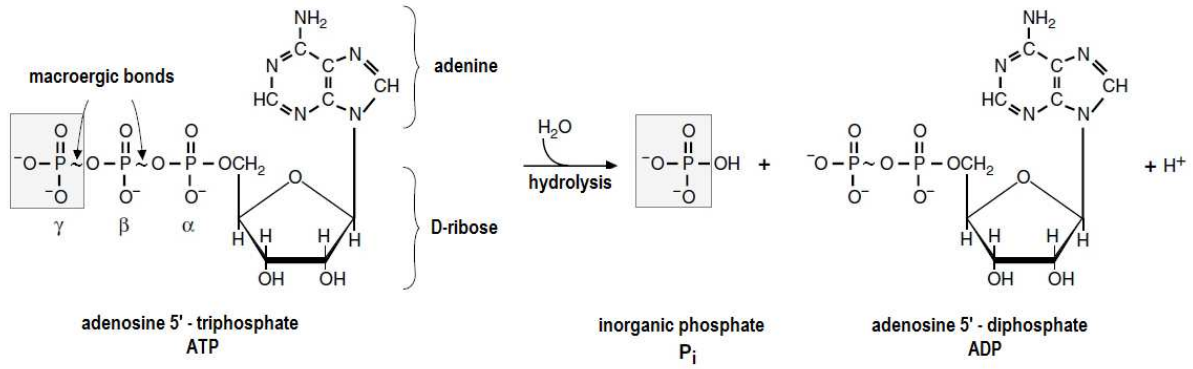


Fig. 5.8. ATP hydrolysis

Although the hydrolysis of ATP is highly exergonic, the molecule is kinetically stable at pH 7 because the activation energy for ATP hydrolysis is relatively high. Rapid cleavage of the phosphoanhydride bonds occurs only when catalyzed by an enzyme which decreases the activation energy of the reaction. The value -30.5 kJ/mol that is generally used for the $\Delta G'^0$ of ATP hydrolysis is thus the amount of energy available from hydrolysis of ATP under standard conditions. But the actual free energy of hydrolysis (ΔG) of ATP in living cells is very different and the cellular concentrations of ATP, ADP, and P_i are not identical and are much lower than the 1.0 M of standard conditions. Furthermore, Mg^{2+} in the cytosol binds to ATP and ADP and form the complexes which partially shield the negative charges and conformation of phosphate groups in ATP and ADP (Fig. 5.9). Thus the final ΔG under intracellular conditions depends on actual concentrations of substrates and pH in the cell. In intact cells, ΔG for ATP hydrolysis is much more negative than $\Delta G'^0$, ranging from -50 to -65 kJ/mol and is often called **the phosphorylation potential**.

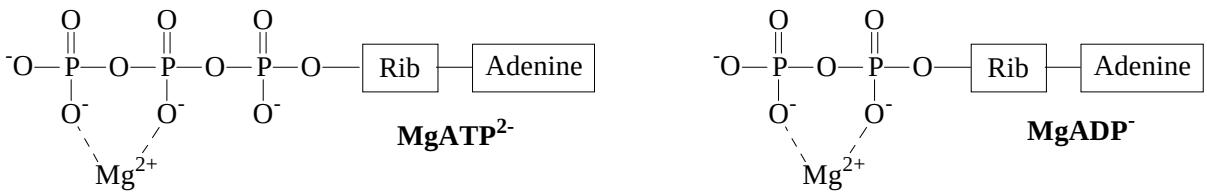


Fig. 5.9. Formation of ATP complexes with Mg^{2+}

Though it is often spoken about ATP hydrolysis in biochemistry, ATP is not hydrolyzed directly in the cell in the majority of cases. Energy released as heat from ATP hydrolysis cannot be transferred into energy-requiring processes such as biosynthetic reactions. In general, it is not ATP hydrolysis but **the direct transfer of a phosphoryl, pyrophosphoryl, or adenylyl group** from ATP to a substrate or enzyme molecule that couples the energy of ATP breakdown to endergonic transformations of substrates. The reactions of ATP are generally S_N2 nucleophilic displacements. Each of the three phosphates of ATP is susceptible to nucleophilic attack and each position of attack yields a different type of product. The nucleophile may be an alcohol (ROH), a carboxyl group (RCOO) or a phosphoanhydride. According to the position of nucleophilic attack and the group which is transferred to an acceptor (Fig.5.10), it is identified:

1. **the phosphoryl transfer** - a nucleophile attacts γ position in ATP, the group transferred from ATP is a phosphoryl ($-PO_3^{2-}$), not a phosphate ($-OPO_3^{2-}$) and ADP is displaced, $\Delta G'^0 \sim 30.5$ kJ/mol
2. **the pyrophosphoryl transfer** – attack at the β phosphate of ATP displaces AMP and transfers a pyrophosphoryl group, $\Delta G'^0 \sim 45.6$ kJ/mol
3. **the adenylyl transfer** – nucleophilic attack at the α position of ATP displaces PP_i and transfers adenylate (5'-AMP) as an adenylyl group. The reaction is called adenylylation, hydrolysis of the α – β phosphoanhydride bond releases considerable amount of energy, $\Delta G'^0 \sim 46$ kJ/mol. Furthermore, the PP_i formed as a byproduct of the adenylylation is hydrolyzed to two P_i by the ubiquitous enzyme inorganic pyrophosphatase, releasing 19 kJ/mol and thereby providing a further energy "push" for the adenylylation reaction. Adenylylation reactions are therefore thermodynamically very favorable and are used to drive a particularly unfavorable metabolic reaction, e.g. fatty acid activation, amino acid activation

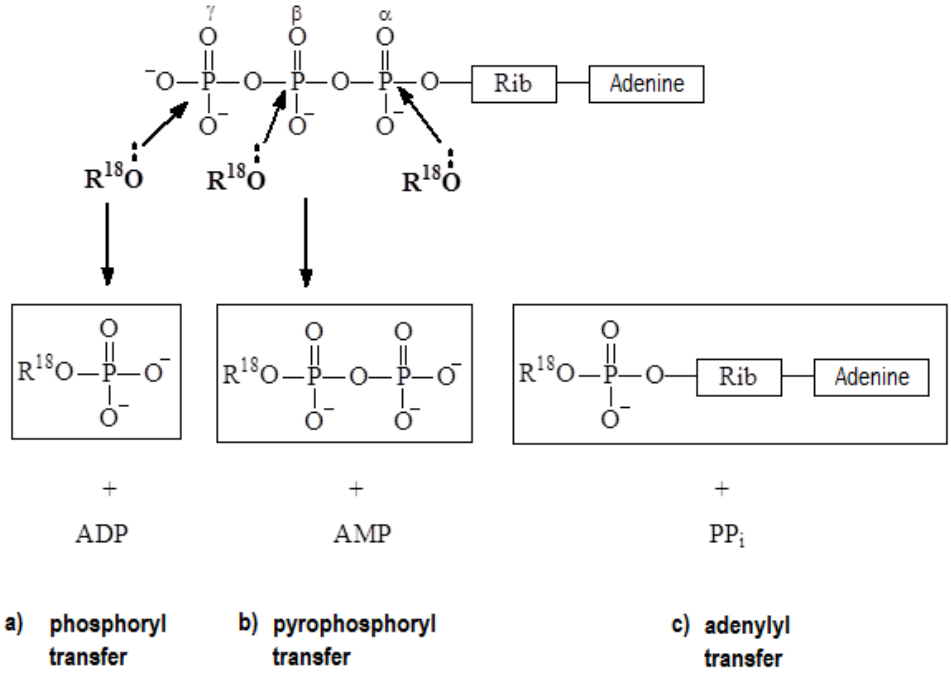


Fig. 5.10. Types of the energy transfer after a nucleophilic attack on ATP

The nucleophilic attack by the labeled nucleophile $R^{18}O$: produces three types of energy transfer (a) when the oxygen of the nucleophile attacks the γ position, the bridge oxygen of the product is labeled, the group transferred from ATP is a phosphoryl transfer (b) attack on the β position displaces AMP and leads to the transfer of a pyrophosphoryl group to the nucleophile (c) attack on the α position displaces PPi and transfers the adenylyl group to the nucleophile.

Nucleotides formed in these reactions are very important regulators of cell metabolism because many reactions in metabolism are controlled by the energy status of the cell. **Energy charge** is an index used to measure the energy status of biological cells. It is related to ATP, ADP and AMP concentrations and it is defined as

$$\frac{[ATP] + {}^1/_2\,[ADP]}{[AMP] + [ADP] + [ATP]}$$

In general, increased concentrations of AMP or ADP result in the stimulation of catabolic reactions, i.e. the reactions which produce ATP and at the same time energy-consuming reactions are blocked. Thus, the increased level of AMP in the cell represents the signal with the final meaning - „starvation“ of the cell.

Phosphoryl group transfers from ATP also result in an accumulation of ADP. ADP contains one **energy rich** phosphate bond therefore the cell is able to generate ATP from ADP in the reaction catalyzed by **adenylate kinase,** the enzyme that transfers a phosphate from one ADP to another ADP

$$2ADP \leftrightarrow ATP + AMP$$

Although ATP is the primary high-energy phosphate compound, several enzymes in the cell can carry phosphoryl groups from ATP to the other nucleotides (UDP, CDP, GDP). Nucleoside diphosphate kinase, found in all cells, catalyzes **the transphosphorylation reaction**

$$ATP + NDP \leftrightarrow ADP + NTP$$

where NDP is nucleoside diphosphate (UDP, CDP, GDP) and NTP is nucleoside triphosphate (UTP, CTP, GTP). Although this reaction is fully reversible, the relatively high [ATP]/[ADP] ratio in cells normally drives the reaction to the right, with the net formation of NTPs. Besides RNA synthesis, the cells use these NTPs to form activated intermediates. Different anabolic pathways use different nucleotides as the direct source of high phophate-bond energy, e.g. UTP is used in sugar metabolism, CTP in lipid metabolism and GTP in protein synthesis.

5.3.2. Other energy rich compounds

Organism, to ensure its energy demands, forms other compounds containing high-energy bonds. They are

- **phosphoenolpyruvate,** where a phosphate ester bond undergoes hydrolysis to yield the enol form of pyruvate, and this direct product immediately tautomerizes to the more stable keto form of pyruvate (Fig.5.11)

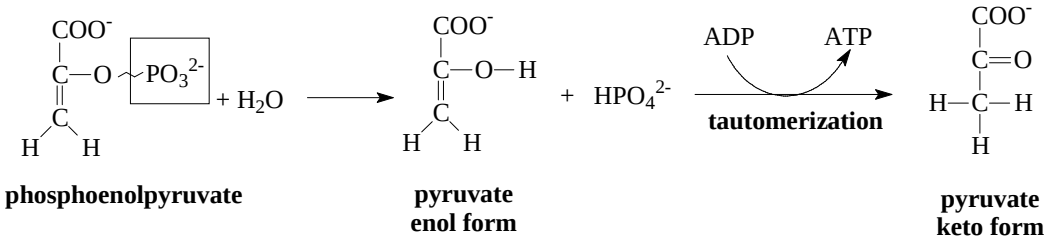


Fig. 5.11. Hydrolysis of phospoenolpyruvate

- **1,3-bisphosphoglycerate,** where hydrolysis of an anhydride bond is accompanied by a large, negative, standard free-energy change ($\Delta G'^{0}$ = - 49.3 kJ/mol) and resonance stabilization of products contributes to the negative free energy change (Fig. 5.12)

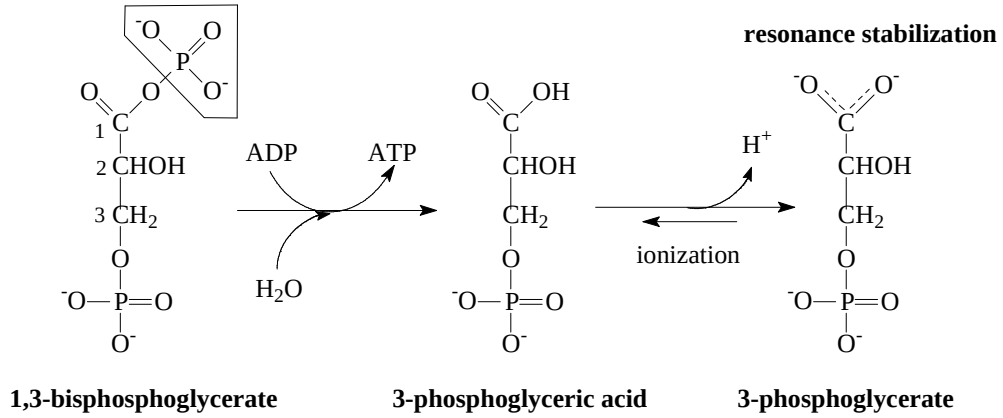


Fig. 5.12. Hydrolysis of 1,3- bisphosphoglycerate

- **thioesters,** in which a sulfur atom replaces the usual oxygen in the ester bond. Hydrolysis of thioester bond also have large, negative, standard free energy ($\Delta G'^{0}$ = - 31.4 kJ/mol). The acyl group in these compounds is activated for transacylation, condensation, or oxidation-reduction reactions. Acetyl-CoA is one of many thioesters important in metabolism (Fig. 5.13)

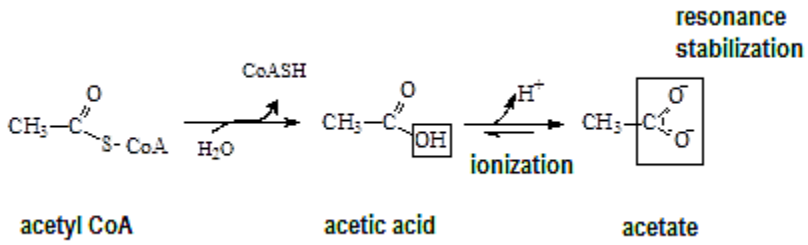


Fig. 5.13. Hydrolysis of acetyl coenzyme A

- **phosphocreatine,** the P-N bond is hydrolyzed to generate free creatine (Cr) and P_i (Fig. 5.14). The release of P_i and the resonance stabilization of creatine result in energy The standard free-energy change of phosphocreatine hydrolysis is again large ($\Delta G'^{0}$ = - 43.0 kJ/mol).

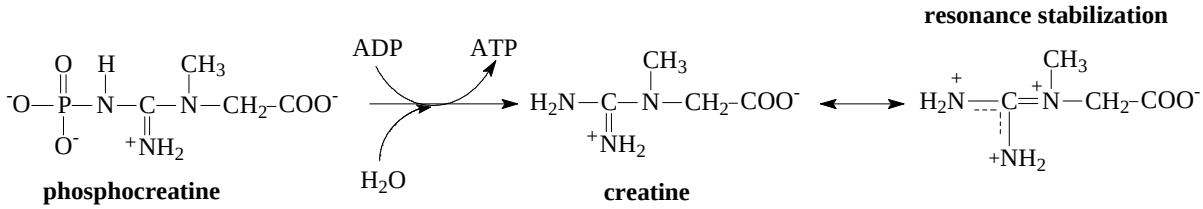


Fig. 5.14. Hydrolysis of phosphocreatine

Phosphocreatine (PCr) serves as a ready source of phosphoryl groups for the quick synthesis of ATP from ADP in the case of extended energy demand such as muscle contraction. The enzyme **creatine kinase** catalyzes the reversible reaction

ADP + PCr ↔ ATP + Cr

When the demand for energy slackens, ATP produced by catabolism is used to replenish the PCr reservoir by reversal of the creatine kinase reaction.

As you could see in this chapter, in the course of evolution an organism has developed highly efficient mechanisms for coupling the energy obtained from sunlight or fuels to the many energy consuming processes it must carry out. ATP as universal energy currency plays the central role in these processes. Cells maintain constant levels of ATP despite fluctuations in the rate of utilization. Therefore, increased utilization of ATP increases the rate of fuel oxidation. Opposite, the less ATP is used, the less fuel will be oxidized. The major control mechanism in regulation of fuel oxidation employs the feedback regulation by ATP levels or by compounds related to the concentration of ATP. Thus based on the validity of the thermodynamic laws, energy received in consumed fuel is either oxidized to meet the energy demands of the basal metabolic rate and exercise, or it is stored as fat.

Control questions:

1. Calculate the equilibrium constant of fumaric acid conversion to malic acid if a change in standard Gibbs energy (ΔG^0 = -880 J/mol, T= 310 K)

2. The highest-energy phosphate bond in ATP is located between which of the following groups?
   a) adenosine and phosphate
   b) ribose and phosphate
   c) ribose and adenine
   d) two phosphate groups

3. Assess the type of a reaction that occurs in the conversion of citric acid to α-ketoglutaric acid, if the standard Gibbs energy of these reactions are:
   citrate $^{3-}$ ↔ isocitrate $^{3-}$ ΔG^0 = +1,490 J/mol
   isocitrate $^{3-}$ + ½ O_2 + H_2 ↔ α-ketoglutarate $^{2-}$ + H_2O + O_2 ΔG^0 = -54,400 J/mol

4. Which of the following statements best describes the direction a chemical reaction will follow:
   a) A reaction with positive free energy will proceed in the forward direction if the substrate concentration is raised enough
   b) A reaction will proceed in the forward direction if the free energy $\Delta G^{0'}$ is positive under the standard conditions
   c) The direction of a reaction is independent of the initial substrate and product concentration because the direction is determined by the change in free energy
   d) The enzyme for the reaction must be working at > 50% of its maximum efficiency for the reaction to proceed in the forward direction

6. ORGANIC COMPOUNDS AND THEIR BIOLOGICALLY IMPORTANT REACTIONS

6.1. General characteristics of organic compounds.

Organic chemistry is the **chemistry** of **carbon compounds**. In organic compounds carbon atoms are **covalently** bonded to each other and to atoms of other nonmetals, such as hydrogen, oxygen, nitrogen, sulfur and the halogens. All other compounds are inorganic, but they include a few carbon compound – oxides of carbon, carbonates, bicarbonates, cyanides, cyanates. While there are known only about 500 thousands of inorganic compounds, natural or synthetic organic compounds are over 15 millions. Almost all reactions in living matter involve organic compounds.

Carbon is of central importance **at the molecular level of life**, because its atoms make up most of the backbones of molecules (other than water) that are in living cells. The major constituents of living matter **- proteins, carbohydrates, lipids, nucleic acids, enzymes, hormones, cell membranes are organic.** These compounds have quite complex structures. To understand them, we will have to study simpler molecules first.

Originally, organic chemistry concerned only the substances obtained from living matter. For years scientists believed that organic compounds may be formed only in living systems. However, in 1828, the German chemist Wöhler prepared urea in laboratory by heating the inorganic compound ammonium cyanate, without the necessity of a kidney. Urea is an organic compound which is formed in the liver and excreted by kidneys (the main nitrogen waste of animals).

$$\underset{\text{Ammonium cyanate}}{NH_4CNO} \longrightarrow \underset{\text{Urea}}{H_2N–CO–NH_2}$$

Organic substances include not only natural products but also synthetic substances. At present, the number of organic compounds that have been synthesized in academic and industrial research laboratories is far greater than the number known natural products. Another reason for synthesis is to create new substances with more useful properties than the natural products. Many compounds used in medicine are synthetic.

6.2. Structure of organic molecules

Carbon atom is in the group 14 (carbon group) of periodic table of elements. With four valence electrons, the valence shell of carbon is half filled. Carbon atom has neither a strong tendency to lose all its electrons nor a strong tendency to gain four electrons. Being in the middle of the periodic table, carbon is neither strongly electropositive nor strongly electronegative. Instead, it usually forms covalent bonds with other atoms by sharing electrons. The sharing of electron pair leads to formation of a **covalent bond.**

Carbon exists in two forms, In basic state (inorganic carbon) its electron configuration is expressed as ${}_6C$ ($1s^2\ 2s^2\ 2p_x^1\ 2p_y^1 2p_z^0$) from which it follows that carbon atom is two – bonded. In excited state electron „jumps“ from *s* orbital to energetically more rich orbital *p*. Electron configuration of that carbon atom (organic carbon) can be written as ${}_6C$ ($1s^2\ 2s^1\ 2p_x^1\ 2p_y^1 2p_z^1$), where is visible that carbon atom in excite state has four non-paired electrons, that is why it will be in its compounds four - bonded - it can take part in the formation of four single bonds.

basic state	↑↓	↑↓	↑	↑	
excited state	↑↓	↑	↑	↑	↑
	1s	**2s**	**$2p_x$**	**$2p_y$**	**$2p_z$**

All bonds are equivalent and symmetrically organized, valence angles in space are approximately 109° (e.g. in methane molecule).

Organic chemistry is a chemistry of covalent bond, characteristic with sharing of one electron pair (simple bond, C – C, with bond angles 109°), two electron pairs (double bond, C = C, with bond angles 120°) or three electron pairs (triple bond, C ≡ C with bond angles 180°) either between carbon atoms or between the carbon atom and another element.

The prevalence of nonmetal atoms in organic compounds means that their molecular structures are dominated by covalent bonds. Most inorganic compounds are ionic. Carbon-carbon and carbon-hydrogen bond are the most

prevalent in organic molecules. Organic compounds are relatively nonpolar, except when electronegative atoms, such as oxygen and nitrogen are present. Most organic compounds are relatively insoluble in water, but many ionic compounds are soluble.

The properties of organic compounds are influenced with polarization of covalent bonds when arrangement of molecules and substituents causes shift of electron density around the atoms. There are formed reactive places in molecules of organic compounds allowing reaction to proceed.

Structure theory – the chemical properties of compounds are determined by different arrangement of atoms in molecule and their mutual relations, i.e. chemical structure. Each compound has certain characterizing structure. The structural features allowing to classify organic molecules are called **functional (characteristic) groups** (Chapter 6.4.1).

6.2.1. Relationship between compounds structure and properties. Isomerism.

The **molecular formula** of a substance tells us the numbers of different atoms present, but a **structural formula** tells us how those atoms are arranged. Molecules that have the same molecular formula (kinds and number of atoms) but different arrangements are called **isomers.** So, isomers are different compounds, that have the same molecular formula, different molecule structure and different chemical and physical properties. Existence of unlimited number of organic compounds results from the existence of isomerism.

When we consider the molecules of nature, such as carbohydrates, lipids, proteins, and nucleic acids, we will see that different isomers have different chemical and physiological behavior. We often find that although there are many possible isomers, only one isomer has physiological function. There is high selectivity in the molecules used in nature.

There are two different types of isomers – **constitutional isomers** and **stereoisomers**. Both types of isomers play key roles in the selectivity of biological processes.

6.2.1.1. Constitutional isomers

Constitutional isomers are different compounds having the same molecular formula but different structural formulas – different connectivity, the order in which atoms are attached to one another. There are types of connectivity differences, any one of which gives rise to constitutional isomers:

a) **carbon chain isomerism** (different carbon skeletons, branched or unbranched chain)

C_4H_{10}	$CH_3 - CH_2 - CH_2 - CH_3$	$CH_3–\underset{\displaystyle CH_3}{\underset{\vert}{CH}}–CH_3$
molecular formula	butane	2-methylpropane (isobutane)

b) **position isomerism** (different placements of the functional groups on the same carbon skeleton)

C_3H_8O	$CH_3 - CH_2 –CH_2 – OH$	$CH_3–\underset{\displaystyle OH}{\underset{\vert}{CH}}–CH_3$
molecular formula	1-propanol	2-propanol

c) **functional group isomerism** (different functional groups together with different carbon skeletons)

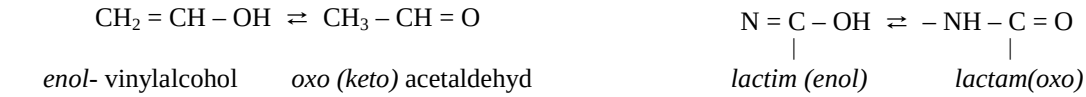
C_2H_6O	$CH_3 – CH_2 – OH$	$CH_3–O–CH_3$
molecular formula	ethanol	dimethyl ether

d) **isomerism of double bonds position** (different position of diouble or triple bond or their number)

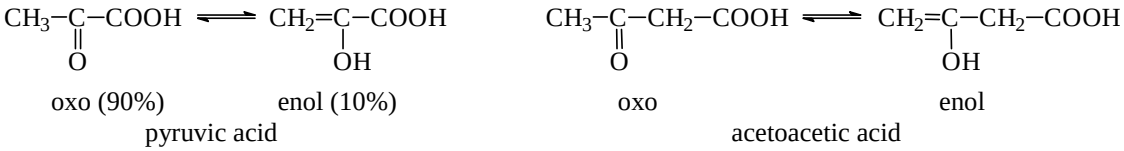
C_4H_8	$CH_2 = CH – CH_2 – CH_3$	$CH_3 – CH = CH – CH_3$
molecular formula	1-butene	2-butene

e) **oxo – enol** and **lactam – lactim** tautomerism (different position of hydrogen atom, which is conditioning position of double bond)

Many aldehydes and ketones actually exist as an equilibrium mixture of two forms called the keto (oxo) form and the enol form. The two forms differ in the location of a proton and a double bond.



Lactim – lactam tautomerism is very important in purine and pyrimidine bases of nucleic acids. Oxo – enol tautomery is exhibited in case of molecules of oxo acids, e.g. pyruvic acid and acetoacetic acid:



6.2.1.2. Stereoisomers

Stereoisomers have the same connectivity but differ in their configurations. **Configuration** describes the relative orientations in space of the atoms of a stereoisomer, independent of changes that occur by rotation about the single bond (they are of different biological activity). There are two classes of stereoisomers:

a) **geometrical stereoisomers**, also called **cis – trans** isomers.

Geometrical isomers are compounds with double bond (as alkenes) and cyclic compounds, when there is bound the same substituent on each of two carbon atoms which are connected by double bond.

Examples of geometrical isomers of compounds with **double bonds** are cis– and trans–2-butene or cis– and trans– butendioic acid:

Geometrical isomery causes different properties of both isomers. The fumaric acid is biochemical important component of citrate cycle, while maleinic acid is a little toxic.

Cis - trans isomerism is important in several biological processes, one of which is the vision. In this process isomerization of highly unsaturated aldehyde of cis–retinal to trans–retinal plays role. Retinal arises by oxidation of alcohol, –OH group of vitamine A to aldehyde group.

Cis –trans stereoisomerism in **cycloalkanes.** The carbon – carbon bonds of a ring, unlike those in acyclic structures, have restricted rotation. The carbon atoms of a ring are not free to rotate relative to each other. Certain cycloalkanes posess geometrical, or cis–trans, stereoisomerism, a type of isomerism different from constitutional isomerism, because of this restricted rotation. Cis–trans stereoisomers have the same substituents attached to the same carbon atoms of the ring – the same connectivity – but the compounds differ in configuration – that is, in the spatial orientation in which the substituents extend from the ring carbons into the regions lying on opposite sides of the plane of the ring. The cis– isomer is the isomer in which two identical substituents on two different ring carbons are on the same side of the ring. The trans– isomer has the two similar substituents on opposite sides of the ring. For example cis– and trans– stereoisomers of cyclopropane:

b) enantiomers, or **optical isomers.**

Optical isomerism is conditioned by presence of **asymmetric (chiral)** carbon atom (C*). Asymmetric carbon atom, called **chiral center**, is defined as a carbon atom with four different groups attached to it.

Enantiomers are isomers that are nonsuperimposable mirror images of each other. The two molecules are mirror images if each one can see the other as its mirror image. The two molecules are nonsuperimposable if they do not become identical. A molecule that is nonsuperimposable on its mirror- image molecule is said to be chiral or to have chirality. Molecules that do not poses chirality are achiral.

Enantiomers have **configuration D- or L-.** Chiral compounds exhibit optical activity, and are said to be optically active. The enantiomers of an enantiomeric pair are identical in all their physical properties (e.g. boiling point, solubility) except optical activity. Optical activity is the ability of a compound to rotate the plane of polarized light. The two enantiomers of a pair of enantiomers rotate the plane of polarized light by the same number of degrees, but in opposite direction. One enantiomer rotates light in the clockwise direction and is called dextrarotatory. The direction of rotation of plane of polarized light is indicated in the names of enantiomers by placing (+) and (-) as prefixes to their names, for example (+) – glyceraldehyde and (-) – glyceraldehyde. The direction of optical rotation (+ or -) is determined with polarimeter. The configuration (whether D- or L-) is determined by X – ray crystallography. There is no general relation between configuration and direction of optical rotation. Some D- enantiomers are dextrarotatory (e.g. D-(+)-glyceraldehyde), other are levorotatory (e.g. D-(-)- fructose, or L-(+)-lactic acid).

D(+) - glyceraldehyde **L(-) - glyceraldehyde**

Equimolar mixture (1:1) of both enantiomers is signed as D,L- (racemate) and is without optical activity.

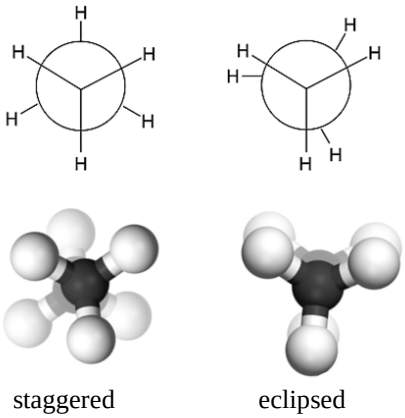
The enantiomers of an enantiomeric pair have different biological activities. Enzymes of saccharide metabolism catalyze reactions only of D(+)-saccharides. Enzymes of mammal catalyze only reactions of L-amino acids. Lactate dehydrogenase oxidizes only L(+) –lactic acid, and cannot change D(-) – lactic acid. One enantiomer of an enantiomer pair can be active medicament (e.g.. (-) – adrenaline), while the other is inactive ((+) – adrenaline). One may be toxic, and the other without this effect.

6.2.1.3. Conformational isomers (conformers)

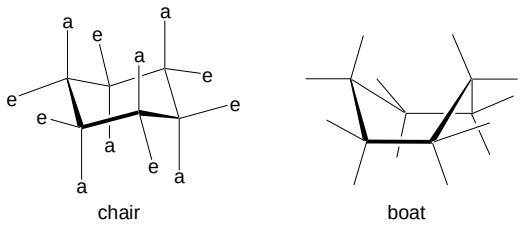
A single bond between any two atoms possesses free rotation, that is, the atoms in the bond are able to rotate freely relative to one another through 360° , and they do it continuously. There is little or no energy rotation about single bonds. Due to the free rotation a molecule can have different **conformations.**

Conformation of molecule is arrangement of a given configuration in space. Conformation is usually critical for large-sized biochemical molecules such as proteins, carbohydrates and nucleic acids. One conformation is favored over others for a large-sized molecule, and this specific conformation determines the molecule's overall shape, which in turn is critical to its physiological function.

Conformation of **alkanes**. For a simple molecule like ethane, for example, an infinite number of structures is possible as a consequence of rotating one carbon atom (and its attached hydrogens) with respect to the other carbon atom. Two of possible conformations of ethane are staggered and eclipsed. In the **staggered** conformation of ethane, each C –H bond on one carbon bisects an H- C –H angle on the other carbon. In the **eclipsed** conformation, C-H bonds on the front and back carbons are aligned. By rotating one carbon 60° with respect to the other, we can interconvert staggered and eclipsed conformations. Between these two extremes there is an infinite number of intermediate conformations of ethane:



Conformations of cycloalkanes. Six-membered rings are common in nature. If **cyclohexane** were a planar hexagon, the internal C–C–C angles would be 120° - quite a bit larger than the tetrahedral angle (109.5°). The strain that would result from such angles prevents cyclohexane from being planar. The most favored conformation of cyclohexane is the **chair conformation**, an arrangement in which all the C-C-C angles are the normal 109.5°, and all the hydrogens on adjacent carbon atoms are perfectly staggered. In the chair conformation, the hydrogens in cyclohexane fall into two sets, called **axial** and **equatorial**. Three axial hydrogens lie above and three lie below the average, or mean, plane of the carbon atoms, the six equatorial hydrogens lie approximately in that plane. By a motion in which alternate ring carbons move in one direction (down) and the other three ring carbons move in the opposite direction (up), one chair conformation can be converted into another chair conformation in which axial hydrogens have become equatorial, and vice versa. Another puckered conformation for cyclohexane, one in which all C-C-C angles are the normal 109.5° is the **boat conformation**. This conformation is very much less stable than the chair conformation:



6.3. Reactions of organic compounds

Reagents in organic chemistry have character of low molecular weight compounds (molecules, ions, radicals) reacting with substrate. We recognize:

a) **homolytic reagents** – they are free radicals – particles containing one or more non paired electrons (e.g. H•, Cl•). They exist only for splits of seconds and have tendency to connect resulting in formation of covalent bond.

b) **heterolytic reagents** – they are ions or polar molecules. In their molecules they contain free electron pairs.
   - **nucleophilic** – reagent is a donor of electron for formation of bond (e.g. OH^- , X^-/halogen/, H_2O, NH_3
   - **electrophilic** – reagent is an acceptor of electrons in reaction (e.g. $^+SO_3H$, $^+NO_2$, Cl^+)

Reaction are classified:

1. according to the nature of bond cleavage to:

a) radical reactions – homolytic cleavage of bond $\mathbf{X\bullet \bullet Y \rightarrow X\bullet + Y\bullet}$

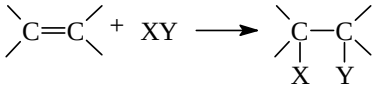
During homolytic cleavage of bond each of atoms connected in covalent bond retains one of valence electrons. Homolytic cleavage of bond of stable compound can be caused with ultraviolet light or effect of a homolytic reagent.

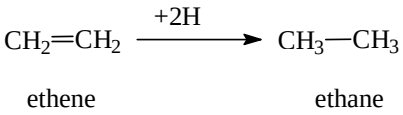
b) ionic reactions – heterolytic cleavage of bond $\mathbf{X\bullet \bullet Y \rightarrow X:^- + Y^+}$

During heterolytic cleavage of covalent bond the complete electron pair stays with one atom. According to the reagent ionic reactions are classified to – <u>nucleophilic</u> – using nucleophilic heterolytic reagent
– <u>electrophilic</u> – using electrophilic heterolytic reagent

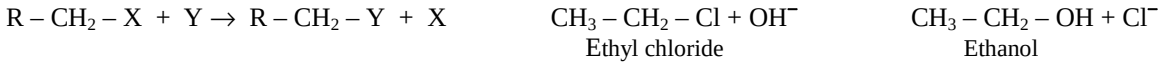
2. according to the structural change of substrate to:

a) addition – double or triple bond change into the simpler one

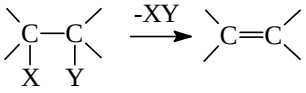


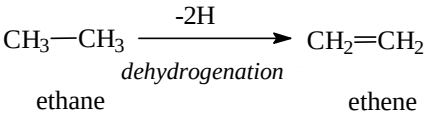


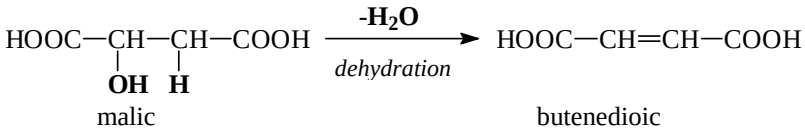
b) substitution – in a substrate molecule (initial compound) atom or group of atoms are substituted with another atom or group of atoms, respectively.



c) elimination ⟶ - (reverse of addition) – from substrate simple molecule (water, hydrogen, ammonia, halogen, hydrogen halide) is eliminated to form product with multiple bond







d) Molecular rearrangements – reshuffle of atoms or group of atoms from one place in molecule of substrate to another one to form more stable product (e.g. isomerization of ammonium cyanate to urea in heating).

6.4. Types of organic compounds and their characteristic groups

The study of organic chemistry is organized around a functional (characteristic) group. Parts of molecules that include nonmetal atoms other than C and H, or that have double or triple bonds, are specific sites in organic molecules most often attacked by chemicals. These small units are called **functional** (characteristic) **groups**, because they are chemically functioning locations. Although over fifteen million organic compounds are known, there are only a handful of groups, and each one serves to define a family of organic compounds (Tab. 6.1.)
Organic compounds according to arrangement of carbon chains are:

1. <u>Acyclic</u>
- unbranched (straight chain)
  e.g. butane $CH_3 – CH_2 – CH_2 – CH_3$
- branched, e.g. isobutane $CH_3 – CH(CH_3) – CH_3$

2. <u>Cyclic</u>
- alicyclic (cyclohexane, cholesterol)
- aromatic – arenes (benzene)
- heterocyclic (pyrrole, nicotine)

Both, cyclic and acyclic compounds, according to present bonds may be saturated or unsaturated. Cyclic compounds that contain rings of carbon atoms are called **carbocyclic**. The smallest possible cyclic ring has three carbon atoms, but carbon rings come in many sizes and shapes. The rings may have chains of carbon atom attached to them and may contain multiple bonds. Five- and six-membered rings are most common. Many compounds with more than one cyclic ring are known.

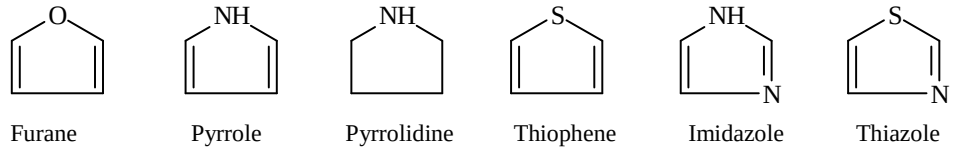
Aromatic compounds

Aromatic compounds (e.g. benzene) are class of cyclic substances with special chemical properties, called aromaticity. They contain conjugated system of double bonds, despite its, they do not behave as unsaturated, their test for unsaturation is negative. They do not undergo the typical addition reactions, like compounds with double bonds. They are not easily oxidized by oxidizing agents (e.g. potassium permanganate, $KMnO_4$). They do not act as unsaturated, because π- electrons of their conjugated double bonds are delocalized – the π- electrons are distributed evenly around the ring.

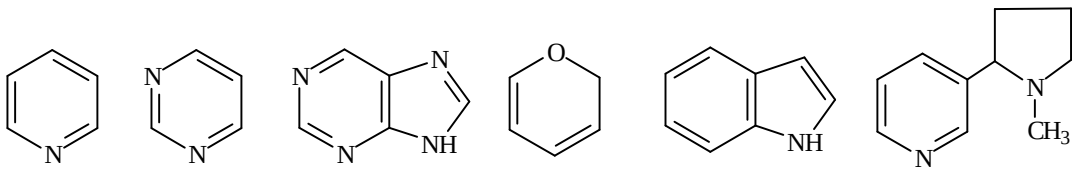
Heterocyclic compounds

Heterocyclic compounds are cyclic compounds, in which at least one atom in the ring must be a **heteroatom,** an atom that is not carbon. The common heteroatoms are oxygen, nitrogen and sulfur, but many hetorocycles with other elements are also known. More than one heteroatom may be present, and, if so, the heteroatoms may be alike or different. Heterocyclic rings come in many sizes, may contain multiple bonds, may have carbon chains or rings attached to them, and in short may exhibit a great variety of structures. The most important are five-, and six – membered with one or more heteroatoms. More than one ring contain fused heterocycles. Heterocycles are components of proteins, nucleic acids, vitamins, and coenzymes. Heterocycles are present in many natural products, which have important physiological effects on humans or play a key role in some biological processes.

The most important heterocycles are following:



Furane Pyrrole Pyrrolidine Thiophene Imidazole Thiazole



Pyridine Pyrimidine Purine Pyran Indole Nicotine

Many heterocyclic compounds are aromatic. One or more carbon atoms of an aromatic ring are replaced with heteroatoms, and the system will still retain its aromatic properties. Except pyrrolidine and pyran, all heterocycles shown above are aromatic. Pyrrole, furan and thiophene are also aromatic, because the unshared electron pair on the heteroatom (nitrogen, oxygen and sulfur) is delocalized as part of the six π -electron aromatic ring.

Different basicities of nitrogen heterocyclic compounds reflect their structural differences. For example, pyrrole is very weakly basic (K_b of 4.10^{-19}), but pyrrolidine is an ordinary amine base (K_b of $1.3.10^{-3}$). Pyrrole is an aromatic compound, the unshared electron pair on the nitrogen is delocalized as part of the six π -electron aromatic ring. In contrast pyrrolidine is an ordinary secondary amine with the unshared electron pair localized on the nitrogen atom.

Pyrrole rings form the building blocks of several biologically important pigments. The **porphin** contains four pyrrole rings linked by one-carbon bridges. The molecule is flat and has a conjugated system comprising 18 π electrons. Its derivatives are **porphyrins** and they form complexes with metallic ions. Porphin itself does not occur in nature, but several metalloporphyrins play key roles in the life processes. For example **heme**, an iron-porphyrin complex present in the red blood pigment hemoglobin, is responsible for oxygen transport.

The **indole** ring contains amino acid **tryptophan**. Decarboxylation of tryptophan gives tryptamine. Many compounds with this skeleton have a profound effect on the brain and nervous system. One example is serotonin (5-hydroxytryptamine), a neurotransmitter and vasoconstrictor active in the central nervous system.

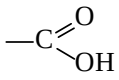
Vitamins **B_3 and B_6** is a relatively simple **pyridine** derivative. The **imidazole** skeleton is present in the amino acid **histidine.** The **pyrimidine** and **purine** ring systems are present in the **DNA** and **RNA** bases. The **barbiturates**, whose uses range from mild sedatives to hypnotics to anesthetics, are **pyrimidine** derivatives. **Pyrimidine** and **thiazole** rings contain **thiamin** (vitamin B_1). Examples of well-known **purines** are **uric acid**

(the end product of nitrogen metabolism of birds and reptiles), **caffeine** (present in coffee, tea) and **theobromine** (found in cocoa).

6.4.1. Functional (characteristic) groups occurring in organic compounds

Although over 15 millions of organic compounds exist they can be classified into several types according to the presence of certain characterizing structure **- characteristic (functional) groups** in their molecules (Tab. 6.1.). **Functional group** is a *group of atoms, which has characteristic chemical behavior in every molecule where it occurs.* These atom groups give the character to the whole molecule and together with its skeleton determine its physical properties, reactivity and the type of potential reactions.

Tab. 6.1. Types of organic compounds and their characteristic groups

Type of compound	Characteristic group	Prefix	Suffix
Halogen compounds	— F, — Cl, — Br, — I	fluoro-, chloro-, bromo-, iodo-	
Nitro compounds	— NO_2	nitro-	
Nitrosocompounds	— NO	nitroso-	
Aldehydes		formyl-	- al
Ketones		oxo-	- one
Carboxylic acids		carboxy-	- ic acid
Alcohols	— OH	hydroxy-	- ol
Thiols	— SH	merkapto- (thio-)	- thiol
Ethers	— O — R	R-oxy	
Sulfides	— S — R	R-thio	
Disulfides	— S — S—		
Sulfonic acids	— SO_3 H	sulfo-	
Amines	— NH_2	amino-	- amine
Imines	=NH	imino-	- imine
Oximes	=N — OH	hydroxylimino-	- oxime
Nitriles	— C≡N	cyano-	- nitrile

6.5. Hydrocarbons

Hydrocarbons are compounds that contain only atoms of carbon and hydrogen**.**

6.5.1. Classification of hydrocarbons

There are three main classes of hydrocarbons: saturated, unsaturated and aromatic. Saturated hydrocarbons (alkanes) contain only carbon-carbon single bonds. Unsaturated hydrocarbons contain carbon-carbon multiple bonds, either double (alkenes) or triple (alkynes) or both. Hydrocarbons may be cyclic or acyclic. Aromatic hydrocarbons are a special class of cyclic compounds related in structure to benzene.

6.5.2. Solubility of hydrocarbons

Hydrocarbons of all types, saturated or not, have common physical properties. They are insoluble in water. The reason is that water molecules are polar, whereas hydrocarbons are nonpolar. Hydrocarbons dissolve in nonpolar

solvents. Hydrocarbons themselves are used as nonpolar solvents. Substances whose molecules are entirely or even mostly hydrocarbon-like are likely to be insoluble in water but soluble in nonpolar solvents. For example, the entire cholesterol molecule is hydrocarbon-like. It has only one polar , the –OH or alcohol group, and this is not enough to make cholesterol sufficiently polar to dissolve either in water, or in blood. It is known that cholesterol can form solid deposits in blood capillaries and may even close them. The heart has to work harder, and a heart attack can occur.

6.5.3. Alkanes

All alkanes fit the general molecular formula C_nH_{2n+2}, where *n* is the number of carbon atoms. Each member of homologous series differ from the next higher and the next lower member by a $–CH_2–$ group (methylene group). Members of such a series have similar chemical and physical properties.

Alkanes are saturated hydrocarbons containing only simple (σ) bonds in their molecules. Four bonds going out of carbon atom contain 109° angle (tetrahedral arrangement).

Formally, removal of one hydrogen atom from hydrocarbon results in formation of alkyl group (one valency group) or **alkyl**, e.g.:

$R – H$	$R –$	$CH_3 – H$	$CH_3 –$	$CH_3 – CH_2 – H$	$CH_3 – CH_2 –$
Alkane	Alkyl	Methane	Methyl	Ethane	Ethyl

Alkanes are nonpolar compounds, therefore they are soluble in nonpolar solvents and insoluble in water. Alkanes are relatively **nonreactive**, and typically take part in **radical substitution** reactions, such as halogenation, e.g. chlorination of methane to mono-, di- tri-, and tetrachloromethane, under UV irradiation or heat:

$$CH_4 \xrightarrow[Cl_2]{-HCl} CH_3Cl \xrightarrow[Cl_2]{-HCl} CH_2Cl_2 \xrightarrow[Cl_2]{-HCl} CHCl_3 \xrightarrow[Cl_2]{-HCl} CCl_4$$

Methane	Chloromethane	Dichlormethane	Trichloromethane (chloroform)	Tetrachloromethane (carbon tetrachloride)

The most important use of alkanes is as fuels. Alkanes (also cycloalkanes) burn in an excess of oxygen to form carbon dioxide and water. Most important, the reactions evolve large quantities of heat, that is, the reaction are exothermic. The **combustion** of methane and butane is expressed in the following reactions:

$$CH_4 + 2\ O_2 \rightarrow CO_2 + 2\ H_2O + 848\ kJ/mol \qquad C_4H_{10} + 6.5\ O_2 \rightarrow 4\ CO_2 + 5\ H_2O + 2\ 800\ kJ/mol$$

These combustion reactions are the basis for the use of hydrocarbons for heat (natural gas or heating oil) and for power (gasoline). The reaction has radical nature, an initiation step is required (usually ignition by a spark or flame). Once initiated, the reaction proceeds spontaneously and exothermically. **Combustion** – complete **oxidation** of hydrocarbons and other compounds is one of the most important of organic reactions. It is not without any problems. If insufficient oxygen is available for complete reaction, partial combustion may occur, and the reaction products, such as carbon monooxide, carbon or oxidized organic compounds such as aldehydes and acids, may become atmospheric pollutants:

$$2\ CH_4 + 3\ O_2 \rightarrow 2\ CO + 4\ H_2O \qquad CH_4 + O_2 \rightarrow C + 2\ H_2O \qquad CH_4 + O_2 \rightarrow CH_2O + H_2O$$

Carbon monoxide — Carbon — Formaldehyde

Toxic carbon monoxide in exhaust fumes, soot emitted copiously from trucks with diesel engines, smog resulting in part from aldehydes, and acids build-up in lubricating oils, these all are prices we pay for being a motorized society.

6.5.4. Alkenes

Alkenes are unsaturated hydrocarbons that contain a carbon-carbon double bond. Double bond consists of one δ bond and one π bond. Double bond has some special features that distinguish it from simple bond. Each carbon atom of a double bond is connected to only three other atoms (instead of four atoms, as with tetrahedral carbon. Such carbon is **trigonal**. The two carbon atoms of a double bond and the four atoms that are attached to them lie in a single plane, and the bond angles are approximately 120°. Although rotation occurs freely around simple

bonds, rotation around double bonds is restricted. Alkenes form homologous series, with general molecular formula C_nH_{2n}.

Unsaturated hydrocarbons have physical properties similar to those of alkanes. They are insoluble in water. As with alkanes, compounds with four or less carbon atoms are colorless gases, and the more common five-carbon and larger homologues are volatile liquids.

Alkenes are far more reactive than alkanes. Double bond is very sensitive to oxidizing agents.

The most common reaction of alkenes is **electrophilic addition** reaction. In this reaction, the π - bond of alkenes is broken and the δ - bond of the electrophilic reagent is also broken. Two new δ bonds are formed. The typical reaction of alkenes is addition of halogens or halogenic acids HX. For example, if bromine adds to ethylene, 1,2 – dibromoethane is formed:

$$CH_2{=}CH_2 + \mathbf{Br_2} \longrightarrow \underset{\mathbf{Br}}{\overset{|}{CH_2}}{-}\underset{\mathbf{Br}}{\overset{|}{CH_2}}$$

In case of assymetric alkenes the addition reaction follows **Markovnikov´s rule**: the hydrogen atom of HX is added to the carbon with the greatest number of hydrogen atoms while the X component is added to the carbon with the least number of hydrogen atoms, e.g. in reaction of hydrochloric acid with propene:

$$CH_2{=}CH{-}CH_3 + \mathbf{HCl} \longrightarrow \underset{\mathbf{H}}{\overset{|}{CH_2}}{-}\underset{\mathbf{Cl}}{\overset{|}{CH}}{-}CH_3$$

Propene — 2-Chloropropane

Addition of water to alkene molecules (the reaction is called **hydration**) produces alcohol, e.g. ethylene hydration produces ethanol. The reaction is used industrially to synthesize alcohols from alkenes. Addition of hydrogen to alkenes (that is called **hydrogenation**) produces saturated hydrocarbons, e.g ethane is formed by hydrogenation of ethene:

$$CH_2{=}CH_2 + \mathbf{H_2O} \xrightarrow{\text{hydration}} \underset{\mathbf{H}}{\overset{|}{CH_2}}{-}CH_2{-}\mathbf{OH}$$

Ethene (ethylene) — Ethanol

$$CH_2{=}CH_2 + \mathbf{H_2} \xrightarrow[\text{hydrogenation}]{\text{catalyst}} CH_3{-}CH_3$$

Ethene — Ethane

Polymerization of alkenes is free radical addition and leads to formation of **polymer**. A polymer is a large molecule, usually with a high molecular weight, built up from small repeating units. The simple molecule from which these repeating units are derived is called a monomer, and the process of converting a monomer to a polymer is called polymerization. Some polymers, such as starch, cellulose, and silk, are natural polymers, they are produced in nature by animals or plants. Synthetic polymers are those made in laboratory, and are called **plastic**:

$$n\ \underset{\text{Ethylene}}{CH_2 = CH_2} \longrightarrow \underset{\text{Polyethylene}}{-[-CH_2 - CH_2-]_n-}$$

n = several tens till thousands of molecules

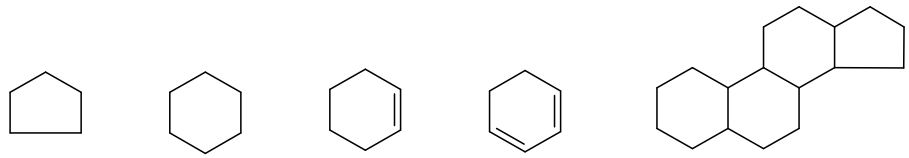
Dienes are alkenes, that contain in molecules two double bonds. Double bonds are said to be **cumulated** when they are right next to one another (C = C = C). When multiple bonds alternate with single bonds, the multiple bonds are **conjugated** (C = C – C = C). When more than one single bond comes between multiple bonds, the latter are **isolated** (C = C – C – C = C). Dienes are reactive, important reaction is – **addition polymerization**. For example, polymerization of isoprene molecules leads to formation of **isoprenoides:**

$$n\ CH_2 = \underset{\displaystyle CH_3}{\overset{|}{C}} - CH = CH_2 \longrightarrow -[-CH_2 - \underset{\displaystyle CH_3}{\overset{|}{C}} = CH - CH_2-]_n-$$

2-Methyl-1,3-butadiene (isoprene) — Polyisoprene (isoprene polymer)

Natural rubber is an unsaturated hydrocarbon polymer. It is obtained commercially from the milky sap (latex) of the rubber tree. When latex is heated in the absence of air, it breaks down to give unsaturated hydrocarbons

isoprene. The five – carbon repeating unit that makes up the natural rubber molecule is called an **isoprene unit**. It consists of a four-carbon chain with a one-carbon branch at carbon – 2. The isoprene units are found in many other natural products besides rubber, such as steroids, terpenes, some vitamins.



6.5.5. Alkynes

Alkynes are unsaturated hydrocarbons, that contain a triple bond. A carbon that is part of a triple bond is attached to only two atoms, and the bond angle is 180°. They have a linear geometry. Alkynes form homologous serie, with general molecular formula C_nH_{2n-2}.

Alkynes are reactive, give **addition reactions**, for example:

$CH \equiv CH + HCl \rightarrow CH_2 = CH - Cl$
Ethine (acetylene) — Vinylchlorid

$CH \equiv CH + HOH \rightarrow [CH_2 = CH - OH] \rightarrow CH_3 - CH = O$
Ethine (acetylene) — Acetaldehyde

6.5.6. Cyclic hydrocarbons

a) Alicyclic

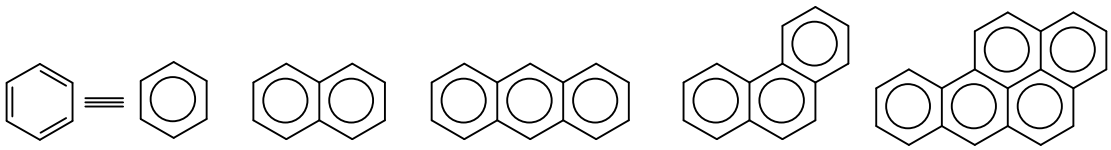
Alicyclic hydrocarbons are cykloalkanes, cykloalkenes and fused - polycyclic hydrocarbons, e.g.:

Cyclopentane Cyclohexane Cyclohexene Cyclohexadiene Cyclopentanoperhydrophenanthrene

From cyclopentanoperhydrophenantrene are derived steroids. Cycloalkanes give radical substitution reactions. Cykloalkenes give addition reactions and are sensitive to oxidizing agents.

b) Aromatic hydrocarbons – arenes

Arenes are cyclic hydrocarbons, which have in ring conjugated π - electrons, their number must be equal to $(4n + 2)$, where n is integer (n= 0, 1, 2 ...).



Benzene Naphthalene Anthracene Phenanthrene Benzopyrene

The basic aromatic hydrocarbon is **benzene.** It formally corresponds to hypothetical 1,3,5-cyclohexatriene, but it is not unsaturated, because its π - electrons are distributed evenly around the ring (delocalized). Benzene ring is planar, with angle bonds of 120°.

Aromacity means the unusual stability of certain fully conjugated cyclic systems. Benzene is aromatic with special chemical properties. Benzene does not behave as unsaturated, it does not undergo the typical addition reactions. It is not easily oxidized. Instead, benzene reacts mainly by **substitution** (nitration, halogenation, sulfonation, alkylation).

Aryl (Ar-) group is formally created by separation of hydrogen atom from an arene. Phenyl and benzyl are two groups the most commonly occurring in arenes:

Benzene Phenyl Toluene (methyl benzene) Benzyl

Arenes, e.g. **benzene** and polycyclic arenes, like **benzopyrene,** are **carcinogenic**. Enzymatic oxidation converts benzopyrene to diol-epoxide, which reacts with cellular DNA, causing mutations. These carcinogenic hydrocarbons are present not only in coal tar but also in soot and tobacco smoke.

Benzene itself is quite toxic to human and can cause liver damage, but **toluene** (methylbenzene) is very much less toxic. To eliminate benzene from the body, the aromatic ring must be oxidized. Benzene ring is stable to oxidation. However, the methyl side chain of toluene can be oxidized to give benzoic acid, which can be eliminated.

6.6. Derivatives of hydrocarbons

They are formed by replacing hydrogen atom in hydrocarbon with another atom or a group, so called functional group (Tab. 6.1.).

6.6.1. Halogen derivatives of hydrocarbons

Halogen derivatives (alkyl- or aryl halides) are derived from hydrocarbons by replacing the hydrogen with halogens (chlorine, bromine, iodine, fluorine). They contain polar covalent bond C $\longrightarrow$ halogen, but they are non polar. Therefore they are insoluble in water, and are soluble in alcohols and ethers. They are used as solvents of nonpolar compounds (CH_2Cl_2, $CHCl_3$, CCl_4).

Typical reactions for alkyl halides are **nucleophilic substitution reactions**. The reaction is **alkylation**, and alkyl halides are used as **alkylation agents.**

For example ethyl bromide reacts with hydroxide ion to give ethyl alcohol and bromide ion:

$$OH^- + CH_3CH_2 - Br \xrightarrow{H_2O} CH_3CH_2 - OH + Br^-$$

Hydroxide ion is the **nucleophile**. It reacts with substrate (ethyl bromide) and replaces bromide ion. The bromide ion is called the **leaving group**. In reaction of this type, one covalent bond is broken and one new covalent bond is formed. The leaving group (bromide) takes with it both of the electrons from the C –Br bond, and the nucleophile (hydroxide ion) supplies both electrons for the new carbon-oxygen bond. A nucleophile is a reagent that can supply an electron pair to make a covalent bond.

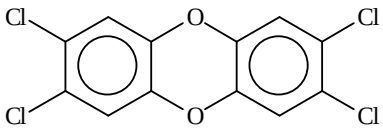
Halogen derivatives of organic compounds are very toxic for living organism, except one – iodide derivative of thyroxine, thyroid gland hormone, thyroxine. Despite this, many halogen compounds have very practical uses – as insecticides, herbicides, fire retardants, cleaning fluids and refrigerants.

Chlorinated methanes are made by chlorination of methane. Carbon tetrachloride (CCl_4) is liquid insoluble in water, but it is a good solvent for oil and greases and is used to remove stains from clothes. Because of its high density and nonflammability, carbon tetrachloride was once widely used as a fire extinguisher. Chloroform ($CHCl_3$) and methylene chloride (CH_2Cl_2) are both widely used as solvents of organic substances.

Chloroform and carbon tetrachloride are suspected carcinogens. CCl_4 with water is decomposed to toxic phosgene ($COCl_2$). **Iodoform** – CHI_3 (yellow powder), with disinfection effects, is used as antiseptic. **Bromoform** - $CHBr_3$ contains some antitussives.

The most important polyhalogenated methanes and ethanes are the chlorofluorocarbons - **freons** (e.g. dichlorodifluoromethane, CF_2Cl_2). They are colorless gases or liquids, that are nontoxic, nonflammable. They are used as refrigerants in air conditioning and deep-freeze units, and as aerosol propellants. Excessive use led to concern that these inert materials might accumulate in the environment and pollute the atmosphere. They can diffuse upward and have a damaging effect on the earth's ozone layer, a section of stratosphere that screens out much of the dangerous ultraviolet radiation coming from the sun. This could result in climate modification, crop damage and additional cases of skin cancer.

Many polyhalogen compounds have been used as insecticides and herbicides. They are toxic, they affect central nervous system. **Tetrachloro-dibenzodioxin** (dioxin) is, in trace amount, a by-product in polyhalogen compounds manufacture. **Dioxin** is one of the most powerful poisons known. It is a carcinogen, a teratogen (substance that causes abnormal growth), and a mutagen (substance that induces hereditary mutations). It is toxic at very low concentrations ($c < 1$ mg.l^{-1}).



Tetrachloro-dibenzodioxin („dioxin")

6.6.2. Hydroxyderivatives of hydrocarbons

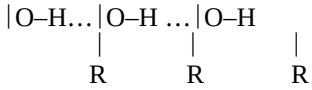
Hydroxyderivatives contain **hydroxyl group –OH** in their molecules**. Alcohols** have the – OH group attached to a saturated carbon. **Phenols** have the same group attached to an aromatic ring.

$R-OH$	CH_3-OH	CH_3-CH_2-OH	C_6H_5-OH	$C_6H_5-CH_2-OH$
An alcohol	Methanol	Ethanol	Phenol	Benzylalcohol

The hydroxyl group is present in many biologically important molecules, such as saccharides, proteins, nucleic acids, steroids (cholesterol), terpenes (geraniol), vitamins (vitamin A – alcohol, vitamin E – phenol)..

6.6.2.1. Alcohols

The bond –O←H bond is highly polar by the high electronegativity of the oxygen atom. This polarization places a high partial positive charge on the hydrogen atom. Because of this charge and its small size, the hydrogen atom can link together two electronegative oxygen atoms. Two or more alcohol molecules become associated through hydrogen bonds. Consequently alcohols have higher boiling points than those of ethers or hydrocarbons with similar molecular weights



Classification of alcohols:

a) according to the kind of carbon that holds the OH group

- **primary** (1- butanol) $CH_3-CH_2-CH_2-CH_2-OH$
- **secondary** (2- butanol) $CH_3-CH_2-CH(OH)-CH_3$
- **tertiary** (tert. butyl alcohol) $CH_3-C(OH)(CH_3)-CH_3$

b) according to the number of OH groups:

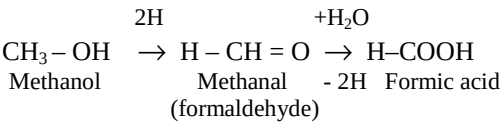
- **monohydric** (ethanol) CH_3-CH_2-OH
- **dihydric** (diols or glycols) (ethanediol or ethylene glycol) $CH_2(OH)-CH_2(OH)$
- **trihydric** (triols) (propanetriols or glycerol) $CH_2(OH)-CH(OH)-CH_2(OH)$
- **polyhydric** (sorbitol)

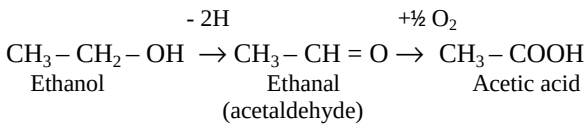
Biologically important reactions of alcohols

a) oxidation (dehydrogenation)

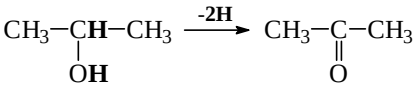
Alcohols with at least one hydrogen attached to the hydroxyl-bearing carbon can be oxidized to carbonyl compounds. An oxidation is the loss of 2 H atoms or gain of O atom by a molecule.

Primary alcohols give **aldehydes**, which may be further oxidized to **carboxylic acids:**





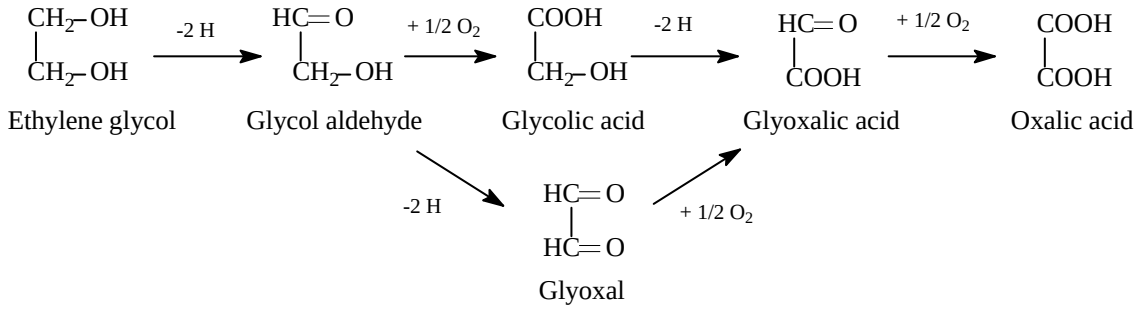
Secondary alcohols give **ketones**:



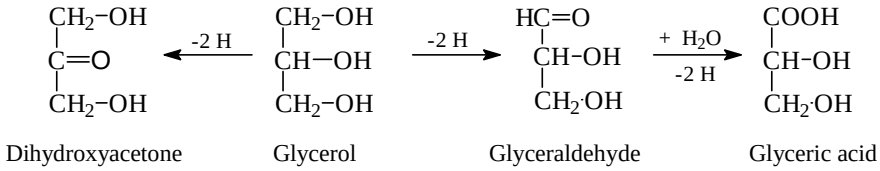
Tertiary alcohols do not undergo this type of oxidation.

Alcohol with more than one hydroxyl group:

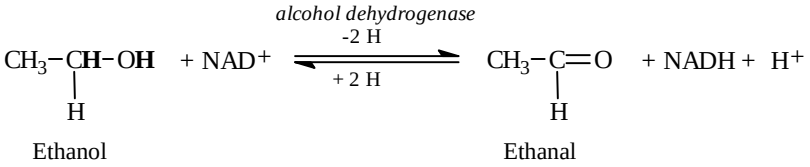
Oxidation of **diols - glycols,** compounds with two adjacent alcohol groups, is expressed by the following equation:



Oxidation of **triols**, compounds with three hydroxyl groups, is expressed by the equation:



In the body, similar oxidations are accomplished by enzymes together with coenzyme, nicotinamide adenine dinucleotide, NAD^+ (see Chapter 12.2.1). NAD^+ dehydrogenates alcohol to aldehyde (or ketone). This reaction takes place in liver. It is a key step in the body´s attempt to get rid of consumed alcohol:



The resulting acetaldehyde, also toxic, is further oxidized to acetic acid and eventually to carbon dioxide and water. Formation of oxidation metabolites causes a **toxicity** of **alcohols.**

Methanol can cause permanent blindness or death, even in small doses (5-100 g). The reason methanol is so dangerous is that human have liver enzymes that oxidize it to formaldehyde ($H_2C=O$). Formaldehyde react rapidly with the components of cells. It causes the coagulation of proteins. This property accounts for the great toxicity of methanol. Formaldehyde is further oxidized to formic acid (HCOOH), that causes acidosis, since it is only very slowly excreted in urine.

Ethanol is also toxic, but to a much lesser degree than methanol. In the body, ethanol is oxidized by the same liver enzymes that operate on methanol. (Ethanol, administered intravenously, has long been used as an antidote for methanol poisoning). Product obtained from ethanol is acetaldehyde. This product is oxidized, in turn, to acetic acid, a normal constituent of cells, that is further oxidized in citrate cycle. When an enzyme, that oxidizes acetalhehyde into acetic acid is missing, acetaldehyde is accumulated in liver and causes liver fibrosis. Excessive ingestion of ethanol over a long period of time leads to deterioration of the liver and loss of memory. Ethanol acts as a mild hypnotic. Ethanol can cause sedation, transquility, lack of coordination, obvious intoxication, unconsciousness and possible death (at blood concentration about 80 mmol/l or 4 promile)

Ethylene glycol is water soluble liquid with sweet taste and is used as antifreeze in automobile radiators (its 50 % solutions don't freeze to –40°C). Ethylene glycol is toxic (lethal dose about volume of 100 ml). The reason of ethylene glycol toxicity lies in oxidation described above. Because its metabolites – glyoxalic and oxalic acid are toxic.

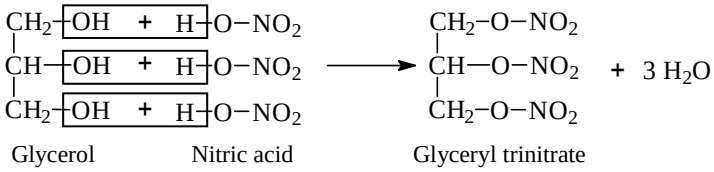
b) Esterification

Esterification is the reaction of either organic or inorganic acids with alcohol.

Esterification of alcohol with inorganic acids

Inorganic esters are compounds in which the proton of an acids is replaced by an organic residue (R–)

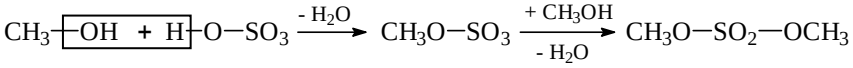
a) by reaction of alcohol with nitric acid, HNO_3 , alkyl nitrates may be prepared:



Glycerol Nitric acid Glyceryl trinitrate

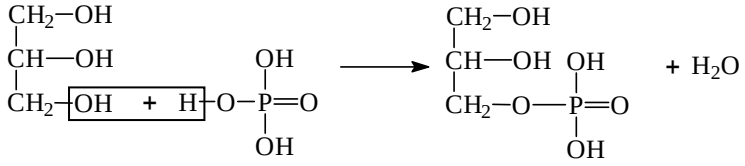
Glyceryl trinitrate is explosive and also is used in medicine as a vasodilator, to prevent heart attacks in patients who suffer from *angina pectoris*.

b) by the reaction of alcohol with sulfuric acid, H_2SO_4 , alkyl sulfates are prepared:



Methanol Sulfuric acid Methyl sulfate Dimethyl sulfate

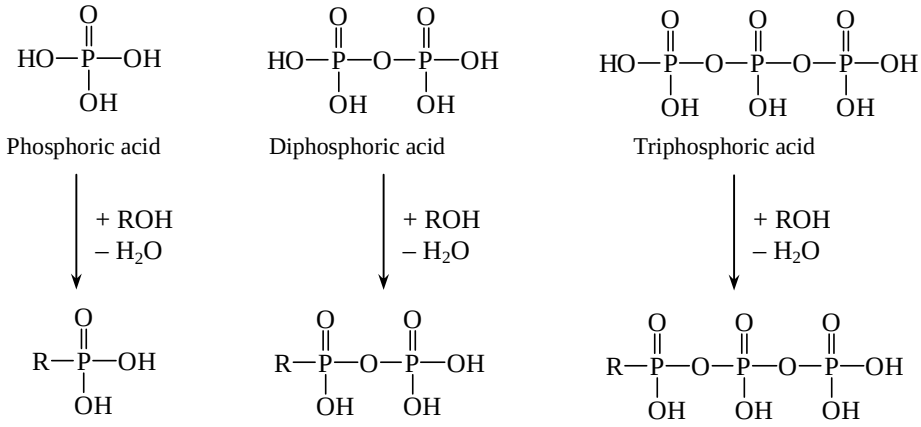
c) by the reaction of alcohol with phosphoric acid, H_3PO_4, alkyl phosphates are prepared :



Glycerol Phosphoric acid Glycerolphosphate

Glycerol phosphate is a structural component of complex lipids.

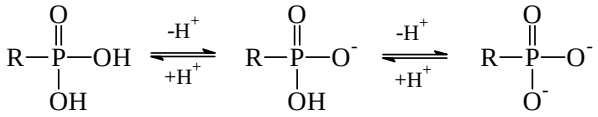
Esters of phosphoric acid (alkyl phosphate), diphosphoric acid (alkyl diphosphate), triphosphoric acid (alkyl triphosphate) are versatile biochemical reactants:



Phosphoric acid Diphosphoric acid Triphosphoric acid

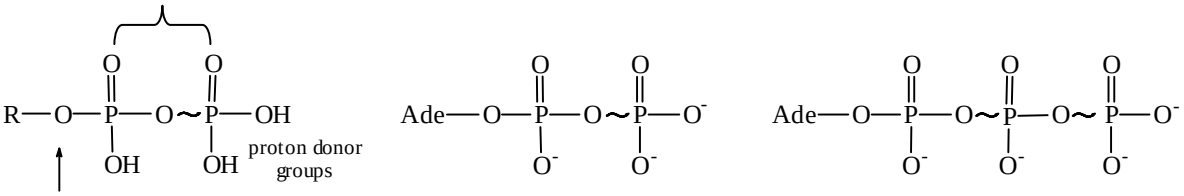
Alkyl phosphate Alkyl diphosphate Alkyl triphosphate

At the pH of most body fluids (slightly more than 7) phosphate esters exist mostly as negative ions:



A di- and triphosphate contain three functional groups: the phosphate ester group, the proton donating – OH group and the phosphoanhydride system:

Phosphoanhydride system



Phosphoester bond ADP (adenosine diphosphate) ATP (adenosine triphosphate)

The **phosphoanhydride system** is a major storehouse of chemical energy in living systems, contains energy rich (mark ~) phosphoanhydride arrangement. The source of internal energy in the triply charged anion of ADP is the tension up and down of the anhydride chain. This central chain bears oxygen atoms with full negative charges, and these charges repel each other. This internal repulsion primes the phosphoanhydride system to break apart exothermically if it is attacked by a suitable reactant. Because adenosine triphosphate, or ATP, has two phosphoanhydride systems in molecule, it is one of the most important energy-rich substances in the body (see Chapter 5.3.). By cleavage of phosphoanhydride system, energy is released:

$$ATP + H_2O \rightleftharpoons ADP + P_i \qquad \Delta G'^{\circ} = -30.5 \text{ kJ/mol} \quad (P_i = \text{inorganic phosphate})$$

ATP is the main source and transporter of chemical energy in living cells.

Macroergic phosphates are energy reach compounds, and they are protected against attack of nucleophiles, because of their negativelly charged oxygen atoms.

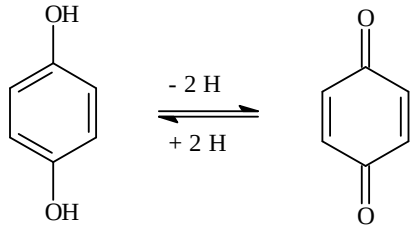
Esterification of alcohols with organic acids (see Chapter 6.6.5.1.)

6.6.2.2. Phenols

Phenols are hydroxyderivatives of arenes, where –OH functional group (or more -OH groups) is attached to an aromatic ring. Phenols are much stronger acids than alcohols.

Phenols have found use as germicides or antiseptics, and as disinfectants. Phenol kills not only undesirable microorganisms, but all types of cells. Phenol is toxic. Applied to the skin, it can cause severe burns. In bloodstream it is a systematic poison. Phenol arises at small quantity in colon, is detoxicated with glucuronic acid (see Chapter 7.3.4.1.).

Phenols are easily oxidized. Biologically important is reversible **oxidation** of **diphenols (dihydroxybenzenes)** into **quinones.** Quinones constitute a unique class of carbonyl compounds. They are cyclic conjugated **diketones** (unsaturated, not aromatic!). The phenols function as **antioxidants,** and are oxidized instead of substances to which they have been added. Also vitamin E (alpha - tocopherol) is a widespread naturally occurring phenol. One of its biological functions appears to be acting as a natural antioxidant.



Hydroquinone
1,4 -dihydroxybenzene

1,4 - Benzoquinone

Biologically very important is reversible oxidation of **hydroquinone**, which is trivial name for **1,4-dihydroxybenzene** into **1,4-benzoquinone.**

This oxidation-reduction reaction also expresses principles of using of hydroquinone as photographic developers. Hydroquinone reduce silver ion that has been exposed to light to metallic silver and in turn is oxidized to quinone.

This reversible reaction plays an important role in several biological oxidation-reduction reactions.

All **quinones** are colored. Many are naturally occurring plant pigments, and they exhibit special biological activity. **Coenzyme Q**, derivative of 1,4-hydroquinone, also known as **ubiquinone,** because of its occurrence in animal and plant cells. Ubiquinones participate in electron transport in mitochondria in the cell that are involved in the metabolism of lipids, saccharides and proteins.

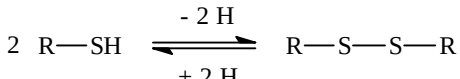
6.6.3. Thiols, disulfides and sulfides

Thiols are sulfur analogs of alcohols and phenols (Ar = aromatic ring)

R – OH	R – SH	$CH_3 – SH$	$CH_3 – CH_2 – SH$	Ar – OH	Ar – SH
Alcohol	Thiol	Methanethiol	Ethanethiol	Phenol	Thiophenol

Both, the –SH group, an easily oxidized group, and the S –S system, an easily reduced group, are present in molecules of proteins. The –SH group is called the thiol group, or the sulfhydryl group. Some very important properties of proteins depend on the presence of the –SH group, which contributes to protein structure by one of the building blocks of proteins, the amino acid called cysteine.

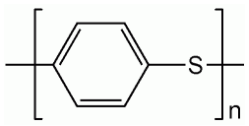
Thiols are easily oxidized to **disulfides** by mild oxidizing agents. Disulfides are reduced to thiols. This reversible oxidation-reduction reaction is biochemically very important.



Thiol Disulfide

Glutathione (see Chapter 9.2.4.) is biologically important thiol, its antioxidative properties are linked with above reversible reaction. Disulfide bond (–S–S–) is important in formation of protein structure. Amino acid cysteine is oxidized, according to the above reversible reaction to cystine. Hair consists of a fibrous protein, **keratin**, which contains an unusually large percentage of sulfur-containing amino acid **cystein** (horse hair, for example contains about 8% of cysteine).

In organic chemistry, "**sulfide**" usually refers to the linkage C-S-C, although the term thioether is less ambiguous. For example, the thioether dimethyl sulfide is CH_3-S-CH_3. **Polyphenylene sulfide** has the empirical formula C_6H_4S. It is a polymer commonly called "Sulfar". Its repeating units are bonded together by sulfide (thioether) linkages.

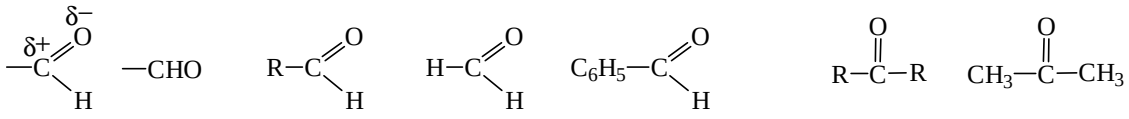


Polyphenylene sulfide is an organic polymer consisting of aromatic rings linked with sulfides. Synthetic fiber and textiles derived from this polymer exhibit excellent resistance to chemical and thermal attack.

6.6.4. Carbonyl compounds

The carbonyl group ─C = O is present in **aldehydes** and **ketones**. These compounds are important in many biological processes.

Aldehydes have at least one hydrogen atom attached to the carbonyl group. In ketones, the carbonyl carbon atom is connected to two other carbon atoms:



Aldehyde group (polarization)

An aldehyde

Methanal (formaldehyde)

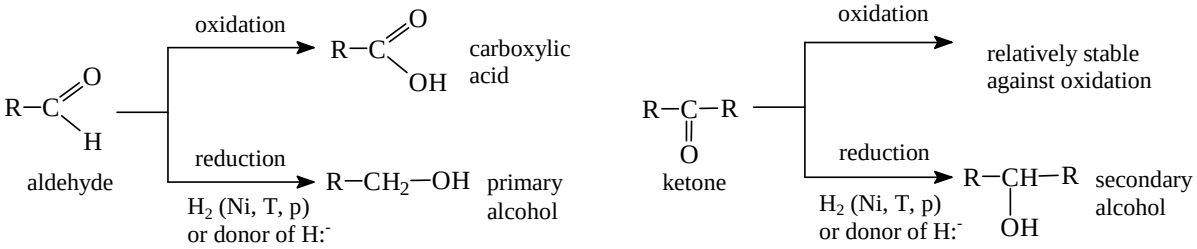
Benzaldehyde (C_6H_5 –phenyl)

Ketone

Propanone (acetone)

The carbon-oxygen double bond consists of a σ bond and a π bond. As accepted for sp^2 hybridization, the three atoms attached to the carbonyl carbon lie in a plane with bond angles of 120°. Oxygen is much more electronegative than carbon. Consequently the electrons in the C = O bond are attracted to the oxygen, and the bond is highly polar. This effect is especially pronounced for the π electrons, which are less firmly held than the sigma electrons. Carbonyl compounds are reactive. As a consequence of this polarization, many reactions of carbonyl compounds involve attack of a nucleophile (a supplier of electrons) on carbonyl carbon atom. Nucleophils attack the carbon atom of a C = O double bond because that carbon atom has a partially positive charge. Ketones are somewhat less reactive than aldehydes toward nucleophiles.

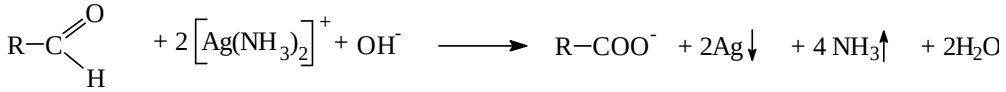
Aldehydes cause irritation of respiratory tract and eyes. **Formaldehyde**, $H_2C{=}O$, has bactericidal properties. Its 37 % aqueous solution called **formalin**, is used as a disinfectant and preservative. **Acetaldehyde**, $CH_3CH{=}O$, is formed during fermentation of glucose. It is a toxic substance, responsible for ethanol intoxication.



6.6.4.1. Chemical reactions of aldehydes and ketones

a) oxidation and reduction

The aldehyde group is easily oxidized to a carboxylic group. Oxidation of an aldehyde gives an acid with the same number of carbon atoms, but the ketone system is difficult to oxidize. Aldehydes have reductive properties, they can reduce e.g. Fehling´s or Tollen´s reagents:



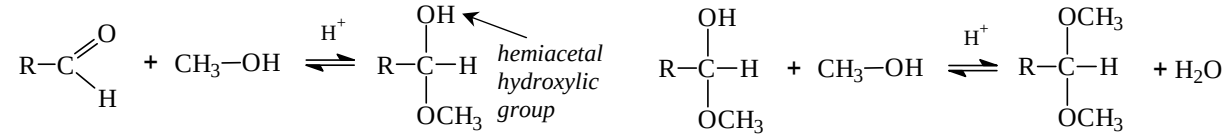
Aldehyde — Silver-ammonia complex ion — Carboxylic acid ion — Silver mirror

Aldehydes and ketones are reduced to alcohols when hydrogen is added to their carbonyl group. Reduction of aldehydes and ketones is either addition of hydrogen or reduction by hydride ion transfer. Donor of hydride ion in laboratory can be a metal hydride, e.g. $LiALH_4$ or in living system NAD:H (coenzyme – nicotinamide adenine dinucleotide). Addition of hydrogen (2 H) to double bond is called **hydrogenation.**

b) nucleophilic addition to carbonyl group and condensation reactions

Addition of alcohols. Formation of hemiacetals and acetals.

Alcohols are oxygen nucleophiles. They can attack the carbonyl carbon of aldehydes or ketones, resulting in addition to the C=O bond. Because alcohols are weak nucleophiles, an acid catalyst is used. The reaction product is a hemiacetal, which contains both an ether and an alcohol functional groups at the same carbon atom. Aldehydes and ketones react with alcohols to form initially hemiacetals (addition of alcohol) and, if excess alcohol is present, acetals (1,1 – diethers).

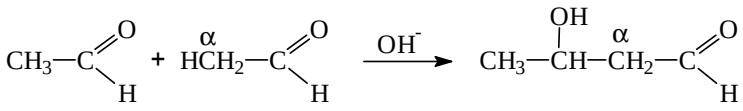


Aldehyde — Alcohol — Hemiacetal — Hemiacetal — Acetal

The hydroxyl group of the hemiacetal is replaced by an alkoxyl group. Acetals have two ether functional groups at the same carbon atom. Each step is reversible. Hemiacetals are unstable, easily can be cleaved to carbonyl compound and alcohol. Relatively stable are cyclic hemiacetal forms of saccharides (intermediates of formation of saccharide acetals – glycosides (see Chapter 7.3.4.4.).

Aldol condensation

The simplest example of an aldol condensation is the combination of two acetaldehyde molecules, which occurs when a solution of acetaldehyde is treated with aqueous base:



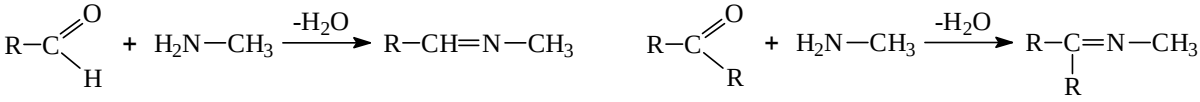
Ethanal (acetaldehyde) Ethanal 3-Hydroxybutanal (an aldol)

The product is called an **aldol, 3–hydroxyaldehyde.** The α hydrogen in carbonyl compounds is more acidic than normal hydrogens bound to a carbon. After removal of α hydrogen as a proton, enolate anion is formed. The α carbon acts as a nucleophile, the product aldol always has just one carbon atom between the aldehyde and alcohol carbons.

Aldol condensation is extremely useful carbon-carbon bond-forming reaction, it takes place in metabolism of glucose (glycolysis).

Addition of nitrogen nucleophiles

Amines have an unshared electron pair on the nitrogen atom and act as nitrogen nucleophiles toward the carbonyl carbon atom. The addition product that is formed first is similar to a hemiacetal, but it has an NH group in place of one of the oxygens. These addition products are normally not stable. They eliminated water to form a product with a carbon – nitrogen double bond. With primary amines, the products are called imines, or **Schiff bases**. Aldehydes give aldimines and ketones give ketimines in this type of reaction.

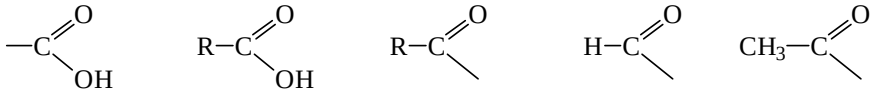


Aldehyde Aldimine Ketone Ketimine

Schiff bases are important intermediates in some biochemical reaction, particularly in binding carbonyl compounds to the free amino groups present in most enzymes. Reaction take place, for example, at non-enzymatic protein glycation (see Chapter 7.3.4.5.).

6.6.5. Carboxylic acids

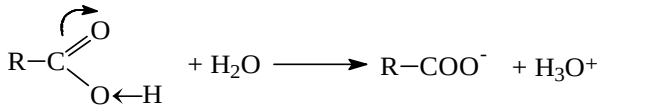
Carboxylic acids are the most important organic acids. Their functional group is the **carboxylic group**:



Carboxyl group Carboxylic acid An acyl group Formyl Acetyl

Acyl groups are single bond groups derived by removing –OH from carboxylic group of acid. Acyl groups are named from the corresponding acid by changing the ending –ic to –yl. Acyl group derived from formic acid is formyl and from acetic acid is acetyl..

Carboxylic acids are polar. Carboxylic acids are acidic, since ionize by losing H^+ from carboxyl group. Carboxylic acids dissociate in water, yielding a **carboxylate anion** and a hydronium ion:



Carboxylate ion Hydronium ion

Carboxylic acids are usually weak acids with values ionization constants K_a about 1.10^{-5}.

According number of their carboxyl groups –COOH are known: **mono-, di- a tricarboxylic acids.** Carboxylic acids may be **saturated** or **unsaturated** (Tab. 6.2. and Tab. 6.3.).

In the nomenclature of carboxylic acids, the carbon of the carboxyl group is numbered 1. In their nomenclature we can designate carbons with letters of the Greek alphabet. For example, the carbon neighboring with the carboxyl group is labeled alpha (α) (and following are β, γ, δ...ε). A common nomenclature is also used in connection with carboxylic acids such as lactic acid.

Formic acid, HCOOH, the simplest carboxylic acid is the strongest monocarboxylic acid, it exerts bactericide effect. It is partially responsible for the irritation of ant bites and bee stings.

Acetic acid, CH_3COOH, constitutes about 6 % of vinegar. It is a common intermediate of metabolism fats, saccharides and proteins.

Oxalic acid, $(COOH)_2$**,** the simplest and strongest dicarboxylic acid. It is crystalline solid. Concentrated form of oxalic acid and its salt calcium oxalate are poisonous. Oxalic acid occurs in many plants and vegetables, such as spinach and rhubarb. Though toxic, it decomposes and is rendered harmless during cooking. The acid acts directly on the mucose and by formation of insoluble calcium oxalate it deprives the body of ionized calcium.

Benzoic acid, (C_6H_5COOH), prevents the grow of fungi and microorganisms. Its sodium salt, sodium benzoate is used as a food preservative. It is a toxic substance for living organisms, and is detoxicated in the liver.

Tab. 6.2. Examples of saturated mono- and dicarboxylic acids

Monocarboxylic acids			Dicarboxylic acids		
formula	Name		formula	Name	
	substitution	common		substitution	common
HCOOH	Methanoic	Formic			
CH_3 COOH	Ethanoic	Acetic	HOOC–COOH	Ethanedioic	Oxalic
CH_3 CH_2 COOH	Propanoic	Propionic	HOOC– CH_2 –COOH	Propanedioic	Malonic
$CH_3(CH_2)_2$ COOH	Butanoic	Butyric	HOOC–$(CH_2)_2$–COOH	Butanedioic	Succinic
CH_3 $(CH_2)_3$ COOH	Pentanoic	Valeric	HOOC–$(CH_2)_3$–COOH	Pentanedioic	Glutaric
CH_3 $(CH_2)_4$ COOH	Hexanoic	Caproic	HOOC–$(CH_2)_4$–COOH	Hexanedioic	Adipic

6.6.5.1. Chemical reactions of carboxylic acids

a) neutralization

Carboxylic acids are neutralized by strong bases to give salts. Salts of carboxylic acids are ionic compounds. This reaction is extremely important at the molecular level of life, because the carboxylic acids produced by metabolism must be neutralized. Almost all carboxylic acids in cell and body fluids are present as **carboxylate anions** ($R—COO^-$) at physiological pH values.

$$\underset{\text{acid}}{R-COOH} + \underset{\text{base}}{NaOH} \longrightarrow \underset{\text{salt}}{R-COO^-Na^+} + H_2O$$

$$\underset{\text{Acetic acid}}{CH_3-COOH} + NaOH \longrightarrow \underset{\text{Sodium acetate}}{CH_3-COO^-Na^+} + H_2O \qquad \underset{\text{Oxalic acid}}{(COOH)_2} + Ca(OH)_2 \longrightarrow \underset{\text{Calcium oxalate}}{(COO)_2Ca} + 2\,H_2O$$

Sodium and potassium salts are well soluble in water. Calcium oxalate is insoluble in water. It is a component of urine stones which may clog the kidney tubules.

Organic acids at pH of cell value 7.4 are dissociated into carboxylate anions **R – COO$^-$** . Summary of important mono-, di- and tricarboxylic acids and their oxygen-containing substitution derivatives are presented in Tab. 6.3. Most of these acids are converted in citric acid cycle.

Carboxylic acids can form a wide range of derivatives, which are classified as:

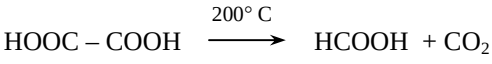
1. **functional** – **salts**, **esters**, **thioesters**, **halogenides**, **amides**, **anhydrides**,
2. **substitution** – **hydroxy acids**, **oxo acids**, **amino acids**, **halogen acids**.

Tab. 6.3. Summary of important acids, their oxygen containing substitution derivatives and their anions

Name of acid		Formula	Name of salt
substitutional	**trivial**	**R-COOH**	**R-COO$^-$**
methanoic	formic	HCOOH	formiate
ethanoic	acetic	CH_3COOH	acetate
ethanedioic	oxalic	HOOC-COOH	oxalate
propanedioic	malonic	HOOC-CH_2-COOH	malonate
butanedioic	succinic	HOOC-$(CH_2)_2$-COOH	succinate
penthanedioic	glutaric	HOOC-$(CH_2)_3$-COOH	glutarate
butenedioic	fumaric (trans) maleinic (cis)	HOOC-CH=CH-COOH	fumarate maleinate
2-hydroxypropanoic	lactic	$CH_3-\underset{\displaystyle OH}{\underset{\vert}{CH}}-COOH$	lactate
3-hydroxybutanoic	3-hydroxybutyric β-hydroxybutyric	$CH_3-\underset{\displaystyle OH}{\underset{\vert}{CH}}-CH_2-COOH$	3-hydroxybutyrate β-hydroxybutyrate
2-hydroxybutanedioic	malic	$HOOC-\underset{\displaystyle OH}{\underset{\vert}{CH}}-CH_2-COOH$	malate
2,3-dihydroxybutanedioic	tartaric	$HOOC-\underset{\displaystyle OH}{\underset{\vert}{CH}}-\underset{\displaystyle OH}{\underset{\vert}{CH}}-COOH$	tartrate
2-hydroxypropanoic-1,2,3-tricarboxylic	citric	$\begin{array}{l} \quad CH_2-COOH \\ HO-\underset{\vert}{C}-COOH \\ \quad CH_2-COOH \end{array}$	citrate
2-oxopropanoic	pyruvic	$CH_3-\underset{\displaystyle O}{\underset{\Vert}{C}}-COOH$	pyruvate
3-oxobutanoic	acetoacetic	$CH_3-\underset{\displaystyle O}{\underset{\Vert}{C}}-CH_2-COOH$	acetoacetate
2-oxobutanedioic (2-oxosuccinic)	oxaloacetic	$HOOC-CH_2-\underset{\displaystyle O}{\underset{\Vert}{C}}-COOH$	oxaloacetate
2-oxopentanedioic	2-oxoglutaric α-ketoglutaric	$HOOC-CH_2-CH_2-\underset{\displaystyle O}{\underset{\Vert}{C}}-COOH$	2-oxoglutarate α-ketoglutarate
3-oxopropane-1,2,3-tricarboxylic	oxalosuccinic	$\begin{array}{l} O=\underset{\vert}{C}-COOH \\ \quad HC-COOH \\ \quad\quad \vert \\ \quad\quad CH_2-COOH \end{array}$	oxalosuccinate

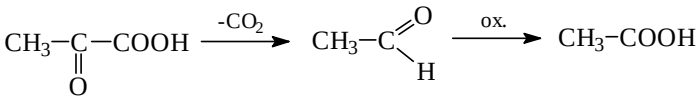
b) ***Decarboxylation***

Decarboxylation occurs when carboxylic acids are heated. Acid loses carbon dioxide (CO_2) from carboxyl group.



Oxalic acid Formic acid

In organism decarboxylation often proceeds as a part of oxidative decarboxylation, in which usually oxo acid is decarboxylated to aldehyde and this can be further oxidized to carboxylic acid. Oxidative decarboxylation of pyruvic acid runs as follows:

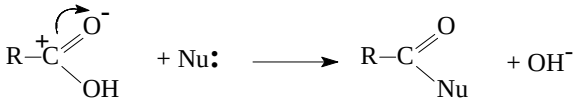


pyruvic acid acetaldehyde acetic acid

Decarboxylation is important reaction in metabolism of amino acids where e.g. biogenic amines can be formed (see chapter *9.1.4.1*)

c) ***Nucleophilic substitutions***

By replacing of –OH group from carboxyl group by nucleophil, functional derivatives of carboxylic acids (esters, amides, acid anhydrides, acyl halides) are formed.

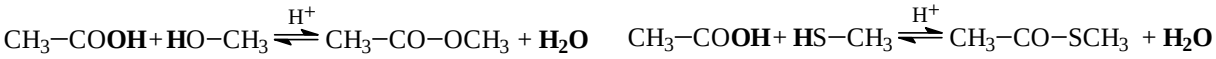


Most reactions of carboxylic acids involve nucleophilic attack on a carbonyl carbon atom:

Biochemists refer to the overall reaction as acyl transfer. The acyl group is transferred to nucleophile in the product.

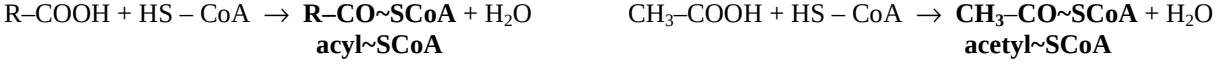
Example of such a reaction is **esterification. Esterification** is the reaction of carboxylic acid with alcohol. When a carboxylic acid and alcohol are heated in the presence of an acid catalyst (usually HCl), an equilibrium is established with the ester and water: –OH group of carboxyl group is replaced by –OR group (e.g. methoxy group $–OCH_3$).

When the alcohol is in an excess, Le Chatelier's principle operates and the equilibrium shifts so much to the right that this is a good method for making an ester. Since esterification is reversible, it is possible to hydrolyze an ester to the corresponding acid and alcohol by using an acid catalyst and a large excess of water. Reverse of acid esterification is ester hydrolysis.



Acetic acid Methanol Methyl acetate Acetic acid Methanethiol Thioester

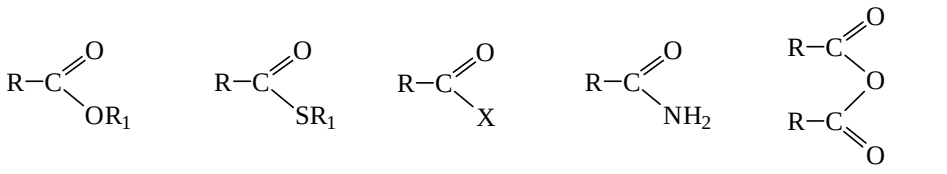
When carboxylic acids react with thiols (sulfur analogue of alcohols), **thioesters** are formed. **Thioesters** represent activated forms of carboxylic acids (acyls) in living cells. Acyl transfer plays an important role in many biochemical processes. The most important thiol to activate acyl groups in the cell is **coenzyme A** which is a complex thiol (see Chapter 12.2.2.). It is usually abbreviated by the symbol **HS–CoA**. Coenzyme A forms thioesters which are the active acyl-transfer agents. Of the thioesters that coenzyme A forms, the acyl ester, called **acetyl-coenzyme A**, abbreviated as **CH_3–CO ~ SCoA**, is the most important.



AcetylCoA reacts with many nucleophils and takes part in reactions of acetyl transfer (acetylations), which are catalyzed with enzymes and run in the organism promptly under normal body temperatures. It is also a substrate of Krebs (citrate) cycle and common intermediate of metabolism of lipids, saccharides and proteins

6.6.6. Functional derivatives of carboxylic acids

Functional derivatives of carboxylic acids are compounds in which the hydroxyl part (–OH) of the carboxyl group (–COOH) is replaced by other groups.



ester | thioester (X = Cl, I) | acyl halide | acid amide | acid anhydride

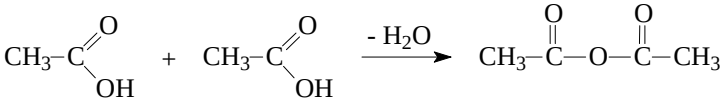
Esters

Esters are derived from acids by replacing the –OH group with –OR group. Esters are named in a manner analogous to salts (e.g. methyl acetate, ethyl acetate, ethyl benzoate). Esters are prepared by esterification from carboxylic acids or better from their functional derivatives, because they are more reactive than acids.

Many esters are rather pleasant-smelling substances with flavor and fragrance of many fruits and flowers. Mixtures of esters are used as perfumes and artificial flavors. Esters of higher carboxylic acids (fatty acids) are fats, cerides and complex lipids. Esters and amides occur widely in nature, and acyl halides are strictly products of the laboratory. (The most important amides are proteins, which contain amide bond –CO–NH–).

Acid anhydrides

Acid anhydrides are derived from acid by removing water from two carboxyl groups and connecting the fragments. For example acetic acid anhydride:

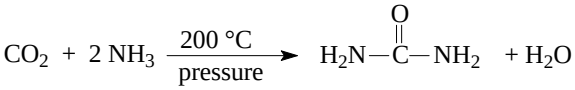


Acetic acid + Acetic acid → Acetic anhydride

Amides

Amides of carboxylic acids are formed by replacing –OH group with $–NH_2$ group. The most important amides are proteins, which contain amide bond –CO–NH– . A special amide, the diamide of carbonic acid (H_2CO_3, inorganic acid) is **urea**.

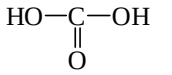
Urea is produced by the reaction of ammonia with carbon dioxide, mainly for use as a fertilizer, and as raw material in the manufacture of certain drugs and plastics:



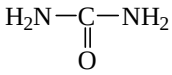
In mammals **urea** is the end product of protein metabolism. It is formed in liver in a metabolic pathway known as the urea cycle, an anabolic process.. The individual atoms that make up a urea molecule come from carbon dioxide, water, aspartate and ammonia. In humans, urea is dissolved in blood (in a concentration of 2.5 - 7.5 mmol/liter) and excreted by the kidney as a component of urine. In addition, a small amount of urea is excreted (along with sodium chloride and water) in sweat. An average adult excretes approximately 30 g of urea in the urine daily. If case of damaged kidneys excretion of urea is depressed and uremia occurs.

Reverse reaction, hydrolysis of urea, is in body catalyzed by urease and produces free ammonia.

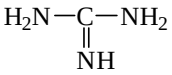
Guanidine is derivative of urea and biochemically derivatives of guanidine are creatine and creatinine. Creatine phosphate plays important role in muscle work and creatinine (anhydride of creatine) is formed from creatine by elimination of water.



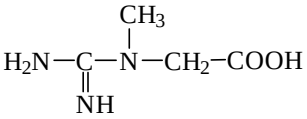
carbonic acid



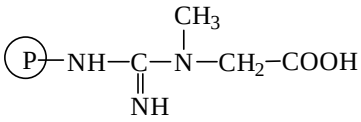
urea



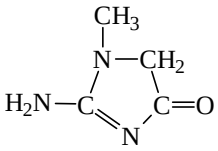
iminourea (guanidine)



creatine



creatine phosphate

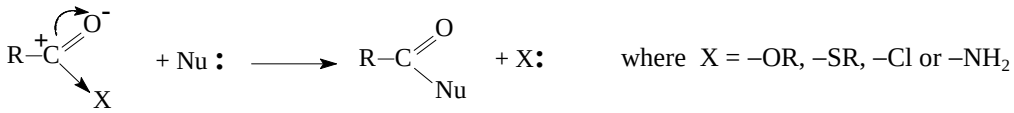


creatinine

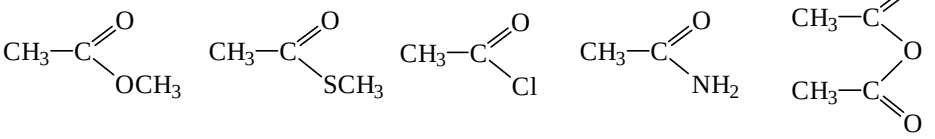
6.6.6.1. Chemical reactions of functional derivatives of carboxylic acids

The functional derivatives of carboxylic acids are more reactive compared to the individual carboxylic acids. Acid halides are most reactive, less reactive are anhydrides, esters and the least reactive are amides.

Functional derivatives are **activated forms of carboxylic acids**. Electrophilicity of the carboxyl carbon is increased by substituent with electron acceptor properties thereby facilitating nucleophilic attack at the carboxy carbon in reactions of functional carboxylic acid derivatives. Examples include acylation which means acyl (R-CO-) transfer to the nucleophile:



where X = –OR, –SR, –Cl or –NH_2



methyl acetate | thioster of acetic acid | acetylchloride | acetamid | acetanhydride

The nature of leaving group can affect the reaction rate. The more electronegative L is, the more positive the carbonyl carbon becomes, and, therefore, the more susceptible is to nucleophilic attack.

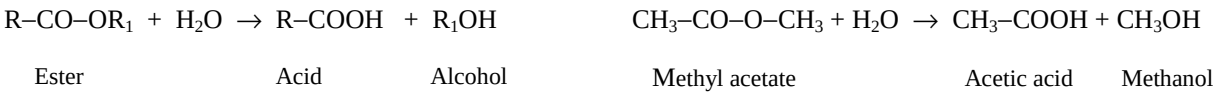
Reactions of carboxylic acid derivatives with some nucleophiles are summarized in Tab. 6.4. The four types of acid derivatives are listed at the left of the table in order of decreasing reactivity toward nucleophiles. Three common nucleophiles are listed across the top. Note that the main reaction product in each column is the same, regardless of which type of acid derivative we start with, whether we start with acyl halide, acid anhydride or acid amide. **Hydrolysis (+ water)** of all acid derivatives gives the corresponding organic acid, **alcoholysis** (+ R–OH) gives an ester, and **ammonolysis** (+ ammonia) gives an acid amide.

Tab. 6.4. Reactions of acid derivatives with some nucleophiles

Acid derivative	**+ nucleophile**		
	HOH (hydrolysis)	**R_1–OH (alcoholysis)**	**NH_3 (ammonolysis)**
R–CO–Cl **Acyl halide**	**R–COOH** + HCl	**R–CO–OR_1** + HCl	**R–CO–NH_2** + NH_4Cl
R–CO–O–CO–R **Acid anhydride**	2 **R–COOH**	**R–CO–OR_1** + R–COOH	**R–CO–NH_2** + R–COOH
R–CO–OR_2 **Ester**	**R–COOH** + R_2–OH	**R–CO–OR_1** + R_2–OH	**R–CO–NH_2** + R_2–OH
R–CO–NH_2 **Amide**	**R–COOH** + NH_3	**R–CO–OR_1** + NH_3	-
Reaction product	**acid**	**ester**	**amide**

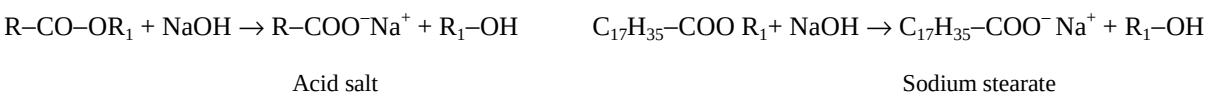
a) Hydrolysis of esters

Hydrolysis of esters is the reverse of direct ester formation, in which carboxylic acid and an alcohol are formed:



Since esterification is reversible reaction, it is possible to hydrolyze an ester to the corresponding acid and alcohol by using an acid catalyst and a large excess of water.

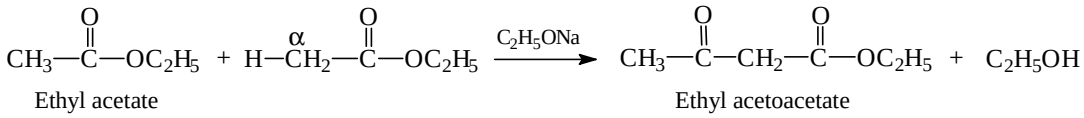
However, esters are more commonly hydrolyzed with a base. It is the nucleophile attack by hydroxide ion on the carbonyl carbon of the ester. Reaction is irreversible. Products of **alkaline hydrolysis** of esters are **salts** of carboxylic acids. **Alkaline hydrolysis** of esters is called **saponification**, because this type of reaction is used to make soaps from fats:



Soaps are sodium or potassium salts of long-chain carboxylic acids.

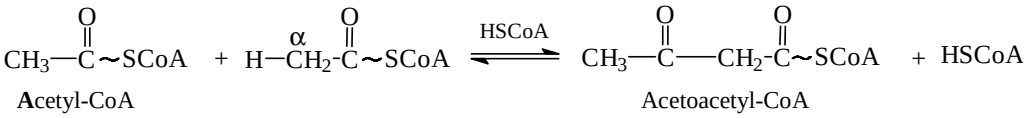
b) Claisen condensation

The Claisen condensation is very similar to the aldol condensation and it is a way of making β-keto esters by reaction of two esters or ester with carbonyl compound. For example, treatment of ethyl acetate with sodium ethoxide in ethanol produces the β- keto ester ethyl acetoacetate:



This reaction is useful for making new carbon-carbon bonds in the laboratory, and it also plays an important role in fat metabolism.

In living cells, the reaction, in which thioesters react (instead of oxygen esters), plays an important role in metabolism of fats during biosynthesis of fatty acids:



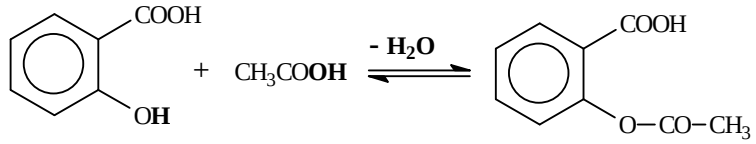
6.6.7. Substitution derivatives of carboxylic acids

Substitution derivatives of carboxylic acids are compounds in which hydrogen atom of the side chain carbon of an acid is replaced by another atom or a group. They are **hydroxy acids, oxo acids, amino acids and halogen acids.** Some important hydroxy and oxo acids are shown in Tab. 6.3..

6.6.7.1. Hydroxy acids and their chemical reactions

Hydroxy acids are formed from carboxylic acids by substitution of hydrogen atom of the side chain by –OH group. Many hydroxy acids occur in nature and are biologically important. The acid, which has its carboxy- and hydroxy- groups attached to the same carbon atom are α–hydroxy acids (e.g. α – hydroxy propionic acid, lactic acid). Of β-hydroxy acids, β-hydroxy butanoic (acetoacetic) is important intermediate in fat metabolism.

To biochemically important hydroxyacids belong glycolic acid, lactic acid, malic acid, citric acid, 3-hydroxy-butyric acid. The most important aromatic hydroxyacid is 2-hydroxybenzoic acid – salicylic acid. The main use of salicylic acid is to prepare aspirin – **acetylsalicylic acid. Aspirin** is widely used as an analgesics and antipyretics. It is a product of esterification of salicylic acid –OH group by acetic acid:



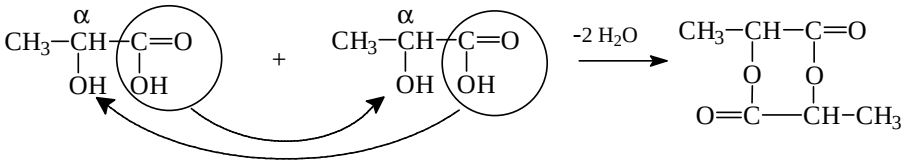
2-Hydroxybenzoic acid (salicylic acid) | Acetic acid | Acetylsalicylic acid

Lactic acid – is an important intermediate in carbohydrate metabolism and it is normal constituent of blood and urine. Lactic acid is formed during muscle activity. With increased muscle activity, oxygen deficiency (ie. myocardial infarction, pneumonia, anemia) or diabetes we find its blood concentration increased. An optical isomer, L(+)-lactic acid is a normal intermediate of anaerobic metabolism of carbohydrates. D, L- (lactic) acid is formed by the action of lactic fermentation of sugars.

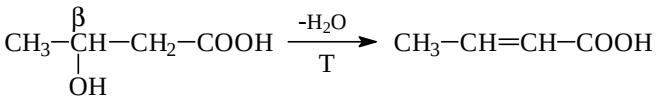
Chemical reactions of hydroxy acids:

a) formation of esters (behavior of hydroxy acids on heating)
Hydroxy acids contain both –COOH and –OH groups. These two functional groups react to form esters. Reaction depends on their relative positions, on the distance between both groups:

α–hydroxy acids react **intermolecularly** to give **cyclic diesters**, called **lactides**.



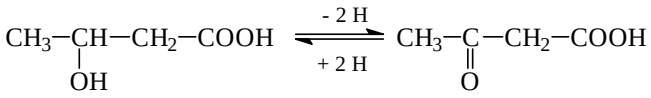
β –hydroxy acids dehydrate on heating to give **unsaturated acids:**



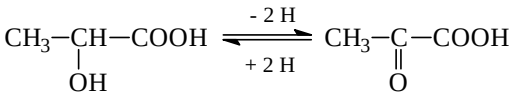
3–Hydroxybutyric acid | 2- Butenoic acid

γ- and δ– hydroxy acids, acids in which both –COOH and –OH groups are separated by three or four carbon atoms, react **intramolecularly** to form **cyclic esters**, called **lactones.** They are important in saccharide chemistry, e.g. ascorbic acid is synthesized by dehydrogenation of γ-lactone of L-gulonic acid, which is catalyzed with *L-gulonolactone dehydrogenase.* During evolution the gene for this enzyme was degenerate in human, what is the reason human can not produce ascorbic acid.

b) oxidation – dehydrogenation (formation of oxo acids)



3-hydroxybutyric acid 3-oxobutyric acid (acetoacetic)



lactic acid pyruvic acid

c) oxidative decarboxylation

- the reaction during which decarboxylation is associated with oxidation (dehydrogenation). The example of oxidative decarboxylation is conversion of pyruvate to acetyl-CoA as well as two reactions in Krebs cycle.

6.6.7.2. Oxo acids (or keto acids) and their chemical reactions

Keto acids, especially those with the ketone group either **α–** or **β–** position related to the carboxyl group, are important intermediates in biological oxidations and reductions. They are important intermediates of metabolism of saccharides, fats and proteins. Biochemically important oxo acids are e.g. **pyruvic acid** and **acetoacetic acid**

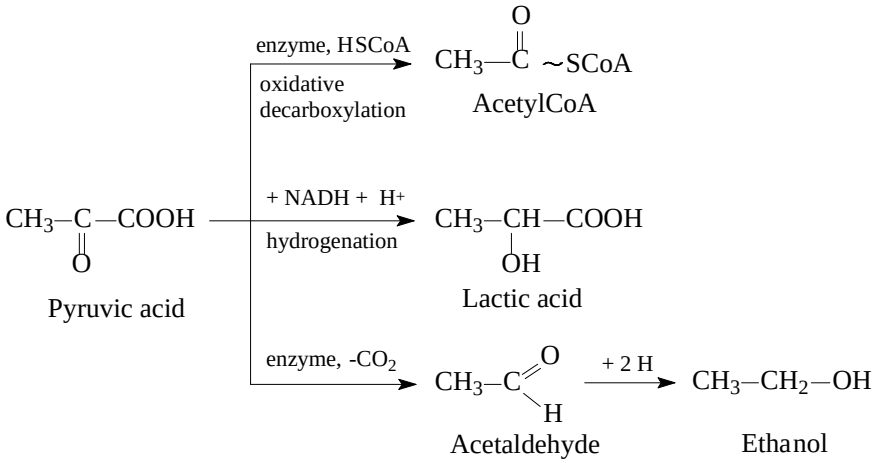
Chemical reactions of oxo acids:

a) oxidation-reduction: hydrogenation, oxidative decarboxylation (2-oxo acids) – activation to thioesters

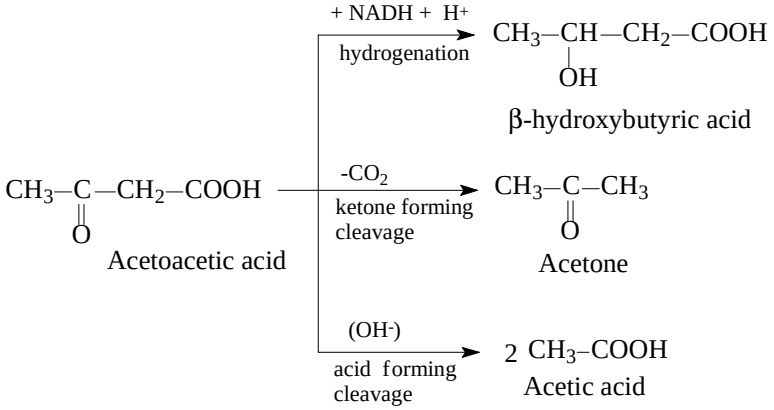
b) acid forming or ketone forming cleavage (3-oxo acids)

c) Claisen condensation (thioesters of 3-oxo acids) as well as reverse reaction - biodegradation, so called β-oxidation of fatty acids

The best known **α– keto acid** is **pyruvic acid**, which plays an important role in carbohydrate metabolism. It is a source of acetyl group for acetyl-coenzyme A. In muscle tissue, pyruvic acid is reduced enzymatically to lactic acid. Pyruvic acid is also a key intermediate in the fermentation of glucose to ethanol. It is converted to ethanol through enzymatic decarboxylation and reduction. These biochemical functions of **pyruvic acid** are summarized in the following scheme:



β– keto acids are important intermediates of fat metabolism. **Acetoacetic acid** is unstable, it is decarboxylated to acetone or hydrogenated to β–hydroxybutyric acid. Biochemical functions of acetoacetic acid are summarized in following scheme:



Acetoacetic acid (acetoacetate), β–hydroxybutyric acid (β–hydroxybutyrate) and **acetone** are called the **ketone bodies**. Acetone arises from acetoacetate by the decarboxylation (loss of carboxyl group). **β–hydroxybutyric acid (β–hydroxybutyrate)** is produced when the keto group of acetoacetate is reduced by NADH (hydrogenation).

Ketone bodies are normal constituents of blood and in absence of other sources of energy acetoacetate and 3–hydroxybutyrate can be used in skeletal muscles, heart muscle and also in brain for energy. When they are produced at a rate higher than the blood buffer can handle or tissues can utilize, ketoacidosis is developed. Normally, the levels of acetoacetate and 3–hydroxybutyrate in the blood are less than 0.4 mmol/l. Under the conditions of prolong starvation or nontreated diabetes, these values can increase as much as 30 times. The condition of excessive levels of ketone bodies in the blood is called **ketonemia.** As acetone is volatile, most of it leaves the body via the lungs and may cause acetone breath, the noticeable odor of acetone in breath.

When there is a combination of ketonemia, ketonuria and acetone breath, the overall state is called **ketosis.**

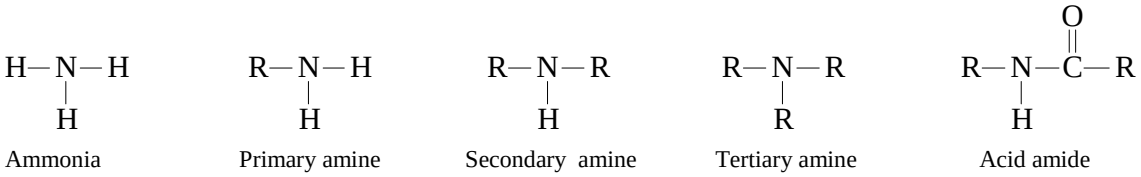
Amino acids – they represent a special group of substitution derivatives of carboxylic acids. For this reason they are described as basic building units of proteins in the individual Chapter 9.

6.6.8. Amines and amides

Amines and amides are two families of nitrogen containing organic compounds**,** that play major roles in biological systems. Amino acids, the building blocks of proteins, are both amines and carboxylic acids (substitutional derivatives of carboxylic acids), the proteins themselves are amides. Nucleic acids contain amine and amide group. Some lipids in biological membranes contain amine groups. Some saccharides and polysaccharides contain amide groups. A wide range of amines and amides are physiologically active. Among them there are hormones adrenaline and melatonin, antibiotics (e.g. penicillin, tetracycline, and a vast number of substances, such as caffeine, nicotine, narcotics, anesthetics, various medications, alkaloids (e.g. opiates, such as morphine, codeine, heroin).

Amines and **amides** are organic derivatives of ammonia in which one or more hydrogen atoms are replaced by organic groups.

Relationship between ammonia, amines and amides illustrates next scheme, where R = alkyl or aryl:



$CH_3 – CH_2 – NH_2$	$CH_3 – NH – CH_3$	$(CH_3)_3 N$	$C_6H_5 – NH_2$
Ethylamine (primary amine)	Dimethyl amine (secondary amine)	Trimethyl amine (tertiary amine)	Phenylamine – aniline (an aromatic amine)

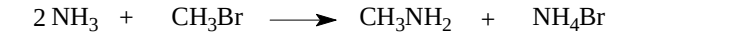
Amines

Amines are the most important type of organic base that occurs in nature. Amino group basicity plays an important role in biochemical processes.

Amines are classified according to the number of carbons directly bound to the nitrogen. Primary, secondary and tertiary amines have one, two and three carbons directly bound to the nitrogen.

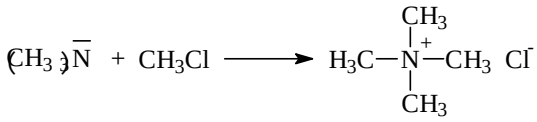
Amines are also classified as **aliphatic or aromatic** (aryl) and **heterocyclic** amines. An **aliphatic** amine is an amine in which aliphatic group is bound to nitrogen. An **aromatic** (aryl) amine is an amine in which at least one benzene or another aromatic group is directly bound to nitrogen. Example of aromatic amine is toxic aniline (aminobenzene), which oxidizes hemoglobin into methemoglobin. **Heterocyclic** amines are cyclic compounds containing one or more nitrogen atoms in the ring (not attached to the ring). Nitrogen atoms are present in the heterocyclic rings of many natural products, which have important physiological effects on humans or play a key role in some biological processes. E.g. alkaloids are amines produced by plants. They are usually heterocyclic amines, taste bitter, with significant physiological effects.

Amines may be prepared by reaction of ammonia with alkyl halide:



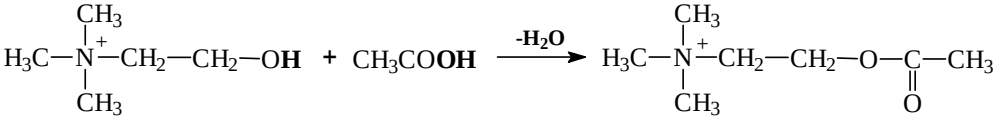
Ammonia Methyl bromide Methylamine Ammmonium bromide

Tertiary amines react with primary or secondary alkyl halides to give **quaternary ammonium salts**, for example:



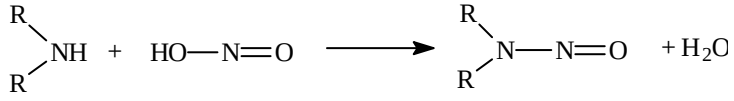
Quaternary ammonium compounds are important in certain biological processes. One of the most common natural quaternary ammonium ion is **choline**, which is present in phospholipids. Choline is not only involved in various metabolic processes, but it is also the precursor of **acetylcholine**, which plays a role in the transmission of nerve impulses.

Acetylcholine arises by esterification of choline with acetic acid:



Choline Acetic acid Acetylcholine

Each class of amines reacts differently with **nitrous acid**. From toxicological viewpoint there is important reaction of secondary amines with nitrous acid (nitrosation) which produces a **nitrosamine**:



Secondary amine Nitrous acid Nitrosamine

Nitrosamines are known to be dangerous carcinogens. Nitrosamines can be formed also in our environment by the reaction of amines with oxides of nitrogen or with sodium nitrite that has been added to meat as a preservative. They have been found also in cigarette smoke.

Control questions

1. Write the reaction of formation of any 3-hydroxybutyric acid ester.
2. Write the formula of hemiacetal formed in the reaction of acetaldehyde and 2-propanol.
3. Write the reaction of imine formation from formaldehyde and ethylamine.
4. Write the reactions of Krebs cycle:
   a. formation of citrate from acetyl CoA and oxaloacetate
   b. dehydration of citrate
   c. formation of isocitrate
   d. dehydrogenation of isocitrate
   e. decarboxylation of oxalosuccinate
   f. oxidative decarboxylation of 2-oxoglutarate
   g. dehydrogenation of succinate
   h. hydration of fumarate
   i. dehydrogenation of malate
5. Explain the principle of methanol toxicity.
6. Illustrate structurally step by step reduction of coenzyme Q from quinone to hydroquinone form.

7. SACCHARIDES

Saccharides represent a large group of compounds in which hydrogen and oxygen are combined with carbon. Although these compounds are not hydrates of carbon, their summary composition can be expressed with formula: $\mathbf{C_n(H_2O)_n}$ (another name is carbohydrates).
From chemical viewpoint saccharides can be defined as **polyhydroxyaldehydes** or **polyhydroxyketones** or substances that form these when hydrolyzed. Saccharides are the most occurring organic compounds in nature. They are produced by green plants and bacteria in process called photosynthesis:

$$\mathbf{n\,CO_2 + n\,H_2O \xrightarrow[\text{chlorophyll}]{h.\nu} C_nH_{2n}O_n + n\,O_2}$$

Yearly about 5.10^{21} kcal of energy stream to Earth from Sun. 1/3 of this energy reflects in dust and clouds back to universe.. Less than 0.05 % of solar energy which attains Earth is used for photosynthesis in plants. **The organism can obtain energy**, which was formed in plants, **through the oxidation metabolic reactions**:

$$\mathbf{C_nH_{2n}O_n + n\,O_2 \longrightarrow n\,CO_2 + n\,H_2O}$$

7.1. Functions of saccharides in the living organism

Saccharides are persistent components of all cells in living organisms. They represent the **main source of energy** – 50 - 60 % of total need (predominantly glucose). Starch and glycogen are **storage energetic molecules**. At the same time saccharides represent **reserve compounds**, which can be metabolized into other necessary molecules. Cellulose and chitin belong to the **building components** of the cells and tissues of plants. Heteropolysaccharides as **proteoglycan components** are basic part of connective tissues, as a **part of glycoproteins** (see Chapter 7.5.2.) they represent functionally important part of molecules with variety of effects (enzymes, blood groups glycoproteins, membrane receptors, transport proteins and others). Besides mentioned some saccharides have **specific effects** (e.g. heparin has anticoagulation effect).

7.2. Classification of saccharides

According to the number of monomeric units saccharides can be divided into three basic groups – **monosaccharides** with one unit, **oligosaccharides** with 2-10 monomeric units (disaccharides, trisaccharides, ...decasaccharides) and **polysaccharides** with many units (homoglycans, heteroglycans). **According to the number of carbon atoms** monosaccharides are divided to trioses, tetroses, pentoses, hexoses, heptoses, octoses and nonoses.

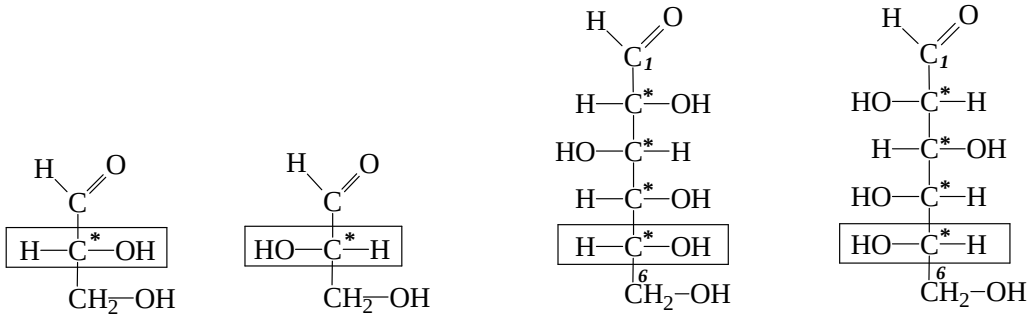
7.3. Structure and properties of monosaccharides

Carbohydrates which cannot be hydrolyzed are called monosaccharides and they are polyhydroxycarbonylic compounds and according to the functional group occurring in their molecule two groups can be recognized – **aldoses** and **ketoses**. The main monosaccharide that is important for nature and living organisms is **glucose**. Glucose is the most occurring organic monomer in nature. It is the building unit of cellulose, polysaccharide, which creates approximately 10 % of all the tree leaves of the world, about 50 % of woody parts of plants and nearly 100 % of cotton. In addition it is a monomer of starch – polysaccharide present in many foods, e.g. in cereals and tubers. In the human organism glucose represents basic physiological monosaccharide. Physiological blood glucose concentration (glycemia) is 3.3 – 5.6 mmol/l. In medical praxis the pure glucose solution is administered to patients in infusions or during determination of glycemic curve (oral glucose tolerance test) .

7.3.1. D- and L- isomers of monosaccharides (enantiomers)

The basic and the simplest monosaccharide, from structure of which other monosaccharides are derived, is called **glyceraldehyde**. It can exist in D- or L- isoform (generally two series of monosaccharides are recognized) and it contains one asymmetric carbon. D- and L- forms of the same monosaccharide are called **enantiomers**. Physiologically monosaccharides are present **in living system in D-isomeric form**. Series of D-aldoses are shown in Fig. 7.3.

D- and L- isomers are as a subject and a picture in the mirror. They have all secondary –OH groups in opposite side. The designation of a monosaccharide isomer as the D form or of its mirror image as the L form is determined according to the position of –OH group on the last optically active carbon, by its spatial relationship to the parent compound of the carbohydrates – glyceraldehyde. The orientation of the –H and –OH groups around the carbon atom adjacent to the terminal primary alcohol carbon (carbon 5 in glucose) determines whether the saccharide belongs to the D or L series. When the –OH group on this carbon (the last optically active carbon) is on the right, the saccharide is D-isomer; when it is on the left, it is the L-isomer.

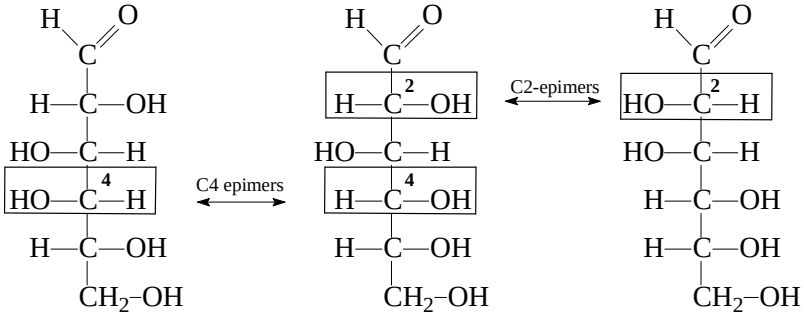


D – glyceraldehyde L- glyceraldehyde D-glucose L-glucose

The number of stereoisomers of monosaccharides with the same number of carbons can be calculated as $\mathbf{n=2^c}$ (c = number of asymmetric carbons).

7.3.2. Epimers

Epimers are isomers of monosaccharides which differ in position/configuration of only one secondary –OH group. For glucose the most important epimers are mannose (C2 epimer) and galactose (C4 epimer):



D - galactose D - glucose D - mannose

7.3.3. Cyclic structure of monosaccharides - formation of anomers

Monosaccharides (higher than tetroses) predominantly occur in **cyclic structures** because of the tendency of carbonyl and hydroxyl groups to react together in intramolecular reaction thus forming **cyclic hemiacetals**. Fig. 7.1.a) shows the arrangement of D-glucose chain, in which –OH group on the carbon C-5 is approaching to carbonyl carbon C-1 in reaction distance. The cyclization of fructose is illustrated at Fig. 7.1. b).

During cyclization of monosaccharide into hemiacetal structure, double bond on carbon of oxo-group is eliminated and this carbon becomes an asymmetric, this means a new chiral center is formed (on **anomeric carbon**). According to the configuration of the new chiral center, two possible hemiacetal structures are recognized. Two monosaccharides differing only in configuration on anomeric carbon are called **anomers** and they are dependent on –OH group position. This group is called **hemiacetal –OH group. Anomers** are designated as **α-** (the same position of hemiacetal –OH group than hemiacetal ring in Tollens formula) and **β-** (the position of hemiacetal group is on the opposite side than hemiacetal ring).

α- and β- **anomers** are differing in physico-chemical properties, e.g. value of optical rotation. The change of optical rotation of monosaccharide solutions after dissolving of monosaccharides is called **mutarotation** (Fig. 7.1.).

The mutarotation is typical for all monosaccharides existing in α- and β- anomeric forms. It is the change in the optical rotation because of the change in the equilibrium between two anomers α- or β- form. Equilibrium mixture of water solution of glucose contains 35 % of α - anomer, 64 % of β - anomer and only 0.003 % of acyclic form. In human organism the majority of glucose is in cyclic form.

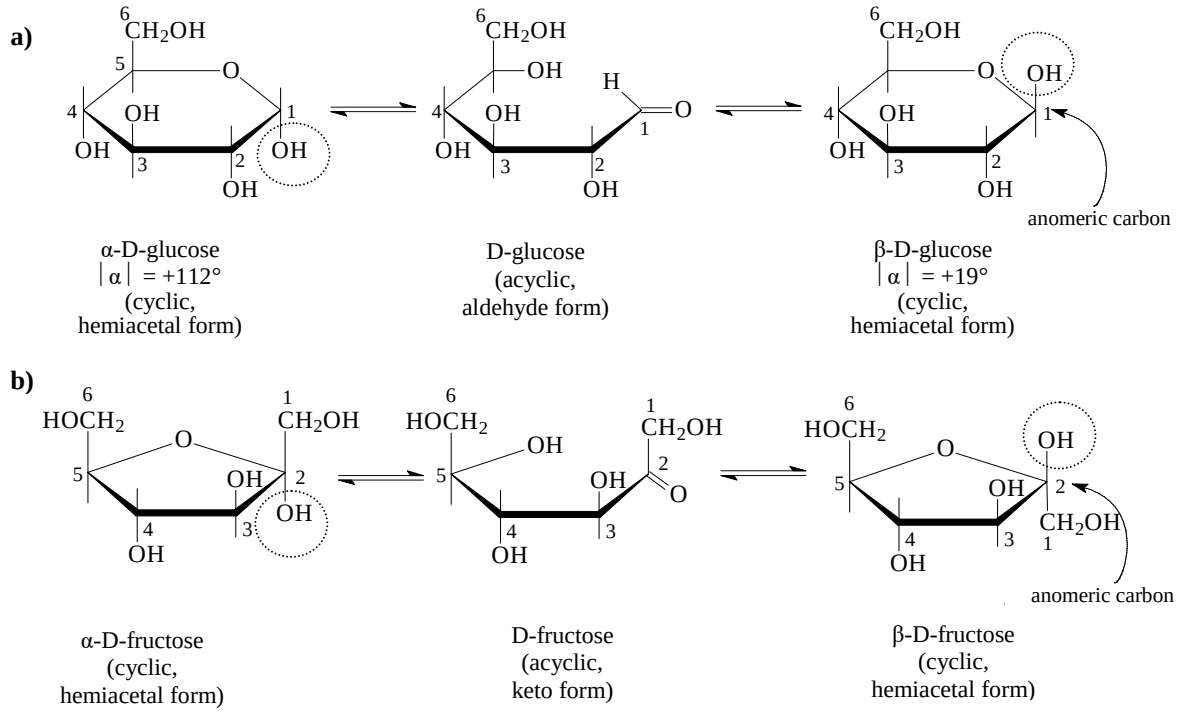
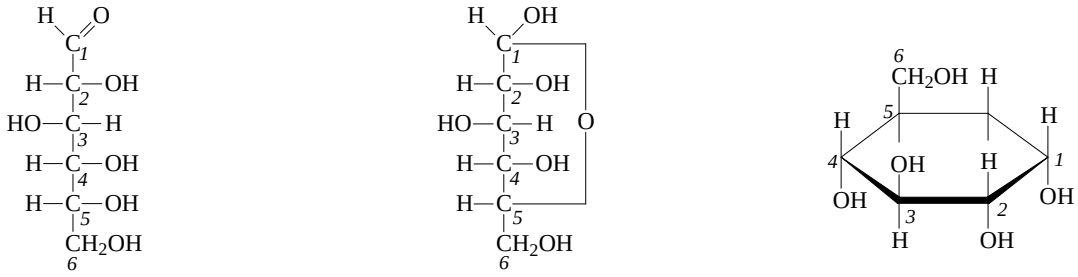


Fig. 7.1. Mutarotation of glucose (a) and fructose (b)

The structure of saccharides can be expressed in several types of formulas – Fischer (acyclic). Tollens and Haworth (cyclic); glucose structures are shown in Fig. 7.2.



D-glucose
(Fischer formula)

α-D-glucopyranose
(Tollens formula)

α-D-glucopyranose
(Haworth projection)

Fig. 7.2. Acyclic and cyclic structures of glucose

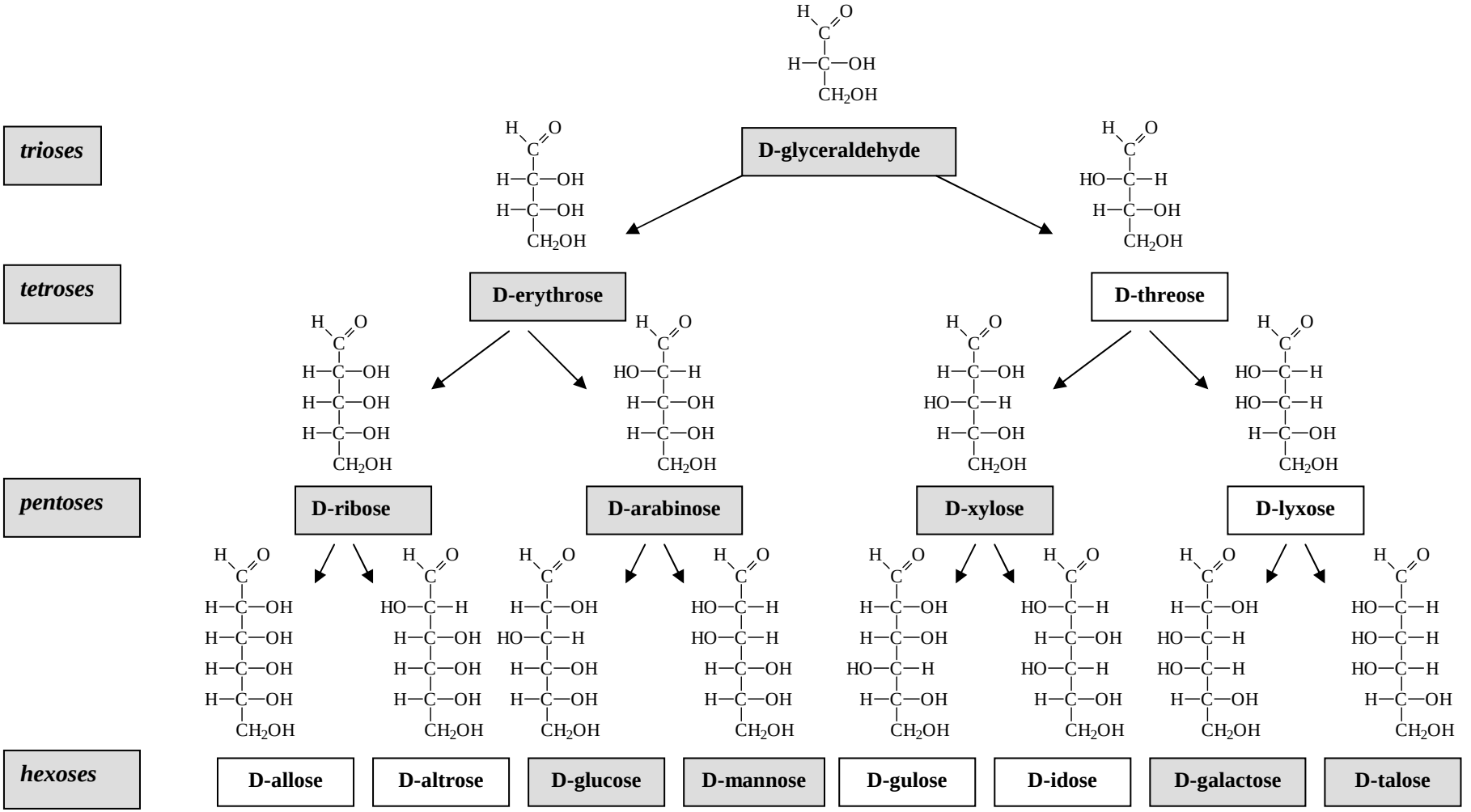


Fig. 7.3. Series of D-aldoses having from three to six carbon atoms (the most common ones in nature are bold typed)

7.3.4. Physical and chemical properties of saccharides

Monosaccharides and oligosaccharides are white crystalline substances of sweet taste (Tab 7.1.), they are water soluble in contrast to most of polysaccharides. They are insoluble in nonpolar solvents.

Tab. 7.1. Comparison of different saccharides sweetness

saccharide	sweetness	saccharide	sweetness
sucrose	100 (reference)	xylose	40
fructose	173	maltose	32
invert sugar	130	galactose	32
glucose	74	raffinose	23
sorbitol	48	lactose	16

Chemical properties of saccharides result from functional groups present in saccharide molecule (hydroxyl. aldehyde, oxo groups). Many of characteristic reactions are provided only by acyclic forms of saccharides because of reactivity of free carbonyl functional groups. These are not important for organism under the physiological conditions due to insignificant amount of acyclic form of monosaccharides. In case of increased acyclic form concentration (e.g. blood glucose during diabetes mellitus) the reactivity of carbonyl group of glucose assumes the pathophysiological importance (see Cahpter 7.3.4.5).

7.3.4.1. Oxidation of monosaccharides

Aldehyde group of aldoses acyclic form can be easily oxidized to carboxylic group resulting in formation of **aldonic acids**. Stronger oxidation reagents (HNO_3) oxidize aldehyde and primary –OH groups to form **aldaric** (dicarboxylic) **acids.** If aldehyde group (hemiacetal –OH group) is protected before the oxidation, only primary –OH group on the last carbon is oxidized and **uronic acids** are formed. The scheme of reactions of glucose oxidation (to simplify in acyclic form) are in the Fig. 7.4.

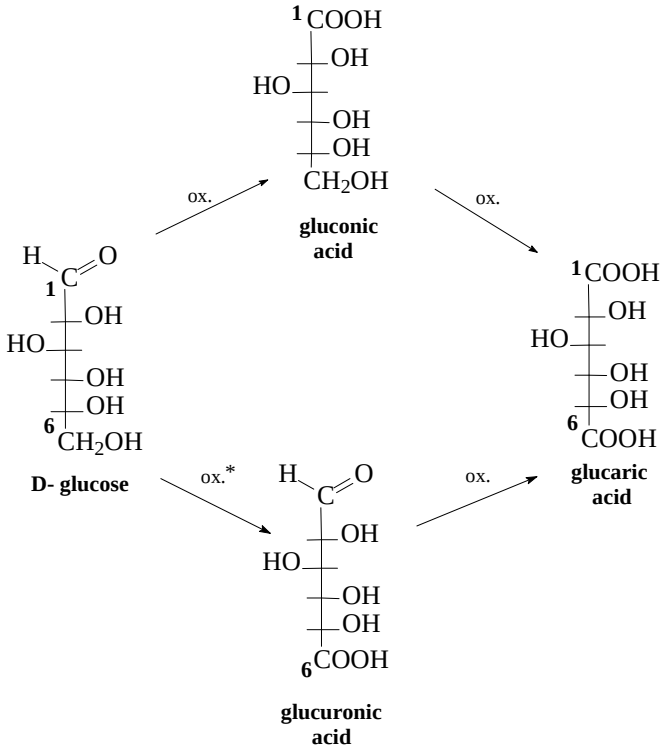


Fig. 7.4. Reaction scheme of glucose oxidation
(- oxidation on the last carbon is proceeding under the special conditions - the most reactive aldehyde group has to be chemically "protected")*

The example of important uronic acid is **glucuronic acid**. Glucuronic acid (in ionized form with COO^- is signed as glucuronate) is bound in polysaccharides (e.g. hyaluronic acid, chondroitine sulphate) and it is a precursor of ascorbic acid biosynthesis in vertebrates. It has detoxication function because it binds toxic exogenous and endogenous compounds (xenobiotics, drugs with aromatic character, steroid hormones, bilirubin) in the form of glycosides, which are excreted by urine.

Glucuronic acid is formed in the organism by the oxidation of uridine diphosphate glucose (UDP-glucose) to UDP-glucuronic acid (Fig. 7.5.) . Uridine diphosphate glucuronate (anion of UDP-glucuronic acid) is an active form of glucuronate, which conjugates with compound to be detoxicated (substrate containing –OH, $-NH_2$, -SH or COOH groups in its molecule) thus forming β-glucoside uronates. Detoxication reaction is predominantly proceeding in liver and it is catalyzed by *UDP-glucoside uronate transferase (UDP-glucuronosyl transferase).* Fig. 7.6. shows reaction of phenol detoxication.

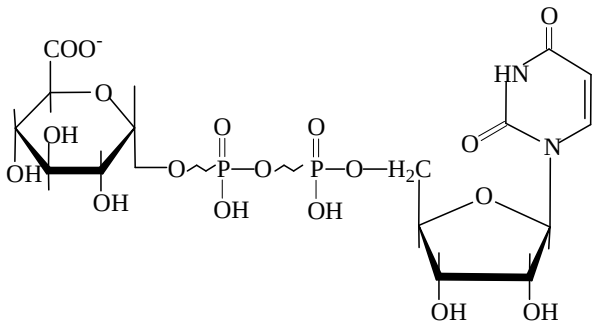


Fig. 7.5. The structure of UDP-glucuronic acid

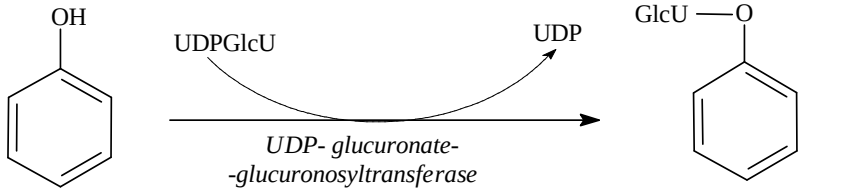


Fig. 7.6. Reaction of phenol detoxication through the formation of O-glycoside with UDP-glucuronic acid

7.3.4.2. Reduction of monosaccharides

Reduction of oxo groups of aldoses and ketoses provides **polyhydroxyhydrocarbons** as products. Aldoses are reduced only to one product and ketoses provide the mixture of two ones because of the formation of new center of asymmetry (marked with * in Fig. 7.7.). Thus glucose is reduced to **glucitol** and fructose is reduced to **glucitol** and **manitol**. D-glucitol (sorbitol) is used as an artificial sweetener, which does not affect glycemia (blood glucose concentration) - this is widely used in diet of diabetic patients.

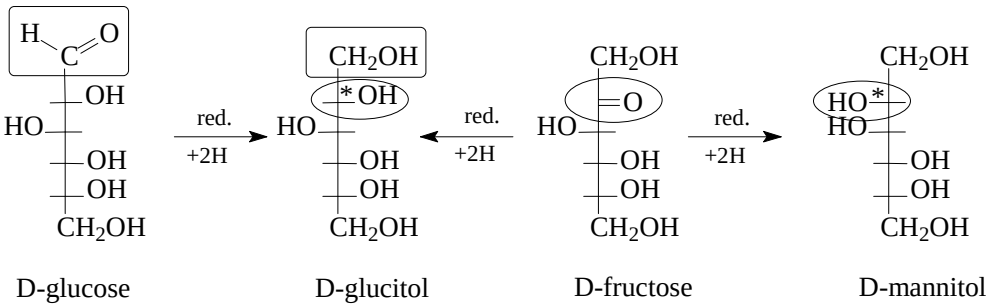
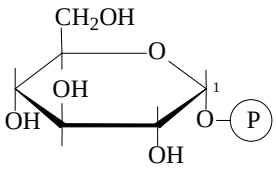


Fig. 7.7. Reactions of glucose and fructose reduction

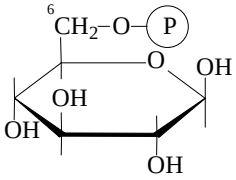
7.3.4.3. Esterification of monosaccharides

Hydroxyl groups of saccharides can react with inorganic as well as with carboxylic acids forming the esters. Biologically important products of reactions of saccharides esterification are the ones with trihydrogenphosphoric acid (H_3PO_4). They are present in all living cells as intermediates of saccharides metabolism as well as the components of DNA and RNA structures. The trihydrogenphosphoric acid residue is usually signed as (P) in the molecules.

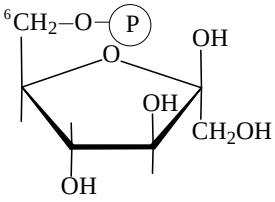
To the biologically important phosphate esters of monosaccharides also following molecules belong:



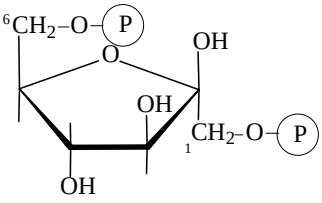
α-D-glucose-1-phosphate
(Cori ester – also β-anomer)



β-D-glucose-6-phosphate
(Robison ester)



β -D–fructose-6-phosphate
(Neuberg ester)



β -D–fructose-1,6-bisphosphate
(Harden-Young ester)

7.3.4.4. Formation of glycosides

Glycosides are the compounds formed through condensation of hydroxyl group of a monosaccharide (or a monosaccharide residue) anomeric carbon and hydroxyl group of another compound, which is (or not) also monosaccharide (Fig. 7.8.).

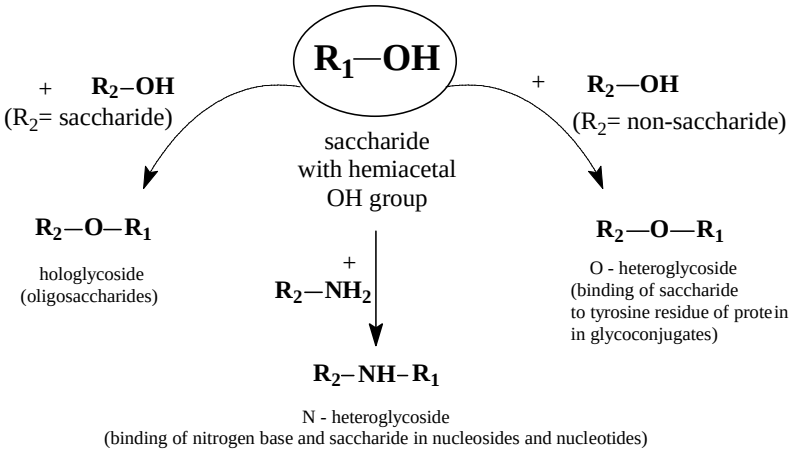


Fig. 7.8. Types of glycosides

If the nonsaccharide molecule creates with saccharide glycosidic bond this molecule is called **aglycon** and **heteroglycoside** is product of the reaction. As aglycon there can be e.g. methanol, glycerol, sphingosine, sterol, phenol or nitrogen base as adenine. Hologlycoside is the glycoside formed of two molecules of saccharides. Hologlycosidic bond is formed e.g. during the formation of oligosaccharides or polysaccharides between the monosaccharide units (Fig. 7.9.).

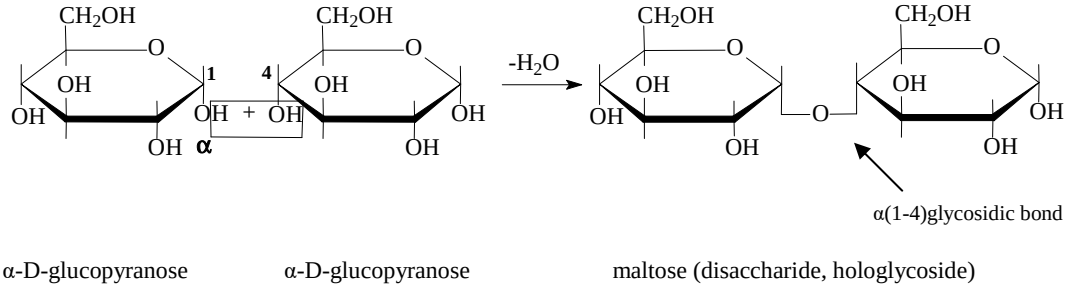


Fig. 7.9. Formation of glycosidic bond between two molecules of α-D-glucopyranose

There are known many glycosides of medicinal importance. Some of them can be the parts of drugs (e.g. antibioticum streptomycine), also so called heart glycosides are important, which contain as aglycons steroids (e.g. ouabaine – inhibitor of Na^+/K^+-ATPase of cell membranes)

7.3.4.5. Reaction with primary amines

The carbonyl groups present in the molecules of acyclic monosaccharides can react with free primary amino groups of proteins (Fig. 7.10.) This reaction has a key role in patients with diabetes mellitus, disease characterized with persistent hyperglycemia (increased concentration of blood glucose). The entire process is called nonenzymatic glycation and there is included not only the reaction of glucose (or of other reducing monosaccharides) with free amino groups of proteins but also consecutive oxidative and dehydration reactions. In 1984 the new term "advanced glycation endproducts" (AGEs) was introduced for the group of brown and in some cases fluorescent molecules, which are formed in those advanced reactions of glycation. AGEs and mainly their overaccumulation take part in the processes, which are closely related to the formation and progression of diabetic complications as well as to the e.g. ageing process.

This follows the assumption that the change of protein structure (proteins with Amadori products bound) results in the change of biological functions. Modification concerns many important enzymes, structural proteins, lipoproteins, etc. The protein studied in detail, function of which is markedly changed during glycation, is hemoglobin. Determination of level of glycated hemoglobin and glycated plasma proteins is widely used in diabetological praxis as a marker of treatment efficiency.

In addition to nonenzymatic glycation, which is basically the manifestation of glucose “toxicity”, free glucose at the increased concentration due to its reducing properties shows the ability to generate superoxide anion radical and consequently hydrogen peroxide, which produces hydroxyl radical. This “autooxidation “ reaction represents the glucose contribution to the total oxidative stress accompanying diabetes mellitus.

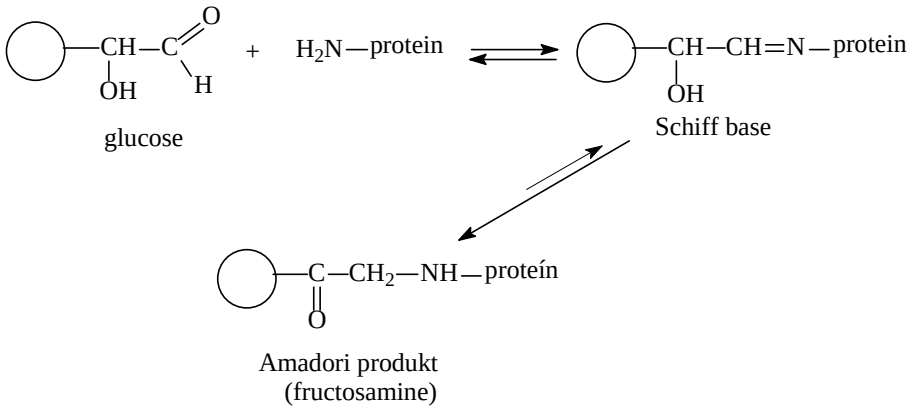


Fig. 7.10. Reaction of glucose molecule with primary amino group of protein

residue of glucose molecule

7.4. Disaccharides

Disaccharides are molecules consisting of two monosaccharide subunits and they are formed by formation of glycosidic bond between the hemiacetal hydroxyl group of one monosaccharide and some of hydroxyl groups of the second monosaccharide (the second one can or cannot be hemiacetal hydroxyl group). Disaccharide can be hydrolyzed back to the initial monosaccharides.

Nutritionally important disaccharides are maltose, lactose and sucrose (Fig. 7.11.).

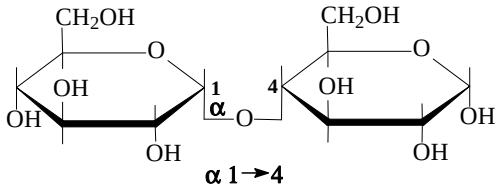
Maltose is formed from **two glucose units**. Maltose (or “malted sugar”) as such is not widespread in nature even it is present in germinating grain. It occurs in corn molasses, which is made from corn starch and formed during hydrolysis of starch with α-amylase.

Lactose (“milk sugar”) consists of **galactose and glucose** molecules. It is the main saccharide of mammalia milk (4 – 6 % in cow's milk, 5 – 8 % in mother´s milk). Galactosemia is the disease characterized with absence of enzyme, which catalyzes isomerization of galactose into glucose and the elimination of milk from food is the prevention of disease symptoms (caused by accumulation of galactose).

Sucrose consists of **glucose and fructose** molecules and it is usually obtained from sugar cane or white beet.

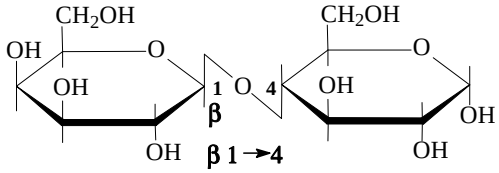
Disaccharides, in which one monosaccharide unit retains its free hemiacetal hydroxy group, can open the ring of this monosaccharide and cyclize it again. It means that aldehyde group can be oxidized through the acyclic form and by this way another molecule can be reduced. Such disaccharide is called reducing disaccharide.

Fig. 7.10. shows the structures of disaccharides mentioned above and from their structures it is known that maltose and lactose are reducing disaccharides and sucrose is nonreducing disaccharide.



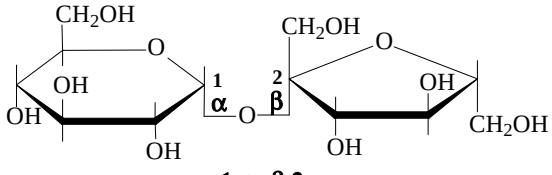
α-D-glucopyranose α-D-glucopyranose

MALTOSE



β-D-galactopyranose α-D-glucopyranose

LACTOSE



α-D-glucopyranose β-D-fructofuranose

SUCROSE

Fig. 7.11. The structure of selected important disaccharides

7.5. Polysaccharides (glycans)

7.5.1. Homopolysaccharides (homoglycans)

Homoglycans are saccharide polymers consisting of more than 10 monosaccharide units of the same type. These units are bound to each other with α- or β- glycosidic bonds.

Starch is a storage plant polysaccharide, which is built from glucose monomers (i.e. it is **glucan**). Through the different intermediates it can be cleaved into the glucose units. Starch has two components: **amylose** (15-20%), and **amylopectin** (80 - 85%). Amylose is linear and coiled into helix and glucose units are bound with α(1→4) glycosidic bonds. Amylopectin has branched structure because in addition to α(1→4) glycosidic bonds it contains also α(1→6) glycosidic bonds in the branching points. α-Amylase of animal origin can hydrolytically cleave the starch step by step to branched and unbranched dextrins. The final product of hydrolysis is disaccharide maltose.

Glycogen (animal starch) is a storage polysaccharide of animal cells and is built also from glucose monomers. It occurs in liver and muscle cells. Glycogen is structurally similar to the amylopectin, but branching is more abundant (from 8 to 10 1→4 glycosidic bonds per one 1→6 bond) (Fig. 7.12.). Organism can cleave the glycogen enzymatically thus obtaining glucose e.g. under conditions of hypoglycemia (decreased concentration of blood glucose).

Cellulose is the most occuring saccharide in the nature. It is a glucan, in which glucose units are bound with β(1→4) glycosidic bonds. Human organism is not able to cleave β(1→4) glycosidic bonds using digesting enzymes (α-amylase).

Inulin is a fructan (with D-fructofuranose as monomer), containing α(2→1) glycosidic bonds and it occurs e.g. in chicory. Inulin is used for determination of kidney function because it is excreted from organism only by glomerular filtration in kidney.

Agar and **pectins** are examples of homoglycans consisting of galactose as a monomer (they are **galactans**). Agar is isolated from sea-grass and used as cultivation medium in form of gel. Pectins (polygalactouronic acids) are components of fleshy part of fruit and vegetable.

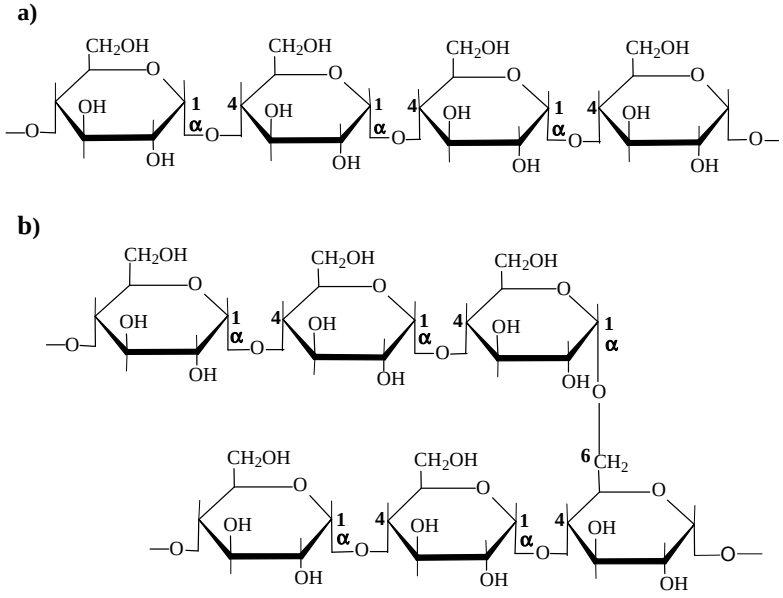


Fig. 7.12. Structure of starch: a) amylose b) amylopectin

Mannans represent homopolysaccharides composed of mannose units, which occur in the yeast, proteoglycans, glycoproteins, some hormones of glycoprotein character (tyreotropic – TSH) and in the different enzymes with glycoprotein character.

Important homopolysaccharide is **chitin** with glucose derivative – N- acetylglucosamine – as a monomer. It is structural (building) polysaccharide of fungi, insect or shellfishes. Liner structure is provided by β(1→4) glycosidic bonds between subunits.

7.5.2. Heteropolysaccharides (heteroglycans)

Heteropolysaccharides are polysaccharides, which are formed minimally from two types of monosaccharides (often from their derivatives – mainly aminosaccharides and uronic acids) (Fig. 7.13.). Many of heteropolysaccharides have the disaccharide composed of aminosaccharide and uronic acid as a building unit and they contain higher amount of sulphur in a form of $-SO_3H$ groups linked by ester bound to the saccharide unit. An example is **heparin** with anticoagulation properties, **chondroitine sulphate** and **keratane sulphate** in cartilage.

Heteropolysaccharide is often bound to the nonsaccharide component thus forming **glycoconjugates**. According to the character and extent of nonsaccharide component, there are recognized **proteoglycans, glycoproteins, glycopeptides and glycolipids.**

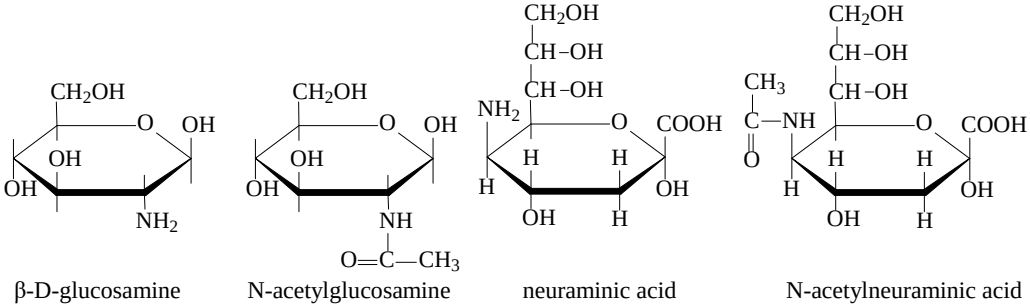


Fig. 7.13. Examples of glucose amino derivatives occuring in the structure of heteropolysaccharides

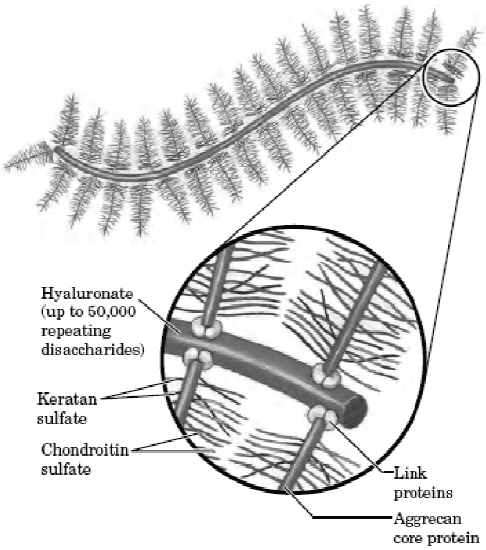
Tab. 7.2. Some important polysaccharides occuring in living organisms

Polysaccharide	Monosaccharide 1	Monosaccharide 2	Linkage	Branching	Occurence	Function
Bacteria						
Murein	D-GlcNAc	D-MurNAc[1)]	β 1→4	—	Cell wall	SC
Dextran	D-Glc	—	α 1→6	α 1→3	Slime	WB
Plants						
Agarose	D-Gal	L-aGal[2)]	β 1→4	β 1→3	Red algae (agar)	WB
Carrageenan	D-Gal	—	β 1→3	α 1→4	Red algae	WB
Cellulose	D-Glc	—	β 1→4	—	Cell wall	SC
Xyloglucan	D-Glc	D-Xyl (D-Gal, L-Fuc)	β 1→4	β 1→6 (β 1→2)	Cell wall (Hemicellulose)	SC
Arabinan	L-Ara	—	α 1→5	α 1→3	Cell wall (pectin)	
Amylose	D-Glc	—	α 1→4	—	Amyloplasts	RC
Amylopectin	D-Glc	—	α 1→4	α 1→6	Amyloplasts	RC
Inulin	D-Fru	—	β 2→1	—	Storage cells	RC
Animals						
Chitin	D-GlcNAc	—	β 1→4	—	Insects, crabs	SC
Glycogen	D-Glc	—	α 1→4	α 1→6	Liver, muscle	RC
Hyaluronic acid	D-GlcUA	D-GlcNAc	β 1→4 β 1→3	—	Connective tissue	SC,WB

SC = structural carbohydrate; RC = reserve carbohydrate; WB = water binding carbohydrate
[1)] N-acetylmuramic acid; [2)] 3,6-anhydrogalactose

7.5.2.1. Proteoglycans

Proteoglycans are molecules or molecule aggregates (Fig. 7.14.), which are formed from larger **saccharide** and less protein parts. Saccharide moiety represents up to 95 % of glycoprotein molecules. The axis of proteoglycan is created by **core protein**, upon which different types of **heteropolysaccharide (glycosaminoglycan - GAG) chains** are bound (through serine or threonine with O-glycosidic bond). These glycosaminoglycan (GAG) chains are long, linear carbohydrate polymers that are negatively charged under physiological conditions, due to the occurrence of sulphate and uronic acid groups.



Proteoglycans are a major component of the animal extracellular matrix, the 'filler' substance existing between cells in an organism. Here they form large complexes, both to other proteoglycans, to hyaluronan and to fibrous matrix proteins (such as collagen). They are also involved in binding cations (such as sodium, potassium and calcium) and water, and also regulating the movement of molecules through the matrix. Evidence also shows they can affect the activity and stability of proteins and signalling molecules within the matrix. Individual functions of proteoglycans can be attributed to either the protein core or the attached GAG chain.

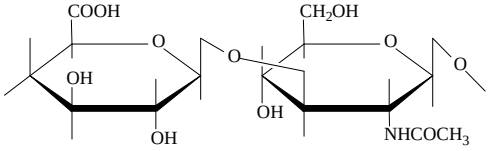
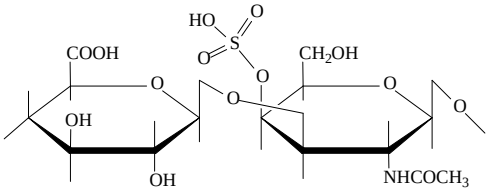
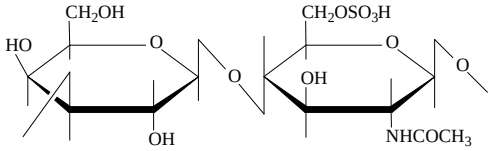
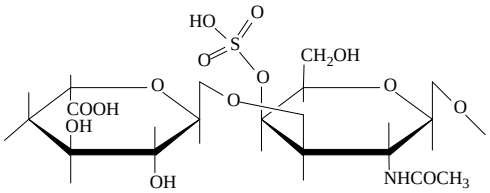
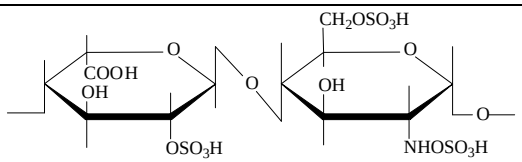
An inability to break down proteoglycans is characteristic of a group of inherited metabolic diseases, called **mucopolysaccharidoses**. The inactivity of specific lysozomal enzymes that normally degrade glycosaminoglycans leads to the accumulation of proteoglycans within cells. This leads to a variety of disease symptoms, depending upon the type of proteoglycan that is not degraded.

Fig. 7.14. Proteoglycan aggregate of the extracellular matrix.

Tab.7.3 Heteropolysaccharides and their localization in the organism

GAG	Localization	Note
Hyaluronate	synovial fluid	big polymers, decreasing of internal friction in joints and protection of cartilage against damage
Chondroitin sulfate	cartilage, bones, heart valves	the most occurring GAG
Heparine	liver, skin, component of intracellular granules in mast cells lining pulmonary arteries	more sulfated than heparan sulfates
Heparan sulfate	basal membranes, component of cell surfaces	higher content of acetylated glucosamine than heparin
Keratan sulfate	cornea, bones, cartilage – aggregates with chondroitin sulfates	
Dermatan sulfate	skin, blood vessels, heart valves	

Tab. 7.4 Structure of selected heteropolysaccharides

Heteropolysaccharide (names of monomers)	Disaccharide unit of heteropolysaccharide
Hyaluronic acid D-glucuronic acid β(1→3) N-acetyl-D-glucosamine	
Chondroitin sulfate D-glucurnic acid β(1→3) N-acetyl-D-galactosamine-4-sulfate	
Keratan sulfate D-galactose β(1→4) N-acetyl-D-glucosamine-6-sulfate	
Dermatan sulfate L-iduronic acid α(1→3) N-acetyl-D-galactosamine-4-sulfate	
Heparin, heparan sulfate L-iduronate-2-sulfate α(1→4) N-sulfo-D-glucosamine-6-sulfate	

7.5.2.2. Glycoproteins

Glycoproteins are heteroproteins that contain oligosaccharide chains (glycans) covalently attached to their polypeptide backbones. The basis of glycoprotein molecule is **protein** (protein portion prevails – in different %), upon to which saccharide part is bound (oligosaccharide) with N- or O-glycosidic bond. On the cell surface glycoproteins, **O-glycosidic** links are found between the carbohydrate part and a serine or threonine residue, instead of *N*-glycosidic links to asparagine residues. This type of link is less common than the *N*-**glycosidic** one.

There are two types of oligosaccharide structure with *N*-glycosidic links, which arise through two different biosynthetic pathways - **mannose-rich** and **complex types**. At the external end of the structure, glycoproteins of the complex type often contain *N*-acetylneuraminic acid residues, which give the oligosaccharide components negative charges (Fig. 7.15.).

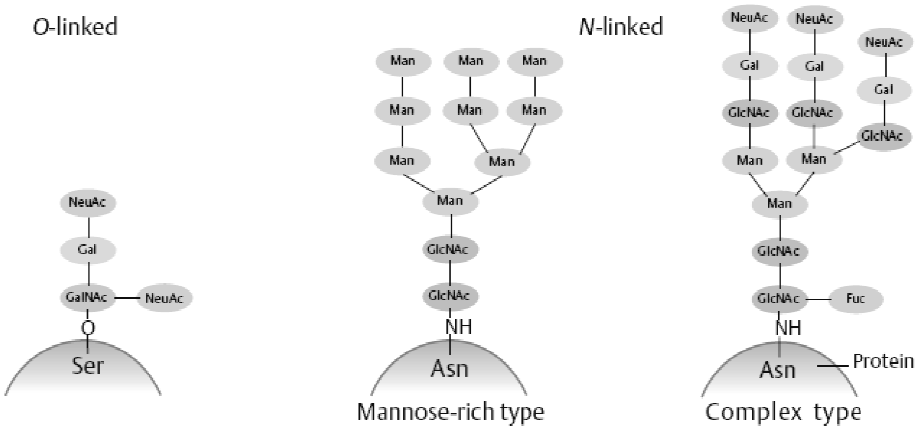


Fig. 7.15. The types of glycoproteins according to the type of linkage between the protein and oligosaccharide and according to the types of monosaccharide composition.

Into this group of glycoconjugates **glycoproteins of blood plasma** and **mucine type glycoproteins** belong. To the blood plasma glycoproteins, proteins with different functions pertain: e.g. ceruloplasmin, plasminogen, prothrombin or immunoglobulins. Some of them are parts of membrane receptors or they can have hormone functions (Tab.7.5.). Glycoproteins of mucine type occur e.g. in mucosal fluid of the epithelial cells and their saccharide part is represented by sialic acids – neuraminic and N-acetyl neuraminic acids.

Tab. 7.5. Some functions served by glycoproteins

Function	Glycoproteins
Structural	Collagens
Lubricant and protective	Mucins
Transport	Transferrin, ceruloplasmin
Immunologic	Immunoglobulins, histocompatibility antigens
Hormonal	Chorionic gonadotropin, thyroid-stimulating hormone (TSH)
Enzymatic	Various, e.g. alkaline phosphatase
Cell attachment-recognition	Various proteins involved in cell-cell (e.g. sperm-oocyte), virus-cell, bacterium-cell, and hormone cell interactions
Antifreezing	Certain plasma proteins of cold water fish
Interaction with specific carbohydrates	Lectins, selectins (cell adhesion lectins), antibodies
Receptor	Various proteins involved in hormone and drug action
Affection of folding of certain proteins	Calnexin, calreticulin
Regulation of development	Notch and its analogs, key proteins in development
Hemostasis (and thrombosis)	Specific glycoproteins on the surface membranes of platelets

Oligosaccharide chain has different functions in glycoprotein:
- Increasing of protein polarity
- Influence on solubility and electrical charge
- Recognizing function – receptors
- Stabilization of structure
- Defense against proteolysis
- Influence on biological half-life of protein

Control questions

1. Illustrate mutarotation of ribose, mark resulting anomers.
2. Write the reaction of mannose with:
   a) Fehling´s reagent
   b) CH_3OH (H^+)
3. Write Haworth formula of :
   a) methyl-α-D-glucopyranose
   b) γ-lactone of gluconic acid
4. Illustrate the equilibrium between α- and β- forms of:
   a) lactose
   b) maltose
5. Write the equation of lactose hydrolysis.
6. Write the reaction of glucose with:
   a) Tollens´s reagent
   b) H_3PO_4
   c) $ArNH_2$ (Ar = aryl)
7. Write the reaction of acid catalyzed hydrolysis of :
   a) maltose
   b) sucrose
8. Explain what hyperglycemia and hypoglycemia mean and which state or diseases can lead to them.
9. Write the Tollens formula of D-glucose and L-glucose.
10. Write the Haworth formula of glucuronic acid and explain its biological importance.

8. LIPIDS, STEROIDS, EICOSANOIDS AND TERPENES

The food we eat is divided into three primary groups: carbohydrates, proteins and **lipids**. Of the three types of foodstuffs, two are classified by functional groups. Carbohydrates are polyhydroxyaldehydes or polyhydroxyketones. Proteins are polyamides. Lipids are not poly-anythings. Many are esters or amides, but that is not what makes a lipid a lipid. A lipid is defined by its **solubility**. Water is the major solvent in living systems, and most reactions of physiological importance take place in aqueous solutions. Thus, water-insolubility is a noteworthy property in biologically important molecules. Lipids (from the Greek word *lipos* – fat) are structurally very diverse groups of natural substances of plant, animal and microbial origin. Lipids are biomolecules that are more soluble in relatively non-polar organic solvents than they are in water.

Lipids vary greatly in function:

- Important source of energy (mainly triacylglycerols – fats) (38 kJ/g fat).
- Energy reserve stored in the fatty tissue of animals and plant oleaginous fruits.
- Source of essential fatty acids.
- Solvent of the lipid-soluble vitamins (A, D, E, K), required for various physiological functions, steroid hormones and other water-insoluble compounds (drugs, etc.).
- Glycerophospholipids, sphingolipids, and cholesterol (together with proteins) are the primary structural components of the membranes that surround all cells and organelles.
- Part of intracellular regulatory mechanisms.
- Lipoproteins are an important structural part of the cell enabling the transport of practically all lipids in the organism.
- Wrap around organs to protect them against mechanical damage.
- Lipids of subcutaneous adipose tissue provide thermal insulation in animals.
- Steroid hormones, including sex hormones, and other hormone-like lipids (eicosanoids) act as chemical messengers, initiating or altering activity in specific target cells.
- Bile salts are needed for the digestion of lipids in the intestinal tract.

Lipids can be classified from different points of view. According to hydrolytic cleavage ability they are classified as hydrolyzable or non-hydrolyzable.

- **Non-hydrolyzable lipids** do not undergo hydrolytic cleavage into considerable smaller compounds (higher carbohydrates, higher carboxylic acids, higher alcohols, steroids, eicosanoids, terpenes, fat-soluble vitamins).
- **Hydrolyzable lipids** undergo hydrolytic cleavage into two or more considerably smaller compounds in the presence of an acid, base or digestive enzymes (triacylglycerols, waxes, sterids, phospholipids, glycolipids).

Another way of lipid classification is according to their chemical structure:

1. **Simple** (non-polar) **lipids** – this group includes free higher alcohols, free fatty acids (FA) and compounds in which they are linked by an ester bond. They are divided to:
   - ***Higher carboxylic acids (fatty acids)***
   - ***Higher alcohols***
   - ***Triacylglycerols***
   - ***Waxes***
   - ***Sterids*** (esters of sterols)
2. **Complex lipids** belong to hydrolysable lipids. In addition to alcohol (glycerol and sphingosine) and fatty acids they contain another, often very polar, parts according to which they can be further divided into:

- ***Phospholipids*** contain alcohol, FA, **phosphoric acid** and some amino alcohols or carbohydrate alcohol inositol. This includes glycerophospholipids, plasmalogens (ethers), inositolphosphatides and sphingophospholipids
- ***Glycolipids*** contain alcohol, FA and **carbohydrate component** in the molecule (e.g. cerebrosides and gangliosides), sometimes contain a residue of sulfuric acid (sulfolipids)

3. **Derived lipids** – in this group all other compounds of lipid character are included with the common physicochemical properties, which can not be included in the foregoing groups and the basic building block is isoprene (e.g. terpenes, steroids and carotenoids).

8.1. Simple lipids

8.1.1. Higher carboxylic acids (fatty acids)

All hydrolyzable lipids contain one or more higher carboxylic acids. If they are lipid components, they are called **fatty acids (FA).**

Fatty acids are long-chain aliphatic monocarboxylic acids, almost all containing an even number of carbon atoms from 4 to 36, but the 16- and 18-carbon acids are the most abundant. A few of them contain hydroxyl group (for example ricinoleic acid [18:1 (9), 12-OH], 2-hydroxy-lignoceric acid [24:0, α-OH], 2-hydroxy-nervonic acid [24:1 (15), α-OH]. According to their carbon chain length they are generally divided into:

- *Lower carboxylic acids* - containing 4-10 carbon atoms per molecule (chains of 4-6 carbons are referred to as a short and with 8-10 carbons as a medium length). These FA are less presented in the lipids.
- *Higher carboxylic acids* - having 12 or more carbon atoms.

Fatty acids may be either **saturated** (Tab. 8.1.) or **unsaturated (**Tab. 8.2.) according to the number of double bonds. Unsaturated fatty acids contain one or more C=C double bonds in ***cis* configuration**. Palmitic (16:0) and stearic acids (18:0) are the most abundant saturated fatty acids. Oleic acid (18:1) is the most abundant unsaturated fatty acids. Two unsaturated fatty acids, linoleic (18:2) and linolenic (18:3), are **essential fatty acids (vitamin F)**, and are synthesized only by plants but are required by animals and must be included in the diets. All other fatty acids are nonessential fatty acids that animals synthesize either from other fatty acids or from other precursors. Arachidonic acid can be formed from linoleic acid by chain elongation (extension) and by two desaturation reactions (dehydrogenation).

Higher saturated carboxylic acids are found mainly in animal fats such as butter or lard. Unsaturated FA are found primarily in vegetable oils, e.g. olive, sunflower, soya, but also in fish oil. Other sources include some crops, mainly almonds, walnuts, sunflower seeds and dry oleaginous fruits and germs of cereals.

The presence of the double bond in the FA allows spatial isomerism, therefore such FA can exist in two isomeric forms, ***cis***- and ***trans***-. Fatty acid [18:1(9)] is in the ***cis configuration***, known as oleic acid and in ***trans configuration*** as elaidic acid (Fig. 8.1.). The spatial arrangement of the *cis* form leads to the bending of the carbon chain based on the number of double bonds which affects the physical and chemical properties of the lipid. Increasing unsaturation of FA in lipids increases their fluidity and decreases the freezing point of triacylglycerols. The double bonds of polyunsaturated fatty acids are almost never conjugated (are separated by at least one methylene group $-CH_2-$) and almost all are in the ***cis configuration***.

Fatty acids with *trans* configuration of double bonds have physical properties much more like those of saturated fatty acids than like *cis* unsaturated fatty acids.

System names of FA are derived from the name of a hydrocarbon with the same number of carbon atoms. On recurring FA trivial names are commonly used. Shorthand designation of FA structure comprises a number of carbon atoms, with the number of double bonds behind the colon. Position of double bonds is expressed according to the rules of organic nomenclature by carbon numerical code, from which the double bond starts (Tab. 8.2.).

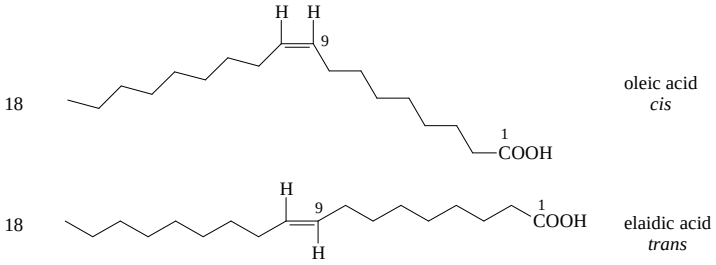


Fig. 8.1. Structure of oleic and elaidic acids

The position of the double bond closest to the methyl group in unsaturated fatty acids is often indicated by an **omega number or n- number (ω-, or n-)**. The ω-1 carbon is the methyl carbon in the fatty acid chain; that is, the carbon atom farthest from the carboxyl group. The omega number of the fatty acid says nothing about the number of double bonds in a molecule but it is the number of the first double bond carbon, counting from the ω-/n- carbon number (Tab. 8.2.). In the literature we can find the term omega (ω) fatty acid (or *n*-fatty acid), particularly omega-3 and omega-6 (n-3 or n-6) fatty acids. Number 3 in the ω-3 refers to the position of the first double bond from the FA omega end. Arachidonic acid [C 20: 4 (5, 8, 11, 14)] is therefore an omega-6 (n-6) fatty acid: it has the first double bond on the sixth carbon atom from omega end. Importance of representation of ω-3 or ω-6 fatty acids in the diet for the prevention of especially cardiovascular diseases is not clear yet.

On one hand polyunsaturated fatty acids are suitable substrates for lipoperoxidation processes (Chap. 13) and on the other hand literature provides information that high level of the omega-3 fatty acids in oils provides protection from cardiovascular diseases and atherosclerosis. It has been accepted that treatment with ω-fatty acids is not harmful in case of sufficient antioxidants level.

Tab. 8.1. Selected saturated carboxylic acids

Acids	Shorthand designation[1)]	Structure	Rational formula
Butyric	4:0	CH_3-$(CH_2)_2$-COOH	C_3H_7COOH
Capric	10:0	CH_3-$(CH_2)_8$-COOH	$C_9H_{19}COOH$
Lauric	12:0	CH_3-$(CH_2)_{10}$-COOH	$C_{11}H_{23}COOH$
Myristic	14:0	CH_3-$(CH_2)_{12}$-COOH	$C_{13}H_{27}COOH$
Palmitic	16:0	CH_3-$(CH_2)_{14}$-COOH	$C_{15}H_{31}COOH$
Stearic	18:0	CH_3-$(CH_2)_{16}$-COOH	$C_{17}H_{35}COOH$
Arachidic	20:0	CH_3-$(CH_2)_{18}$-COOH	$C_{19}H_{39}COOH$
Behenic	22:0	CH_3-$(CH_2)_{20}$-COOH	$C_{21}H_{43}COOH$
Lignoceric	24:0	CH_3-$(CH_2)_{22}$-COOH	$C_{23}H_{47}COOH$
Cerotic	26:0	CH_3-$(CH_2)_{24}$-COOH	$C_{25}H_{51}COOH$

[1)] See explanation in Tab. 8.2.

Tab. 8.2. Selected mono- and polyunsaturated carboxylic acids

Acids	Shorthand designation[1)] and ω- number	Structure	Rational formula
Palmitoleic	16:1 (9), ω−7	CH_3-$(CH_2)_5$-CH=CH-$(CH_2)_7$-COOH	$C_{15}H_{29}COOH$
Oleic	18:1 (9), ω−9	CH_3-$(CH_2)_7$-CH=CH-$(CH_2)_7$-COOH	$C_{17}H_{33}COOH$
Linoleic	18:2 (9,12), ω−6	CH_3-$(CH_2)_3$-$(CH_2$-CH=CH$)_2$-$(CH_2)_7$-COOH	$C_{17}H_{31}COOH$
Linolenic	18:3 (9,12,15), ω−3	CH_3-CH_2-(CH=CH-$CH_2)_3$-$(CH_2)_6$-COOH	$C_{17}H_{29}COOH$
Arachidonic	20:4 (5,8,11,14), ω−6	CH_3-$(CH_2)_3$-$(CH_2$-CH=CH$)_4$-$(CH_2)_3$-COOH	$C_{19}H_{31}COOH$
Eicosapentaenoic	20:5 (5,8,11,14,17), ω−3	CH_3-CH_2-(CH=CH-$CH_2)_5$-$(CH_2)_2$-COOH	$C_{19}H_{29}COOH$
Docosahexaeonic	22:6 (4,7,10,13,16,19), ω-3	CH_3-CH_2-(CH=CH-$CH_2)_6$-CH_2-COOH	$C_{21}H_{31}COOH$
Nervonic	24:1 (15), ω−9	CH_3-$(CH_2)_7$-CH=CH-$(CH_2)_{13}$-COOH	$C_{23}H_{45}COOH$

[1)] In the shorthand designation, the first mentioned number is the number of carbon atoms and the the second number is a number of double bonds. In brackets there are carbon numbers underlying double bonds. Behind the brackets there is indicated the type of ω-fatty acids.

Tab. 8.3. Trivial and systematic names of selected carboxylic acids

Trivial name of saturated FA	Systematic name of saturated FA	Trivial name of unsaturated FA	Systematic name of unsaturated FA
butyric acid	butanoic acid	palmitoleic acid	9-hexadecenoic acid
caproic acid	hexanoic acid	oleic acid	9-octadecenoic acid
lauric	dodecanoic acid	linoleic acid	9, 12-octadecadienoic acid
myristic	tetradecanoic acid	α-linolenic acid	9, 12, 15-octadecatrienoic acid
palmitic acid	hexadecanoic acid	γ-linolenic acid	6, 9, 12-octadecatrienoic acid
stearic acid	octadecanoic acid	arachidonic acid	5, 8, 11, 14-eicosatetraenoic acid
arachidic acid	eicosanoic acid	EPA	5, 8, 11, 14, 17-eicosapentaenoic acid
behenic acid	docosanoic acid	DHA	4, 7, 10, 13, 16, 19-docosahexaenoic acid
lignoceric acid	tetracosanoic acid	nervonic	15-tetracosenoic acid

Properties of fatty acids:

- prevails non-polar character
- non-soluble in water and polar solvents – ethanol
- soluble in non-polar solvents (ether, chloroform)
- without colour, taste or smell
- lighter than water
- high surface tension
- melting point of higher carboxylic acids (and lipids which contain them) is elevated with increasing number of carbon atoms and is reduced with the increasing number of double bonds
- presence of double bonds increase their reactivity with free radicals

8.1.2. Higher alcohols

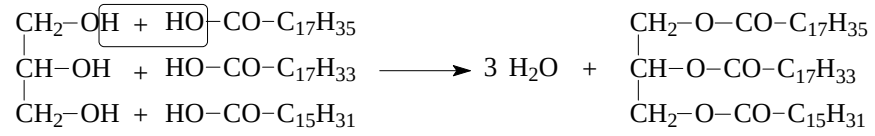
To this group mono- or dihydric alcohols with long chains belong, e.g. saturated cetyl alcohol (C-16), stearyl alcohol (C-18), ceryl alcohol (C-26) or melissyl alcohol (C-30).

8.1.3. Triacylglycerols

Triacylglycerols (TAG), neutral fats (older name triglycerides) are esters of trihydric alcohol glycerol (1,2,3-propanetriol) and fatty acids. Triacylglycerols make up about 90 % of our dietary lipid intake. All **animal fats** such as beef, butter, pork, and poultry fats and all **plant oils** (liquid fats are called oils) such as corn, olive, peanut, and soybean oils are triacylglycerols. They are esters (in fact – triesters) of glycerol.

By the number of esterified OH groups of glycerol, we can identify the **mono-**, **di-** and **triacylglycerols**.

If the fatty acids are all the same, the fat is a **simple triacylglycerol**. Most naturally occurring fats contain different fatty acid units in the same molecule. These fats are called **mixed triacylglycerols**:



Glycerol **Fatty acids** **1-stearoyl-2-oleoyl-3-palmitoylglycerol**

Fats and oils are rather mixtures in which the acid fraction of the molecule varies in chain length and degree of unsaturation. Animal fats generally contain both saturated and unsaturated fatty acid units. At room temperature, animal fats are usually solids. At the body temperature of warm-blooded animals, though, these fats are apt to exist in the liquid state. Plants, on the other hand, are not warm-blooded. They keep their fats in the liquid state by incorporating a higher proportion of unsaturated acid units. Human fat looks like tallow. It is yellow-colored and contains mainly palmitic, stearic, oleic acid and a small amount of linoleic, linolenic and arachidonic acids. The yellow coloring indicates the presence of carotenes. Properties and melting point of human fat are changed depending on their habitat in the body, as well as nutrition. Tab. 8.4. compares the fatty acid content of selected fats and oils.

Tab. 8.4. Fatty acids distribution (as percentages) in several fats and oils

Fat or oil	$<C_{14}$	Myristic	Palmitic	Stearic	Oleic	Linoleic	Linolenic	Others
Animal fats								
Beef tallow	0.2	2 − 5	24 − 34	15 − 30	35 − 45	1 − 3	1	1
Butter	14	8 − 13	25 − 32	8 − 13	22 − 29	3	−	6 − 15
Lard	−	1 − 2	25 − 30	12 − 16	41 − 54	3 − 8	−	4 − 8
Human		3	24	8	4	10	−	3
Whale	−	5 − 10	10 − 20	2 − 5	33 − 40	−	−	50
Sardine	−	5 − 6	14 − 16	2 − 3	9	9	−	> 32
Plant oils								
Olive	−	0 − 1	5 − 15	1 − 4	67 − 84	8 − 12	−	−
Peanut	−	−	7 − 12	2 − 6	30 − 60	20 − 38	−	−
Corn	−	1 − 2	7 − 11	3 − 4	25 − 35	50 − 60	−	−
Soybean	−	1 − 2	6 − 10	2 − 4	20 − 30	50 − 58	5 − 10	−
Cottonseed	−	1 − 2	17 − 23	1 − 3	23 − 31	34 − 55	−	0 − 1
Linseed	−	−	4 − 7	24	14 − 30	14 − 25	45 − 60	−

Chemical properties of triacylglycerols are determined by the properties of bound FA, especially unsaturated (lipid peroxidation, hydrogenation and fat rancidity), or by general characteristics of esters (hydrolytic cleavage).

Lipid peroxidation

Lipid peroxidation is the oxidative damage to fatty acids in lipids, mainly membrane structural lipids and lipids present in lipoproteins. Most susceptible to oxidative damage are polyunsaturated FA. The process is usually initiated by the hydroxyl radical (see Chapter 13).

Hydrogenation

The hydrogenation (addition of hydrogen to alkene double bonds in the presence of metal catalyst such as Ni, Pt) of vegetables oils gives solid margarine and other products. We say that the double bonds become *saturated*. When hydrogen is made to add to some of the double bonds in vegetable oils, the oils become like animal fats, both physically and structurally. The oils change from being liquids to solids at room temperature.

Rancidity

Butter, salad and cooking oils, fatty meats can become **rancid**, developing unpleasant odors and flavors. Rancidity is due to a combination of two reactions:

1. bacterial hydrolysis of ester groups
2. air oxidation of alkene double bonds. It is the auto-oxidative process in which peroxides of unsaturated FA and various aldehydes are formed.The rancidity reactions are slowed by refrigeration. Fats and oils stored at room temperature, such as salad oil, should by tightly capped to impede the entry of moisture, bacteria, and air − all of which are required for the hydrolysis and oxidation reactions. Some manufactures of vegetable oils and other products add antioxidants to inhibit the oxidation reactions (see Chapter 13).

Hydrolysis

Triacylglycerols undergo hydrolysis at the ester groups to produce glycerol and fatty acids. Hydrolysis in the laboratory requires an acid or base: acidic hydrolysis yields the fatty acids (mixture) and basic hydrolysis (saponification) yields a mixture of the salts of fatty acids (sodium or potassium). These salts are soaps.

In the process of digestion in GIT (exogeneous) or when mobilized from fat tissue (endogeneous), TAG are hydrolyzed by special enzymes – **lipases**. This *in vivo* hydrolysis is more complicated than hydrolysis in the laboratory.

During digestion of TAG by lipases with the help of bile salts mixture of products is formed, most of which are monoacylglycerols and free fatty acids and some diacylglycerols and glycerol. The enterocytes absorb fats in that form and then rebuild TAG and package them with proteins, into lipoprotein particles called **chylomicrons**, which are transported through the lymphatic system to the bloodstream. The blood carries the chylomicrons to various tissues where the TAG is separated from the protein and hydrolyzed to FA and glycerol, which are then metabolized to generate energy (β-oxidation). Fatty acids which are not needed for immediate energy generation are reconverted into TAG and stored as fat droplets in **adipocytes**. Such cells make up most of the animal fats.

During fasting or exercise, when we need the chemical energy of the stored TAG, they are again hydrolysed by lipase in process of lipolysis.

8.1.4. Waxes

Biological waxes are esters of long-chain (C-14 to C-36) saturated and unsaturated fatty acids with long-chain (C-16 to C-30) alcohols having one –OH group. The acids and alcohols usually contain an even number of carbons. The most commonly present alcohols are cetyl alcohol, ceryl alcohol and melissyl alcohol. Wax alcohols have more than 22 carbon atoms in the chain.

Waxes have a variety of protective functions in plants and animals. Many fruit, vegetable, and plant leaves are coated with waxes that protect against parasites and mechanical damage and prevent excessive water loss. The skin glands of many vertebrates secrete waxes that protect hair, furs, feathers, and skin, keeping them lubricated, pliable, and waterproof. Bees secrete wax to construct the honeycomb in which eggs are laid and new bees

develop. In some marine organisms notably the plankton, waxes are used instead of triacylglycerols for energy storage.

Biological waxes find a variety of application in the pharmaceutical, cosmetic, and other industries. Bee wax CH_3-$(CH_2)_{12}$-CO-O-$(CH_2)_{25}$-CH_3, carnauba wax CH_3-$(CH_2)_{24}$-CO-O-$(CH_2)_{29}$-CH_3, from Brazilian palm tree, wax extracted from spermaceti oil CH_3-$(CH_2)_{14}$-CO-O-$(CH_2)_{15}$-CH_3 (whale oil), lanolin (from lambs wool), are widely used in the manufacture of lotions, creams, and polishes.

8.1.5. Sterids (esters of sterols)

Sterids are esters of fatty acids with sterols (steroid alcohols). They usually contain animal zoosterols (cholesterol) and plant phytosterols (sitosterol). The most common FAs are linoleic and palmitic acids. Sterids usually occur together with wax and free sterols.

8.2. Complex lipids

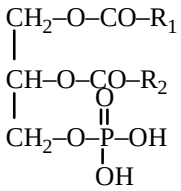
Complex lipids are esters, ethers or amides of fatty acids and alcohols. In addition to these essential components they also contain other compounds. There are two broad groups of complex lipids: those based on glycerol, the **glycerolipids**, and based on sphingosine, the **sphingolipids**. Complex lipids can be classified according to the character of non-lipid part occurring in their molecules into ***phospholipids,*** which contain phosphoric acid and ***glycolipids***, which contain saccharide. They are predominant lipids of cell membranes because of their ***amphipathic*** character (one part of the molecule is hydrophobic and another part is hydrophilic). All of them are hydrolyzable lipids. Most phospholipids and glycolipids are called by trivial names.

8.2.1. Phospholipids

Phospholipids are esters of glycerol or amides of sphingosine with higher carboxylic acids. In the molecule there is bound phosphoric acid residue. According to the basic alcohol in their structure they are classified to ***glycerophospholipids*** and ***sphingophospholipids***.

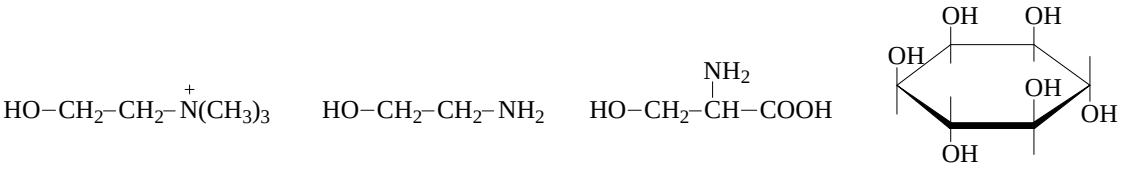
8.2.1.1. Glycerophospholipids

Phosphatidic acid is a compound formed if two hydroxyl groups of glycerol (the first and second carbons) are esterified with carboxylic acids (C-1 usually saturated, C-2 usually unsaturated), and one hydroxyl group with phosphoric acid.



Phosphatidic acid

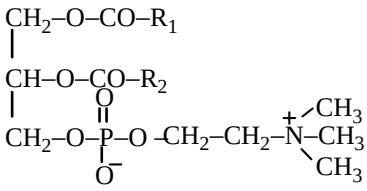
At physiological pH, phosphatidic acid exists in an ionized phosphatidate form. Phosphatidic acids are esterified with a small alcohol molecule to form esters called phosphatides (phosphodiester). The phosphatides contain the alcohol choline, ethanolamine, or inositol or the amino acid serine (Fig. 8.2.). Glycerophospholipids are named as derivates of the parent compound phosphatidic acid, according to the polar alcohol in the head group: **phosphatidylcholine** (lecithin), **phosphatidylethanolamine** (cephalin), **phosphatidylserine, phosphatidyl-inositol**. In **cardiolipin**, two phosphatidic acids share a single glycerol.



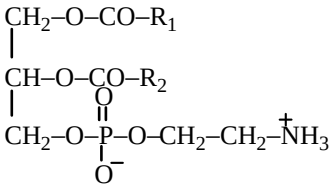
Choline **Ethanolamine** **Serine** **Inositol**

Fig. 8.2. Alcohols bound to phosphatidic acid

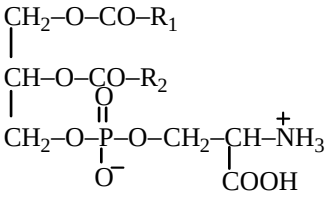
Phosphatidylcholines, trivial name **lecithins**, include choline in the molecule. They differ by FA bound to glycerol. There are predominantly saturated FA on the first carbon atom and predominantly unsaturated FA on the second carbon. Lecithins are present in almost all plant and animal cells as the most common phospholipids. **Phosphatidylethanolamines (cephalins)** bind ethanolamine instead of choline and occur together with lecithin. In **phosphatidylserines**, present mainly in the brain tissue, amino acid serine is bound to the phosphatidic acid. Inositol is present in **phosphatidylinositol**s. Phosphatidylinositol takes part in lipid anchor which is responsible for binding some proteins in extracellular side of membranes. **Cardiolipins** (polyglycerol phospholipids) are compounds without a molecule of the second alcohol. In cardiolipins two phosphatidic acid moieties connect with a glycerol backbone in the center to form a dimeric structure. They occur in the heart muscle, particularly in mitochondrial membranes.



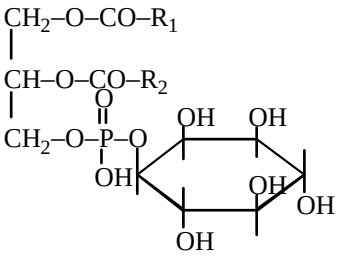
Phosphatidylcholine (Lecithin)



Phosphatidylethanolamine (Cephalin)



Phosphatidylserine



Phosphatidylinositol

Lysolecithins are compounds formed from the lecithins by *phospholipase A*. FA is bound to one carbon (C-1 or C-2) of glycerol by ester bond, while the hydroxyl group at another position is free (the effect of phospholipase is to cleave off FA). According to this we can distinguish 1-lysolecithin resulting from the effect of *phospholipase* A_1 (cleaving off the FA on the first glycerol carbon) and 2-lysolecithins that are generated by *phospholipase* A_2 (cleaving off the FA on the second glycerol carbon). Lysolecithins are generated in the lipid metabolism in very low concentrations, but if they are formed in higher amount, cause lysis of erythrocytes.

Lysolecithin may also be formed by *lecithin:cholesterol acyltransferase (LCAT)*. This enzyme catalyzes the transfer of FA residue from position 2 of lecithin to cholesterol to form cholesterol ester and lysolecithin. LCAT is responsible for the formation of cholesterol esters in plasma lipoproteins, which allows the reverse cholesterol transport from extrahepatic tissues to the liver (see Chapter. 8.3.).

Most cells continually degrade and replace their membrane lipids. For each hydrolyzable bond in the glycerophospholipid, there is a specific hydrolytic enzyme in the lysosome. They are classified according to ester bond in the molecule, which is cleaved (Fig. 8.3.):

- *Phospholipase A_1* - hydrolyzes the ester bonds of intact glycerophospholipids at C-1 of glycerol, producing 1-lysolecithins and acyl residue of fatty acid,
- *Phospholipase A_2* - hydrolyzes the ester bonds of intact glycerophospholipids at C-2 of glycerol, producing 2-lysolecithins and acyl residue of fatty acid. When one fatty acid is removed by *phospholipase A*, the second fatty acid is cleaved off by *lysophospholipase* (*phospholipase B*),
- *Phospholipase C* - splits diacylglycerol residue,
- *Phospholipase D* - splits phosphatidate residue.

Some *phospholipases* act on only one glycerophospholipid, such as phosphatidylinositol 4,5-bisphosphate or phosphatidylcholine; others are less specific (Fig. 8.3.).

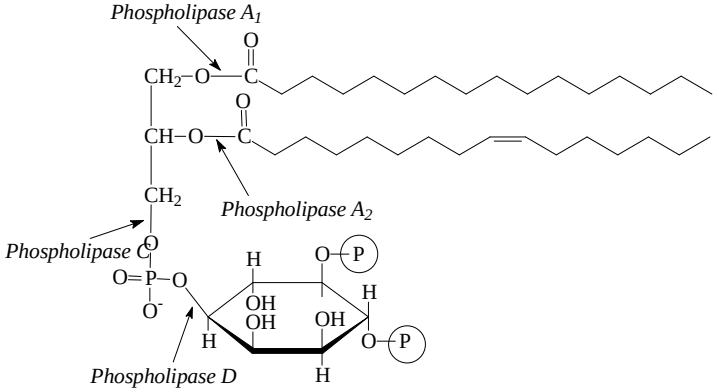
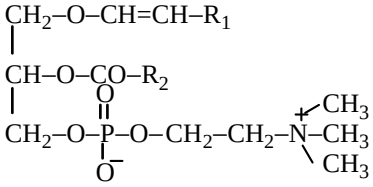


Fig. 8.3. Specificities of *phospholipases*

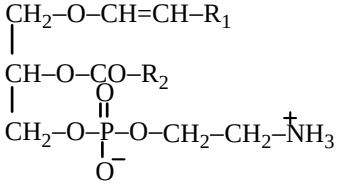
Some *phospholipases* have a regulatory role. *Phospholipase A_2* catalyzes the release of arachidonic acid which is a precursor of tissue hormones, eicosanoids (see Sec. 8.5.). They also have an important role in cell signaling, affect the composition of cell membranes and cell death. *Phospholipase C* hydrolyses the bond between glycerol and phosphate in glycerolphospholipids, such as phosphatidylinositol 4,5-bisphosphate to release inositol 1,4,5-triphosphate (IP 3) and diacylglycerol - second messengers in cell signaling.

Phospholipase A_2 is present also in the venom of Indian cobra and rattlesnake diamond. After the snake bite levels of the enzyme in blood increases and causes formation of 2-lysolecithin, leading to extensive hemolysis, which can endanger human life.

The **plasmalogens** make up another family of glycerophospholipids. They occur widely in the vertebrate heart tissue, membranes of both the nerve and muscle cells. They differ from the phosphatides by the presence of an unsaturated *ether group* instead of an acyl group at one end of the glycerol unit, bound by ***vinyl etheric bond***. The resulting structure is referred to as plasmenic acid. Choline, ethanolamine or serine create esteric bond with phosphoric acid in the molecule and plasmenylcholine, plasmenylethanolamine or plasmenylserine are formed. Plasmalogen molecules, like phosphatides, also carry electrically charged atoms as well as long hydrocarbon chains. To this group also the lipid platelet activating factor (PAF) can be included, which is derived from cholinergic plasmalogen. PAF is a potent mediator of inflammation, allergic response, shock and blood coagulation.



Plasmenylcholine



Plasmenylethanolamine

Properties of gycerophospholipids

Phospholipids isolated from the biological material do not constitute a homogenous fraction with a completely identical structure. Each type of phospholipids is actually a mixture of very similar substances differing in fatty acids composition.

Physicochemical properties of phospholipids are determined by the presence of two different parts of the molecule (Fig. 8.5.). Phospholipids have amphiphilic or **amphipathic properties.** One part of each molecule of phosphatidylcholine, phosphatidylethanolamine and phosphatidylserine is very polar because it carries full electrical charges (the **polar** sites are called **hydrophilic groups**). These charges are partly responsible for the phosphatides being somewhat more soluble in water than triacylglycerols. The remainder of a phosphatide molecule is nonpolar and hydrocarbon-like. The **nonpolar**, hydrocarbon sections are called **hydrophobic groups.** This fact is the reason why phospholipids form a lamellar or micellar structures in water: they arrange into lipid bilayers in such a way that the polar parts are on the outer and internal side of the bilayer and the non-polar parts (hydrophobic chains of fatty acids) within the bilayer. Bilayer formation from phospholipid molecules is essential for the formation of biological membranes. The presence of hydrophilic and hydrophobic parts in the e.g. lecithin molecule, is the basis for its important property as the cell emulsifier and surfactant (liquid produced by lung cells, Fig. 8.4.).

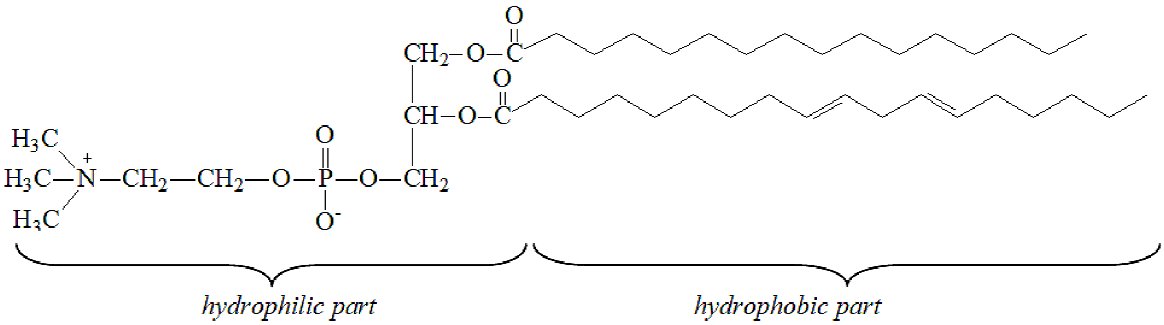
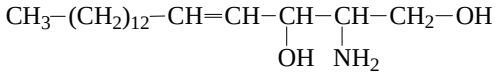


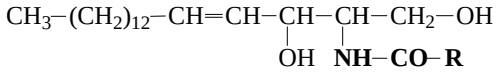
Fig. 8.4. Structure of lecithin

8.2.1.2. Sphingophospholipids

Sphingophospholipids are the second large class of membrane lipids. They contain a long-chain unsaturated aminoalcohol called **sphingosine** (1,3-dihydroxy-2-amino-4-octadecene).

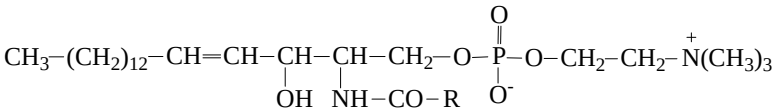


Sphingosine



Ceramide

Carbons C-1, C-2 and C-3 of the sphingosine molecule are structurally analogous to the three carbons of glycerol in glycerophospholipids. Conversion of the amine $-NH_2$ of sphingosine (C-2) into an **amide** with a fatty acid yields a **ceramide**. Ceramide is the structural parent of all sphingolipids. Esterification of the primary alcohol group (C-1) of a ceramide with phosphoric acid and choline (or ethanolamine) results in a **sphingophospholipid** (**sphingomyelin**):



Sphingophospholipid (sphingomyelin)

Sphingomyelins are compounds found in the plasma membrane of animal cells and are especially prominent in myelin sheath surrounding nerve fibers and insulates the axons of some neurons. Sphingomyelins have fatty acid residues that are 20 – 26 carbon atoms long. These long chains intertwine and wrap around each other to form a very stable coating for the sensitive nerve fibers. In individuals with some genetic diseases, the carbon chains are shorter and defects result in the myelin sheath reducing and damaged nerve fibers do not fulfill their function.

8.2.2. Glycolipids

Glycolipids are amphiphilic lipids. Carbohydrate (monosaccharide, disaccharide or oligosaccharide), which is the hydrophilic part of the molecule, is bound to the hydrophobic lipid component by a glycosidic bond.

8.2.2.1. Glyceroglycolipids

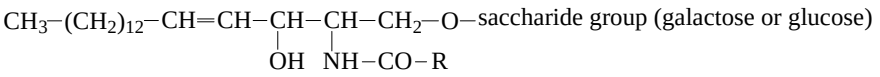
Glycolipids have bound lipid as well as saccharide components in their molecules. **Glyceroglycolipids** have a relatively simple structure. The basic structure is 1,2-diacylglycerol, which has a monosaccharide or disaccharide bound at C_3 position. They occur mainly in germs.

8.2.2.2. Sphingoglycolipids

Sphingoglycolipids are similar to sphingophospholipids − they contain sphingosine and a fatty acid residue bound as an amide in a ceramide. However, sphingoglycolipids contain no phosphate. They contain a saccharide unit at C_1, joined through a glycosidic linkage to the primary alcohol oxygen atom of the ceramide. In terms of organic chemistry glycolipids can be included to the acetals. We can subdivide the sphingoglycolipids into the following groups:

- cerebrosides
- sulfolipids
- globosides
- gangliosides

Cerebrosides have a single sugar linked to ceramide; those with galactose (galactocerebrosides) are characteristically found in the plasma membranes of cell in neural tissue, and those with glucose (glucocerebrosides) in the plasma membranes of cell in nonneural tissues. As FA usually bind stearic acid, cerebronic acid, nervonic acid, and others.



Cerebrosides

Globosides are neutral (uncharged) glycosphingolipids with two or more saccharides, usually D-glucose, D-galactose, or N-acetyl-D-galactoamine. Cerebrosides and globosites are sometimes called **neutral glycolipids**, as they have no charge at pH 7.

The **sulfolipids** (sulfo-glycosphingolipids, cerebroside sulfates) are acid glycolipids. In sulfolipids the monosaccharides (mainly galactose) are esterified with sulfuric acid at the carbon atom C-6 (sometimes C-3). By ionization sulphuric acid obtaines a negative charge. This is a diffence from neutral glycolipids.

Gangliosides, the most complex sphingolipids, have oligosaccharides (consisting of glucose, galactose, galactoamine) and one or more residues of N-acetylneuraminic acid as their polar head groups, also called sialic acid, at the termini. Sialic acid gives gangliosides the negative charge at pH 7 that distinguishes them from globosides.

Cerebrosides and gangliosides are found in the myelin sheath. Cerebrosides are in the white matter of the central nervous system, whereas gangliosides occur in the gray matter of the brain. Although glycolipids are found in all tissues, we consider them as typical lipids of the nerve tissue. Glycolipids also play an important role in immunological processes at the cell surface.

The saccharide moieties for certain sphingolipids define the human blood groups and therefore determine the type of blood that individuals can safely receive in blood transfusions. The kinds and amounts of gangliosides in the plasma membrane change dramatically with embryonic development, and tumor formation induces the synthesis of a new complement of gangliosides.

8.3. Lipoproteins

Lipids are virtually insoluble in water and they are transported in blood as particles called the **lipoproteins**. Lipoproteins are a large group of macromolecules in which proteins bind lipids by non-covalent bonds. There are several kinds of lipoproteins, each with its own function, and they are classified according to their densities (Tab. 8.5.) to **chylomicrons, very low density lipoproteins (VLDL), low density lipoproteins (LDL) and high density lipoproteins (HDL)**.

Tab. 8.5. Characteristics and composition of plasma lipoproteins

Characteristics	Chylomicrons	VLDL	LDL	HDL
Density ($g.cm^{-3}$)	< 0.95	0.95 – 1.006	1.006 – 1.063	1.063 – 1.21
Molecular weight	$10^9 – 10^{12}$	$8 – 35.10^6$	$2 – 4.10^6$	$2 – 4.10^5$
Mean of particles (nm)	75 – 1200	30 – 80	18 – 25	5 – 12
Triacylglycerols (%)	80 – 95	60	10	5
Cholesterol (%)	5	12	50	20
Phospholipids (%)	3	18	15	25
Proteins (%)	1 – 2	10	25	25
Half-life	10 – 15 min	several hours	3 days	4 days

The lipoproteins with the lowest density are called **chylomicrons**. They are the largest lipoprotein particles. Their main lipid components are triacylglycerols and the biological function is the transport of exogenous lipids from the gut to the peripheral tissues (mainly muscle and adipose tissue), and cholesterol to the liver. They include 2 % proteins or less. After their assembly within the cells of the intestinal membrane, chylomicrons are delivered to the lymph, which carries them to the bloodstream. When they enter the capillaries embedded in muscle and adipose tissue, chylomicrons encounter binding sites and are held up. An enzyme, *lipoprotein lipase*, now catalyzes the hydrolysis of the chylomicrons' triacylglycerols to fatty acids and monoacylglycerols, which are promptly absorbed by the nearby tissue.

The liver is a site where breakdown products from saccharides are used to make fatty acids and, from them, triacylglycerol. Lipids made in the liver are called *endogenous lipids.* Two similar lipoproteins are used to transport endogenous lipids in the bloodstream: ***very-low-density lipoproteins***, and ***low-density lipoproteins***. These lipoproteins also carry cholesterol. LDLs are formed as a product of VLDL metabolism (the degradation of VLDL directly in the blood). LDLs are responsible for cholesterol transport from the liver to the cells of extrahepatic tissues. Other lipoproteins, **high density lipoproteins**, carry the cholesterol released from tissues back to the liver. They are the smallest lipoprotein particles. They are formed in the liver and small intestine, and have a dual role: they can prevent the entry of LDL into the cells, on the other hand they are able to transport the excess and unused cholesterol from cells to the liver where it is metabolized to bile acids. HDL lipoprotein particles in the body transport esterified cholesterol. It is less polar than free cholesterol, and therefore occurs in the core of HDL particles, which also allows to receive another free cholesterol and esterify it. This is called the reverse cholesterol transport and is responsible for the anti-atherosclerotic effect of HDL lipoproteins.

Size of lipoprotein particles decreases from chylomicrons towards HDL.

The most abundant proteins which are part of lipoproteins are apoprotein A, apoprotein B, apoprotein C, apoprotein E etc.

8.4. Steroids

Steroids are tetracyclic organic compounds that contain the **steroid ring structure**, consisting of four fused rings: three six-membered rings (A, B, C) and one five-membered ring (D). Each ring is numbered by a standard system. They occur in the plant and animal kingdom.

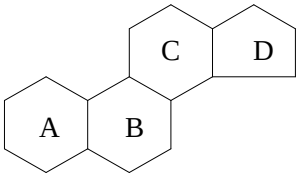


Fig. 8.5. Steroid nucleus (sterane)

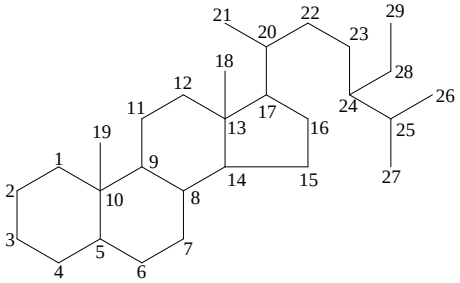


Fig. 8.6. Numbering of carbon atoms in steroids

Steroids contain a variety of functional groups such as hydroxyl, carbonyl, carboxylic, and carbon-carbon double bonds. The combination of the cycles of the steroid backbone extends the possibility of the stereoisomers. There are asymmetric (chiral) carbon atoms in the all backbone nodes. The steroids are also different in length and the structure of the side chain at carbon atom C-17. The numbering of the carbon atoms of the natural steroids is in Fig. 8.6.

Nomenclature of particular steroid groups results from the names of basic saturated hydrocarbons derived from cyclopentanoperhydrophenanthrene (sterane). The main saturated hydrocarbons from which the steroids are derived, are: **estrane** (C-18), **androstane** (C-19), **pregnane** (C-21), **cholane** (C-24), **cholestane** (C-27), **ergostane** (C-28) and **stigmastane** (C-29).

Classification of steroids according to ***functional significance***:

1. sterols (steroid alcohols),
2. bile acids,
3. sex hormones and adrenal cortex hormones,
4. cardiotonic steroids,
5. steroid saponins,
6. steroid alkaloids,
7. provitamins D (vitamins D have B ring split).

Classification of steroids according to ***the number of carbon atoms***:

C-18 steroids (female sex hormones estrogens)

C-19 steroids (male sex hormones androgens)

C-21 steroids (hormones of corpus luteum – gestagens, female sex hormones and adrenal cortex hormones – corticoids)

C-23 steroids (aglycons of cardiotonic steroids)

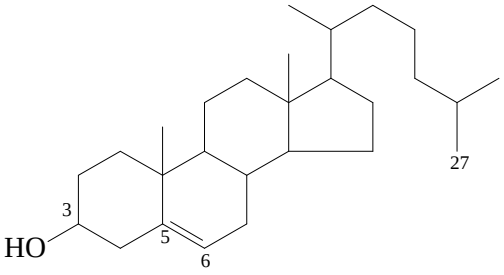
C-24 steroids (bile acids)

C-27 steroids (animal sterols, steroid sapogenins)

C-28 and C-29 steroids (sterols, mainly of plant origin)

8.4.1. Sterols

Cholesterol (5-cholesten-3β-ol) is a well-known animal steroid with 27 carbons and eight-carbon branched alkyl is bound to the C-17. It occurs in amounts up to 25 % in cell membranes of animals, 10 % in the brain, in human plasma (free or esterified). In the blood stream it is transported as cholesteryl esters in the lipoprotein particles. The body gets cholesteryl esters via diet, but up to 800 mg per day can normally be synthesized in the liver. Cholesterol is critical to many physiological functions. It is a substrate for synthesis of all steroid hormones, including the sex hormones and corticoids, bile acids (salts) vitamin D and others.



Cholesterol (5-cholesten-3β-ol)

Although it is a substance essential for life, it can also act contradictory. If cholesterol level in blood is increased, it tends to be deposited in the arterial walls leading to their damage and thus reducing their diameter and their throughput, causing a condition known as atherosclerosis. High cholesterol increases the risk for myocardial infarction, arterial thrombosis, and insufficient blood supply in the limbs. In addition, cholesterol is a major component of certain types of gallstones.

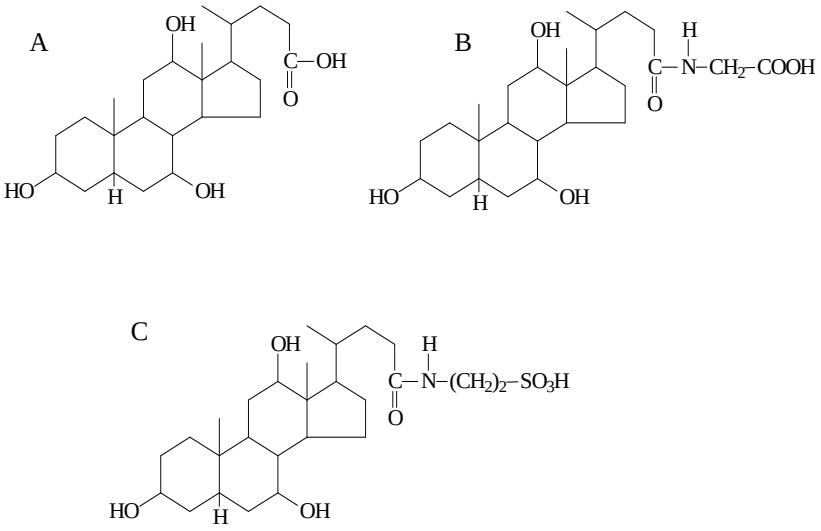
Phytosterols are plant sterols. **Ergosterol** (C-28) is an ergocalciferol (vitamin D_2) provitamin.

8.4.2. Bile acids

Bile acids are derivatives of **cholane** (C-24). The basic bile acid is cholic acid which conjugates with glycine or taurine to form glycocholic or taurocholic acids, from which bile acid salts are formed (sodium glycocholate and sodium taurocholate). Bile acids are synthesized from cholesterol in liver and stored in gall bladder as a solution called bile, which is a mixture of bile salts, cholesterol, and pigments from the breakdown of red blood cells. They play a crucial role in lipid digestion, in the absorption of fats in the intestine, where they function as extracellular emulsifiers. Synthesis of bile acids is also a major route of cholesterol secretion from the body.

Bile is secreted into the small intestine after a fatty meal and participates in the digestion of hydrolyzable lipids. Dietary lipid arrives in the intestine in the form of insoluble lipid globules. Hydrolysis by lipases can take place only at the surfaces of the globules. The larger the globules, the smaller the total surface area of lipid available for digestion and the slower the rate of digestion by the lipases. Bile salts contain a large hydrophobic part (steroid ring system) and a small hydrophilic part (carboxylate). Bile salts break up large lipid globules into much smaller ones, greatly increasing the surface area available to lipases, and this increases the rate of digestion.

Bile salts are also needed for the efficient intestinal absorption of the fat soluble vitamins (A, D, E, and K).



A. Cholic acid; B. Glycocholic acid; C. Taurocholic acid

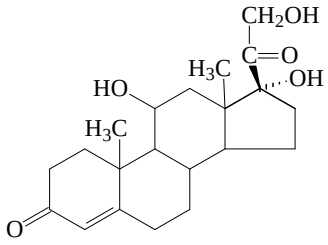
8.4.3. Steroid hormones

The steroid hormones are just one group of hormones, substances synthesized in the endocrine glands from which they are excreted and then transported in the bloodstream to target tissues, where they regulate a variety of cell functions. Hormones are often called chemical messengers because they relay messages between different parts of the body. They are effective at very low concentration. The major groups of steroid hormones are the hormones produced by the adrenal cortex and male and female sex hormones.

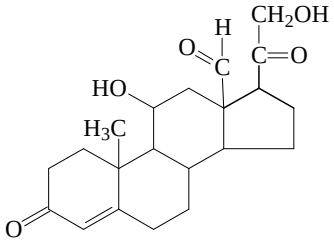
Steroids produced in the adrenal cortex are called **corticosteroids** (C-21). The corticosteroids are of the two types: *glucocorticoids and mineralocorticoids*.

Glucocorticoids are involved, along with insulin, in controlling the glucose balance in the body, they affect metabolism of lipids and proteins. Glucocorticoids such as ***cortisol*** promote gluconeogenesis and the formation of glycogen, whereas insulin facilitates the use of glucose by cell. These two substances must be in balance for the body's sugar to be properly metabolized. Cortisol is the best known of the corticosteroids because of its use in medicine. Since the blood-glucose is needed for brain function, cortisol stimulates other cells to decrease their use of glucose and, instead, metabolize fats and proteins for energy. Cortisol also blocks the immune system, because a stress situation is not the time to feel sick or have an allergic reaction. It is an effective anti-inflammatory and anti-allergic agent.

Mineralocorticoids affect the electrolyte balance of body fluids and hence the water balance. ***Aldosterone***, secreted by the adrenal cortex, is the most active of the mineralocorticoids. This hormone causes the kidney tubules to reabsorb Na^+, Cl^-, and HCO_3^-. The water balance of the body is essentially under the control of this hormone.



Cortisol



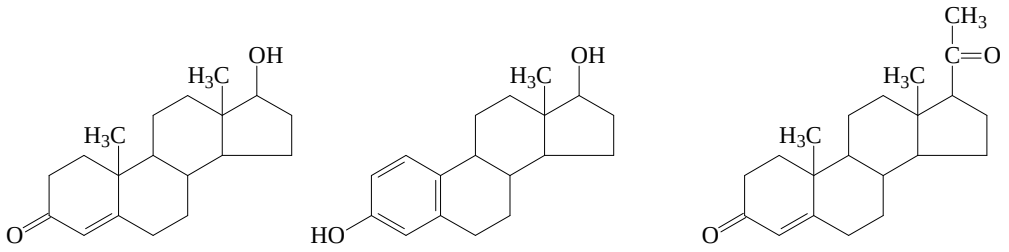
Aldosterone

Sex hormones

The testes of males and the ovaries of females produce steroidal sex hormones that control the growth and development of reproductive organs, the development of secondary sex characteristics, and the reproductive cycle.

Male sex hormones are **androgens** (C-19), which are produce mainly in the testes and in smaller amount in the adrenal cortex. ***Testosterone***, the most important androgen, stimulates production of sperms by the testes and promotes the growth of the male sex organs. Testosterone also is responsible for muscle development. Male sex steroids are responsible also for secondary sex characteristics in males such as deep voices and beards. **Anabolic steroids** are synthetic substances related to testosterone that are used by some athletes to promote muscle development. Their use is banned by most athletic unions.

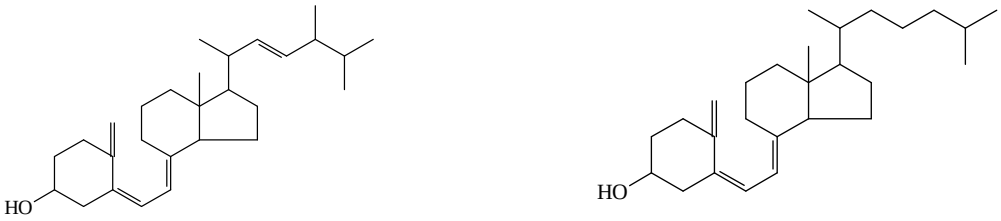
There are two kinds of *female* sex hormones: the **estrogens** (C-18) and **gestagens** (progestins) (C-21). The mainly ovaries produce estrogens, such as ***estradiol*** and ***estrone***. Gestagens are the hormons of corpus luteum, for example ***progesterone***. Progesterone together with estradiol regulates the menstrual cycle. The estrogens cause the growth of tissues in female sexual organs. Although estrogens are secreted in childhood, the rate of their secretion increases by 20-fold after puberty. The fallopian tubes, uterus, and vagina all increase in size. The estrogens also initiate growth of the breast and the breasts' milk-producing ductile system.



Testosterone **Estradiol** **Progesterone**

8.4.4. Vitamins D

Vitamin D exists in a number of forms. ***Vitamin D$_2$***, also called ***ergocalciferol***, and ***vitamin D$_3$***, also called ***cholecalciferol,*** are most often naturally occurring vitamin D forms. Vitamins D are formed by the action of ultraviolet radiation on the 5, 7-unsaturated sterols (provitamins, which belong to steroids), resulting in cleavage of the B ring, thereby losing steroid character. Vitamin D_2 is formed from ergosterol (C-28) of yeast and fungi and vitamin D_3 is formed in the skin from 7-dehydrocholesterol (C-27) in a photochemical reaction driven by the UV component of sunlight. These two are equally useful in humans, and either can be changed in the body to the slightly oxidized forms acting as hormones stimulating absorption and use of calcium and phosphate iones. Lack of vitamin D leads to deficiency disease known as rickets a bone disorder. Eggs, butter, fatty fish, and fish oils such as cod liver oil are good natural sources of vitamin D precursors (see Chapter 11).



Ergocalciferol (vitamin D$_2$) **Cholecalciferol (vitamin D$_3$)**

8.5. Eicosanoids

During oxidation of arachidonic acid, biologically highly effective compounds with an exceptionally wide range of actions are formed in many tissues. This group of compounds − **eicosanoids** (prostanoids) − includes *prostaglandins*, *prostacyclins*, *thromboxanes*, *leukotrienes*, and *epoxides*. The scheme of eicosanoid formation is shown in Fig. 8.7.

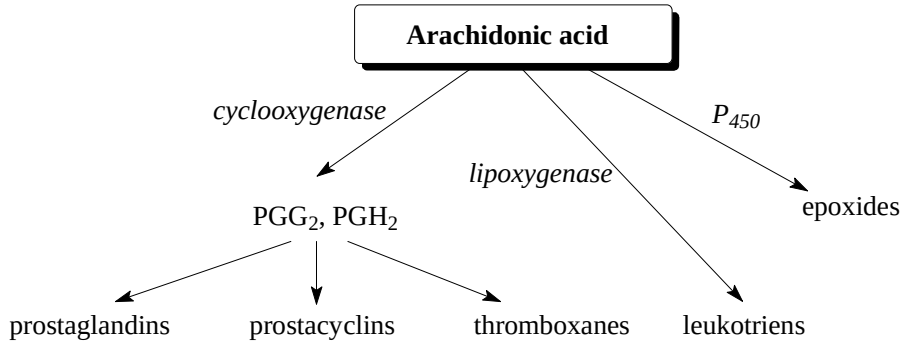


Fig. 8.7. Scheme of eicosanoids formation

By action of the enzyme ***cyclooxygenase*** endoperoxides (PGG_2 and PGH_2) are formed as precursors, from which prostaglandins (PG), thromboxanes (TX) and prostacyclins (PGI) are produced during further enzymatic

reactions. Leukotriene biosynthesis also starts from arachidonic acid. They are, however, formed by the action of the enzyme ***lipoxygenase***. Enzymes synthesizing the above compounds have been detected in the microsomal fraction of cells.

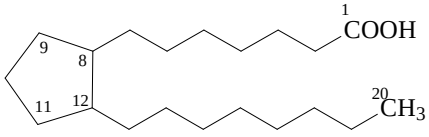
Tab. 8.6. Main biological effects of eicosanoids

Eicosanoid	Effect
Prostaglandins (PGD_2, $PGE_{2\alpha}$, PGF_2)	vasodilatation, pain, inflammation, uterine contractions
Thromboxanes (TXA_2)	vasoconstriction, thrombus formation
Prostacyclins (PGI_2)	vasodilatation, thrombolysis
Leukotriens (LTB_4)	control functions of polymorphonuclear leukocytes in inflammation
Leukotriens (LTC_4, LTD_4, LTE_4)	slow reactant of anaphylaxis

Arachidonic acid cascade is stimulated, also by oxygen-derived free radicals and by the myeloperoxidase system (which is known to play an important role in phagocytosis). By their action, cells release several enzymes, including *phospholipase* A_2, cleaving off the free arachidonic acid from phospholipids of cell membranes. Main biological effects of eicosanoids are listed in the Tab. 8.6.

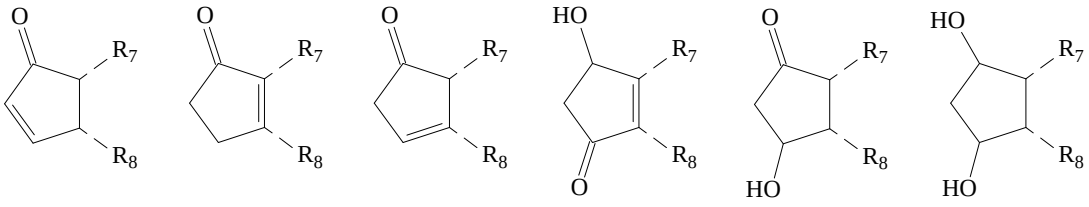
8.5.1. Prostaglandins

Prostaglandins were the first eicosanoids that have been recognized. They are biological derivates of arachidonic acid and are formed via the 20-carbon **prostanoic acid**, which contains a 5-member cyclopentane ring:

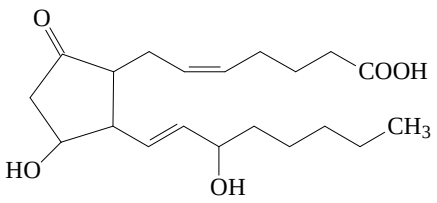


Prostanoic acid

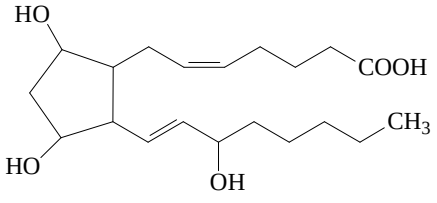
Prostaglandins are classified according to the number and arrangement of double bonds and hydroxyl and ketone groups. Individual series are denoted PGA, PGB, PGC, PGD, PGE, PGF. The numerical index in the abbreviation gives the number of double bonds, e.g. PGE_3 has 3 double bonds:



PGA **PGB** **PGC** **PGD** **PGE** **PGF**



Prostaglandin E_2 (PGE_2)



Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$)

The prostaglandins, which were originally isolated from the prostate gland, occur in many body tissues and are physiologically active at very low concentrations. The prostaglandins are like hormones in many ways, except that they do not act globally, that is, over the entire body. Prostaglandins are generated directly in the cells of the tissues based on nerve, chemical and mechanical stimuli. They do their work within the cells where they are made or in nearby cells, so they are sometimes called *local hormones*. This is perhaps why the prostaglandins have such varied functions; they occur and express their roles in such varied tissues. They work together with hormones to modify the chemical messages that hormones bring to cells. In some cells, the prostaglandins inhibit enzymes and in others they activate them. In some organs, the prostaglandins help to regulate the flow of blood within them. In others, they affect the transmission of nerve impulses.

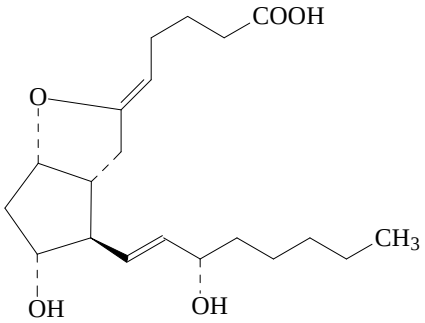
Some prostaglandins enhance inflammation in a tissue, and it is interesting that the acetylsalicylic acid (aspirin), an inflammation reducer, does exactly the opposite. This effect is caused by aspirin's ability to inhibit the activity of an enzyme needed in the synthesis of prostaglandins (*cyclooxygenase*). *Cyclooxygenase* is also inhibited by another anti-inflammatory drug, *indomethacin*. Acetylsalicylic acid, however, is not an inhibitor of *lipoxygenase*, and therefore it does not block formation of leukotrienes.

The molecular basis of prostaglandin activity is still not well understood. However, there is considerable clinical interest in these compounds. Regulation of menstruation, control of fertility, contraception, control of blood pressure, and blood clot prevention are all potential uses of prostaglandins. For this reason, many prostaglandins have be synthesized in the laboratory for pharmacological use (*synthetic prostaglandins*). Natural prostaglandins cannot be taken orally because they are rapidly degraded and do not survive long enough for effective action. Thus, one research goal is to develop modified prostaglandins that can be administered orally.

In experiments that use prostaglandins as pharmaceuticals, they have been found to have an astonishing variety of effects. One prostaglandin induces labor at the end of a pregnancy. Another stops the flow of gastric juice while the body heals an ulcer. Other possible uses are to treat high blood pressure, rheumatoid arthritis, asthma, nasal congestion, and certain viral diseases.

8.5.2. Prostacyclins

Prostacyclins contain in their molecule, in addition to the cyclopentane ring, another five-membered ring, formed by an epoxide bond between C_6 and C_9. The effects of prostacyclins are opposite to that of thromboxanes. They have vasodilatation effects and stimulate thrombolysis.

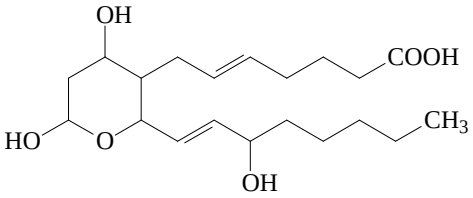


Prostacycline I_2 (PGI_2)

8.5.3. Thromboxanes

Thromboxanes are synthesized in thrombocytes. Instead of the cyclopentane ring they contain a six-membered oxane ring. Thromboxanes induce thrombocyte aggregation and vasoconstriction, resulting in induction of thrombus formation.

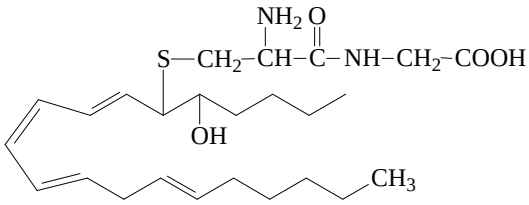
The two major thromboxanes are thromboxane A_2 and thromboxane B_2. Thromboxane TXA_2 is unstable, as it has another ring formed by an oxygen bridge, therefore it is promptly reduced to the more stable TXB_2:



Thromboxane B_2 (TXB_2)

8.5.4. Leukotriens

Leukotrienes are formed *via* a biochemical pathway different from the one mentioned above. The term "leukotrienes" is derived from their chemical structure − they contain a conjugated triene group (three double bonds in conjugation). They are produced from the intermediate 5-HPETE (5-**h**ydro**p**eroxy-**e**icosa**t**etra**e**noic acid). First LTA is synthesized, and next it is converted into LTB, LTC, LTD, LTE, LTF. Leukotrienes A and B differ only in hydroxyl groups in their basic chain. Other leukotrienes contain, in addition, glutathione or cysteine units (LTD_4 is an example).



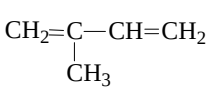
Leukotriene D_4 (LTD_4)

Biological effects of leukotrienes are diverse. Leukotriene LTB_4 mainly regulates functions of polymorphonuclear leukocytes and eosinophils during phagocytosis, and inflammation. Leukotrienes LTC_4 and LTD_4 are mediator compounds of anaphylaxis. In concentration thousand times smaller than histamine they induce contraction of bronchial musculature and edema of bronchial mucosa. They constrict airways and change permeability of blood vessels. They are released during asthma attacks and other allergic reactions.

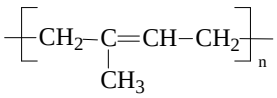
8.6. Terpenes

Terpenes are substances mainly of vegetable origin. They are essential components of plant essential oils (volatile fragrances of flowers, fruits, leaves or other parts of plants) and plant resins (oxidation products of essential oils produced in areas with damaged bark of conifers). Terpenes include also balms, which are semi-liquid mixture of resins and essential oils, as well as turpentine oil.

Terpenes are compounds which consist of **isoprene** units (2-methyl-1,3-butadiene). These substances are therefore also called isoprenoids.



Isoprene



Isoprene units

For animals, some of them are important as basic structural components of cells, as vitamins and hormones, or their significant precursors. Isoprenoids are formed by polymerization. They are secondary metabolites that are formed during degradation of certain substances in the body. Terpenes have a lipophilic character – they are insoluble in water but soluble in fat.

The biosynthesis of isoprenoids starts from acetic acid, more particularly, from its active form - acetyl-coenzyme A, which generates active isoprene (isopentenyl pyrophosphate) via several by-stages leading to the formation of all isoprenoids.

The isoprene units are joined together and general formula is $(C_5H_8)_n$. According to the number of isoprene units, terpenes can be classified to:

monoterpenes	(n = 2)	$C_{10}H_{16}$
sesquiterpenes	(n = 3)	$C_{15}H_{24}$
diterpenes	(n = 4)	$C_{20}H_{32}$
triterpenes	(n = 6)	$C_{30}H_{48}$
tetraterpenes	(n = 8)	$C_{40}H_{64}$
polyterpenes		$(C_5H_8)_n$

Terpenes may have different degrees of unsaturation, and a variety of functional groups may be present (for example –OH, >C=O, –CHO).

Terpenes, which are abundant in the oils of plants and flowers, have distinctive odors and flavors. They are responsible for the odors of pine trees and for the color of paprika (capsicum), carrots, and tomatoes. Vitamins A, E, and K are terpenes.

8.6.1. Monoterpenes

The monoterpenes (C 10) are found in nature in the form of alcohols and aldehydes. They are the simplest terpene class, containing two isoprene units. They occur mainly in plants, but they can be found also in germs and animals. We recognize aliphatic, monocyclic and bicyclic monoterpenes.

Examples of monoterpenes are: ***geraniol*** (geranium, eucalypt, rose oils), ***citral*** (orange oil), menthane, ***menthol*** (used in medicine as moderate antiseptic and as additive to tooth-pastes and mouthwash), ***limonene*** (citrus oil), ***carvone*** (oil of caraway), ***borneol*** (lavender, rosemary oils), ***camphor*** (mainly in essentials oils of camphor tree). Because of its antiseptic properties, camphor is used in medicine (Camphora) as a part of several ointments (in rheumatic diseases) (Fig. 8.8.).

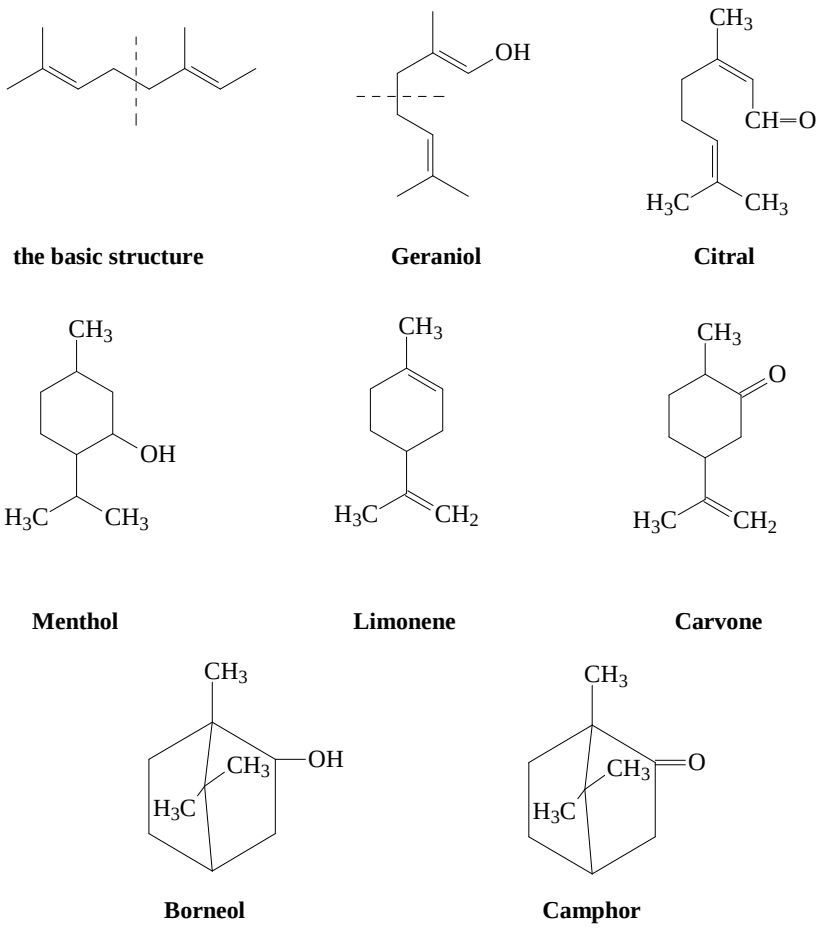
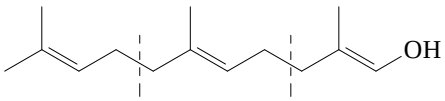


Fig. 8.8. Structure of some monoterpenes

8.6.2. Sesquiterpenes

Sesquiterpenes (C_{15}) consist of three isoprene units. They can be aliphatic and monocyclic. Several of these compouds have –OH group, for example ***farnesol***:



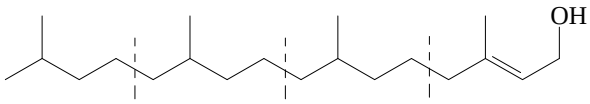
Farnesol

Farnesol is naturally widespread, it is an important metabolite for the synthesis of other terpenes. ***Difarnesyl***, which is a part of vitamin K_2 (Chap. 11) is derived from two molecules of farnesol.

8.6.3. Diterpenes

Diterpenes (C_{20}) are composed of four isoprene units. ***Phytol*** is biochemically important aliphatic diterpene, which is primary alcohol with one double bond. Phytol is an ester bound to chlorophyll. Diterpenes form the basis of biologically important compounds such as retinol (vitamin A_1) or retinal (Chap. 11).

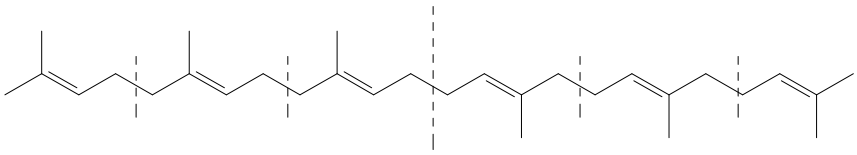
After the breakage of –OH group the rest of phytol molecule is also a part of vitamin K_1 (phyloquinone) and vitamin E (Chap. 11).



Phytol

8.6.4.Triterpenes

Triterpenes (C_{30}) consist of six isoprene units. The linear triterpene *squalene*, the major constituent of shark liver oil, is derived from the reductive coupling of two molecules of farnesyl pyrophosphate. Squalene is a structural precursor of cholesterol from which all steroids are synthesized.

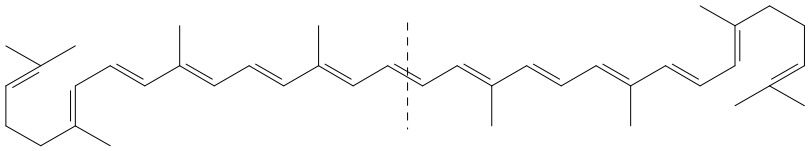


Squalene

The vitamin K_2 (farnoquinone) (Chap. 11), an important molecule in blood clotting and sapogenin (cyclic triterpene), which forms a nonsaccharide part of heteroglycosides (saponins), are built from 6 isoprene units.

8.6.5. Tetraterpenes

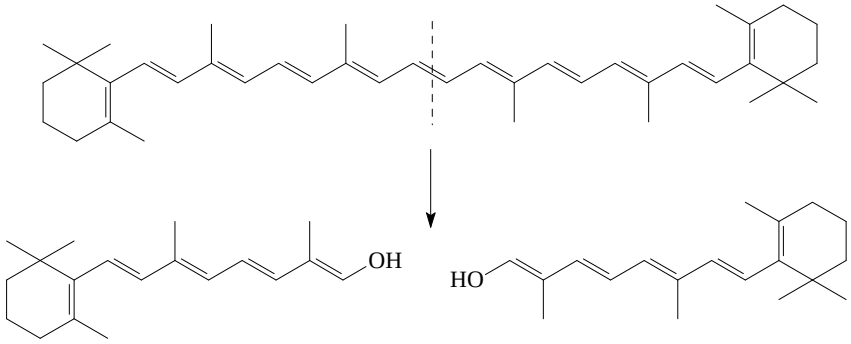
Tetraterpenes (carotenoids, C_{40}) contain eight isoprene units. Carotenoids can be considered derivates of ***lycopene***, found in fruits and flowers. Its long straight chain is highly unsaturated and composed of two identical units joined by a double bond between carbon 15 and 15'. Biologically important carotenoids may be acyclic (lycopene) or cyclic (mono- or bi-, alicyclic or aryl) with or without oxygen atom in their molecule. The monocyclic tetraterpene is γ-carotene, and the bicyclic ones are α-carotene and β-carotene. All three isomers in their molecules comprise a β-ionone ring.



Lycopene

The color of fruits and flowers, for example tomatoes and carrots comes from carotenoids. These compounds absorb light because they contain extended network of single and double bonds and are important pigments in photosynthesis. They are yellow, red and violet pigments of plant and animal organisms. Colour of carotenoids is conditioned by polyene structure of their molecule.

Among the represented molecules, **β-*carotene*** is probably the most important as a precursor of vitamin A which is formed from β-carotene by central cleavage into aldehyde retinal, alcohol retinol (vitamin A_1) and retinoic acid (Chap. 11):



β-Carotene → 2 Molecules of retinol (*vitamin A_1*)

8.6.6. Polyterpenes

Polyterpenes are macromolecules consisting of long chains of isoprene units. The most important compounds in this group are rubber and gutta-percha. Natural ***rubber latex*** is extracted from the rubber tree. It consists of polyisoprene in which the double bonds are in *cis* configuration and is characterized by elasticity. Some plants produce a polyisoprene with *trans* double bonds, known as ***gutta-percha***. It is not elastic.

8.7. Biological membranes

Biological membranes separate cells (as well as individual organelles in the cytoplasm) from their surrounding. Cell or organelle membranes are not inert barriers, they are barriers with very selective permeability, which regulate the molecular and ionic composition inside the cell and organelles:

- Membranes control the entry of substrates needed for the synthesis of biomolecules as well as other molecules that are going to be modified or broken down in the cell organelles.
- Membranes control the release of products from organelles so they can be used anywhere in the cell, and the release from cells so they can be used anywhere in the organism, as well as the release of waste products from cellular processes.
- Membranes play a central role in cell recognition.
- They are involved in cellular communication. The receptor molecule of the membrane obtains information from other cells via e.g. hormones, and transforms it into molecular changes within the cell
- Membranes keep different distribution of ions on either side of the membrane. These concentration gradients are used in the formation of chemical or electrical signals in nerve impulse transmission and muscle contraction and in the transport of ions across the cell membrane (secondary active transport).

Cell membranes are about 7.5 nm thick and are composed mainly of lipids (phospholipids, glycolipids, and cholesterol) and proteins. The relative distribution of lipids is changed according to the type of the membrane. Most membranes of the same tissues (e.g. plasmatic membrane of human erythrocytes), contain the same amount of lipids and proteins. A typical membrane contains 2 - 10% of carbohydrates.

The membrane lipids are **amphiphilic (amphipathic)** lipids, characterized by:

- long **hydrophobic** (nonpolar) part, or tail - hydrocarbon chains of glycerophospholipids, sphingolipids or cholesterol esters.
- short **hydrophilic** (polar) part, or head - phosphodiester group of glycerophospholipids and sphingophospholipids, carbohydrate component of sphingoglycolipids, or hydroxyl group of cholesterol.

Cell membrane structure is uniform. Currently the "**fluid mosaic model**" is accepted (SJ Singer and GL Nicholson, 1972). In this model (Fig. 8.9.) the membrane is formed by membrane lipids, organized into the **lipid bilayer** and *peripheral proteins, proteins bound through the lipid anchor* and the *integral proteins* (see Chapter 9.3.). The basic membrane component is a phospholipid bilayer with embedded derivatives of sterols and polyisoprene derivates. Bilayer has two layers, and each has a hydrophilic and hydrophobic side, which are arranged in such a way that the hydrophobic sides touch and the hydrophilic sides face the the internal and external aqueous environment of the membrane. The driving force for the formation of the lipid bilayer is composed of secondary attractive and repulsive forces. Molecules of phospholipids within the membrane layer are oriented with their hydrophobic acyl chains towards the center and the polar groups towards the surface of the bilayer.

Properties of the membrane are determined by the type FA and a type of polar groups in phospholipids. Components and organization of the membrane differs on both sides, on the outer and inner surfaces. For example, phosphatidylcholine and sphingomyelin are usually found on the outside surface, while the phosphatidylserine and phosphatidylethanolamine are normally on the inner surface. Cholesterol molecules help to keep a membrane from being too fluid.

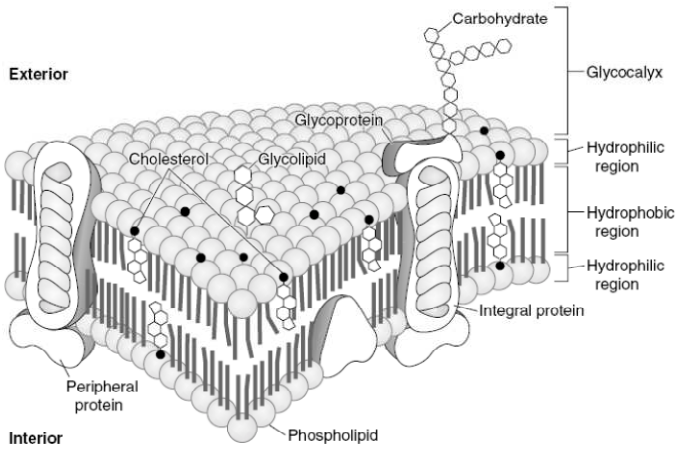


Fig. 8.9. Fluid mosaic model of the cell membrane

Membrane saccharides are on the extracellular hydrophilic surface. They are covalently bonded to membrane lipids and proteins such as glycoproteins or glycolipids. Carbohydrate part of glycolipids and glycoproteins acts as a receptor site for the cell recognition processes and cellular communications.

Lipid bilayer mosaic is formed of various lipids and proteins. The membranes are structurally and functionally asymmetric. The outer and inner surfaces have different components and functions, and each surface is asymmetric with various components and functions in various locations. Because the lipid bilayer is fluid the individual lipid and protein molecules have lateral mobility: they can move sideways in the plane of each layer. This mobility is possible because of attractive forces (not covalent) between lipids and proteins.

The precise **fluidity** needed for a membrane depends on its function. The relative amounts of cholesterol, saturated chains, and unsaturated chains vary among species of organisms, among cell and organelle membranes in the same organism, and even in the same type of membrane over time with changes in temperature and dietary lipid composition.

Processes that induce degradation of the membrane structure may play a role in the pathogenesis of various diseases. In addition to phospholipid hydrolysis by the *phospholipases*, **lipid peroxidation**, initiated by free oxygen radicals, is considered to be, the main cause of impairment of membrane structures and their functions (Chap. 13).

Membrane rafts

Cell membrane lipids, in addition to the structural function, take part in signal transmission from the outside to the inside of the cell. It seems that many functions of cell membranes are closely associated with various

specialized microdomains, called **rafts**. These small sections of membranes differ in their structure and properties. They are rich in sterols and sphingolipids and contain specific signaling proteins.

Control questions:

1. Provide basic classification of lipids.
2. Describe the lipid components and the bonds, by which they may be bound in lipids.
3. Write at least three alcohol formula, which occur in lipids.
4. Which fatty acids are referred to as the vitamin F? Write their structure.
5. Explain what omega fatty acids are.
6. Write the reaction and conditions of triacylglycerol hydrolytic cleavage.
7. Write the formula of ceramide and the name of bond between an alcohol and a fatty acid.
8. Explain the effect of snake venom in hemolysis of erythrocytes.
9. Indicate what lipoproteins are, their classification and function in lipid transport.
10. Explain the term "reverse cholesterol transport".
11. Divide the steroids to the main groups of hydrocarbons according to the number of carbon atoms in the molecule and write examples of compounds which belong to them.
12. Write reaction catalyzed by *cyclooxygenase* and product names.
13. Indicate, which group of substances are called tissue hormones and why.
14. Write the groups of terpenes and examples of compounds which belong to them.
15. Which of isoprenoid compound is a precursor of cholesterol synthesis in the body? In which group of terpenes is it included?

9. AMINO ACIDS, PEPTIDES AND PROTEINS

9.1. Amino acids

Amino acids (AA) are organic compounds and from chemical viewpoint they are **substitutive derivatives of carboxylic acids.** They have one **–NH_2 group** bound to α-carbon (C-2) in proteinogenic AA and exceptionally on β-carbon (in some peptides – carnosine, anserine) and one **–COOH group**. Besides these basic functional groups they could have another –NH_2 group in the molecule (or other group of basic character) and the second carboxylic group. AA has optically active carbon (except glycine) and can occur in two optical isomeric forms, L- and D-. Proteinogenic AA are present in human in L-isomeric form. Amino acids in nature are colourless crystalline compounds, well soluble in water, many of them (L-AA) are sweet and some of them are tasteless.

9.1.1. The functions of amino acids in living organism

In organism most amino acids are occurring **in peptides and proteins as their building units**. In natural sources there were found about 250 amino acids but **only 21** of them occur in proteins (**proteinogenic amino acids**). Some portion of amino acids can exist in the cells and tissues in their free, chemically unbound form. These ones are initial compounds in the biosynthesis of non-protein nitrogen containing compounds (e.g. β-alanine as a part of coenzyme A) or they are products of their metabolism.

9.1.2. Classification of amino acids

Proteinogenic amino acids are classified according to several aspects:

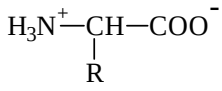
a) **according to the character and number of functional groups** into
- monoaminomonocarboxylic (glycine, alanine, valine, leucine, isoleucine)
- monoaminodicarboxylic (aspartic acid, glutamic acid)
- diaminomonocarboxylic (lysine, arginine)
- cyclic - aromatic (phenylalanine, tyrosine)
  - heterocyclic (tryptophan, histidine)
  - proline, hydroxyproline
- hydroxy amino acids (serine, threonine)
- sulphur containing amino acids (cysteine, methionine);

b) **according to the character of side chain** into
nonpolar, polar, acidic, basic and aromatic;

c) **according to their physiological significance** into
essential (Val, Leu, ILe, Thr, Met, Lys, Phe, Trp and His, Arg in children)
and nonessential (others)

9.1.3. The structure of amino acids

From general formula of amino acids (carbon atom binds four different substituents) results that all α-amino acids except glycine have a chiral character. In the human organism there are exclusively L-amino acids. In solution amino acids exist only in the form of **zwitterions (amphoteric ion)**. It is a particle with zero net charge, even though it contains two almost completely ionized groups in these conditions.



Final charge of the molecule is affected with pH of solution what is resulting from ionic character of free amino acids. pH value, at which amino acid exists mainly in its neutral form, is called **pI – isoelectric point**. At pH lower than pI dissociation of carboxylic group is depressed (it is protonated) and amino acid obtains character of cation, at pH higher than pI quaternary amino group is deprotonated and amino acid exists in form of anion (Fig. 9.1.). pI of neutral amino acids is situated according to their structure in the range of pH 5.0 – 6.3; for basic AA the interval for pI is 7.6 – 10.8 and for acidic AA this interval is pI 2.7 – 3.2.

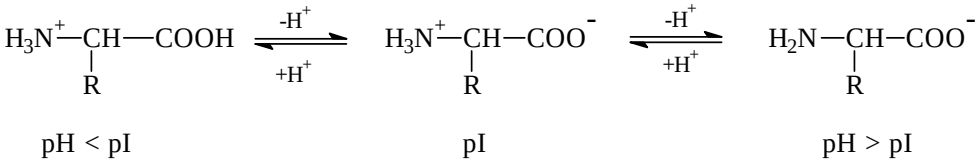
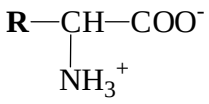
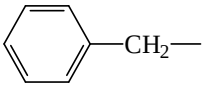
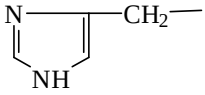
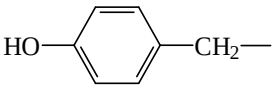
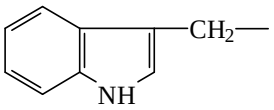
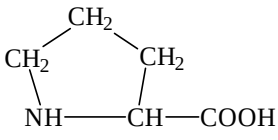


Fig. 9.1. The equation of the amino acid ionization depending to pH

Tab. 9.1. The structures of proteinogenic amino acids



R —	Name of amino acid (abbreviation)	R —	Name of amino acid (abbreviation)
H—	Glycine (Gly)	HO−CH_2—	Serine (Ser)
CH_3—	Alanine (Ala)	HO−CH(−CH_3)—	Threonine (Thr)
H_3C, H_3C >CH—	Valine (Val)	H_2N−C(=O)−CH_2—	Asparagine (Asn)
H_3C, H_3C >CH−CH_2—	Leucine (Leu)	H_2N−C(=O)−CH_2−CH_2—	Glutamine (Gln)
H_3C, H_3C-CH_2 >CH—	Isoleucine (Ile)	HO−C(=O)−CH_2—	Aspartic acid (Asp)
HS−CH_2—	Cysteine (Cys)	HO−C(=O)−CH_2−CH_2—	Glutamic acid (Glu)
H_3C−S−CH_2−CH_2—	Methionine (Met)		
	Phenylalanine (Phe)		Histidine (His)
	Tyrosine (Tyr)	H_2N−CH_2−CH_2−CH_2−CH_2—	Lysine (Lys)
	Tryptophan (Trp)	H_2N−C(=NH)−NH−CH_2−CH_2−CH_2—	Arginine (Arg)
			Proline (Pro) (whole structure)

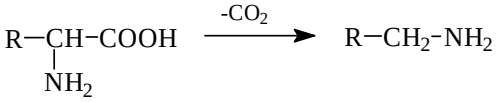
9.1.4. Reactions of amino acids

The types of reactions that amino acids provide result from the functional groups occurring in their molecules. Many of these reactions are important from biochemical viewpoint, because they take part in metabolic processes in human organism. In addition, many of them are the basis of analytical procedures used in clinico-

biochemical diagnostics. The most often occurring reactions of amino acids are **decarboxylation, deamination** and **transamination**.

9.1.4.1. Decarboxylation of amino acids

Product of decarboxylation of amino acids is the primary amine while CO_2 is eliminated:



Reaction is catalyzed with ***decarboxylase***, coenzyme of which is ***pyridoxal phosphate*** (it contains carbonyl functional group). The basis of reaction mechanism is formation of Schiff base between amino group of amino acid and carbonyl group of the coenzyme (see subchapter 6.6.3.1.).

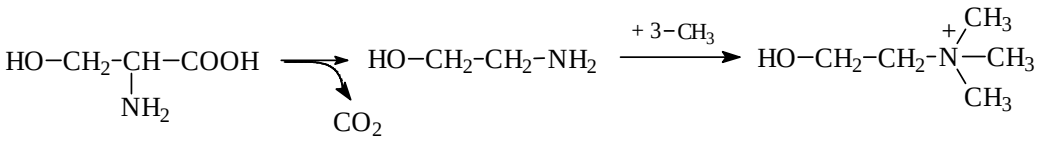
The process of decarboxylation is very important, because it produces very effective compounds – **biogenic amines**. Many of them have considerable pharmacological effects, they can act as hormones or their precursors, others are components of coenzymes and other biologically important substances (Tab.9.2).

Tab. 9.2. Products of decarboxylation of selected amino acids

Amino acid	Primary amine	Biological importance
serine	ethanolamine	component of phosphatides
threonine	isopropanolamine	cobalamin part
cysteine	cysteamine	coenzyme A part
aspartic acid	β - alanine	coenzyme A part
glutamic acid	γ - amino butyric acid	brain metabolism
histidine	histamine	regulation of blood pressure
tyrosine	tyramine	catecholamines biosynthesis
5-hydroxythryptophan	serotonin	neurotransmitter

From general reactions there are important **reactions of the carboxylic group**, which can change amino acids into derivatives of acids – mainly esters and amides.

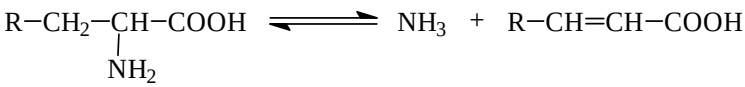
In complete methylation of amino group **betains** are formed. They are quaternary ammonium bases occurring in plants as well as in organism in liver and kidney. Decarboxylation and subsequent methylation of serine results in formation of **choline** (present in phospholipids molecules):



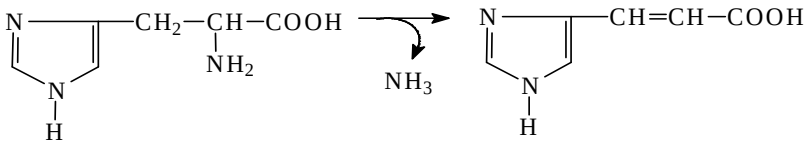
serine ethanolamine choline

9.1.4.2. Deamination of amino acids

a) Direct deamination (elimination of ammonia) of amino acid resulting in formation of unsaturated amino acid is the simplest way of nitrogen elimination (**desaturation deamination**):

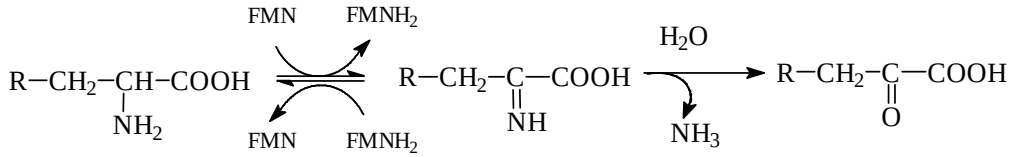


The reaction is moderately exergonic and in organism it proceeds only in case of some amino acids, e.g. histidine:



histidine urocanic acid

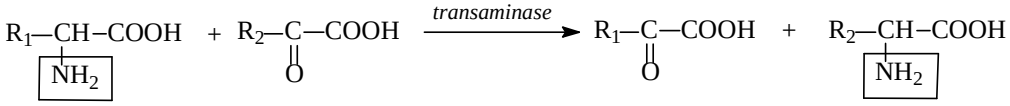
b) When the elimination of ammonia from the molecule of amino acid realizes through the oxidative processes, this is called **oxidative deamination.** In the first step of reaction imino acid is formed which is in the second step hydrolytically decomposed to oxo acid and ammonia:



mino acid imino acid oxo acid

9.1.4.3. Transamination of amino acids

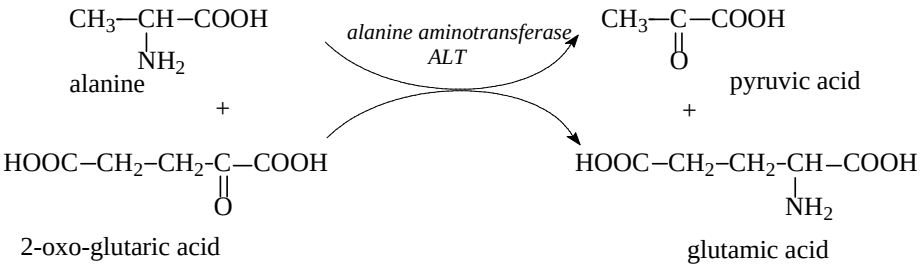
Transamination is reaction in which amino acid (nitrogen donor) reacting with oxo acid (nitrogen acceptor) is changed into equivalent oxo acid. At the same time oxo acid receiving amino group is changed to the equivalent amino acid. This type of reaction is catalyzed with enzyme ***transaminase*** (*aminotransferase*), which has pyridoxal phosphate as coenzyme. It means that the transamination mechanism is also proceeding through the formation of Schiff bases. *Transaminases* are enzymes specific only for one pair of α-amino acid and α-oxo acid.



amino acid-1 oxo acid-2 oxo acid-1 amino acid-2

The main function in the transamination reactions have glutamic and 2-oxoglutaric acids that are most occurring in transamination reactions as donor or acceptor of nitrogen, respectively.

From the large number of transaminases in clinico-biochemical praxis there are two very important enzymes: *L-alanine-2-oxoglutarate aminotransferase* (ALT) (Fig.9.2.) and *L-aspartate-2-oxoglutarate aminotransferase* (AST). Increased concentration of ALT in blood can be found in case of hepatocytes (liver cells) damage and concentration of AST in blood is increased if myocardial cells are damaged (e.g. infarct of myocardium).

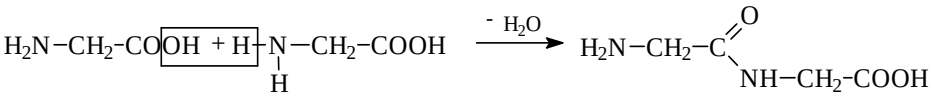


2-oxo-glutaric acid glutamic acid

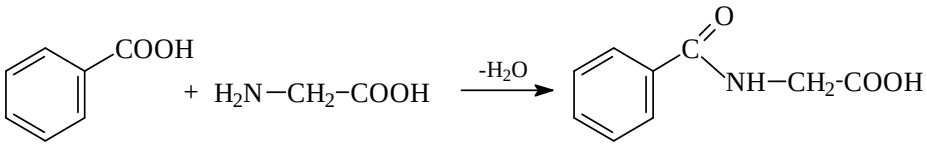
Fig. 9.2. Transamination reaction catalyzed with ALT

9.1.4.4. Acylation reactions

In acylation reactions hydrogen in amino group in one amino acid is substituted with unit (acyl) of another acid (e.g. amino acid) thus forming peptide bond or acyl derivatives. Two molecules of glycine thus create peptide bond in dipeptide:



The acylation reaction is also the basis of some detoxication mechanisms in organism, e.g. formation of hippuric acid (benzoylglycine) that is excreted into urine as product of detoxication of aromatic carboxylic acids mainly of plant origin.

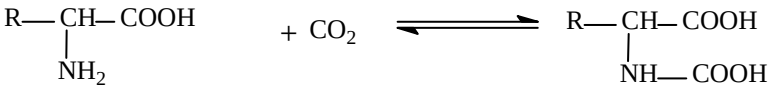


benzoic acid glycine hippuric acid

This reaction is used in the test of liver function. Benzoate is administered to patient and amount of hippuric acid excreted in urine is measured.

9.1.4.5. Formation of carbamine acids

The important reaction is formation of carbamine acids. Amino acids have ability to bind reversibly CO_2 to NH_2 group to form carbamine acids. In organism the reaction is taking part in transport of CO_2 from the tissues to lungs bound to hemoglobin and its release in lungs.



carbamine acid

R = rest of hemoglobin peptidic chain

9.1.5. Nitrogen flow in amino acids catabolism

The animals excrete nitrogen from amino acids and other sources in a form of one of three products: ammonia (NH_3), uric acid (urate) or urea. Many terrestrial organisms (human including) are ureotelic. It means they excrete urea - in water very well soluble and non-toxic compound. Fig. 9.3 shows the flow of nitrogen in amino acids catabolism. Amino acids transfer nitrogen to oxo acids (mainly to 2-oxoglutaric acid) in transamination reactions. Since one of the main acceptor of nitrogen in the transamination is 2-oxoglutarate, amino nitrogen is concentrated particularly in glutamate, and glutamate is then oxidatively deaminated. This is important, because the L-glutamate is the only amino acid that in mammalian tissues undergoes oxidative deamination by sufficient speed. Ammonia released by oxidative deamination of glutamate is directed from extrahepatic tissues to the liver, to be incorporated into a molecule of urea in the urea cycle. The most important transport form of ammonia in the blood is glutamine which is formed in the tissue by binding ammonia to the glutamate. In the liver glutamine is hydrolyzed by *glutaminase* into ammonia and glutamate. Subsequently ammonia directly enters the first reaction of urea biosynthesis together with CO_2.

The urea formed in this biochemical pathway is excreted by urine from organism.

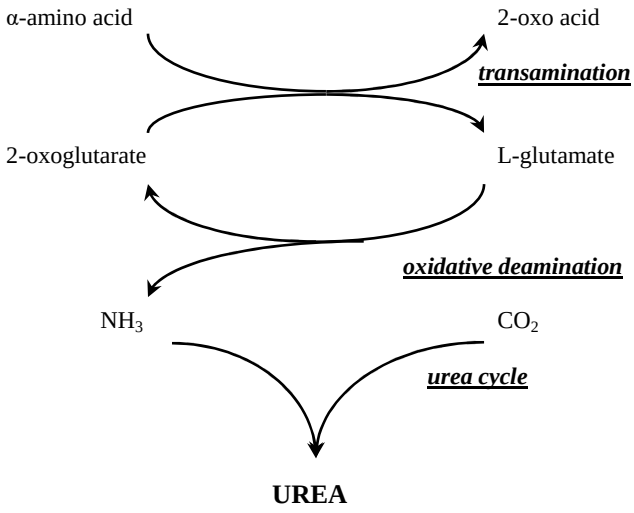
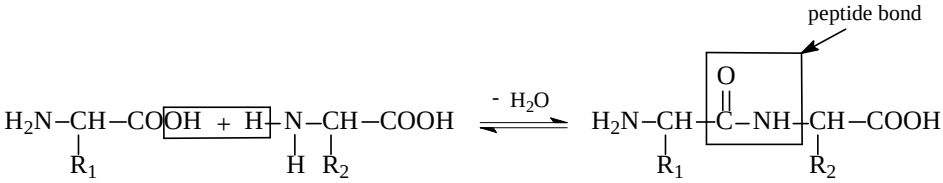


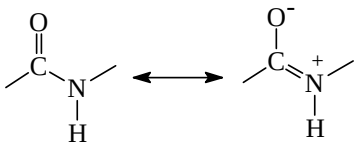
Fig. 9.3. Overall flow of nitrogen in amino acid catabolism

9.2. Peptides

From chemical viewpoint peptides are composed of the **rests of amino acids connected by peptide bond**. Peptide bond is formed in reaction of amino group of one amino acid and carboxylic group of another amino acid. Thus in each peptide there is remaining one amino acid residue with free –COOH group (C-terminal amino acid) and one amino acid residue with free $–NH_2$ group (N-terminal amino acid). Peptide bond can be chemically or enzymatically cleaved.



Atoms –CO–NH– are located in the same plane in the *trans* configuration, while the electrons of the double bond between C and O are delocalized. Delocalization of electrons causes the character of a partial double bond between C and N in the peptide bond:



In organism there are several types of peptides with important biological effects – hormonal, antibiotic, toxic and other.

9.2.1. Peptide hormones

Hormones are physiologically effective compounds released by specific tissues into the blood stream, which transports them to the target tissue where they perform their biological function. One group of hormones has chemical character of peptides.

Hypothalamus produces two cyclic **nonapeptides** (composed of 9 amino acids) – **vasopressin** and **oxytocin.** The structure of both hormones is similar but the physiological function is different. While vasopressin increases blood pressure by increasing tonus of peripheral vessels and water reabsorption in kidney, oxytocin causes contraction of smooth muscles (uterus, mammary gland).

Insulin (51 amino acids) is produced in β-cells of the islets of Langerhans in pancreas. It arises from the precursor molecule *proinsulin* by enzyme-catalyzed hydrolytic cleavage of an internal sequence called C-peptide. The insulin molecule is composed of the two polypeptide chains A and B (A has 21 and B 30 amino acids), which are connected by two disulphide bridges. At low concentration in the blood it occurs as a monomer, whereas the higher concentrations forms a dimer, of which in the presence of Zn^{2+} hexamers may be formed. The insulin secreted at higher concentrations of glucose stimulates intake of glucose by the cells (muscle and fat tissue), and its utilization in tissues (glucose metabolism and the synthesis of glycogen). Insulin therefore has a hypoglycemic effect, reducing the blood glucose concentration. In addition to the role in glucose metabolism insulin interferes also with other metabolic processes as lipolysis and lipogenesis (synthesis of higher carboxylic acids).

Glucagon (29 amino acids) is produced in α-cells of the islets of Langerhans in pancreas and it stimulates cleavage of glycogen and proteins and slows down glycogen synthesis. By this way the glucose concentration in blood is increased (antagonist of insulin), therefore glucagon has hyperglycemic effect.

β-endorphins consist of 31 amino acids and they are **precursors of enkephalins**. **Enkephalins** are **pentapeptides occuring in 2 forms - Met-enkephalins (Tyr-Gly-Gly-Phe-Met) and Leu-enkephalins (Tyr-Gly-Gly-Phe-Leu**), they were found in brain and bind to the same receptors of central nerve system as morphine opiates thus playing the role in endogenic pain perception. Enkephalins have higher analgesic efficiency (18-30 times higher at the molecular level) than morphine has. In addition they take part in sleep regulation, food intake, sex as well as in regulation of behavior, study or memory. Their practical application is impossible because of incapability to permeate the structures of hematoencephalic barrier to the brain.

Adenohypophysis produces many other peptide hormones, e.g. **corticotropin, tyreotropin, melanotropins, lipotropins**.

9.2.2. Peptide antibiotics

Hundreds of peptide antibiotics have been described in the past half-century. These inhibit growth and reproduction of microorganims, mainly of bacteria. They fall into two classes, non-ribosomally synthesized peptides, such as the gramicidins, valinomycin, actinomycin polymyxins, bacitracins, glycopeptides, etc., and ribosomally synthesized (natural) peptides. The former are often drastically modified and are largely produced by bacteria, whereas the latter are produced by all species of life (including bacteria) as a major component of the natural host defense molecules of these species. The natural peptides represent a new opportunity for the medicinal chemistry with emphasis on the role in natural host defenses (as nature's antibiotics) and the clinical potential of peptides derived from these natural peptides. Penicillines may be considered as derivatives of peptides.

9.2.3. Toxic peptides

Peptide poisons (toxins) are produced by different animals and plants and have a great efficiency. **Plant toxins** are e.g. **phallotoxins** (bicyclic heptapeptide phalloidin and another peptides occurring in *Amanita phalloides* fungus) which are rapidly-acting and interrupt actin polymerization or impair cell membrane function, or **amatoxins** (cyclic **oktapeptide α-amanithin**), which are about 10-times more toxic than phallotoxins and interfere with RNA polymerase II and prevent the transcription of DNA. **Animal peptide toxins** are e.g. **cobratoxin** (snake venom), which is a mixture of toxins and different enzymes used for other purposes like increasing the prey's uptake of toxins and **botulinum toxin** with neurotoxic effects produced by the bacterium *Clostridium botulinum*.

9.2.4. Glutathione and other biochemically important peptides

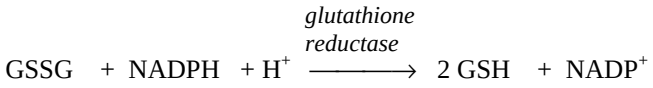
Glutathione is one of the most widely distributed peptides in nature. It can be found practically in all cells (with the exception of some bacteria). It is tripeptide (γ-glutamyl-cysteinyl-glycine) belonging to the typical low molecular intracellular antioxidants.

Glutathione:

a) is cofactor of some enzymes, which take part in antioxidative mechanisms (glutathione peroxidase, *glutathione transferase, dehydroascorbate reductase)*
b) takes part in transport of amino acids across the cell membrane
c) is direct scavenger of OH radical and $^1O_2$,detoxicates H_2O_2 and lipoperoxides by GPx

d) can reduce tocopherol radical
e) protects -SH groups in protein molecules from oxidation (redox buffer)
f) takes part in detoxification of xenobiotics (in cooperation with *S-glutathione transferase*).

The ratio of physiological concentrations GSH:GSSG is 10-100:1. GSSG can be reduced by *glutathione reductase* to regenerate GSH in reaction:



Glutathione as redox system is structurally illustrated in the figure 9.4:

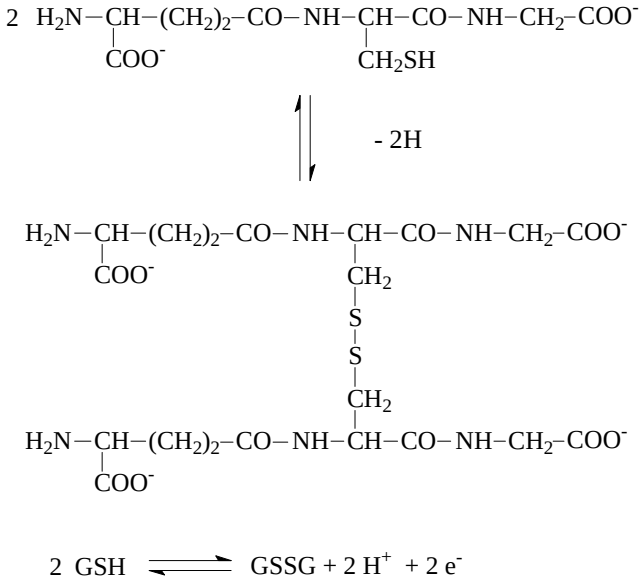


Fig. 9.4. Gluthione as redox system
(GSH – reduced glutathione, GSSG – oxidized glutathione)

Oxidized glutathione at higher (non-physiological) concentration can oxidatively damage many enzymes (*adenylate cyclase, phosphofructokinase*). Probably it reacts with thiol groups of proteins and creates mixed disulfides GSSR.

Carnosine is another important peptide – β-alanylhistidine. It occurs in human skeletal muscles. N-methyl-carnosine – **anserine** – is not present in humans and it occurs in skeletal muscles characterized by rapid contractile activity (rabbit limb, bird pectoral muscle). Carnosine and anserine activate myosin *ATPase* activity. Both dipeptides can also chelate copper and enhance copper uptake.

9.3. Proteins

Proteins occur in each cell and form almost half of the body weight. They give strength and elasticity to the skin and blood vessels. Other proteins have defence functions (antibodies) or they serve as transporters of some molecules (lipids, oxygen, …) for long distances. Almost all enzymes, some hormones and cell membrane receptors are proteins that control reparation, construction, communication and energy transformation in the organism. No other class of compounds has such heterogeneous functions inevitable for life.

9.3.1. Protein structure

Proteins are macromolecules consisting at least from one peptide chain that is formed by polymerization of 100 and more amino acid units connected with peptide bonds.

From chemical composition viewpoint proteins contain in average about 55 % carbon, 21 % oxygen, 17% nitrogen, 7% hydrogen and small amounts of sulphur and phosphorus. Human organism has no ability to synthesize proteins from basic substrates (inorganic nitrogen compounds, CO_2, H_2O) unlike some microorganisms and plants. That is why it is inevitable for human to receive proteins from external sources – vegetal and animal proteins. They undergo the process of digestion, during which they are cleaved into amino acids that are used for biosynthesis of specific own organism proteins.

Primary structure

Primary structure of protein is defined as a sequence of amino acid units in the polypeptide chain. The sequence of amino acids determines physical and chemical properties of proteins and is crucial in formation of all higher types of structures. The change in amino acid sequence (e.g. substitution of only one amino acid) can cause the change or complete damage of biological function of protein molecule (see subchapter 9.3.2.3).

Secondary structure

Secondary structure of protein is characterized with geometric arrangement of polypeptide chain. According to the mutual position of peptide bond planes there is recognized **structure of α-helix** and **β-structure** (pleated sheet structure) (Fig. 9.5.). These structures are stabilized with hydrogen bonds between –C=O and –NH groups of peptide bonds.

Tertiary structure

Tertiary structure is spatial arrangement of polypeptide chain of protein molecule and mutual spatial arrangement of all chains in molecule. Tertiary structure is stabilized by different types of bonds between the functional groups of side chains of amino acids: **disulfide bonds, hydrogen bonds, ionic interactions and hydrophobic interactions** (Fig. 9.6.). The final spatial shape of polypeptide chain is very important for biological activity of protein.

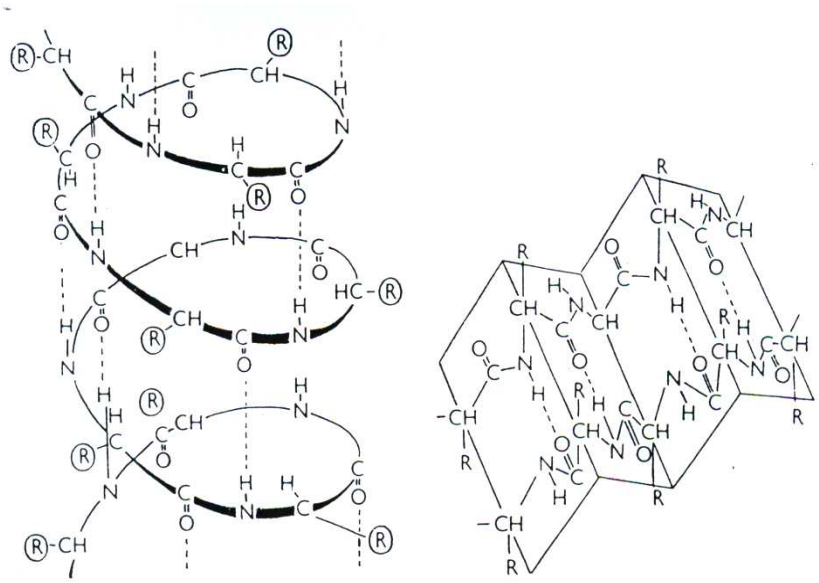
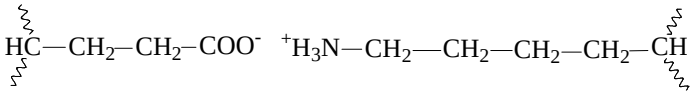


Fig. 9.5. Secondary structure of proteins
(left – α-helix, right – β-structure), R – side chains of AA

disulfide bonds ***hydrogen bonds***

Cys Cys Asp Ser

ionic interactions



Glu Lys

hydrophobic interactions

Leu Leu Phe Phe

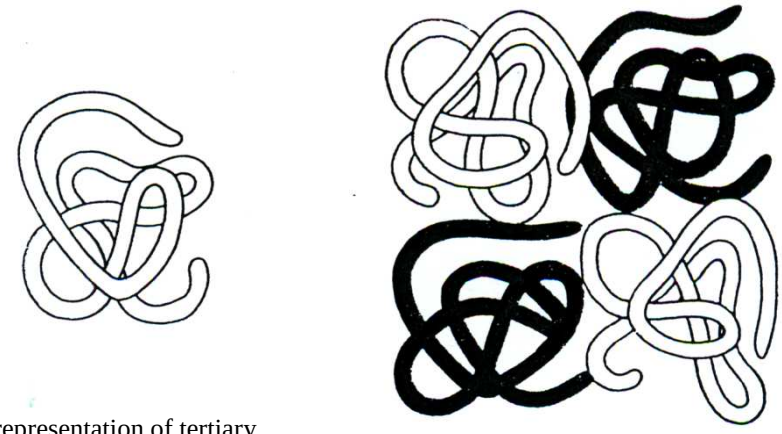
Fig. 9.6. The types of bonds stabilizing tertiary structure of proteins

According to the tertiary structure proteins can be divided into two basic classes:

a) ***globular***, which have peptide chain coiled into globular (spheroidal) shape suggestive of clew (e.g. enzymes); members of this class have regulatory, maintenance and catalytic roles in living organisms. They include hormones, antibodies and enzymes. These proteins are generally more sensitive to temperature and pH change than their fibrous counterparts.
b) ***fibrous***, which are aligned along an axis, have repeating elements, and are extensively linked to each other through hydrogen bonds (e.g. collagen, keratin, elastin). As the name implies, these substances have fibre-like structures, and serve as the chief structural material in various tissues. Corresponding to this structural function, they are relatively insoluble in water and unaffected by moderate changes in temperature and pH.
Subgroups within this category include:
- collagens and elastins - the proteins of connective tissues, tendons and ligaments.
- keratins - proteins that are major components of skin, hair, feathers and horns.
- fibrin - a protein formed when blood clots.

Quaternary structure

Quaternary structure represents the mutual arrangement of several subunits of proteins with already created tertiary structure. From composition of the peptide chain and character of higher structures viewpoint the subunits can be the same or not. They are bound to each other with electrostatic or hydrophobic interactions. The example of multiunit protein is hemoglobin (it is created by 2 α- and 2 β-subunits, i.e. tetramer) (Fig. 9.7.).



representation of tertiary structure of simple polypeptide chain

representation of quaternary structure (of oligomeric protein)

Fig. 9.7. Tertiary and quaternary structures of proteins

9.3.1.1. Denaturation

The natural or native structures of proteins may be altered, and their biological activity changed or destroyed by treatment that does not disrupt the primary structure. This denaturation is often done deliberately in the course of separating and purifying proteins. For example, many soluble globular proteins precipitate if the pH of the solution is set at the pI of the protein. Also, **addition of trichloroacetic acid or the urea is commonly used to** induce protein precipitation. Following denaturation, some proteins will return to their native structures under proper conditions; but extreme conditions, such as strong heating, usually cause irreversible change.

Some treatments known to denature proteins are listed in Tab. 9.3.

Tab. 9.3. The ways of denaturation of proteins

Denaturing Action	**Mechanism of Operation**
Heat	hydrogen bonds are broken by increased translational and vibrational energy (coagulation of egg white albumin on frying)
Ultraviolet Radiation	similar to heat (sunburn)
Strong Acids or Bases	salt formation; disruption of hydrogen bonds (skin blisters and burns, protein precipitation)
Urea Solution	competition for hydrogen bonds (precipitation of soluble proteins)
Some Organic Solvents (e.g. ethanol & acetone)	change in dielectric constant and hydration of ionic group. (disinfectant action and precipitation of protein)
Agitation	shearing of hydrogen bonds (beating egg white albumin into a meringue)

9.3.1.2. Isoelectric point of proteins

Molecules of proteins in solution are electrically charged particles. Carriers of charges are functional groups of side chains of amino acid residues – particularly $-COO^-$ and $-NH_3^+$ groups. The charge of these groups is dependent on the degree of their ionization and **every protein** (like amino acids) **has own pH value at which it is externally electroneutral**. This pH value is called "**isoelectric point**" (**pI**). At this pH proteins do not move in electric field. The value of pI for neutral amino acids is given as $pI = (pK_1 + pK_2) / 2$, where K_1 and K_2 are ionization constants of –COOH and $-NH_2$ groups. In the dependence on pH a protein obtains positive (when pH < pI) or negative (when pH > pI) charge.

The charge of a protein is very important for its activity. It influences hydration of a protein, solubility, viscosity, stability of protein solution, etc.

9.3.2. Classification of proteins

Proteins can be formed only from polypeptide chains. In this case they are signed **as holoproteins**. In **heteroproteins** a non-protein part is linked with a polypeptide chain.

Proteins are macromolecules with wide scale of properties, structures and functions and that is the reason why they are classified according to different criteria:

a) according to the structure into ***fibrou***s, ***globular*** and ***membrane***

b) according to solubility into ***albumins, globulins, glutelins, gliadines*** (prolamins), ***histons*** and ***protamines***

c) according to a nonprotein part into ***simple (holoproteins)*** (without a nonprotein component) and ***complex (heteroproteins)*** (nucleoproteins, glycoproteins, phosphoproteins, chromoproteins, lipoproteins, metaloproteins)

d) according to status into ***natural (native)*** (they have full biological activity), ***denaturated*** (with changed or completely destructed former activity) and ***modified*** (they have chemically bound usually low molecular weight compounds)

e) according to the biological function into ***structural, catalytic*** (enzymes), ***transport, motional, defense*** (antibodies), ***storage, nutritional, senzoric*** and ***regulation***.

Currently, the most often used classification of proteins is according to predominant outer shape to the fibrous (fibrous), globular and membrane. The interface is sometimes not completely sharp, e.g. some globular proteins form fibrous aggregates and some fibrous proteins can have quite extensive globular domain.

9.3.2.1. Globular proteins

Globular proteins are a very large group of proteins with various biological functions. They are usually soluble in water (unlike fibrous proteins) and in solution exist as free, independent molecules. They have a round or a spherical shape. The main polypeptide chain and the majority of nonpolar side chains are located in the compact core while on the surface of the molecule, in contact with water are present mainly polar chains. The conformation of globular proteins or their domains are generally created by the relatively low number of building elements. Protein domains are protein regions with a characteristic primary, secondary and tertiary structure, which determine the specific function of the protein segment. The interaction between domains of the protein molecule is the basis of its biological function.

According to domains which prevail, proteins can be classified into structural classes:

- α-proteins – α-helixes dominate in them. Their portion in overall conformation is very different and not necessarily high. E.g. in myoglobin and hemoglobin 75 % of all amino acid residues participate in the eight α-helixes, in lysozyme 30 % and in cytochrome even only 10 %.
- β-proteins have a significant portion of associated β-structures. To this group belongs for example the carbonic anhydrase. Associated β-chains are also found in the domains of immunoglobulins or in acidic and serine *proteases* present in digestive system (*pepsin*, *trypsin* and *chymotrypsin*).
- α/β-proteins – in their conformation are significantly present the structures, which are formed by the association the α-helixes and β-chains. There are many enzymes belonging to this group (*carboxypeptidase kinase, aldolase*)
- (α+β)-proteins contain in their molecules α-helixes as well as β-chain segments, but they do not interact with each other. These are for example *lysozyme*, *ribonucleases*, insulin or catalytic domain of the NAD^+-*dependent dehydrogenases*.

9.3.2.2. Fibrous proteins

Fibrous proteins are a group of proteins that form microscopic fibers (fibrils). They form the basis of the internal structure of the cells and cytoskeleton, they endow connective tissue with cohesion, tensile strength or flexibility. They give the resistance to the body surface and are components of contractile elements. They are usually water insoluble, some of them are extremely chemically resistant. Structure of fibrous proteins is very heterogeneous, in some α-helical structure is predominant (keratin, tropomyosin, myosin), for collagen triple helix is typical (composed of chains with the secondary structure of α-helix) and in another proteins β-structure predominates (fibroin).

A typical representative of the α-helical fibrous protein is **α-keratin**, which is the primary ingredient of mammals cornified surface layer of the skin and skin derivatives (hair, body hair, nails, horns and hooves). α-keratins are weakly basic or neutral and form a right-handed helix structure. The basic of their structure are α-helix dimers, which coil in two-strand helixes. A pairs of helixes form a fibrous formations – protofibrils – and several protofibrils produce microfibrils. α-keratin chains are rich in cysteine residues, which are connected to adjacent polypeptide chains by disulfide bonds.

Collagen is the most abundant extracellular water-insoluble protein that is the basic building material of the supporting tissues (loose binder, tendons, cartilage and bone as it has a high tensile strength). In Mammal it makes up 25-30% of all proteins. In the form of fibers, collagen is the most important component of the extracellular matrix. At present we know of at least 27 different types of collagen, referred as I - XXVII. High proportion of proline and glycine residues produces characteristic secondary structure of helix, which is stabilized by hydrogen bridges between the three chains to form tropocollagen units. Each subunit contains 1050 amino acids, and when the chain is coiled the helix is about 300 nm long. Tropocollagen units (triple-stranded left-handed helix) are bound to form the microfibrils. In microfibrils adjacent tropocollagen units are shifted by a quarter of their length (64 nm) (in the electron microscopy we can see the cross-bands). The microfibrils are further stabilized by intramolecular cross-covalent bonds, which are formed mostly by reaction of lysine side chain residues (Fig. 9.8).

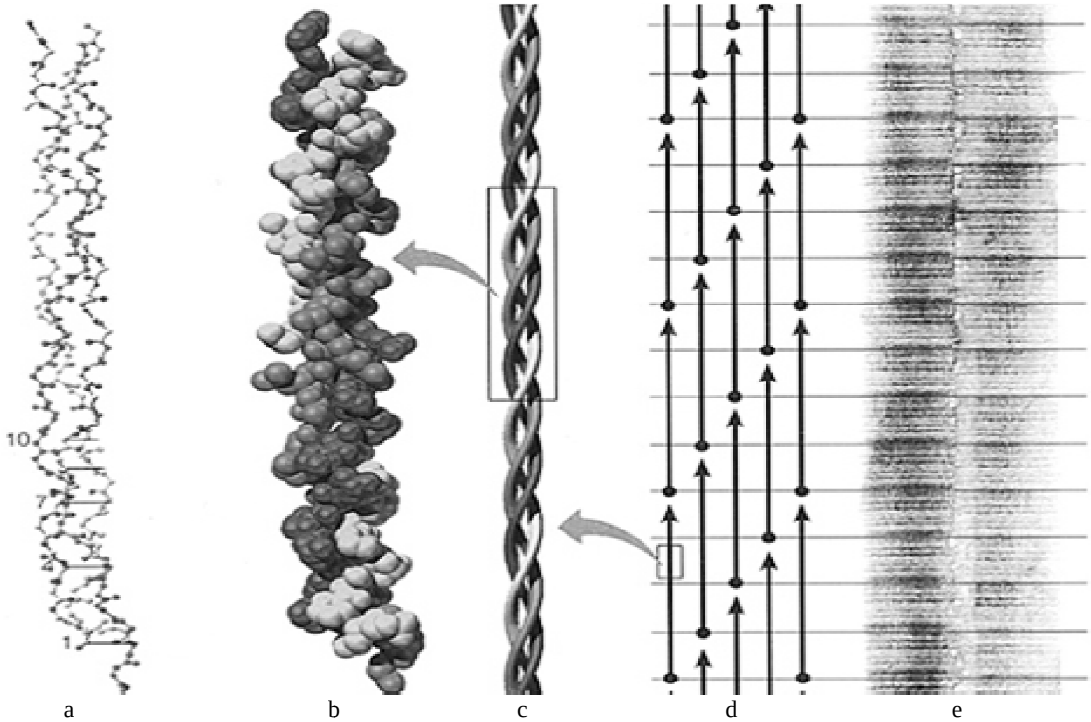


Fig. 9.8 The structure of collagen

(a-c) three subunits of collagen coiled into triplex (triple helix); (d-e) adjacent collagen molecules overlap at fibrils ends (overlap by 67 nm), resulting in a visible bands.

Elastin is a fibrous protein that in some types of supportive tissue prevails collagen (elastic connection, arteries, lungs, skin). Elastin is not soluble in any standard solvent, heat does not cause its denaturation and it exhibits rubber-like elasticity. Polypeptide chains of a soluble tropoelastin form helical regions rich in glycine. Among them there are numerous short segments with the alanine and lysine residues. The flexibility of elastin is given by crosslinking of tropoelastin chains through the lysine side chains, which are bound by strong covalent bonds within a molecule as well as intermolecularly.

Fibroin is keratin-like protein of silk fiber. In its primary structure amino acid residues of glycine, alanine and serine prevail, for the secondary structure anti-parallel β-chains are characteristic. These structures are stabilized by the hydrophobic interactions between the side chains. Fibroin fibers are firm and well-flexible, but they have little elasticity.

9.3.2.3. Membrane proteins

The proteins are inseparable part of biological membranes, basis of which is lipid bilayer. Some of membrane proteins only stabilize the membrane structure, however many of them perform various biological functions - facilitated transport of substances across the membrane, the catalytic function, specific binding of signal molecules and transmission of signals through the membrane, or they are the carriers of antigenic determinants, etc. On the outside of the plasma membrane these proteins are often glycosylated (e.g., glycoproteins). Glycoproteins, together with glycolipids, form the protective glycocalyx on the cell surface.

Membrane proteins are divided into three classes according to their interaction with the lipids in the lipid bilayer:

1. Integral (transmembrane) - the protein chains pass across the membrane
2. Bound via the lipid anchor
3. Peripheral

Integral proteins are more or less immersed in the hydrophobic interior of the lipid bilayer, possibly penetrate it (transmembrane, penetrating proteins) and consist of three parts - domains. Cytosolic and extracellular domains have a hydrophilic character and their amino acid composition and structure are similar to other water-soluble proteins. The third domain that penetrates the 3 nm thick membrane contains many hydrophobic amino acids, which interact with the hydrocarbon core of the bilayer. This domain has usually α-helical secondary structure with several α-helixes. Hydrophobic interactions are responsible for quite tight binding of integral proteins and membrane. Isolation of integral proteins from membrane is difficult and it usually causes loss of their biological functions. Integral proteins are usually glycoproteins. They contain oligosaccharides that are above the surface of the membrane and often they are a part of receptors for various hormones, drugs or bioregulatory compounds as well as receptors by which the cells can recognize each other and mutually interact. Integral proteins are antigen-specific, and therefore significantly affect cell surface recognition by the immune system of the host.

The examples of integral transmembrane proteins are: complexes of *oxidoreductases* present in respiratory chain located in the inner mitochondrial membrane, components of *monooxygenases* transporting electrons in membranes of endoplasmic reticulum with the participation of cytochrome P450, Na^+/K^+*-ATPases*, receptors for neurotransmitters or hormones present in cytoplasmic membranes.

Proteins bound via the lipid anchors

These proteins bind covalently to one or more lipid molecules. The hydrophobic carbon chain of lipid is tightly bound in a single membrane layer and anchors the protein to membrane, while the polypeptide chain does not enter the lipid bilayer.

Lipid anchor types:

- **simple higher carboxylic acids** (myristic and palmitic acids). They are bound with protein via a terminal $-NH_2$ group of glycine forming amide bond. In this manner cytosolic protein Src is bound.
- **unsaturated isoprenoids** (farnesyl and geranyl). They are bound with protein via two cysteine residues closed to the C-end (e.g. cytosolic proteins Ras and Rab).
- **glycosylphosphatidylinositol** (GPI). This anchor comprises two chains of fatty acids, which bind to the lipid bilayer. Through –OH group of the inositol there is bound oligosaccharide which bind the protein. This type of anchor is typical for binding the proteins to extracellular side of membrane (e.g. erythrocyte *acetylcholinesterase*, *alkaline phosphatase* in the gut and placenta, or clathrin, applied in endocytosis).

Peripheral membrane proteins are bound to membrane by rather weak, mostly electrostatic interactions with polar groups of the membrane surface (integral membrane proteins or the polar part of the phospholipid). In contrast to the integral proteins they can be separated *in vitro* easily by changing pH or ionic strength. Peripheral membrane proteins are often close to the globular proteins. In some membranes they build up the structure of the membrane, e.g. spectrin in the erythrocyte membrane. Another peripheral membrane protein is the cytochrome c, one of electron carriers in the mitochondrial respiratory chain. One of important biological function of peripheral membrane proteins is participation in intracellular signal transduction.

9.3.3. Heteroproteins

Proteins containing also a non-protein component in addition to the polypeptide chain, are called heteroproteins or conjugated proteins. If this non-protein component is indispensable for the function of the protein and it is tightly bound we call it prosthetic group. Binding of prosthetic groups per molecule of protein is generally covalent and can be removed only by protein denaturation. A number of proteins contain more than one prosthetic group. According to the chemical nature of non-protein part we can classified heteroproteins into several groups that have important biological functions:

a) ***nucleoproteins*** – complexes of proteins with nucleic acids play an important role in the storage and transmission of genetic information, but also in proteosynthesis

b) ***glycoproteins*** – contain covalently bound carbohydrate ((subchapter 7.5.2.2.). To this group includes e.g. immunoglobulins, which are an important part of the immune system of the organism, but also peripheral and integral proteins of the cell membrane, to serve as receptors, transport molecules, and antigen.

c) ***phosphoproteins*** – contain phosphoric acid, which is bound to −OH groups of the side chains of amino acids such as serine, threonine and tyrosine by ester bond (e.g. casein, a phosphoprotein present in milk and cheese provides enough phosphorus for the growth of a young organism). Phosphorylation / dephosphorylation of proteins is an important regulatory mechanism.

d) ***metalloproteins*** – proteins containing at least one metal ions. Metal ions are usually coordinated by four sites consisting of the protein´s nitrogen, sulphur and/or oxygen atom. They are used either for the storage of metal ions (Fe-ferritin), or have a catalytic function (ceruloplasmin, Cu, Zn-superoxide dismutase, Fe-cytochrome oxidase, Cu-ascorbate oxidase, Zn-alcohol dehydrogenase, Mo-xanthine oxidase, etc.). To metalloproteins belongs also hemoproteins (e.g. hemoglobin, myoglobin), whose prosthetic group - heme, is color and contains iron ion bound by complex bond). They have a great importance and multiple biological functions, so they are often considered as a separate group.

e) ***lipoproteins*** – particles consisting of lipids and proteins and serve for transport of lipids in blood (subchapter 8.3.)

f) ***flavoproteins*** – proteins containing flavin as prosthetic group (riboflavin). These include e.g. enzymes from the group of oxidoreductases (subchapter 12.3.)

From viewpoint of ability to absorb visible light the specific type of conjugated proteins are known. They are called ***chromoproteins*** and they contain in their structure chromophores absorbing in the visible light area, which means they are "colored". Their prosthetic group often contains a metal element (Fe, Cu, Mg) bound to a chelating chromogen (these chromoproteins belong to metalloproteins), or they have only color chromogen (e.g. flavoproteins).

9.3.3.1. Immunoglobulins (Ig)

Immunoglobulins are glycoprotein molecules that are produced by plasma cells in **response to an immunogen** and which function as antibodies. The immunoglobulins derive their name from the finding that they migrate with globular proteins when antibody-containing serum is placed in an electrical field.

All immunoglobulins have a four chain structure as their basic unit. They are composed of two identical **light chains** (23kD) and two identical **heavy chains** (50 - 70kD). The heavy and light chains and the two heavy chains are held together by **inter-chain disulfide bonds** and by non-covalent interactions. The number of inter-chain disulfide bonds varies among different immunoglobulin molecules. Within each of the polypeptide chains there are also **intra-chain disulfide bonds** (Fig. 9.9.).

The region at which the arms of the antibody molecule forms a Y is called the **hinge region** because there is some flexibility in the molecule at this point.

The immunoglobulin molecule structure is not straight, but it is folded into globular regions each of which contains an intra-chain disulfide bond. These regions are called **domains:**

- Light Chain Domains – V_L and C_L
- Heavy Chain Domains – V_H, C_{H1} - C_{H3} (or C_{H4})

Carbohydrates are attached to the C_{H2} domain in most immunoglobulins. However, in some cases carbohydrates may also be attached at other locations.

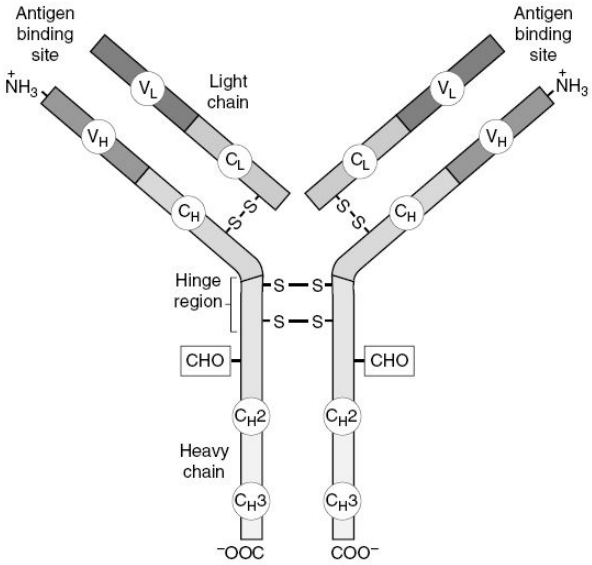


Fig. 9.9 Structure of immunoglobulin molecule

The immunoglobulins can be divided into **five different classes**, based on differences in the amino acid sequences in the constant region of the heavy chains, according to number of basic units ("Y"), according to antigen and other biological functions. All immunoglobulins within a given class will have very similar heavy chain constant regions. These differences can be detected by sequence studies or more commonly by serological means (i.e. by the use of antibodies directed to these differences).

1. **IgG**
   - is most versatile immunoglobulin because it is capable of carrying out all of the functions of immunoglobulin molecules.
   - is the major Ig in serum (12 500 mg/l), where it forms 70 - 75% of serum Ig.
   - its basic function is to bind to cells - macrophages, monocytes, PMN's and some lymphocytes which have receptors for IgG and it allows strong adhesion between phagocyte and phagocytized object.
   - is the only class of Ig that crosses the placenta and sets up the immunity of the fetus at the time its own immune mechanisms are not sufficiently developed.
   - it easily penetrates into extravascular space, where neutralizes bacterial toxins.
   - is a good opsonin (the term **opsonin** is used to describe substances that enhance phagocytosis).
   - fixes complement.

2. **IgA**
   - is the 2nd most abundant serum Ig (2500 mg/l).
   - exist in two forms – as serum IgA and secretory IgA, which is dimer of serum form (symbol SIgA).
   - is the major class of Ig in secretions - tears, saliva, colostrum, mucus. Since it is found in secretions secretory IgA is important in local (mucosal) immunity.
   - normally does not fix complement, unless aggregated.
   - can bind to some cells - PMN's and some lymphocytes.

3. **IgM**
   - is the third most abundant serum Ig (1100 mg/l).
   - as a consequence of its pentameric structure, IgM is a good complement fixing Ig, so IgM antibodies are very efficient in the lysis of microorganisms.
   - is the first Ig to be made by the fetus and the first Ig to be made by a virgin B cells when it is stimulated by antigen.

4. **IgD**
   - its role in serum is not yet clear completely
   - is a main immunoglobulin with function of antigen receptor located in the membrane of immunocompetent lymphocytes (special type of lymphocytes, which after contact with respective antigen start immune response)
   - is found in low levels in serum (50 mg/l)
   - does not bind complement

5. **IgE**
   - is Ig with the lowest level in serum (0,15 mg/l).
   - is bound very tightly to Fc receptors on basophils and mast cells even before interacting with antigen.
   - also plays a role in parasitic helminth diseases.
   - is involved in allergic reactions.
   - does not bind complement.

9.3.3.2. Cu/Zn-Superoxide dismutase

Cu/Zn Superoxide dismutase is categorized as an oxidoreductase class of enzyme and specifically catalyzes dismutation of the superoxide radical to non-radical molecules O_2 and H_2O_2 (subchapter 14.2.1.). In active site of this enzyme the zinc ion is bound to three histidine residues and one aspartate residue. The copper atom is bound to four histidine residues. The two metal ions are connected via a histidine bridge (Fig. 9.10.). Zinc atom does not take part in reaction mechanism, its function is stabilization of enzyme structure and activity in pH range 5 to 9.5. Copper atom is directly taking part in dismutation reaction.

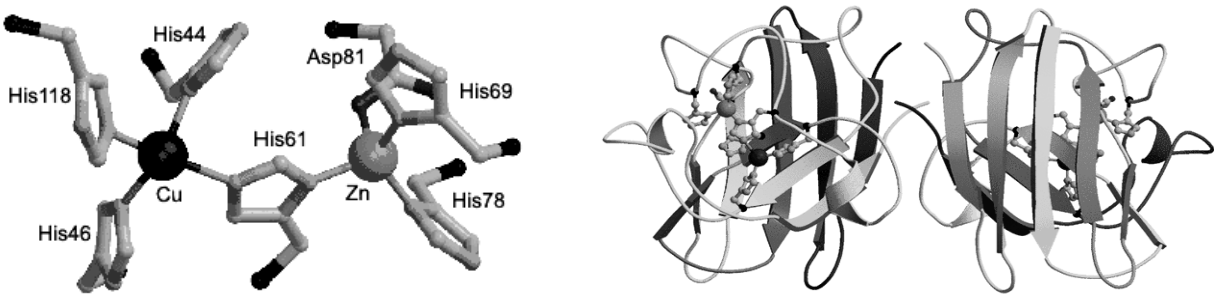


Fig. 9.10. Structure of *Cu/Zn superoxide dismutase* (active site – left; dimeric structure of enzyme – right)

9.3.3.3. Hemoproteins

Hemoproteins belong to chromoproteins. In this group there are included many complex proteins, but the most important of them are hemoglobin and myoglobin, which participate in transport of molecular oxygen in all vertebrates and in man. Myoglobin is very important especially for the life of aquatic animals. It mediates transport of oxygen in the muscles (heart and skeletal) and also serves as a reservoir of oxygen in the case of temporary deficiency.

Nearly all the oxygen carried by whole blood in animals is bound and transported by hemoglobin in erythrocytes (red blood cells). Hemoglobin (*M*r 64.500; abbreviated Hb) is roughly spherical, with a diameter of nearly 5.5 nm. It is a tetrameric protein consisting of four subunits of very similar but not completely identical types α and β chains (α2β2): α-chain consists of 141 amino acid residues and β-chain of 146 amino acid residues (Fig. 9.11).

Each subunit binds one heme molecule containing iron with oxidative number +2 (ferro forms), and with a coordination number 6. Iron is bound to four nitrogen atoms of the pyrrole rings (porphine). The fifth coordination bond is linked to the protein component (globin) through N atom of the histidine side chain. Sixth coordinate bond remains free for reversible binding of oxygen or other molecules such as H_2O, CO, –CN, etc. Oxygen free hemoglobin is denoted as hemoglobin or ***deoxyhemoglobin*** (***HHb***), oxygen carrying hemoglobin (oxygenated) is denoted as ***oxyhemoglobin*** (***OHb***. If Hb binds CO, ***carbonylhemoglobin*** (or ***carboxy-hemoglobin***, ***COHb***) is formed, and if –CN is bound, ***cyanohemoglobin is formed***. Oxidation number of iron in

the hemoglobin is +2 but it can be also +3, when the prosthetic group generally denotes ***hemin*** and after its binding to globin ***ferrihemoglobin*** is formed (often named by older name - ***methemoglobin).*** Oxygen molecules can be reversibly bound only by ferro forms (with Fe^{2+}).

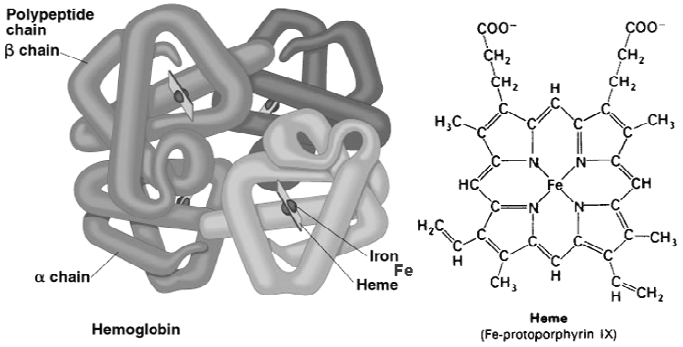


Fig. 9.11. Structure of hemoglobin and heme

The quaternary structure of hemoglobin features strong interactions between unlike subunits. In addition to **carrying nearly all the oxygen required by cells from the lungs to the tissues**, hemoglobin **carries** two end products of cellular respiration, **H^+ and CO_2, from the tissues to the lungs and the kidneys**, where they are excreted. The CO_2, produced by oxidation of organic fuels in mitochondria, is hydrated to form bicarbonate (subchapter 5.5.2.) Hemoglobin transports about 40 % of the total H^+ and 15 – 20 % of the CO_2 formed in the tissues to the lungs and the kidneys. The remainder of the H^+ is absorbed by the plasma bicarbonate buffer; the remainder of the CO_2 is transported as dissolved HCO_3^- and CO_2. The binding of H^+ and CO_2 is inversely related to the binding of oxygen.

In principle, myoglobin and hemoglobin have the same essential function (transfer of O_2), based on their very similar structure. However, the basic difference is that the myoglobin molecule consists only of a single polypeptide chain, and contains only one heme, while the hemoglobin molecule consists of four chains comprising a total four hems. Myoglobin is a monomer (MW = 17 kD) and hemoglobin tetramer (Mr = 68 kD). Myoglobin constitutes the primary, secondary and tertiary structure, while the hemoglobin also quaternary, indicating that the hemoglobin is allosteric protein. Creating a tetramer hemoglobin gains completely new biological properties, which are not observed in myoglobin. The bonds between chains have non-covalent nature.

The binding of oxygen to hemoglobin is affected by allosteric effectors, ions or molecules such as H^+, CO_2 and organic phosphates like 2,3-bisphosphoglycerate (BPG). The binding of oxygen to hemoglobin depends on the pH, while the binding capacity of hemoglobin for O_2 is affected by CO_2 and organic phosphates regulate the affinity of hemoglobin for oxygen. Dissociation curve of hemoglobin is sigmoidal in contrast to the course of myoglobin (Fig. 9.12), what is typical for allosteric interactions. It can be said that, although the O_2, H^+, CO_2 and BFG bind to spatially different locations of hemoglobin, they communicate with each other due to conformational changes which are proceeding within the protein molecule. Unlike the myoglobin molecule hemoglobin one can change the structure responding to their surroundings.

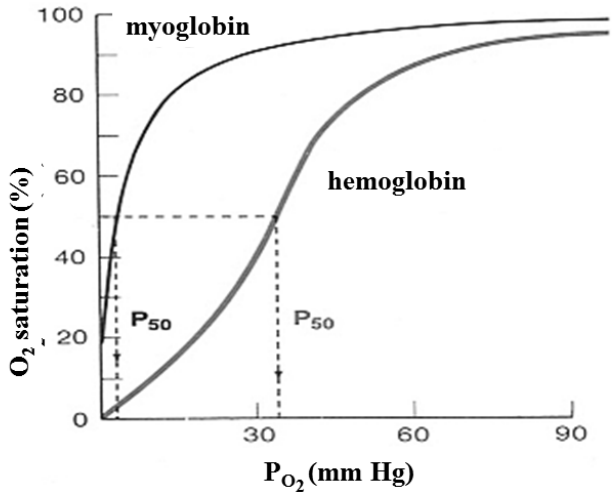


Fig. 9.12 Saturation curve of myoglobin and hemoglobin

Hb may be influenced by various other external factors - oxidative factors, blood poisons and certain drugs (sulfonamides, antipyrine, nitrites, nitrobenzene, etc.)

During the development of human three types of hemoglobin are physiologically formed and they are differing in amino acid composition of their chains:

- **embryonal hemoglobin** (Hb E): Gower I ($\zeta_2\varepsilon_2$), Gower II ($\alpha_2\varepsilon_2$), Portland ($\gamma_2\zeta_2$) – it is formed before the birth
- **fetal hemoglobin** (Hb F) ($\alpha_2\gamma_2$) – it is a dominant type of hemoglobin in the fetus body and is characterized by higher affinity for oxygen, which makes the binding oxygen from mother´s body easier.
- **adult hemoglobin** (Hb A) – it is formed after birth and is the most widespread human hemoglobin. Hb A_1 ($\alpha_2\beta_2$) represents 97.5 % and Hb A_2 ($\alpha_2\delta_2$) 2.5 % of body hemoglobin.

There are also abnormal hemoglobins, for example Hb S in sickle cell anemia (Chap. 9.3.3.4).

9.3.3.4. Sickle cell anemia and changed hemoglobin

The basis of the disease is a change in primary structure, thereby in all other structural characteristics of hemoglobin. Sickle-cell disease occurs more commonly in people (or their descendants) from parts of the world such as sub-Saharan Africa, where malaria is or was common, but it also occurs in people of other ethnicities.

This disease is on the molecular level caused by a **point mutation in the β-globin chain of hemoglobin**, replacing the amino acid glutamic acid with the less polar amino acid valine at the sixth position of the β chain. This means that the $–COO^-$ group of the side chain, which is electrically charged and hydrophilic, is replaced by isopropyl side chain, which is neutral and hydrophobic (see Chapter 10.6.3.).

Normal hemoglobin (Hb A) and sickle cell hemoglobin (Hb S) therefore have a different type of electric charges. Both have almost the same solubility in a well oxygenated blood, but deoxygenated Hb S accumulates within the red cells and precipitates. This accumulation deforms the cells to the typical sickle shape, which makes the movement of erythrocytes through the capillaries difficult, worsens the blood circulation and burdens the heart with increased strain (Fig. 9.13).

Sickle cell anemia provides certain resistance to malaria, which explains, why this inherited feature survives mostly where malaria is most common. The parasite that causes malaria, guests in erythrocytes. However it cannot survive for a very long time inside of the curved cell, since it has a high need for potassium ions, but the membrane of curved cell allows to excessive amount of K^+ to leak from the cell. Thus the people with sickle cell disease may survive for a long time and can give birth to children, to which inherited feature is transferred again.

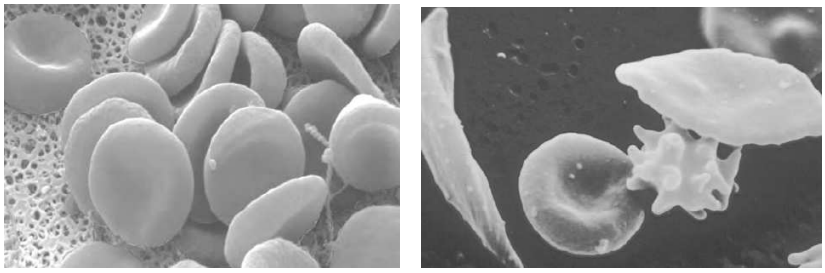


Fig. 9.13. Normal erythrocytes (left) and sickle (right) (pictures from electron microscope))

9.3.4. Blood plasma proteins

Proteins represent quantitatively the most important component of blood. In the whole blood hemoglobin is at the highest concentration, 2.2 – 2.5 mmol/l. Total concentration of plasma proteins is not determined in the whole blood but always only in blood plasma or serum. There are many different proteins in blood plasma as well as in serum (7.0 – 7.5 % of total weight) while the other dry matter components form only 1.5 %.

The main protein components of plasma are **albumins** and **globulins**.

Albumins represent 55 – 60 % of plasma proteins with different functions:

- maintaining of osmotic pressure (oncotic part of blood osmotic pressure)
- transport of compounds
- buffer functions (together with other proteins)
- reserve proteins (e.g. during starving)
- antioxidative ability

Globulins are highly heterogeneous group of proteins, they have mainly character of glycoproteins and some fractions have specific functions:

- hemocoagulation
- immunological function (antibodies)
- part of complement (defense mechanisms of organism)
- binding and transport of some compounds like metals, oxygen, lipids and steroids
- they have properties of enzymes or inhibitors of enzymes

Tab 9.4. summarizes some proteins of globulin character and their functions.

Tab. 9.4. Selected plasma proteins of globulin character

Protein	Biological function
transferrin	binds and transport plasma iron
ceruloplasmin	binds most of plasma copper
fibrinogen, prothrombin	coagulation factors
haptoglobins	bind hemoglobin up to concentration 1g in 1 liter of blood
immunoglobulins	antibodies, participation in defence mechanisms of organism
lipoproteins	transport of lipids (insoluble in water) in blood

In blood there are occasionally (e.g. in case of some bone marrow tumors) occurring anomalous proteins (paraproteins), which are not found in healthy human at all. Albumins concentrations are decreased in some diseases. This “hypoalbuminemia” is very noticeable in some kidney and liver diseases.

Control questions

1. Which of biogenic amino acids can create connection between carbohydrate and protein component of glycoproteins via an O-glycosidic bond?
2. Write the equation of cysteine ionization in an alkaline medium.
3. Write the equation of lysine ionization in an acidic medium.
4. Write general reaction of carbamine acid formation. What is the biological importance of the reaction?
5. Write the equation of oxidative deamination and subsequent decarboxylation of alanine. Name the products.
6. Write the equation of transamination reaction of aspartic acid and 2-oxoglutaric acid and name the products.
7. Write the reaction of dipeptide carnosine formation and indicate its biological function.
8. Describe the composition and biological role of the most important tripeptide.
9. Which amino acid is involved in the stabilization of the tertiary structure via disulfide bond? Express structurally formation of such bond.
10. Explain the concept of allosteric protein, give example.
11. Provide structural and functional differences between myoglobin and hemoglobin.
12. Name the most famous fibrous proteins, their structure and biological importance.
13. Which coenzyme comprises in its structure a biogenic amine, which is produced by decarboxylation of aspartic acid.

10. NUCLEOTIDES AND NUCLEIC ACIDS

One of the most remarkable properties of living cells is their ability of self reproduction. This ability continues almost with absolute perfection for hundreds and thousands of generations. Some organisms are so complex, that it is difficult to imagine the fact of preservation of genetic information in so small objects as nucleus of germ cells (sperm and ova). For reproduction biopolymer compounds – **nucleic acids** are responsible**.** Nucleic acids were discovered in 1869 by Johann Friedrich Miescher (1844-1895), who called the material "nuclein" since it was found in the nucleus of animal cells. It was discovered later than prokaryotic cells, which also contain nucleic acids even though they do not have a nucleus. .

Nucleic acids are natural macromolecular compounds with a specific biological function. Nucleic acids store and regulate the transmission of genetic information. They determine genetic properties of living organism, affect its organization and reproduction, carry information necessary for all life processes.

In the organism, nucleic acids are primarily found in the form of complexes with proteins as **nucleoproteins**. They can be found not only in the nucleus, but also in the cytoplasm and other subcellular structures, such as ribosomes, mitochondria and chloroplasts.

Each biological species differs from the others because of structural differences in their proteins.

Chromosomes, located in the nuclei of cells, contain the hereditary information that directs the synthesis of approximately 100.000 proteins unique to a human being. The sum of all human proteins is called the **human proteome**. All human cells except germ cells are diploid, what means that they contain two copies of each chromosome. Germ cells are haploid cells with only one copy of each chromosome.

Every chromosome contains a large number of **genes**, the fundamental **units of heredity**. Genes are responsible for both the traits common to a species and for the unique traits of individual members of that species. Each gene carries the information for synthesizing one or more polypeptides, which are responsible for those hereditary features.

At the molecular level, the growth and reproduction of organisms are directed and carried out by two types of nucleic acids – **ribonucleic acids (RNAs)** and **deoxyribonucleic acids (DNAs)**. Chromosomes contain DNA molecules. Each gene is just a part of a DNA molecule. DNA contains the hereditary information and directs its own reproduction and the synthesis of RNA. RNA molecules leave the cell nucleus and regulate the synthesis of proteins in ribosomes, which are organelles in the cytosol. The cytosol is the region of the cell outside of all the organells in the cell.

Nucleic acids are polymeric molecules of which the basic unit is a nucleotide.

10.1. Structural units of nucleotides

Nucleotides can be hydrolysed into three components (Fig. 10.1.):

a) a heterocyclic nitrogen base (adenine, guanine, cytosine, thymine, and uracil, resp.),
b) a pentose saccharide (D-ribose or 2-deoxy-D-ribose),
c) phosphoric acid (H_3PO_4).

They are present at a ratio of 1 : 1 : 1.

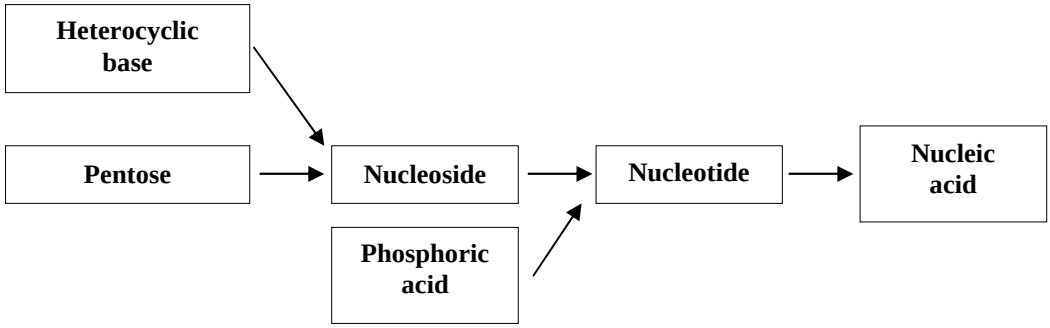


Fig. 10.1. Relation of components of nucleotides to nucleic acid

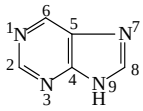
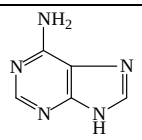
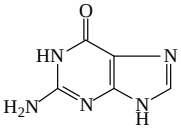
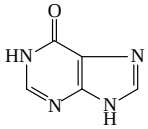
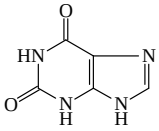
10.1.1. Nitrogen heterocyclic bases

Nitrogen heterocyclic bases are classified into two types: purine and pyrimidine bases. The **purine bases** belong to the double-ringed class of heterocyclic molecules (pyrimidine + imidazole) involving **adenine** and **guanine**, while **pyrimidines** contain six-membered rings. **Cytosine** and **thymine** belong to this group. A fifth pyrimidine base, named **uracil** (present in RNA), usually replaces thymine (present in DNA) and differs from thymine by the lack of a methyl group at the 5 position in its ring (Tab. 10.2.). Uracil is not usually found in DNA, it occurrs only as a product of cytosine deamination. In contrast, following synthesis of certain RNA molecules, a significant number of the uracils are converted to thymines by the enzymatic addition of the missing methyl group. This occurs rather in structural and enzymatic RNAs than in transfer RNAs and ribosomal RNAs.

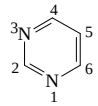
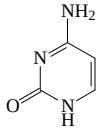
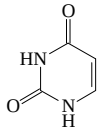
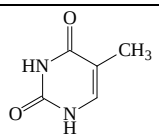
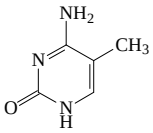
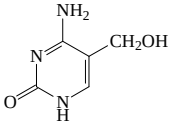
The symbol of each of the five bases is the first letter of its name – A, G, C, T and U for adenine, guanine, cytosine, thymine, and uracil, respectively.

The molecules of nucleic acids contain a certain amount of the **minority bases**, which are chemical modifications of the basic nitrogen bases mentioned above. Their molecules usually contain a methyl (5-methyl cytosine, 1-methyladenine, 7-methylguanine…) or hydroxyl (5-hydroxylmethylcytosine) group. More than 30 various minority bases have been isolated so far. The complete view of purine and pyrimidine bases, their structure and names are presented in the Tab. 10.1. and Tab. 10.2.

Tab. 10.1. Nitrogen bases and nucleosides derived from purine

Base		Formula	Nucleoside	Symbol
Purine				
Adenine (6-aminopurine)	*Basic bases*		**Adenosine**	**A**
Guanine (2-amino-6-oxopurine)			**Guanosine**	**G**
Hypoxanthine (6-oxopurine)	*Minority bases*		**Inosine**	**I**
Xanthine (2, 6-dioxopurine)			**Xanthosine**	**X**

Tab. 10.2. Nitrogen bases and nucleosides derived from pyrimidines

Base		Formula	Nucleoside	Symbol
Pyrimidine				
Cytosine (2-oxo-4-aminopyrimidine)	*Basic bases*		**Cytidine**	**C**
Uracil (2, 4-dioxopyrimidine)			**Uridine**	**U**
Thymine (2,4-dioxo-5-methyl pyrimidine)	*Minority bases*		**Thymidine**	**T**
5-methylcytosine (2-oxo-4-amino-5-methyl pyrimidine)			**5-methycytidine**	**McC mC**
5-hydroxymethylcytosine (hmC)			**5-hydroxy-methylcytidine**	**hMcC hmC**

According to the newest nomenclature, the use the three-letter abbreviations for nitrogen bases is recommended (similarly to amino acids):

Purines	Pyrimidines
***Ade* - Adenine**	***Thy* - Thymine**
***Gua* - Guanine**	***Cyt* - Cytosine**
***Xan* - Xanthine**	***Ura* - Uracil**
***Hyp* - Hypoxanthine**	***Oro* - Orotate**

Free purine and pyrimidine bases have very similar properties. Only few of them are soluble in water and most of them have the ability to form tautomeric forms – lactam (oxo-form) and lactim (enol-form). Only adenine which does not contain oxygen has not this feature, but it exhibits amino-imino tautomerism (Fig. 10.2). In neutral environment lactam form predominates (Tab. 10.1. and 10.2.). This organization is very important for hydrogen bonds formation between so called complementary bases resulting in formation of the secondary structure (Fig. 10.12.).

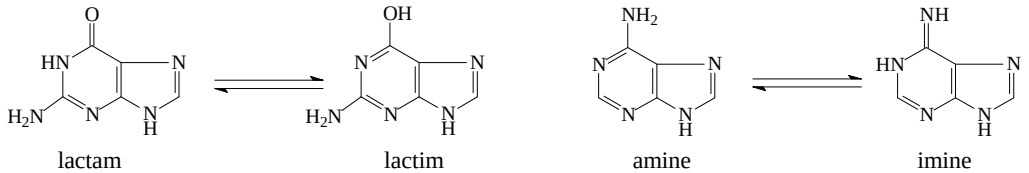


Fig. 10.2. Tautomeric forms of guanine and adenine

Atoms of nitrogen, which are incorporated in the structure of heterocycles, have weak basic character. Structure of them is nearly planar and causes absorption of UV light with absorption maximum at 260 nm. This property is used for spectrophotometric determination of nucleic acids.

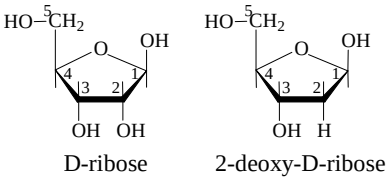
10.1.2. Saccharide component of nucleotides

After complete hydrolysis of nucleic acids, presence of D-ribose and 2-deoxy-D-ribose (Fig. 10.3.) was proven. According to saccharide unit we distinguish two types of nucleotides:

a) **deoxyribonucleotides** – contain 2-deoxy-D-ribose,
b) **ribonucleotides** – contain D-ribose.

The saccharide (pentose) unit occurs in nucleotides in the cyclic form (β-ribofuranose or β-deoxyribofuranose), capable to form β-N-glycosidic bond with pyrimidine and purine bases.

Presence of saccharide units causes increased solubility of nucleosides in comparison with free bases. In acidic environment they hydrolyse relatively fast (pyrimidine nucleotides are more resistant).



D-ribose 2-deoxy-D-ribose

Fig. 10.3. Monosaccharides present in nucleic acid

10.1.3. Phosphoric acid

Phosphoric acid is bound with pentose by ester bond. Dehydration between phosphoric acid and deoxyriboses takes place at C-5 or C-3 of pentose ring, in case of ribose reaction through C-2 is possible.

10.2. Nucleosides

Nucleosides are heteroglycosides. They are formed by dehydration reaction between a saccharide unit (ribose, deoxyribose) and a heterocyclic base while the **β-N-glycosidic bond** is formed (Fig.10.4. and Fig. 10.5.). Exception is the bond in pseudouridine, nucleoside of uracile, which is bound with ribose by –C–C– bond. During reaction –OH group of the hemiacetal carbon (C-1) of the pentose reacts with >NH hydrogen of the heterocyclic base with concomitant release of one molecule of water. In pyrimidine bases, it is the hydrogen at position N-1, in purine bases at position N-9.

To avoid confusion in atoms numbering of the nucleosides, the atoms of the saccharide are marked with numbers followed with an apostrophe. The nitrogen base is always attached to the 1´carbon atom of the saccharide and there is a primary hydroxyl group located at the 5´carbon atom. Ribonucleosides have secondary hydroxyl groups at the 2´- and 3´-carbon atoms, while deoxyribonucleosides have the secondary hydroxyl group only at the 3´-carbon atom.

The name of nucleoside is formed from the type of base present in it. Purine nucleosides are named by replacing the ending –**ine** of the base by –**osine**. Thus adenine + saccharide = **adenosine** and guanine + saccharide = **guanosine**. The pyrimidine bases – cytosine, thymine, and uracil – combined with the saccharide are named **cytidine**, **thymidine**, and **uridine**, respectively. It means they have the ending –**idine**. When saccharide is deoxyribose, the prefix **deoxy**- is used (e.g. deoxyadenosine, deoxyguanosine, deoxythymidine, deoxycytidine).

For ribonucleosides three-letter abbreviations are used:

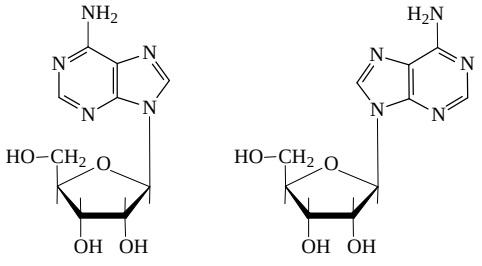
Ado - Adenosine
Guo - Guanosine
Ino - Inosine
Xao - Xanthosine
Puo - Purine nucleoside generally
Thd – Ribosyl thymidine
Cyd - Cytidine
Urd - Uridin
Ψrd - Pseudouridine
Pyd - Pyrimidine nucleoside generally

Nucleosides with deoxyribose have lowercase "*d*" before abbreviation, e.g.:

dAdo - deoxyadenosine
dGuo – deoxyguanosine
dCyd - deoxycytidine
dThd - deoxythymidine

Free nitrogen bases and nucleosides are present only in the „minute" concentrations in the cells. Nucleosides are produced as the second step in nucleic acid degradation, whereby nucleotidases break down nucleotides (such as the thymine nucleotide) into nucleosides (such as thymine) and phosphate. The nucleosides, in turn, are subsequently broken down in the lumen of the digestive system by nucleosidases into nitrogenous bases and ribose (or deoxyribose), and inside the cell by nucleoside phosphorylases into nitrogenous bases, and ribose-1-phosphate (or deoxyribose-1-phosphate).

Nitrogenous heterocyclic bases are planar and are oriented perpendiculary to the furanose ring of deoxyribose. These bases are not free to rotate around the glycoside bond due to deoxyribose. For steric barriers purine and pyrimidine nucleosides may exist only in two defined stable conformations ***syn*** and ***anti*** (Fig. 10.4), according to which the base is turned over deoxyribose planar circle, or on the opposite side. Pyrimidines are usually found in the *anti* conformation because of steric interference between the carbohydrate and the nitrogen on the C-2 pyrimidine. In natural double-stranded DNA *anti* conformation of purines as well as pyrimidines is predominant (*syn* conformation is present only in the Z-DNA).



Syn ***Anti***

Fig. 10.4. Adenosine in the syn and anti conformation

10.3. Nucleotides

Nucleotides are esters of nucleosides (acting as an alcohol) with phosphoric acid. Phosphoric acid is bound by **esteric bond** to the hydroxyl groups of ribose (Fig.10.5.).

In contrast to nucleosides, free nucleotides are present in the cells in relatively high concentrations. This is the **intracellular pool** of free nucleotides. In most animal and bacterial cells the deoxyribonucleotide pool is only 1 % of the ribonucleotide pool. In the processes of biosynthesis, 5'-nucleotides play the most important role; in degradation processes, 3'-nucleotides are often present.

The two protons of the monophosphate ester are ionized at physiological pH, and the ester exists in a solution as an ion with a charge -2.

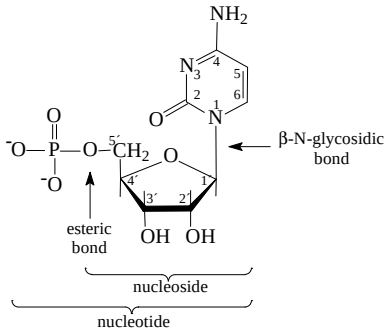


Fig. 10.5. Bonds in nucleotides

Nucleotide synthesis is a complex process, which requires a metabolic energy. Synthetic reactions involve two dehydrations among the three components: dehydration between phosphoric acid and the pentose saccharide and between the pentose saccharide and the heterocyclic base. This reaction proceeds as follows (Fig. 10.6.):

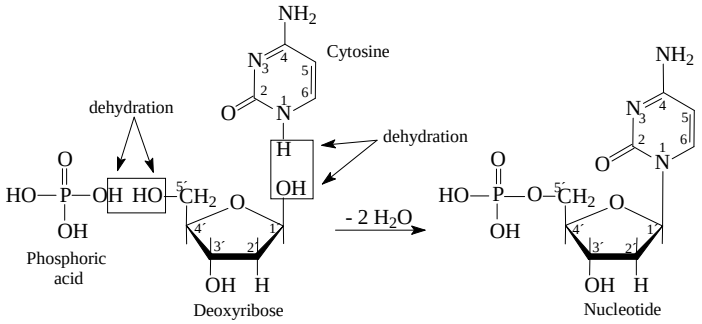


Fig. 10.6. Nucleotide synthesis

Nucleotides are named according to the nucleoside base, in conjunction with the number of bound phosphates e.g.:

- Adenine bound to ribose forms the nucleoside adenosine.
- Adenosine bound to a phosphate forms adenosine monophosphate (AMP).
- As phosphates are added, adenosine diphosphate (ADP) and adenosine triphosphate (ATP) are formed, in sequence.

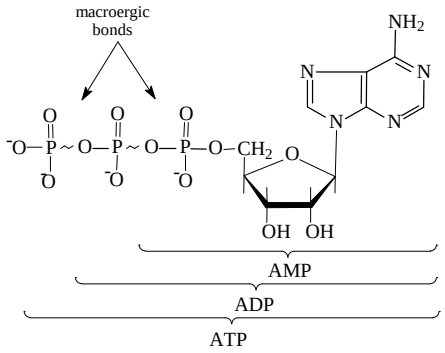


Fig. 10.7. Adenosine 5´-triphosphate

Nucleotide names are usually abbreviated. For example, the prefix **deoxy-** is cut to **d-** and is followed by the one–letter symbol for the base (A, G, C, T and U) and MP for 5´-monophosphate. Thus, the nucleotide containing deoxyribose and cytosine, which structure is shown in Fig. 10.6., is named deoxycytidine 5´-monophosphate or dCMP.

The number "-5'-" in the name indicates the position of that carbon in pentose from which the ester bond with phosphoric acid comes out. In case of binding of the phosphoric acid to the fifth carbon of pentose, the label of the position is not stated in abbreviation (e.g. AMP, CMP, dGMP, dTMP). On the contrary, if the ester bond with phosphoric acid occurs at different position of the pentose, then the position of the ester bond is stated in the abbreviation (e.g. A-3'-MP, C-3'-MP).

10.3.1. Names of nucleotides found in DNA and RNA

DNA

deoxyadenosine 5'-monophosphate (dAMP)	deoxyadenosine-5'-phosphoric acid	5'-deoxyadenylic acid
deoxyguanosine 3'-monophosphate (dGMP)	deoxyguanosine-3'-phosphoric acid	3'-deoxyguanylic acid
deoxythymidine 5'-monophosphate (dTMP)	deoxythymidine-5'-phosphoric acid	5'-thymidylic acid
deoxycytidine 5'-monophosphate (dCMP)	deoxycytidine-5'-phosphoric acid	5'-deoxycytidylic acid

RNA

guanosine 3'-monophosphate (GMP)	guanosine-3'-phosphoric acid	3'-guanylic acid
uridine 5'-monophosphate (UMP)	uridine-5'-phosphoric acid	5'-uridylic acid
cytidine 5'-monophosphate (CMP)	cytidine-5'-phosphoric acid	5'-cytidylic acid

It is possible to express the structure of a nucleotide schematically as follows:

heterocyclic base - saccharide - phosphate
nucleoside
nucleotide

10.3.2. The most important nucleotides from functional point of view

In addition to being building units of nucleic acids, nucleotides exhibit other important and specific biological functions in the cells and tissues of the organism. They act as coenzymes taking part in transport of hydrogen atoms (hydrogenation and dehydrogenation reactions), in transport of groups of atoms, e.g. phosphate groups (phosphorylation reactions), in the transfer of chemical (free) energy and other biochemical processes. In cells, nucleotides play important roles in metabolism and signalling.

From a functional point of view the most important nucleotides include:

a) Nucleotides carrying free energy which are also a source of macroergic phosphate. Nucleoside polyphosphates are nucleosides having a higher number of phosphoric acid units linked to each other by phosphoanhydride bond to a 5'-carbon of saccharide attached. Phosphoanhydride bond is formed in reaction of two molecules of H_3PO_4, releasing one molecule of water. It is energy-rich bond (Fig. 10.7) called macroergic bond (sign ~) and during its hydrolytic cleavage a large amount of energy (30.5 kJ mol^{-1}) is released. Nucleoside diphosphates contain two residues of H_3PO_4 (ADP, GDP, UDP, CDP) and nucleoside triphosphates three H_3PO_4 (ATP, GTP, UTP, CTP). They belong to the group of so called macroergic compounds. They contain one or two macroergic bonds and have an important role in energetic metabolism. The most versatile from the group of triphosphoric esters is adenosine triphosphate (ATP), in which three phosphate groups are linked by two phosphoanhydride macroergic bonds (see Chapter 5). This predetermines it to be a good source and transmitter of energy in the cells. The main process of ATP synthesis is the oxidative phosphorylation coupled with the respiratory chain. In addition, ATP during reactions of phosphorylation/dephosphorylation transfers the rest of phosphoric acid H_3PO_4. In the metabolism also other nucleotide triphosphates are of the great importance as macroergic compounds such as:

- GTP is involved in the citrate cycle and in the synthesis of proteins,
- UTP is essential for the glycoside bond synthesis in carbohydrates. UTP is used by the organism for the activation of monosaccharides (UDP-glucose, UDP-galactose, etc. are formed).
- CTP is important in complex lipids synthesis: in activation of non-lipid components e.g. CDP-choline, CDP-ethanolamine are formed, whereas during activation of lipid components CDP-diacylglycerol arises.

To this group of nucleotides adenosine 5´-triphosphate (ATP) (Fig. 10.7.), uridine 5´-triphosphate (UTP), cytidine 5´-triphosphate (CTP), guanosine 5´-triphosphate (GTP) belong. Structure of them is similar to ATP.

b) Nucleotides, which have a function of coenzymes in free or complex structures (see Chapter 13):

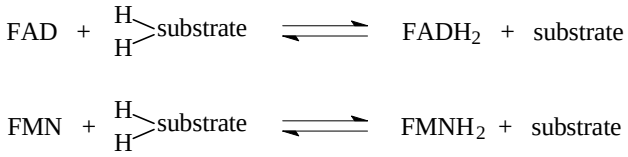
1. **Nicotine nucleotides**: nicotineamide adenine dinucleotide (NAD^+) (Fig. 12.8.), and nicotineamide adenine dinucleotide phosphate ($NADP^+$) (Fig. 12.9.). They are derivatives of nicotinic acid (vitamin B3). They constitute a part of the enzymes dehydrogenases.

The nucleotides take two hydrogen atoms of the substrate. One hydrogen atom is bound to the pyridine core in the form of a hydride ion ($H^- = H + e$) and thus cancels its aromatic character and the positive charge of the molecule as well. The second hydrogen is released into the environment in the form of H^+:

$$NAD^+ + \begin{matrix} H \\ H \end{matrix}\!\!>\text{substrate} \rightleftharpoons NADH + H^+ + \text{substrate}$$

2. **Flavine nucleotides**: flavine mononucleotide (FMN) (Fig. 12.11.) and flavine adenine dinucleotide (FAD) (Fig. 12.10.). They are both riboflavin (vitamin B_2) derivatives, hydrogen-transferring coenzymes. The nucleotides take a hydrogen of the substrate and bind it to the nitrogen atoms of isoalloxazine ring at N-1 and N-10 positions.

To summarize, substrate oxidation can be expressed as follows:



3. **Coenzyme A (CoA)** (Fig. 12.16.) is a nucleotide, whose part is adenosine diphosphate. It is a component of the enzymes transacylases, i.e. it transfers units of carboxylic acids. It has an important role in the transfer of two-carbon residues, in the oxidative decarboxylation of α-keto acids, as well as in the degradation of fatty acids. It is called "active acetic acid" in the form of acetyl-CoA.

Special relevance have cyclic nucleotides adenosine-3´,5´-monophosphate (cyclic AMP, cAMP) and guanosine-3´,5´-monophosphate (cyclic GMP, cGMP) (Fig.10.8.). Cyclic AMP is actually AMP, in which phosphoric acid is bound by ester bonds to two carbon atoms (the third and fifth) of one molecule of ribose. Phosphodiester bond is formed within one nucleotide. cAMP, resp. cGMP are synthesized in the organism from ATP, resp. GTP by the action of the enzyme adenylate cyclase (guanylate cyclase) in the presence of Mg^{2+} ions or other compounds (e.g. NO), while diphosphate (pyrophosphate) is cleaved off (Fig. 12.40).

It is supposed, that cyclic nucleotides (called the second messengers) take part in intracellular regulatory mechanisms. They mediate the transport of the information brought to the surface of the target cell by hormones. They can regulate secretion of compounds, cell differentiation and replication, mobilisation and storage of saccharides and lipids, induction of protein synthesis, cell membrane permeability, transport of ions, membrane potential, neurohumoral transfer, etc. In some cases cAMP and cGMP have antagonistic effects.

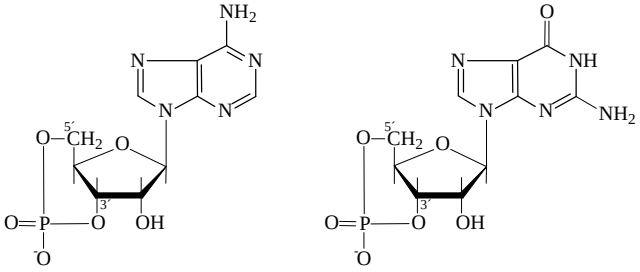


Fig. 10.8. Cyclic nucleotides

S-adenosylmethionine is an alkylating agent present in cells. It transfers methyl group, which is the most reduced form of carbon. Although methyl group can be transmitted also by tetrahydrofolate, the potential of this cofactor is not sufficient for most of biosynthetic reactions. S-adenosylmethionine is the preferred cofactor for the transport of the methyl group in biological systems. It is synthesized from ATP and methionine in a reaction catalyzed by *methionin adenosyltransferase.*

3'-phosphoadenosine-5'-phosphosulfate is a derivative of adenosine monophosphate which is phosphorylated at 3' position and has the sulfate group bound to a 5' phosphate. In the organism it acts as *sulfotransferases* coenzyme, which catalyzes the transfer of the sulfo group on -OH or $-NH_2$ group of the acceptor molecule. A similar anion is adenosine 5'-phosphosulfate which is not phosphorylated at 3' position.

10.3.3. Synthetic nucleotide analogues – used in medicine

Synthetic analogues of purines, pyrimidines, nucleosides, and nucleotides altered in either the heterocyclic ring or the sugar moiety have numerous applications in clinical medicine. Their toxic effects involve either inhibition of enzymes essential for nucleic acid synthesis or their incorporation into nucleic acids with resulting disruption of base-pairing. Oncologists employ 5-fluoro- or 5-iodouracil, 3-deoxyuridine, 6-thioguanine and 6-mercaptopurine, 5- or 6-azauridine, 5-or 6-azacytidine, and 8-azaguanine (Fig. 10.9.), which are incorporated

into DNA prior to cell division. The purine analogue allopurinol, used in treatment of hyperuricemia and gout, inhibits purine biosynthesis and *xanthine oxidase* activity (Fig. 12.28.). Cytarabine is used in chemotherapy of cancer. Finaly, azathioprine, which is catabolized to 6-mercaptopurine, is used during organ transplantation to suppress immunologic rejection.

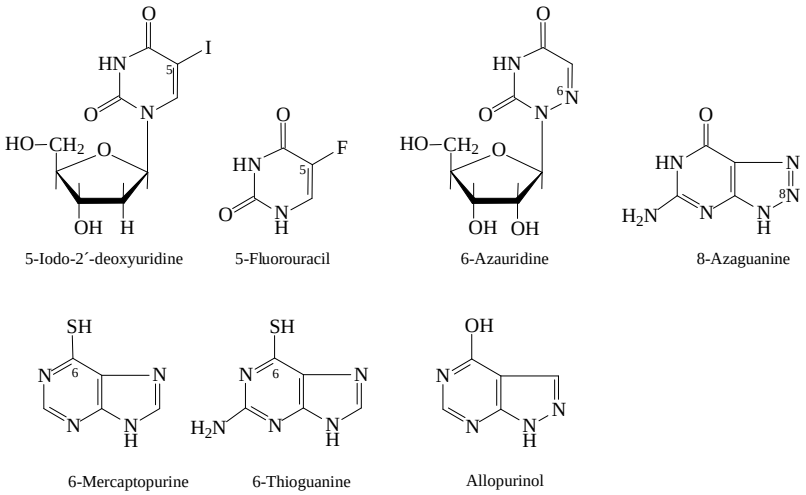


Fig. 10.9. Selected synthetic pyrimidine and purine analogues

10.4. Nucleic acids

Chemical structure of nucleic acids is very complex. Their molecular weight varies in the range of 10^3 to 10^{10}. The molecule of nucleic acid is an unbranched polynucleotide chain, composed of the basic structural units - **nucleotides.**
According to the nature of the saccharide unit nucleic acid are classified to deoxyribonucleic acid (DNA) and ribonucleic acid (RNA).

10.4.1. Nucleic acid formation from nucleotides

As already mentioned, nucleic acids are polynucleotides. RNA is formed from ribonucleotides, and DNA is formed from deoxyribonucleotides. Nucleotides bound through **phosphodiester** bonds with the participation of enzymes (*polymerases*) form the primary structure of both RNA and DNA molecules. Similarly to nucleotides, the synthesis of nucleic acid is a complex process. Products of nucleic acid synthesis are those that would be produced by a simple dehydration reaction between the –OH of the phosphate group at C-5´of one nucleotide molecule and the –OH at C-3´of another nucleotide molecule and diphosphate is released. The group formed in this reaction is called a **phosphodiester group**, joins one residue to another one. For example, the dinucleotide UMP-CMP is produced by the reaction of UMP with CMP through dehydration between the –OH group at C-3´ of UMP and the phosphate group at C-5´ of CMP (Fig. 10.10.). Note that each phosphodiester has one acidic hydrogen atom, however, at physiological pH nucleic acids are ionized.

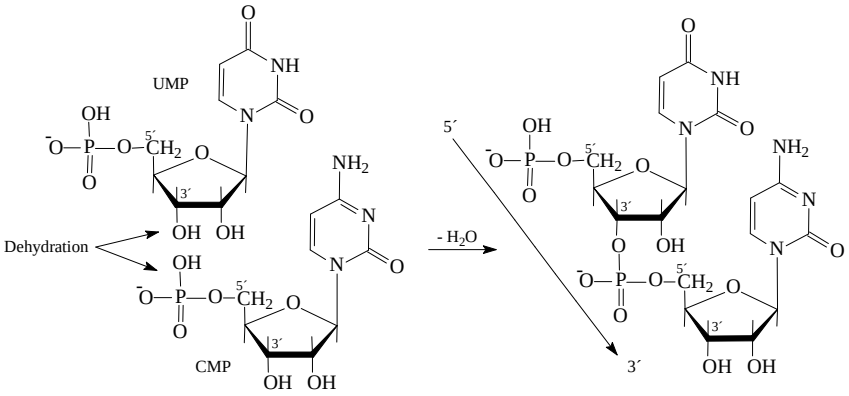


Fig. 10.10. Formation of phosphodiester bond between the nucleotides

By convention, nucleotide sequences are named in the 5´⟶ 3´direction. A nucleic acid has one 5´-end and one 3´-end. The **5´-end** has a phosphate group at C-5´ that is attached to only one pentose group. All other phosphate groups are attached to two different pentose rings. The **3´-end** has a pentose ring with an unreacted –OH group at C-3´. The nucleotide residues in a nucleic acid are named by proceeding from the 5´-end to the 3´-end and commonly one-letter abbreviations are used. For RNA the convention is:

A – C – G – … – U
5´⟶ 3´
RNA

The same process is used for DNA, but a lowercase **d** is placed at the left of the first base to indicate the deoxyribose in the backbone.

dT – C – G – … – A
5´⟶ 3´
DNA

All of these representations emphasize the key feature of nucleic acids – the nucleotide sequence, which is critical to its function.

10.4.2. Deoxyribonucleic acid

Deoxyribonucleic acid, or **DNA**, is a nucleic acid molecule that contains the genetic instructions used in the development and functioning of all known living organisms. The main role of DNA is the long-term storage of information and it is often compared to a set of blueprints, since DNA contains the instructions needed to construct other components of cells, such as proteins and RNA molecules. The DNA segments that carry this genetic information are called **genes**, but other DNA sequences have structural purposes, or are involved in regulating the use of this genetic information. Macromolecules of nucleic acid are characterized by high relative molecular weight (M_r = about 10^9). Polynucleotide chain can include about 200 000 building blocks. Polynucleotide chain of 23 pairs of human diploid cells chromosomes contain a total of 6 x 10^9 pairs of nitrogen bases. DNA is a huge molecule and its sequence was solved and published in 2002.

Primary structure of DNA

Primary structure of nucleic acid is given by the sequence of nucleotides in the polynucleotide chain (Fig. 10.11.). The primary structure of a nucleic acid, the sequence of bases along the saccharide-phosphate polymer chain, determines its higher-level conformational structure (secondary, tertiary, quaternary). The situation is analogous to proteins except of existence of only a few different types of three-dimensional structures for nucleic acids, corresponding to their limited range of functions. The nucleotide sequence in DNA determines: (I) the sequence of α-amino acid residues in a polypeptide, what determines the conformation of protein and its biological function, and (II) the structure of all non-coding RNA.

The content of nitrogen bases in DNA is not accidental. By using quantitative analysis of DNA appears, that molecular ratio of adenine and thymine is 1:1 and in the same molecular ratio occur guanine and cytosine.

According to discoverer this principle is named Chargaff´s rules. So presence of nitrogen bases follows Chargaff´s rules:

- DNA isolated from different animal species contains qualitatively the same bases (A, G, C, T).
- The amounts of adenine and thymine are equal (A = T), as are those of guanine and cytosine (G = C) in all DNAs, it is true that A + G = T + C.
- The quantitative abundance of individual bases (A, G, C, T) is species dependent.
- The base abundance in DNA of individual species is independent of age, diet, or changes in the environment.

Later it was found that the DNA has only one variable element, the ratio (A + T) / (G + C). Representation of nitrogen bases along the chain is not regular, but is strictly specific and constant for one species. In higher animals, the ratio is equal to 1.3 to 1.5, while in the lower forms (viruses, bacteria) it is inverse.

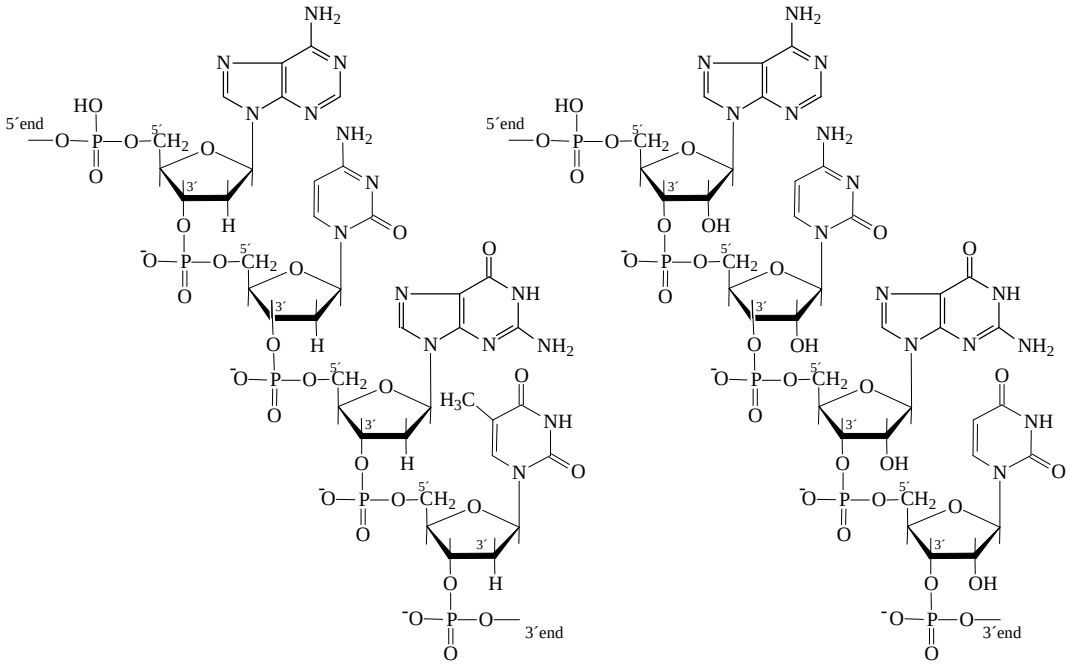


Fig. 10.11. Scheme of the primary structure of one strand of DNA and RNA

Spatial, three-dimensional structure of DNA

Biological properties of nucleic acids depend on their particular spatial arrangement (conformation).

According to roentgen-structural analysis of Maurice Wilkins and Rosalind Franklin and based on the chemical analysis (Erwin Chargaff), American geneticist James Watson and british physicist Francis Crick in 1953 proposed a three - dimensional model of the right-handed **double helix of DNA,** for which they received the Nobel Prize. Discovery of DNA structure belongs to the most important discoveries of 20th century. In the DNA, A is always paired with T and G with C. The length of both pairs is identical (1.085 nm). Between the A and T two hydrogen bonds are formed, whereas between the G and C three hydrogen bonds occur (Fig. 10.12.). The philosophy of Watson - Crick model is based on an assumption, that two nitrogen bases connect together by hydrogen bonds. The match of bases through hydrogen bonds is called **complementary base pairing**. By this way two polynucleotide chains are connected coiled along common axis forming the double strand (helix), which is considered the secondary structure of DNA. A pyrimidine is paired with a purine all the time, and the long dimensions of both pairs are identical (1.085 nm). For each C in one strand of DNA there is a complementary base G in the second strand of DNA molecule, as well as for each A there is a complementary base T:

Adenine – Thymine (connection by two hydrogen bonds),
Guanine – Cytosine (connection by three hydrogen bonds).

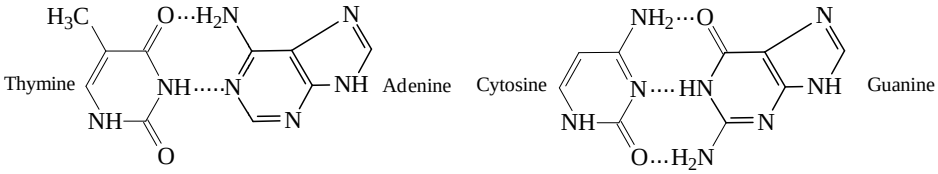


Fig. 10.12. Hydrogen bond pairing

The studies, which have measured the distance between the nucleotides and the α-helix turn were crucial for understanding the secondary structure of DNA. The measurements have shown that one turn of the α-helix contains 10 nucleotides (in B type of DNA – see below), the DNA chain is 2.2 to 2.6 nm wide, and one nucleotide unit is 0.34 nm long.

Subsequent measurements revealed 10.5 base pairs, or 3.6 nm per turn.

Every DNA strand is composed of alternating phosphoric acid and deoxyribose units. Deoxyribose orientation indicates the direction of chain which is called **polarity**. DNA chains have different polarity, we say they are antiparallel to each other. Hence, DNA molecules are composed of two antiparallel polynucleotide chains: the one strand is arranged in the 5'→3' and the other in the 3'→5' direction. Out from the strand, bases bound through the 1' carbon of deoxyribose project to the side. The nitrogenous bases are localised in the inner space of double helix and they are perpendicularly oriented to axis of double helix. Hydrogen bonds occur between the base pairs, but these bonds are no longer regarded as the primary source of the stability of the double helix structure. Hydrogen bonds determine which bases can pair, but hydrophobic interactions stabilise DNA duplex.

The rings of the bases themselves are planar and unsaturated, like benzene rings, and relatively nonpolar despite the N atoms in the ring. In an aqueous medium there is a natural tendency of these rings to stack closely together because they cannot offer much hydrogen bonding alternatives to water molecules. They form a hydrophobic interior of the double helix. Water repels them, so they stack together, what is exactly what the DNA duplex structure portrays.

This structure is stabilized by hydrogen bonds between complementary nitrogen bases of one and a the second chain, but these bonds are not considered to be a primary source of the DNA structure stability. DNA duplex is stabilized also by van der Waals hydrophobic interactions of π-π aromatic rings of bases of one chain (interaction of π-electron systems) above the other, which are called the stacking or surface forces. In addition, the stability of the DNA molecule is also affected by certain metal ions (Mg^{2+}) as well as its interaction with histone molecules in deoxyribonucleoproteins.

A brief demostration that summarises the stechiometric relationships between bases:

5′——————————→ 3′

dT – … – G – C – A

A – … – C – G – dT

3′←—————————— 5′

The two strands of DNA are not functionally equivalent. The **template strand**, the (–) strand or **noncoding strand,** is the one that is read during the synthesis of RNA. Its sequence is complementary to the synthesised RNA. The **sense strand**, the (+) strand or **coding strand**, has the same sequence as the RNA, except that T is replaced by U. It is agreed that ***gene sequences are expressed by reading the sequence of the sense strand in the 5′→3′ direction.***

The double helix is a right-handed spiral. As the DNA strands wind around each other, they leave gaps between each set of phosphate backbones, revealing the sides of the bases inside. There are two of these grooves twisting around the surface of the double helix: one groove, the **major groove**, is 2.2 nm wide and the other, the **minor groove**, is 1.2 nm wide (Fig. 10.13.).

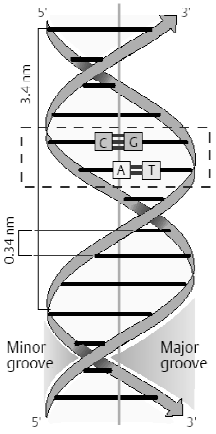


Fig. 10.13. DNA double helix

The narrowness of the minor groove means that the edges of the bases are more accessible in the major groove. As a result, dissociated phosphate groups by their negative charges can bind with basic amino acids in the proteins like transcription factors that can bind to specific sequences in double-stranded DNA usually make contacts to the sides of the bases exposed in the major groove. In major groove, there is a disclosure of the edge bases, what allows binding of the protein to a specific double stranded DNA sequence and can be important in regulation of the expression of genetic information.

On the surface of a DNA molecule there are a lot of negatively charged oxygen atoms that are able to form hydrogen bonds with water molecules, to react with divalent ions (Mg^{2+}), polyamines (spermines, spermidines) or proteins – histones, which also help to stabilize the tertiary structure.

Alternative double-helical structures

There are found many significant deviations from the Watson-Crick DNA structure in cellular DNA, of which some or all may be important in DNA metabolism. These structural variations generally do not affect the key properties of DNA defined by Watson and Crick: strand complementarity, antiparallel strands, and the requirement for A – T and G – C base pairs.

The Watson-Crick structure is also referred to as the **B form of DNA**, or B-DNA. The B form is the most stable structure for a random-sequence of DNA molecule under physiological conditions and is therefore the standard point of reference in any study of the DNA properties. Two structural variants that have been well characterized in crystal structures are the **A** and **Z forms**. These three DNA conformations are shown in Fig. 10.14. The **A** form is favoured in many solutions that are relatively devoid of water. **A** form of DNA arises from **B** form, when water content is decreased by about 75 %. This change is reversible. The DNA is still arranged in a right-handed double helix, but the helix is wider and the number of base pairs per helical turn is 11, rather than 10.5 as in B-DNA. Some properties of these forms of DNA summarize Table 10.3.

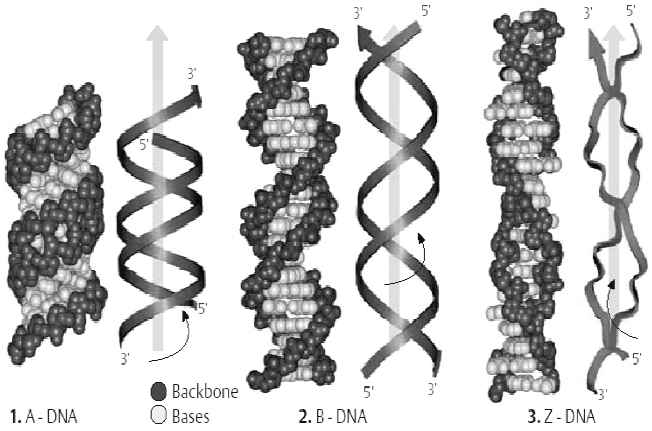


Fig. 10.14. Comparison of A, B, and Z forms of DNA

Tab. 10.3. Properties of three DNA forms

	A form	***B form***	***Z form***
Shape	broadest	intermediate	narrowest
Helical sense	right handed	right handed	left handed
Diameter	2.6 nm	2.0 nm	1.8 nm
Base pairs per helical turn	11	10.5	12
Helix rise per base pair	0.26 nm	0.34 nm	0.37 nm
Base tilt normal to the helix axis	20°	6°	7°

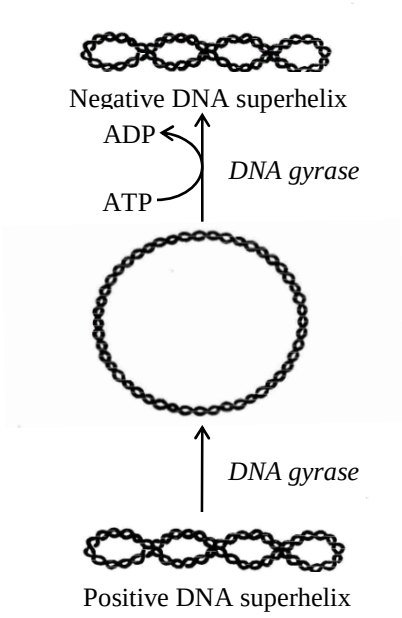
The plane of the base pairs in **A**-DNA is tilted about 20º with respect to the helix axis. It probably does not occur in the cell. In the **Z**-form of DNA, which can occur within GC-rich regions of **B**-DNA, the organization of nucleotides is completely different. In this case, the helix is **left-handed,** and the backbone adopts a

characteristic **zig-zag** conformation (hence "**Z**-DNA"). There are 12 base pairs per helical turn, and the structure appears more slender and elongated. **Z** form of DNA has only one groove.

The place of transition of a right-handed segment into a left-handed one has to be uncoiled and this is of advantage for mounting of the copying enzymes during replication. DNA segments in the Z conformation have probably physiological significance in gene expression, in DNA-DNA, DNA-RNA and DNA-protein interactions. The type of formed DNA conformation depends on the sequence of nucleotides in DNA, the amount and direction of supercoiling, chemical modifications of the bases and also on solution conditions, such as the concentration of metal ions and polyamines.

Supercoiling

DNA is not an inert molecule. From chemical, as well as from structural points of view, it is one of the most variable molecules. It is a polymorphous molecule, which is able to exist in several structural forms.



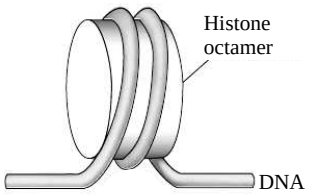
DNA, generally, can be **linear** or **circular**. The DNA molecules in human chromosomes are linear and can be twisted in a process called DNA supercoiling. With DNA in its "relaxed" state, a strand usually circles the axis of the double helix once every 10.4 base pairs, but if the DNA is twisted the strands become more tightly or more loosely wound. If the DNA is twisted in the direction of the helix, this is a **positive supercoiling**, and the bases are held more tightly together. If they are twisted in the opposite direction, this is a **negative supercoiling**, and the bases come apart more easily. There is one turn of the superhelix per 15 turns of the original helix. This superhelix form is more often found in circular DNA than in linear DNA. Circular form is typical for mitochondrial and chloroplast DNA as well as for bacterial plasmids and mammalian viruses. These DNAs can exist in the form of normal circular helix as well as in the form of right-handed and left-handed superhelixes. The isomers are denoted as topological isomers (Fig. 10.15.). In nature, most DNA has slight negative supercoiling that is introduced by enzymes called *topoisomerases* for concurrent ATP consumption. The best characterized *topoisomerase* is the bacterial *DNA gyrase*.

Fig.10.15.Topological isomers of circular helix

These enzymes are also needed to relieve the twisting stresses introduced into DNA strands during processes such as transcription and DNA replication. Supercoiling is biologically important for two reasons. First, a supercoiled DNA molecule has a more compact shape than its relaxed counterpart does. Second, supercoiling may hinder or favor the capacity of the double helix to unwind and thus affect the interaction between DNA and other molecules.

Arrangement of DNA in the cell

DNA molecule is extremely large, and thus it has to be arranged in the cell in a very rational way. It forms a nucleoprotein complex – **chromatin**, which consists of very long double-stranded **DNA molecules** and small basic proteins termed **histones** as well as some **nonhistone proteins** (most of which are acidic and larger than histones) and a small quantity of **RNA**. The double-stranded DNA helix in each chromosome has a length thousands times the diameter of the cell nucleus. One purpose of the complex comprising chromatin is to condense the DNA. Importance of binding the DNA molecule in nucleosomes is based on rational spatial condensation (40x).



Electron microscopic studies of chromatin have demonstrated dense spherical particles called **nucleosomes,** which are approximately 10 nm in diameter and connected by DNA filaments. Nucleosomes are composed of DNA wound around a collection of histone molecules, which are in form of octamer $(H2A, H2B, H3, H4)_2$ (Fig. 10.16.).

Fig. 10.16. Nucleosome

Histones are wrapped by the double helix (165-175 nucleotide pairs). The connection between nucleosomes is provided by histones H1, by non-histone proteins and by a 20-base-pair segment of DNA double helix. One gene has to be composed of several nucleosomes.

The nonhistone proteins include enzymes involved in DNA replication, such as *DNA topoisomerases*. There are included also proteins involved in transcription, such as the *RNA polymerase* complex.

Physical properties of DNA

The double-stranded structure of DNA can be separated into two component strands in solution by increasing the temperature or decreasing the salt concentration. The strands of a given molecule of DNA separate over a temperature range. A temporary damage of DNA secondary structure is called **nucleic acid denaturation**. The midpoint is called the **melting temperature, or T_m**. The T_m is influenced by the base composition of the DNA, their possible modifications and by the salt concentration of the solution. DNA rich in G–C pairs, which have three hydrogen bonds, melts at a higher temperature than the one rich in A–T pairs, which have two hydrogen bonds.

Concomitant with this denaturation of the DNA molecule is an increase in the optical absorbance (DNA has a characteristic absorption spectrum with a maximum at 260 nm) of the purine and pyrimidine bases – a phenomenon referred to as **hyperchromic effect** of denaturation and it is an indicator of the damage to hydrogen bonds between base pairs.

The rate of **reassociation** depends upon the concentration of the complementary strands. **Reassociation** of the two complementary DNA strands of a chromosome after DNA replication is a physiologic example of renaturation. At a given temperature and salt concentration, a particular nucleic acid strand will associate tightly only with a complementary strand.

10.4.3. Ribonucleic acids

Ribonucleic acid or **RNA** is a nucleic acid polymer consisting of nucleotide monomers that plays several important roles in the processes that translate genetic information from deoxyribonucleic acid (DNA) into protein products.

RNA can perform following biological roles:

- RNA may constitute the genetic material (viral RNA),
- mediates the transfer of information from DNA to RNA (messenger RNA),
- in the proteosynthesis mediates the transfer of an amino acid to the ribosome (transfer RNA), it is a part of the ribosomes (rRNA).

RNA is very similar to DNA, but differs in a few important structural details: RNA nucleotides contain ribose saccharides while DNA contains deoxyribose, phosphate backbone and four different bases: adenine, guanine, cytosine, and uracil. The first three are the same as those found in DNA, but in RNA thymine is replaced by uracil as the base complementary to adenine. Uracil is energetically less expensive to produce than thymine, which may account for its use in RNA. In DNA, however, uracil is readily produced by chemical degradation of cytosine. Thus, uracil is appropriate for RNA, where quantity is important but lifespan is not, whereas thymine is appropriate for DNA where maintaining sequence with high fidelity is more critical.

However, there are also numerous modified bases and saccharides found in RNA that serve many different roles. Chargaff rules do not hold for RNA molecules. Pseudouridine (Ψ), in which the linkage between uracil and ribose is changed from a C–N bond to a C–C bond, and ribosylthymidine (T), are found in various places (most notably in the TΨC loop of tRNA). Another notable modified base is hypoxanthine (a deaminated guanine base which nucleoside is called inosine). There are nearly 100 other naturally occurring modified nucleosides. The specific roles of many of these modifications in RNA are not fully understood.

Single stranded RNA exhibits a right-handed stacking pattern that is stabilized by base stacking.

The most important structural feature of RNA, indeed the only consistent difference between the two nucleic acids, that distinguishes it from DNA is the presence of a hydroxyl group at the 2'-position of the ribose sugar. The presence of this functional group causes the helix to adopt the **A**-form geometry rather than the **B**-form most commonly observed in DNA. This results in a very deep and narrow major groove and a shallow and wide minor groove. A second consequence of the presence of the 2'-hydroxyl group is that in conformationally flexible

regions of an RNA molecule (that is, not involved in formation of a double helix), it can chemically attack the adjacent phosphodiester bond to cleave the backbone.

Basic classes of RNA

- messenger (mediator, information) – mRNA
- ribosomal – rRNA
- transfer – tRNA
- nuclear – nRNA
- viral RNA

Individual RNAs differ in their molecular weight, structure, localization in the cell, and biological function. The properties of the most important forms of RNA are summarized in the Tab. 10.4. Fig. 10.17. gives an idea of the secondary structure of these molecules.

Tab. 10.4. Properties of basic classes of RNA

Type	**mRNA**	**rRNA**	**tRNA**	**snRNA**
Species per cells	> 1 000	4	> 50	~ 10
Length (bp)	400 – 6 000	120 – 5 000	74 – 95	100 – 300
Proportion	5 %	80 %	10 – 20 %	< 1 %
Lifespan	Short	Long	Long	Long
Function	Translation	Translation	Translation	Splicing

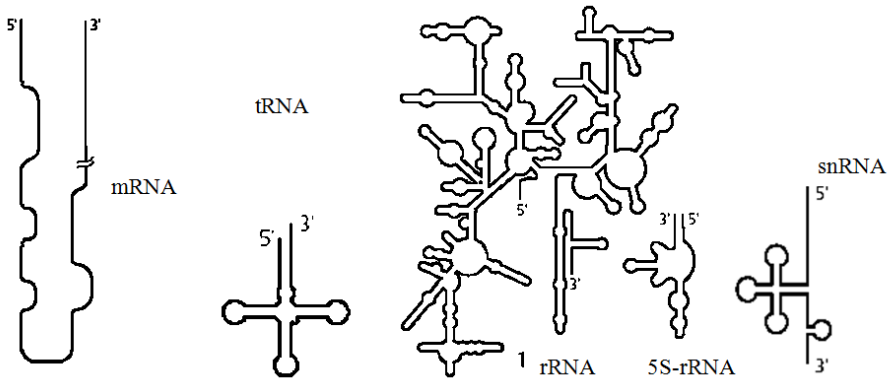


Fig. 10.17. Structures of RNAs

snRNA= small nuclear RNA, bp – base pairs (pairs of heterocyclyc nitrogen bases)

Messenger RNA

Messenger RNA (mRNA) is formed by one unwinded polynucleotide chain. It arises by transcription of some part of DNA molecule (gene) to molecule mRNA. Messenger RNA transfers genetic information from the cell nucleus to the cytoplasm. The instructions or directions for protein synthesis it carries in a code or codon composed of three nitrogen bases. The molecular weight of mRNA depends on the length of the protein which synthesis it must direct. There must be three nucleotides for each amino acid in the protein to be formed. From the theory of the transcription of genetic information follows that the mRNA must have at least 3 times more nucleotides than the number of amino acids in the peptide chain is. mRNA synthesis is carried out after histone dissociation directly on the DNA molecule in the nucleus, by complementary transcription of the nucleotide sequence.

5´——————————————→ 3´
dA – T – A – G – C – C – G – A – T – A template DNA strand
U – A – U – C – G – G – C – U – A – U mRNA strand
3´←—————————————— 5´

Each messenger RNA is complementary to one gene. The primary transcript is dysfunctional. It is denoted as **heterogenous nuclear RNA (hnRNA)**. This molecule is much larger than proper mRNA and has to be subjected to splicing or process called RNA maturation: a part of nucleotides, called "**introns**", has to be cut out. Special enzymes catalyse this reactions and splice together just the units corresponding to the **exons** (hnRNA segments that carry genetic information) of the divided gene. Introns are not transcribed into the peptide they probably have a regulatory function. Exons are bound together after splicing and create the mRNA. The result is a much shorter mRNA molecule. In this molecule occurs a sequence of bases complementary just to the exons of genes, thus RNA now carries the unsplit genetic message. Next, both ends of mRNA have to be modified. At the 3' end of RNA polyadenylated chain consisting of 50-200 adenosines (poly(A)-end, polyadenylate tail) is synthesized. 7-metylguanosine is added to the 5' end by 5',5'-triphosphodiester bond. This post-transcriptional modification of the 5' end is called the cap. Thus, at both ends there are differently modified non-coding sequences which are not encoded by the DNA and probably are involved in the stabilization of the molecule structure (they protect mRNA against enzymatic destruction) and the regulation of its function. After relocation from the nucleus into the cytoplasm, mRNA connects to ribosomes, where the protein synthesis is performed.

Since mRNA has to be read codon by codon in the ribosome, they must not form a stable tertiary structure. This is ensured in part by the attachment of RNA-binding proteins, which prevent base pairing. Lifespan of mRNA is usually short, as they are quickly broken down after translation.

Each group of three adjacent bases on a molecule of mRNA constitutes a unit of genetic information called a codon. Thus it is a sequence of codons on the mRNA backbone more that a sequence of individual bases that now carries the genetic information.

Ribosomal RNA

Ribosomal RNA combines with about fifty different proteins to form a complex of nucleoprotein structures called ribosomes which molecular weight is about three million. Ribosomes are the site of protein synthesis. Eukaryotic ribosomes contain four different rRNA molecules: 18S, 5.8S, 28S, and 5S rRNA. rRNA size is characterized by the ability of ribosomes to sediment in a gravitational field with the sedimentation constant **S** (Svedberg constant). Three of the rRNA molecules are produced from DNA by transcription in the nucleolus, and one is synthesized elsewhere. rRNA molecules are extremely abundant and make up at least 80% of the RNA molecules found in a typical eukaryotic cell. It is synthesized as precursor molecules (pre-rRNA), which are further processed. Except in a few viruses, rRNA is single-stranded, but its molecules often have hairpin loops in which base pairing occurs.

The mammalian ribosome has a sedimentation rate 80S. It contains two major nucleoprotein subunits - a larger one (60S) and a smaller subunit (40S). The 60S subunit contains a 5S rRNA, a 5.8S rRNA, and a 28S rRNA, there are also probably more than 50 specific polypeptides. The 40S subunit is smaller and contains a single 18S rRNA and approximately 30 distinct polypeptide chains. All of the ribosomal RNA molecules except the 5S rRNA are processed from a single 45S precursor RNA molecule in the nucleolus. 5S rRNA is independently transcribed. The highly methylated ribosomal RNA molecules are packaged in the nucleolus with the specific ribosomal proteins. In the cytoplasm, the ribosomes remain quite stable and capable of many translation cycles. The functions of the ribosomal RNA molecules in the ribosomal particle are not fully understood, but they are necessary for ribosomal assembly and seem to play key roles in the binding of mRNA to ribosomes and its translation.

Transfer RNA

Transfer RNA (tRNA) is a small RNA chain of about 74 - 95 nucleotides that delivers individual activated amino acids from the amino acid pool to the site of protein synthesis. More than 60 tRNAs, all of which can be found in the cytoplasm, are known. A specific tRNA carries just one type of amino acid. However, as will be shown later several different tRNA molecules can transport the same amino acid. tRNA is also synthesized as a large precursor, which has to be spliced to about 40 nucleotides.

Each tRNA has an amino acid attachment site and a template recognition site. Each 3´end has a –C–C–A sequence that is the amino acid attachment site. The amino acid binds to adenylic acid. The amino acid is bound to the 3′ carbon of the ribose of the last nucleoside, adenosine by ester bond between the hydroxyl group of ribose and the carboxyl group of amino acid.

The first entire nucleotide sequence of tRNA was determined for alanine in 1965 by Holley. He suggested that the single strand molecule could form intra chain hydrogen bonds between some complementary base pairs. By constructing the maximum number of hydrogen bonds, he postulated a “cloverleaf” model for tRNA of alanine (Fig. 10.18.). The base pairings have been confirmed experimentally, but the actual shape of the three-dimensional molecule is not a planar cloverleaf.

All tRNA molecules contain four main arms or loops. The **acceptor arm** terminates at 3′-end the nucleotides –C–C–A. The 5' end of the tRNA usually contains G. One of the loops, **anticodon loop** involved in protein synthesis has an important three-nucleotide sequence called an anticodon. An anticodon is complementary to a codon located in mRNA, what ensures the order of amino acids in the peptide chain written in the genetic information of DNA (sequence of codons in DNA encoding a particular amino acid).

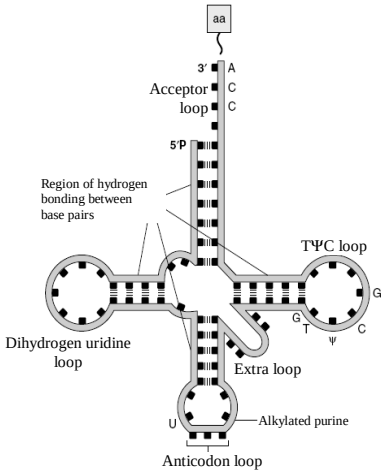


Fig. 10.18. Cloverleaf model for structure of tRNA (aa – amino acid)

For each amino acid there are at least two tRNA in cell, able to transfer them from the cytoplasm to the ribosomes. Other loops are **dihydrogenuridine loop,** containing a larger number of 5,6-dihydrogenuridine and **pseudouridine loop** containing the triplet TΨC in each tRNA. There are present ribosylthymine, as the only exception of thymine presence in RNA and a nucleotide with pseudouridine. It is a type of non-coding RNA.

Nuclear RNA

Nuclear RNAs are found in the nucleus, partly as a precursor of mRNA, rRNA, and tRNA, partly as a separate nuclear RNA (nRNA). Nuclear RNAs include **small nuclear RNA (snRNA)** species that are not directly involved in protein synthesis but play pivotal roles in RNA processing, editing and have regulatory function. These relatively small molecules vary in size from 90 to about 300 nucleotides**. Chromosomal RNAs** (cRNA) are found in chromatin in a complex with non-histone proteins, and are gene activators or repressors.

Viral RNA

The genetic material in some animal and plant viruses is RNA rather than DNA. Although some RNA viruses never have their information transcribed into a DNA molecule, many animal RNA viruses, specifically, the retroviruses, are transcribed by an RNA-dependent DNA polymerase, the so-called **reverse transcriptase,** to produce a double- stranded DNA copy of their RNA genome. In many cases, the resulting double-stranded DNA transcript is integrated into the host genome and subsequently serves as a template for gene expression from which new viral RNA genomes can be transcribed.

Non-coding RNA

RNA genes (sometimes referred to as non-coding RNA or small RNA) are genes that encode RNA that is not translated into a protein. The most prominent examples of RNA genes are transfer RNA (tRNA) and ribosomal RNA (rRNA), both of which are involved in the process of translation. However, since the late 1990s, many new RNA genes have been found, and thus RNA genes may play a much more significant role than previously thought.

Catalytic RNA

Some RNA molecules have intrinsic catalytic activity. The activity of these **ribozymes** often involves the cleavage of a nucleic acid, as well as cutting and ligating other RNA molecules and also the catalysis of peptide bond formation in the ribosome. An example is the role of RNA in catalyzing the processing of the primary transcript of a gene into mature messenger RNA. Much of the RNA synthesized from DNA templates in eukaryotic cells, including mammalian cells, is degraded within the nucleus, and it never serves as either a structural or an informational entity within the cellular cytoplasm.

10.5. DNA, RNA and genetic information flow

10.5.1. The Central Dogma

DNA and RNA are long linear polymers that transmit information in a form that can pass from generation to generation. Genetic information is stored in the sequence of nitrogen bases in the polynucleotide chain. The nitrogen bases have special ability to form specific pairs between each other which are stabilized by hydrogen bonds. The pairing of bases results in the formation of double helix, and at the same time it provides a mechanism to copy the genetic information of an existing nucleic acid chain to form a new chain. Although in the evolution probably RNA played a role of the genetic material, genes of all current cells and many viruses have their genetic information stored in the form of DNA. Prior to cell division, DNA in the cell nucleus replicates by the effect of *DNA polymerases*. These strictly specific enzymes replicate the sequence of the template DNA with a great precision (incorrect inclusion of nucleotide occurs in less than one case of 100 million). Genes define the exact type of protein produced by cells, but DNA is not the direct template for protein synthesis. The template for the protein molecule is RNA, the messenger RNA. Other types of RNAs, such as transfer RNA and ribosomal RNA, actively participate in subsequent steps of proteosynthesis. All types of RNA are synthesized by *RNA polymerases*, which get instructions from DNA templates. The information for protein synthesis is transmitted from DNA to RNA (mRNA) in a process named transcription. This process is followed by translation, protein synthesis according to the instructions given by mRNA templates. In translation information is therefore translated from the nucleotide sequence to corresponding amino acid sequences in the protein chain. The flow of genetic information or gene expression taking place in normal cells can be depicted as follows:

$$\mathbf{DNA} \xrightarrow{\text{transcription}} \mathbf{RNA} \xrightarrow{\text{translation}} \mathbf{protein}$$

The flow of information depends on the genetic code, which defines the relationship between the sequence of the DNA bases (or their mRNA transcript), and the sequence of amino acids in the protein. The record of genetic information is expressed in an alphabetical order of four bases letters A, T (or U), G and C. The polypeptide chain can be written by 21-letters alphabet, (21 proteinogenic amino acids).

To translate from a 4-letter language to a 21-letter language the 4 bases letters are required to be used in groups of a minimum 3 letters. From 4 nucleotides, 64 triplets can be constructed, which are sufficient to encode 21 amino acids. The mRNA codons are read sequentially by tRNA molecules which are used in the transport of amino acids. Protein synthesis takes place on ribosomes, which are composed of rRNA and more than 50 proteins. Biosynthesis of proteins is one of the most complicated synthesis process.

The theory of the transmission of genetic information and protein synthesis is called the central dogma of molecular biology. The principle, according to which this assign is carried out, is denoted as the **genetic code**.

The central dogma is a theory of the transmission of hereditary information and protein synthesis. The priciples of this dogma are:

1. DNA stores and transmits all hereditary information.
2. DNA is replicated in the cell nucleus before cells division. **Replication** *is a copying process by which DNA is supplied to the new cells formed by cell division.*
3. Information for protein synthesis is passed from DNA to a form of RNA called messenger RNA (mRNA). *The transmission of information is called* **transcription** (Fig. 10.19.)**.**
4. mRNA directs the synthesis of proteins by a process called translation. **Translation** *is a conversion of information from the sequence of nucleotides into a sequence of amino acids in a protein chain*. Amino acids are brought to ribosomal RNA, the site of protein synthesis, by transfer RNA (Fig. 10.19.).

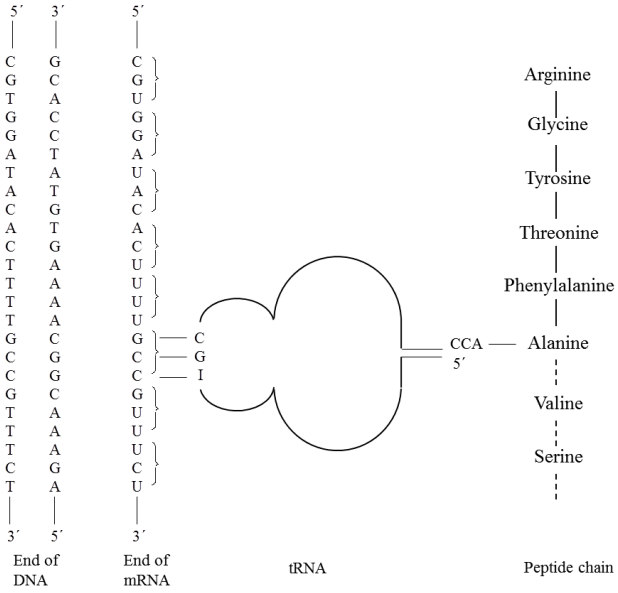


Fig. 10.19. Scheme of transcription and translation of genetic information

10.6. Mutations

Mutation is a change in the nucleotide sequence of the genome. Its principles are nucleotide substitutions, deletions or insertions.

Living organism is exposed to various chemical, physical and biological factors of the environment named **mutagens**. Some of them are of natural origin, the others appeared by deterioration of the environment by human activities. Even though the frequency of spontaneous mutation is very low (estimated to be 10^{-5} – 10^{-10} in the cell during its existence), the mutations are the most important processes of evolution. They lead to the expansion of the genomic spectrum and take place in all living organisms without exception.

10.6.1. Physical mutagens

Ionizing radiation (roentgen X-rays, protons, and electrons with high energy content) applies to the hydrogen atoms. An electron from H acts directly on the DNA (direct effect) or cleaves the water to form the hydroxyl radical which is detrimental to the structure of DNA (indirect effect). Direct and indirect effects of ionizing radiation lead to the same changes in the structure of DNA. Crosslinking, which are the covalent bonds between the opposed nucleotides, and the breaks in one or both DNA strands are formed. About 65 % of the damage is caused by the hydroxyl radical, and 35 % by the direct ionisation.

Ultraviolet radiation (UV radiation) is a type of non-ionizing radiation, which is absorbed by molecules and not by atoms (in contrast with ionizing radiation). In the cell, the most sensitive molecules to the effect of UV radiation are nucleic acids due to the presence of conjugated double bonds. Only molecules with double bonds are able to absorb UV rays. Each molecule like this has its specific absorption spectrum with a maximum at certain wavelengths. Nucleic acids have absorption maximum at 260 – 280 nm. Ultraviolet rays in this wavelength range have direct effect on nucleic acids resulting in dimerization of two adjacent pyrimidine molecules (Fig. 10.20.). Thymine dimer is formed in DNA between two adjacent thymine molecules by breaking down double bonds and creating a cyclobutane ring. Dimers that are formed this way are chemically stable, and they are lethal when not removed, because they restrain replication and transcription. A dimer between thymine and cytosine can be created in a similar manner. UV radiation can lead to mutations by so-called indirect way through oxidation processes in the organism (see Chap. 13).

Radiation of this wavelength does not cause ionization, but excitation of atoms, i.e. skipping of electrons to energetically higher levels, resulting in formation of unstable tautomeric forms. This is a probable mechanism of the increase of spontaneous mutation frequency.

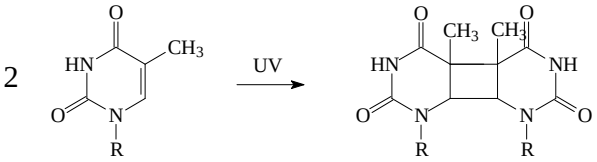


Fig. 10.20. Formation of thymine dimer

10.6.2. Chemical mutagens (chemomutagens)

The effect of chemical mutagens is particularly manifested by modification of heterocyclic bases. Heterocyclic bases determine the specific properties of nucleic acids. They contain reactive groups, which can be modified by effect of various chemical agents. The reactivity of monomer units in the structure of the polynucleotide (biopolymer) are greatelly influenced by spatial factors. Reactivity of heterocyclic bases is dependent on the nature and the reaction conditions and in case of polynucleotide, on its structure. Modification of heterocyclic bases may cause change in base pairing and following mutation.

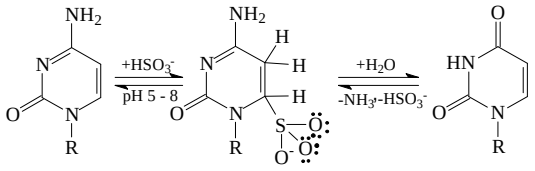
Among chemical mutagens following compounds can be included:

Analogs of nitrogenous bases. Purine and pyrimidine derivatives structurally similar to nitrogen bases are normal constituents of nucleic acids and may be incorporated into nucleic acids instead of them. Very effective mutagen is for example 5-bromouracil. It is the analog of thymine, which means that is incorporated into DNA and replaces thymine. 5-bromouracil, however, pairs with guanine, and so in the process of replication AT base pair is changed by GC base pair. Thus, mutation is induced by transition process. The transition is also caused by 2-aminopurine, which is an analogue of adenine.

Acridines are basic dyes (e.g. proflavine), which are able to incorporate into DNA, thereby causing changes leading to a shift in the reading of the genetic code, so-called frameshift mutations. Their structure is similar to purine – pyrimidine structure, and because of their size they are able to be inserted precisely into the gap between two adjacent bases (intercalation).

Nitrous acid (HNO_2) is a potent mutagen. Heterocyclic bases of nucleic acids underlie the oxidative deamination by HNO_2, resulting in conversion of cytosine to uracil, adenine to hypoxanthine or guanine to xanthine. Deamination leads to a change in the base pairing through substitution transition.

Hyposulfites (HSO_3^-) can be reversibly added to a double bond of cytosine or uracil, resp. to form the 5,6-dihydrogen derivates. The increased reactivity of the amino group of these derivatives causes its easy exchange by various nucleophilic agents. Replacement of the amino group with oxo group allows the specific conversion of cytosine to uracil, which then pairs with adenine, so it occurs GC → AT transition.

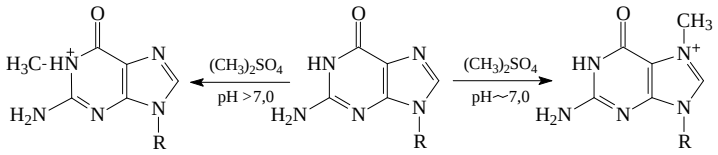


The **hydroxylamine** (NH_2OH) reacts with cytosine, resulting in the replacement of amino group by hydroxyamino group (–NHOH) at the C-6. The modified cytosine pairs with adenine, what leads to the GC → AT transition. Hydroxylamine is more efficient to the single-stranded DNA than to double-stranded.

Methoxyamine ($H_2N–O–CH_3$) reacts with cytosine and adenine. The reaction is based on the substitution of a hydrogen of the amino group by methoxyamino group –NH–O–CH_3. Substitution leads to the GC → AT transition.

Alkylating agents are divided into alkylsulphates that bind preferentially to nitrogen atom and nitroso compounds which bind preferentially to oxygen. N-alkyl-N-nitrosamines are mutagens which require metabolic activation before impact. Such mutagens are named *promutagens* resp. *procancerogens*. Metabolic activation can provide e.g. cytochrome P450 system. The most famous examples of promutagens and procancerogens are N-acetyl-2-aminofluorene, benzopyrene, aflatoxins, N-methyl-N-nitro-N-nitrosoguanidine, dimethylnitrosamine, N-methylnitrosourea and nitrates. Well studied is the reaction with dimethylsulphate $(CH_3)_2SO_4$. Entry of the

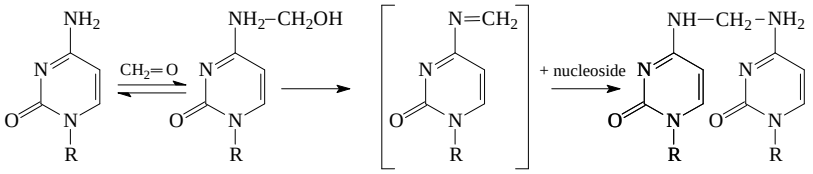
methyl group determinates the nature of the heterocyclic base and reaction conditions. E.g. guanine is alkylated under neutral conditions preferably at the carbon C-7, while in basic conditions at the nitrogen N-1:



In adenine three nitrogen atoms can be methylated, the reaction rate decreases in the order N-1 > N-7 > N-3. The product of cytosine methylation is a 3-methylcytosine.
In DNA molecule, the N-3 atoms of adenine and N-7 atom of guanine are methylated preferentially and the presence of the methylated forms limits to some degree the efficiency of binding between complementary bases.

Psovalens (furocoumarins with the tricyclic planar configuration) are able to intercalate into DNA between two adjacent nucleotides, and then be photoreactivated by ultraviolet light, resulting in the formation of adducts through the 5,6-double bond of thymine.

Formaldehyde (CH_2=O) reacts with the amino group of heterocyclic bases of nucleic acids, resulting in the formation of hydroxymethylamino compounds. The reaction is reversible, after removal of the aldehyde from the reaction mixture substrates are rapidly regenerated. Under basic conditions the intermediate Schiff base is formed which can react with an amino group of another nucleoside to form a stable compound methylene-bis nucleoside:



10.6.3. Molecular basis of point mutations

Substitution of bases leads to amino acid alteration in the protein chain. The classical example of point mutation is a *sicle cell anemia* in which a modified nucleotide can cause profound changes in the structure, and thus in the capacity of protein chain of hemoglobin. Human hemoglobin is composed of two α- and two β-chains. In position 6 of the β-chain of normal hemoglobin glutamic acid is present, whereas in hemoglobin S which causes *sickle cell anemia* valine can be found:

Hb A: val – his – leu – thr – pro – **glu** – glu – lys –
Hb S: val – his – leu – thr – pro – **val** – glu – lys –

Glutamic acid is encoded by the triplets GAA, GAG, and valine is encoded by the triplets GUU, GUC, GUA, GUG. The mutation can lead to exchange of one nucleotide in a codon (e.g. A in normal hemoglobin is replaced with U) resulting in amino acid substitution during synthesis of a polypeptide chain. Molecular basis of the mutation can be expressed as follows:

Val	G U A	G U G
	↑	↑
Glu	G A A	G A G

Point mutation is also a base of other groups of diseases - thalassemia. Substitution of one nucleotide in a codon in *β-thalassemia* yields in formation of so-called UAA or UAG "termination codon". Following process of translation will stop at this codon and abnormal shorter polypeptide of hemoglobin β-chain will be formed (144 amino acid residues instead of 146). In *α-thalassemia* abnormally longer α-chains of hemoglobin are synthesized (173 amino acid residues instead of 141). The molecular basis of α-thalassemia is the mutation in the "stop codon" while triplets CAA (encoding glutamine), AAA (encoding lysine), GAA (encoding glutamate) and UCA

(encoding serine) are formed. Both changes in the nucleotide sequences of the DNA lead ultimately to a change in the structure of hemoglobin, thus to a change of its properties (see chap. 9).

10.6.4. Genetic risk of nucleic acids modification

Modification of heterocyclic bases represent a genetic risk. Genetic risk of chemicals is associatedwith their late effects consisting of the ability to induce changes in the genetic material. A mutation is represented by a change in the DNA structure, or by conversion of the nucleotide sequence. A mutation means a change of information. The genetic load of the population is manifested by increased frequency of metabolic disorders and by susceptibility to certain diseases.

Compounds with mutagenic effects occur in living and working environment due to the contamination with industrial wastes. The most prevalent air pollutants include sulphur compounds that arise from combustion of coal and oil. In addition to SO_2, H_2SO_3, H_2SO_4 and H_2S also the organic sulphur compounds reach the atmosphere.

The most harmful nitrogen oxides are NO and NO_2. NO gets into the atmosphere as a product of biological and combustion processes in industry and traffic. They contribute to the formation of photochemical smog, and at higher concentrations react with water to form HNO_3 and HNO_2. NO and NO_2 are found in large concentrations in cigarette smoke. Excessive use of nitrogen fertilizers results in contamination of surface water. The excess of nitrates in drinking water and food threatens human health. The action of certain microorganisms in the gut can reduce nitrates to nitrites, or into nitrosamines and nitroso amides. These substances damage the liver and exhibit mutagenic and carcinogenic effects.

A highly reactive compound under conventional conditions in the gas phase is **formaldehyde.** Its worldwide production is approximately 7,000,000 tons per year. To formaldehyde (CH_2=O) are exposed individuals in living and working environments and in interior ambience due to its evaporation from the building and insulating materials, and various consumer goods (urea-formaldehyde binder based on phenol formaldehyde resins). Formaldehyde is a part of photochemical smog. Chronic exposure to low concentrations of formaldehyde in interiors affects the damage to the genetic material of somatic cells.

Mutagenic effects were confirmed in numerous **metallic compounds**, which contaminate the environment. These are organic (methyl mercury) and inorganic mercury compounds, certain compounds of lead, nickel, chromium (Cr^{6+}), cadmium, and arsenic (see chap. 2).

Recently, attention has been focused on organohalogens that contaminate food. These include **dioxins** above all, such as 2,3,6,7 - tetrachlorodibenzodioxin (see chap. 6). They are formed in small amounts as a side-product of polyhalogenated compounds production (e.g. in the production of PVC). The highest concentrations of these compounds in foods are found in fish and fish products and in the dairy and meat products. Studies in humans have shown carcinogenic, teratogenic and mutagenic effects of dioxins. They are harmful even at very low concentrations (< 1 ppm).

In order to ensure the healthy development of population it is essential to protect the environment from contamination with industrial waste containing genotoxic substances, i.e. substances resulting in a change of hereditary characteristics.

Control questions:

1. Explain why nitrogen bases may not occur in the Nucleic acids in lactim form.
2. Indicate which bases are complementary:
   A - U mA - T mG - C mA - R mI - mC A - G I - C
3. Nucleic acids can be found:
   a) only in the nucleus
   b) in the nucleus, cytoplasm and mitochondria
   c) in chloroplasts and mitochondria
4. Write the complementary DNA sequence in the direction 5'→ 3'.
   a) 5'-G A T C A A-3'
   b) 5'-T C G A A C-3'
   c) 5'-A C G C G T-3'
   d) 5'-T A C C A T-3'
5. Write the sequence of mRNA that is synthesized from the DNA template:
   5'-A T C G T A C C G T T A-3'
6. The molar composition of one strand of the DNA helix is: [A] = 0.3 and [G] = 0.24.
   a. What can you say about [T] and [C] of the same chain
   b. What can you say about [A], [G], [T] and [C] of the complementary strand
7. The molar ratio of (G + C) / (A + T) after the isolation of the DNA was equal to 1.4. Can you determine from which cells the DNA was isolated? From the cells of higher organisms, bacteria or viruses?
8. tRNA has following anticodons:
   3'-G C A C C U A U G U G A A A A-5'.
   Write a nucleotide sequence of the DNA template in the direction of 3 '→ 5'.
9. Write the reaction of adenine with HNO_2 and indicate the name of the product.
10. Write the reaction of complete hydrolysis of adenosine 5'-monophosphate (AMP).
11. 5-fluorouracil-2-deoxyribonucleotide is a molecule with anti-cancer and antiviral activity. Write the formula.

11. VITAMINS

History of medicine recognizes certain diseases which can be prevented by some foodstuffs. Why and how some food substances help to keep the health of the organism? In 1912 the Warsaw-born biochemist Casimir Funk (1884-1967) at the Lister Institute in London, isolated a substance that prevented nerve inflammation (neuritis) in chickens raised on a diet deficient in that substance. He named the substance "vitamin" (from „vital amines“) because he believed it was necessary to life and it was a chemical amine. The "e" at the end was later removed when it was recognized that vitamins need not to be amines. The letters (A, B, C and so on) were assigned to the vitamins in the order of their discovery. The one exception was vitamin K which was assigned its "K" from "Koagulation" by the Danish researcher Henrik Dam.

What are the vitamins?

Vitamins are organic compounds that cannot be produced by the body (there are a few exceptions such as vitamin H, B_{12}, folic acid synthesized by the gut microflora) and must be obtained through the diet or supplements. They are required by living organisms in relatively small amounts to maintain normal health. They are synthesized in plants and microorganisms. Animals consume them in the final form or in the form of so called **provitamins** which can be converted to their final form in the animal's body. Some vitamins can be produced in the body but in amounts not sufficient for the metabolism in situations such as pregnancy, breast-feeding, growth, infection diseases. Consequently, they must be obtained from the diet.

Classification of vitamins

Vitamins can be classified into two groups:

1. Fat soluble vitamins – A, D, E, K, F

2. Water soluble vitamins - vitamin B complex group and ascorbic acid

Fat soluble vitamins can be stored in the organism for months. Neither water soluble vitamins need to be taken every day. Their supplies can last for a few days and in some cases for a few months or even years. Fat soluble vitamins require healthy liver, gall bladder, healthy digestion and absorption of fat, while water soluble vitamins can be absorbed into the blood from the gut directly or by the help of some enzymes or transportation systems. The excess of water soluble vitamins the body can compensate into some extent by excretion (excess of vitamins B and C can be also harmful), fat soluble vitamins can be stored in the organism and may become hazardous – developing signs of toxic hypervitaminosis.

Function of vitamins

Vitamins are necessary for normal metabolism in animals, but either are not synthesized in the body or are synthesized in inadequate quantities. **Water-soluble** vitamins work together with enzymes as so called **co-enzymes** (substances that assist enzymes). Coenzymes combine with a protein to form enzymes which promote release and utilization of energy. The **fat soluble** vitamins act as regulators of specific metabolic activity. Each vitamin has specific functions. If levels of a particular vitamin are inadequate, a deficiency disease results. Vitamins are **essentia**l for life. A substance that prevents a vitamin from exerting its typical metabolic effects is called **antivitamin.** Each vitamin has one or more antivitamins. They can be classified into following classes:

1. Enzymes decomposing vitamins (*tiaminase, ascorbase*)
2. Compounds producing non-effective complexes with vitamins (avidin)
3. Compounds resembling vitamins with their structure (PABA – para aminobenzoic acid)

Antivitamins are used in medical praxis especially in chemotherapy of various infection diseases.

Avitaminosis is any disease caused by chronic or long-term vitamin deficiency or caused by a defect in metabolic conversion, such as tryptophan to niacin. They are designated by the same letter as the vitamin.

Avitaminoses include:

- **vitamin A** deficiency causes **xerophthalmia** or **night blindness**
- **thiamine** (vitamin B_1) deficiency causes **beri-beri**
- **niacin** (vitamin B_3) deficiency causes **pellagra**
- **vitamin B_{12}** deficiency leads to **pernicious anemia**
- **vitamin C** deficiency leads to **scurvy**
- **vitamin D** deficiency causes **rickets**
- **vitamin K** deficiency causes **bleeding**

However, most common is **hypovitaminosis** caused by relative deficiency of one or more vitamins as a result of non-healthy diet. Conversely **hypervitaminosis** is caused by over-retention of fat-soluble vitamins in the body. Standards and guides have been established for estimating the amount of vitamins which individuals should get per day. The **Recommended Dietary Allowances (RDA's)** provide the best scientific information on vitamin requirements.

11.1. Fat soluble vitamins

11.1.1.Vitamin A – Retinol

Vitamin A is the collective name for a group of fat-soluble vitamins. The most useable form of the vitamin is retinol (Fig. 11.1.). Retinol is the immediate precursor to two important active metabolites: **retinal**, which plays a critical role in vision, and **retinoic acid** (Fig. 11.1.), which serves as an intracellular messenger that affects transcription of a number of genes. Retinol, retinal, retinoic acid and related compounds are known as retinoids.

Vitamin A does not occur in plants, but many plants contain **carotenoids such as beta-carotene** that can be converted to vitamin A within the intestine and other tissues. Beta-carotene (Fig. 11.1.) and other carotenoids are referred to as **provitamin** A (vitamin A precursor). Animals are incapable of synthesizing carotenoids, and must obtain them through their diet. Carotenoids are organic pigments that are naturally occurring in plants and some other photosynthetic organisms. There are over 600 known carotenoids. Carotenoids belong to the category of tetraterpenoids (i.e. they contain 40 carbon atoms). Structurally they are in the form of a polyene chain. In photosynthetic organisms, carotenoids play a vital role in the photosynthetic reaction centre. In non-photosynthesizing organisms, carotenoids have been linked to oxidation-preventing mechanisms. Carotenoids are efficient free-radical scavengers (**antioxidants**), and they enhance the vertebrate immune system.

Retinol is ingested in a precursor form; animal sources (milk and eggs) contain retinyl esters, whereas plants (carrots, spinach) contain pro-vitamin A carotenoids. Hydrolysis of retinyl esters results in retinol while **pro-vitamin A carotenoids can be cleaved to produce retinal**. Retinal, also known as retinaldehyde, can be reversibly reduced to produce retinol or it can be irreversibly oxidized to produce retinoic acid. The best described active retinoid metabolites are 11-*cis*-retinal and the all-*trans* and 9-*cis*-isomers of retinoic acid. 11-*cis*-retinal isomer is the chromophore of rhodopsin, the vertebrate photoreceptor molecule.

Physiologic Effects of Vitamin A

Vitamin A and its metabolites retinal and retinoic acid appear to serve a number of critical roles in physiology, as evidenced by the myriad of disorders that accompany deficiency or excess states. In many cases, precise mechanisms are poorly understood. Some of the well-characterized effects of vitamin A include:

- **Vision:** Retinal is a necessary structural component of **rhodopsin** or *visual purple*, the light sensitive pigment within rod and cone cells of the retina. If inadequate quantities of vitamin A are present, vision is impaired due to inability to synthesize adequate quantities of rhodopsin. Moderate deficiency leads to deficits in vision under conditions of low light ("night blindness"), while severe deficiency can result in severe dryness and opacity of the cornea (xerophthalmia).
- **Vision cycle**: The molecule that takes part in the initial step in the vision process, rhodopsin has two components called 11-*cis* retinal and opsin. Retinal is a light-sensitive derivative of vitamin A and opsin is a protein molecule. Rhodopsin is found in the rod cells of the eye. 11-*cis* retinal is a powerful absorber of light because it is a polyene; its 6 alternating single and double bonds make up a long unsaturated electron network. When no light is present, the 11-*cis* retinal molecule is found in a "bent configuration" (Fig.11.2.) and as such it is attached to the opsin molecule in a stable arrangement. When light strikes the retina, after the retinal molecule absorbs a photon into one of the pi bonds found between the eleventh and twelfth carbon atoms, the **11-*cis* retinal is transformed into the all-*trans* retinal** (Fig.11.2.) in a straightened configuration. The all-*trans* retinal configuration, subsequently, does not fit into the binding site of the opsin molecule; as a result, upon isomerization, the trans isomer separates from the protein, which triggers a G protein signaling pathway including transducin that results in the generation of an electrical impulse, which is transmitted through the optic nerve to the brain for processing. It takes a minimum of five photons to trigger a nerve impulse. In the absence of light, enzymes mediate the isomerization of all-*trans* back to the 11-*cis* configuration, and rhodopsin is regenerated by a new formation of a Schiff base linkage, which actuates the binding of the *cis* isomer to opsin. This is the basic mechanism of the vision cycle (Fig. 11.3.).

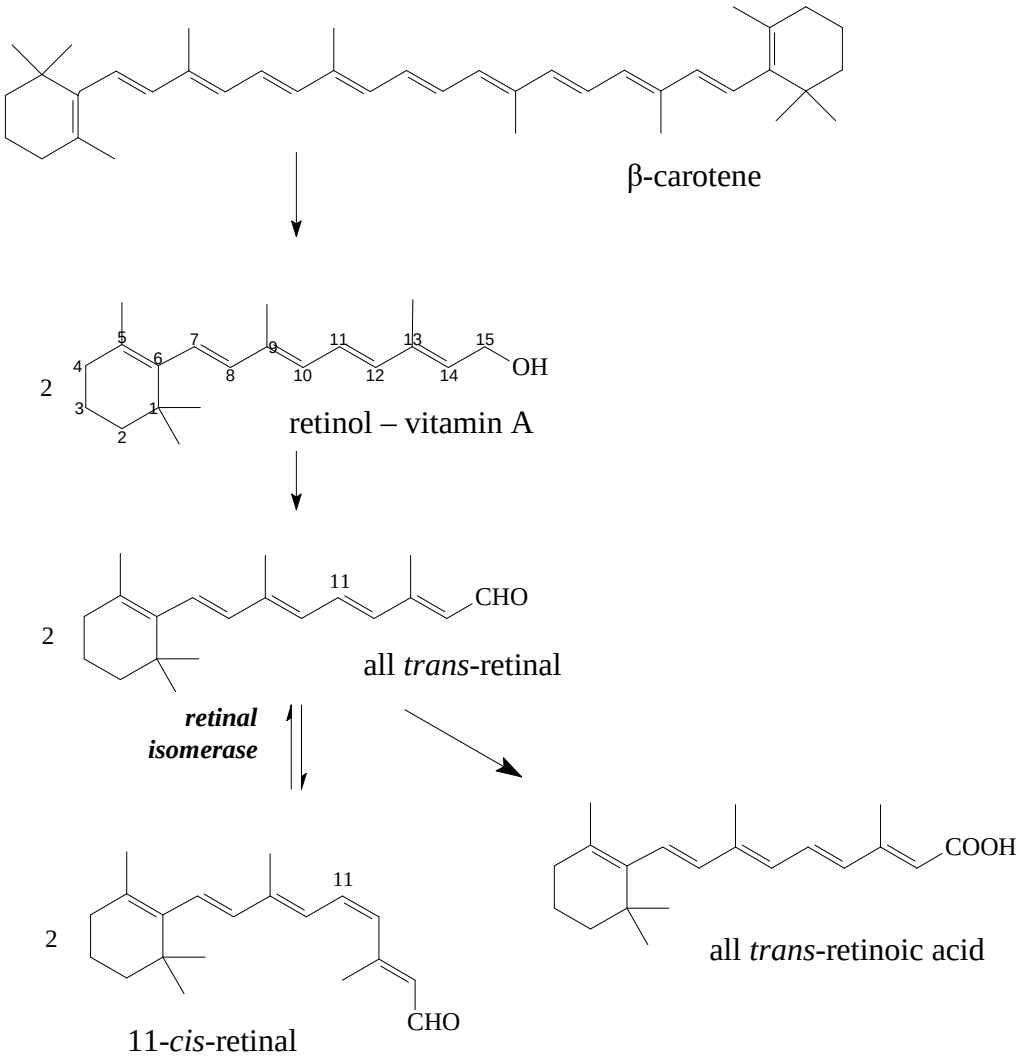


Fig. 11.1. Beta-Carotene. Molecule of β-carotene splits to two molecules of retinol which yields the final retinal or is irreversibly oxidized to retinoic acid.

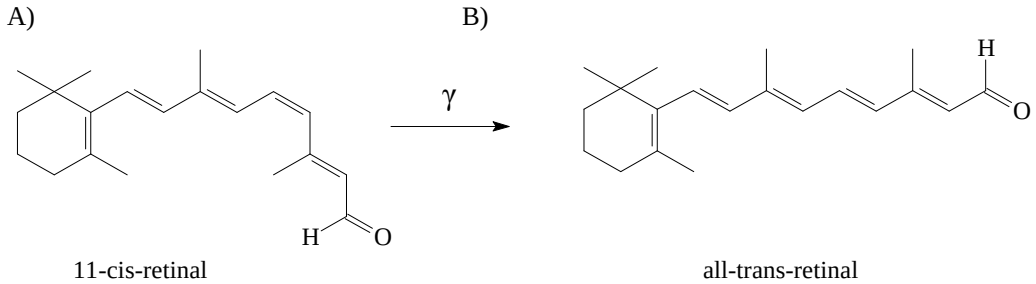


Fig. 11.2. Retinal molecule (A) – straightens (B) in response to a photon γ (light), of the correct wavelength

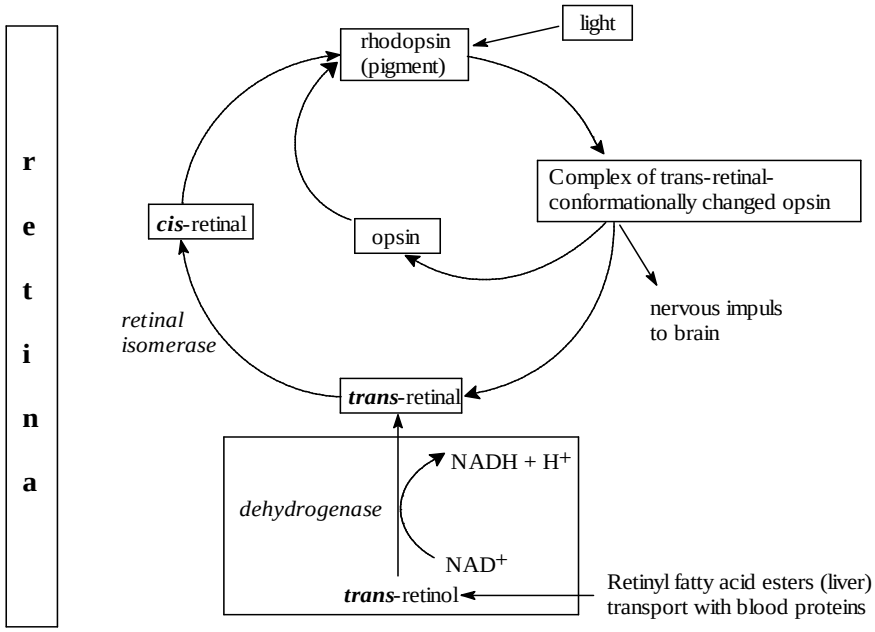


Fig. 11.3. Vision cycle

Many of the non-visual functions of vitamin A are mediated by **retinoic acid**, which regulates gene expression by activating intracellular retinoid acid receptors. The non-visual functions of vitamin A are essential in **the immunological** function, **reproduction** and **embryonic development** of vertebrates as evidenced by the impaired growth, susceptibility to infection and birth defects observed in populations receiving suboptimal vitamin A in their diet.

- **Resistance to infectious disease:** In almost every infectious disease studied, vitamin A deficiency has been shown to increase the frequency and severity of disease. Many infections are associated with inflammatory reactions that lead to reduced synthesis of retinol-binding protein and thus, reduced circulating levels of retinol.
- **Epithelial cell "integrity":** Lack of vitamin A leads to dysfunction of many epithelia - the skin becomes keratinized and scaly, and mucus secretion is suppressed.
- **Bone remodeling:** Normal functioning of osteoblasts and osteoclasts is dependent upon vitamin A.
- **Reproduction:** Normal levels of vitamin A are required for sperm production, reflecting a requirement for vitamin A by spermatogenic epithelial (Sertoli) cells. Similarly, normal reproductive cycles in females require adequate availability of vitamin A.

Vitamin A deficiency usually results from malnutrition, but can also be due to abnormalities in intestinal absorption of retinol or carotenoids. Because the liver stores rather large amounts of retinol, deficiency states typically take several months to develop.

Excess States

Both too much and too little vitamin A are well known causes of disease in man and animals. **Vitamin A excess states**, not as common as deficiency, also lead to disease. Vitamin A and most retinoids are **highly toxic** when taken in large amounts, and the most common cause of this disorder is excessive supplementation. Symptoms of a vitamin A overdose include tiredness, discomfort, lethargy, upset stomach, decreased appetite, vomiting, slow or decreased growth, joint soreness, irritability, headache, drying and cracking of the lips and skin, hair loss, and yellowing of the skin. In contrast, excessive intake of carotenoids are not reported to cause disease. The body cannot be intoxicated by carotenoids – organism can produce only so much of vitamin A from carotenoids that is necessary to meet its needs.

Both hypovitaminosis A and hypervitaminosis A are known to cause congenital defects in animals and likely to have deleterious effects in humans. Pregnant women are advised not to take excessive vitamin A supplements.

Sources

Vitamin A is present in many **animal tissues**, and is readily absorbed from such dietary sources in the terminal small intestine. Liver is clearly the richest dietary source of vitamin A.

Plants do not contain vitamin A, but many dark-green or dark-yellow plants (including carrots) contain carotenoids such as beta-carotene that serve as provitamins because they are converted within the intestinal mucosa to retinol during absorption.

Vitamin A is stored in the liver as **retinyl esters** and, when needed, exported into blood, where it is carried by **retinol binding protein** for delivery to other tissues.

11.1.2. Vitamin D – Calciferol

Vitamin D refers to a group of fat-soluble **prohormones**, the two major forms of which are vitamin D_2 (or **ergocalciferol**) and vitamin D_3 (or **cholecalciferol**). **Vitamin D_2** is derived from **ergosterol (provitamin)** present in fungal and plant sources after exposure to UV light, and is not produced by the human body. **Vitamin D_3** is derived from animal sources and is made in the skin when **7-dehydrocholesterol (provitamin)** reacts with UV light. In this reaction, the B ring of the sterol molecule is opened (Fig. 11.4. – first reaction).

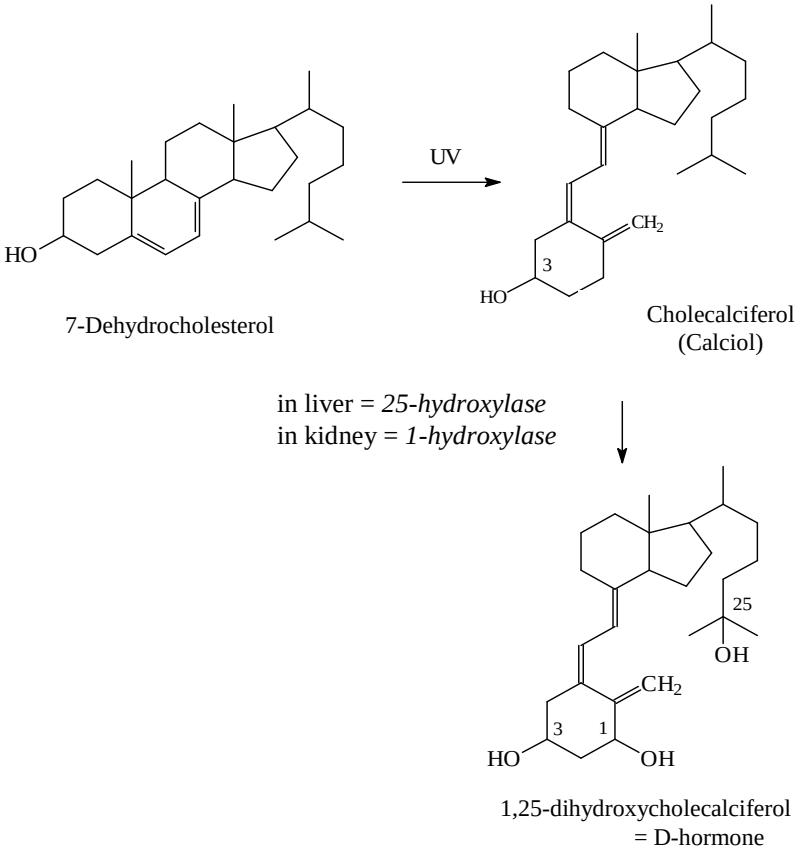


Fig. 11.4. Transformation of 7-dehydrocholesterol to its active form of 1,25-dihydroxycholecalciferol (calcitriol)

Vitamin D, as either D_3 or D_2, does not have significant biological activity. Rather, it must be metabolized within the body to the hormonally-active form known as 1,25-dihydroxycholecalciferol (calcitriol). This transformation occurs in two steps, as depicted in the Fig. 11.4.:

1. **within the liver**, cholecalciferol is hydroxylated to 25-hydroxycholecalciferol by the enzyme *25-hydroxylase*.
2. **within the kidney**, 25-hydroxycholecalciferol serves as a substrate for *1-alpha-hydroxylase*, yielding 1,25-dihydroxycholecalciferol **(calcitriol)**, the biologically **active form**.

Vitamin D is thus not a true vitamin, because individuals with adequate exposure to sunlight do not require dietary supplementation. Active vitamin D functions as a hormone because it sends a message to the intestines to increase the absorption of calcium and phosphorus. Each of the vitamin D forms is hydrophobic, and is

transported in blood bound to carrier proteins. The major carrier is called **vitamin D-binding protein.** The halflife of 25-hydroxycholecalciferol is several weeks, while that of 1,25-dihydroxycholecalciferol is only a few hours. Vitamin D is stored in the human body as calcidiol (25-hydroxycholecalciferol).

Adequate amounts of vitamin D_3 can be made in the skin only after 10 -15 minutes of sun exposure at least two times per week to the face, arms, hands, or back **without sunscreen**. With longer exposure to UVB rays, an equilibrium is achieved in the skin, and the vitamin simply degrades as fast as it is generated.

Physiologic Effects of Vitamin D

Vitamin D plays an important role in the maintenance of several organ systems.

- Vitamin D **regulates** the **calcium** and **phosphorus** levels in the blood by promoting their absorption from food in the intestines, and by promoting re-absorption of calcium in the kidneys
- It promotes **bone formation and mineralization** and is essential in the development of an intact and strong skeleton
- It **inhibits parathyroid hormone** secretion from the parathyroid gland
- Vitamin D affects the **immune system** by promoting immunosuppression and anti-tumor activity

Deficiency

Vitamin D deficiency is known to cause several bone diseases including:

- **Rickets** a childhood disease characterized by impeded growth, and deformity, of the long bones.
- **Osteomalacia** a bone-thinning disorder that occurs exclusively in adults and is characterized by proximal muscle weakness and bone fragility.
- **Osteoporosis**, a condition characterized by reduced bone mineral density and increased bone fragility

Vitamin D malnutrition may also be linked to an increased susceptibility to several chronic diseases such as high blood pressure, tuberculosis, cancer, multiple sclerosis, chronic pain, depression, schizophrenia, seasonal affective disorder and several autoimmune diseases.

Vitamin D Excess States

Vitamin D toxicity can cause nausea, vomiting, poor appetite, constipation, weakness, and weight loss. Too much of vitamin D can make the intestines absorb too much of calcium. This may cause high levels of calcium in the blood. High blood calcium can lead to calcium deposits (**calcinosis**) in soft tissues such as the heart and lungs. This can reduce their ability to function.

Sun exposure is unlikely to result in vitamin D toxicity. Diet is also unlikely to cause vitamin D toxicity, unless large amounts of cod liver oil are consumed. Vitamin D toxicity is much more likely to occur from high intakes of vitamin D in supplements.

Food Sources

Vitamin D is found in the following foods: dairy products (cheese, butter, cream), fatty fish (cod liver oil, salmon, mackerel, tuna, sardines) eggs, beef liver.

11.1.3. Vitamin E – tocopherol (antisterile vitamin)

Natural vitamin E exists in eight different forms or isomers, four tocopherols (Fig. 11.5.) and four tocotrienols. All isomers have a chromanol ring, with a hydroxyl group which can donate a hydrogen atom to reduce free radicals and a hydrophobic side chain which allows for penetration into biological membranes. There is an alpha, beta, gamma and delta form of both the tocopherols and tocotrienols, determined by the number of methyl groups on the chromanol ring. Alpha-tocopherol is the only form of vitamin E that is actively maintained in the human body.

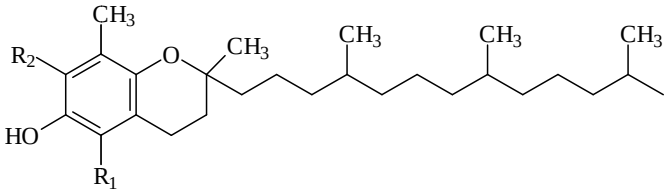
Physiologic Effects of Vitamin E

The main function of alpha-tocopherol in humans appears to be that of an **antioxidant**. Free radicals are formed primarily in the body during normal metabolism and also upon exposure to environmental factors such as cigarette smoke or pollutants. Lipids, which are an integral part of all cell membranes, are vulnerable to destruction through oxidation by free radicals. The fat-soluble vitamin, alpha-tocopherol, is uniquely suited to intercepting free radicals and preventing a chain reaction of lipid destruction. Aside from **maintaining the**

integrity of cell membranes throughout the body, alpha-tocopherol also **protects the fats in low density lipoproteins (LDLs) from oxidation.** When a molecule of alpha-tocopherol neutralizes a free radical, it is altered in such a way that its antioxidant capacity is lost. However, other antioxidant, such as **vitamin C, is capable of regenerating** the antioxidant capacity of **alpha-tocopherol** (see chapter 13.2.2. Fig. 13.2).

Several other functions of alpha-tocopherol have been identified, which likely are not related to its antioxidant capacity:

- vitamin E is important in the formation of red blood cells and helps the body to use vitamin K
- at lower levels, vitamin E may help protect the heart
- it affects the expression and activity of immune and inflammatory cells
- it inhibits platelet aggregation
- it enhances vasodilation
- it inhibits the activity of *protein kinase C*, an important cell signaling molecule



	R_1	R_2
α-tocopherol	$-CH_3$	$-CH_3$
β- tocopherol	$-CH_3$	-H
γ- tocopherol	-H	$-CH_3$
δ- tocopherol	-H	-H

Fig. 11.5. Tocopherol (Vitamin E)

Deficiency

Severe vitamin E deficiency results mainly in neurological symptoms, including impaired balance and coordination (ataxia), injury to the sensory nerves (peripheral neuropathy), muscle weakness (myopathy), and damage to the retina of the eye (pigmented retinopathy).

Food sources

Major sources of alpha-tocopherol include vegetable oils (olive, sunflower), nuts, whole grains, and green leafy vegetables.

11.1.4. Vitamin K – Quinones

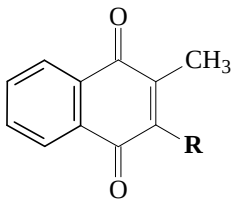
Vitamin K is a fat-soluble vitamin. The "K" is derived from the German word "koagulation". Coagulation refers to blood clotting, because vitamin K is essential for the functioning of several proteins involved in blood clotting.

Plants synthesize **phylloquinone**, also known as **vitamin K_1** (Fig. 11.6). Vitamin K_1 was named phylloquinone since it is an indirect product of photosynthesis in plant leaves where it occurs in chloroplasts and participates in the overall photosynthetic process. The methyl naphthoquinone ring has a phytyl side chain. Bacteria synthesize a range of vitamin K forms, using repeating 5-carbon units in the side chain of the molecule. These forms of vitamin K are designated **menaquinone-n** (MK-n) (Fig. 11.6.), where n stands for the number of 5-carbon units. MK-n are collectively referred to as **vitamin K_2.**

Menadione is a polycyclic aromatic ketone, based on 1,4-naphthoquinone, with a 2-methyl substituent. It was formerly sometimes called vitamin K_3, although derivatives of naphthoquinone without the sidechain in the 3-position cannot exert all the functions of the K vitamins. Menadione is a vitamin precursor of K_2 which utilizes alkylation in the liver to yield menaquinones (K_2 vitamins), and hence, is better classified as a provitamin. Although the natural K_1 and K_2 forms are nontoxic, the synthetic form K_3 (menadione) has shown toxicity.

Many bacteria, such as *Escherichia coli* found in the large intestine, can synthesize vitamin K_2 (menaquinone), but not vitamin K_1 (phylloquinone).

Although vitamin K is a fat-soluble vitamin, the body stores very little of it, and its stores are rapidly depleted without regular dietary intake. Perhaps, because of its limited ability to store vitamin K, the body recycles it through a process called the **vitamin K cycle**. The vitamin K cycle allows a small amount of vitamin K to function in the gamma-carboxylation of proteins many times, decreasing the dietary requirement.



(a) **R** = $CH_2-CH=\overset{\overset{CH_3}{|}}{C}-CH_2-(CH_2-CH_2-\overset{\overset{CH_3}{|}}{C}H-CH_2)_3-H$

(b) **R** = $(CH_2-\overset{\overset{CH_3}{|}}{C}H=C-CH_2)_n-H$

(c) **R** = H

Fig. 11.6. Basic structure of vitamins K and side chains of vitamin K_1 (a); K_2 - n = 6, 7 or 9 (b); menadione (c)

Function

The only known biological role of vitamin K is that of the required **coenzyme for** a vitamin K-dependent **carboxylase** that catalyzes the carboxylation of the amino acid, glutamic acid, resulting in its conversion to gamma-carboxyglutamic acid (Gla). Gla-residues are usually involved in binding calcium. At this time 14 human Gla-proteins have been discovered, and they play key roles in the regulation of three physiological processes:

- **blood coagulation** (prothrombin (factor II), factors VII, IX, X, protein C, protein S and protein Z)
- **bone metabolism** (osteocalcin, also called bone Gla-protein and matrix Gla protein)
- **vascular biology** – it helps against vascular thrombosis

Antagonists

Two vitamin K antagonists are **dicumarol** and **warfarin**. Dicumarol is an anticoagulant and has been used to prevent thrombosis in animals and man. Another anticoagulant, warfarin, is a common rat poison which has five to ten times the anticoagulant activity of dicumarol. The effects of warfarin can also be reversed by administration of vitamin K. Warfarin prevents the recycling of vitamin K.

Deficiency

Vitamin K deficiency is uncommon in **healthy adults** for a number of reasons: **1)** vitamin K is widespread in foods, **2)** the vitamin K cycle conserves vitamin K, and **3)** bacteria that normally inhabit the large intestine synthesize menaquinones (vitamin K_2), though it is unclear whether a significant amount is absorbed and utilized. Adults at risk of vitamin K deficiency include those taking vitamin K antagonist anticoagulant drugs and individuals with significant liver damage or disease. Symptoms of vitamin K deficiency include easy bruising and bleeding that may be manifested as nosebleeds, bleeding gums, blood in the urine, blood in the stool, or extremely heavy menstrual bleeding.

Newborn babies that are exclusively breast-fed are at increased risk of vitamin K deficiency for the following reasons: 1) human milk is relatively low in vitamin K compared to formula, 2) the newborn's intestine is not yet colonized with bacteria that synthesize menaquinones, and 3) the vitamin K cycle may not be fully functional in newborns, especially premature infants. In infants, vitamin K deficiency may result in life-threatening bleeding within the skull (intracranial hemorrhage).

Sources of Vitamin K

Vitamin K is found in green leafy vegetables such as spinach and lettuce; Alfalfa leaves are among the best sources of vitamin K; vegetables such as kale, cabbage, cauliflower, broccoli, and Brussels sprouts, cereals, some fruits, such as kiwifruit, meats, cow milk and other dairy products, eggs, soybeans, and other soy products.

11.2. Water-soluble vitamins

Water-soluble vitamins include ascorbic acid and eight well-recognized members of the vitamin B complex: thiamine, riboflavin, niacin, pantothenic acid, pyridoxine, biotin, vitamin B_{12} and folic acid.

11.2.1. Thiamine (Aneurine) – Vitamin B_1

Thiamine (Fig.11.7.) is a water-soluble B-complex vitamin, previously known as vitamin B_1 or aneurine. Thiamine occurs in the human body as free thiamine and its phosphorylated forms: thiamine monophosphate (TMP), thiamine pyrophosphate (TPP), which is also known as thiamine diphosphate and thiamine triphosphate (TTP). Thiamine consists of a pyrimidine ring and a thiazole ring connected by a one carbon link. The nitrogen in the thiazole ring has a charge of +1. This nitrogen atom serves as an important electron sink in thiamine pyrophosphate mediated reactions.

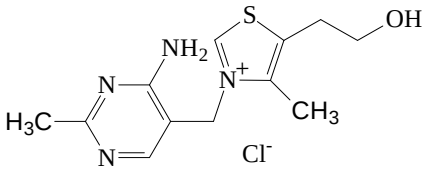


Fig. 11.7. Thiamine

Coenzyme function

Thiamine in the form of **thiamine pyrophosphate** (Fig.12.15.) functions in all cells as the **coenzyme of oxidative decarboxylation of 2-oxoacids.** It participates in the oxidative decarboxylation of pyruvic acid to acetate for entry into the tricarboxylic acid cycle. Thiamine pyrophosphate is also a coenzyme of the **transketolase** system by which direct oxidation of glucose occurs in the cytoplasm via the pentose phosphate pathway.

Non-coenzyme function

Thiamine triphosphate (TTP) is concentrated in nerve and muscle cells. Research in animals indicates that TTP activates membrane ion channels, possibly by phosphorylating them. The flow of electrolytes like sodium or potassium in or out of nerve and muscle cells through membrane ion channels plays a role in nerve impulse conduction and voluntary muscle action. Impaired formation of TTP may play a role in the neurologic symptoms of severe thiamine deficiency.

Deficiency

Thiamine deficiency may result from inadequate thiamine intake, an increased requirement for thiamine, excessive loss of thiamine from the body, consumption of anti-thiamine factors in food, or a combination of factors. **Beri-beri** is the disease resulting from severe thiamine deficiency. Thiamine deficiency affects the cardiovascular, nervous, muscular, and gastrointestinal systems.

Sources

Whole grain cereals, beans, lentils, nuts, lean pork, and yeast are rich sources of thiamine.

11.2.2. Riboflavin – Vitamin B_2

Riboflavin (Fig.11.8.) is stable to dry heat but is irreversibly decomposed on irradiation with ultraviolet rays or visible light, breaking down to lumiflavin.

Positive functions

Riboflavin functions in the tissues in the form of flavin adenine dinucleotide (FAD) (Fig.12.10.) or as flavin mononucleotide (FMN). They function as **coenzymes for** many **oxidases** and **reductases.**

Sources

Riboflavin is widely distributed in plants and in animal glandular tissues. Milk, liver, kidney, heart, yeast, grains, peanuts, soybeans and eggs are rich sources. Keeping feeds from sunlight or intense artificial light is necessary to minimize loss of the vitamin by conversion to lumiflavin.

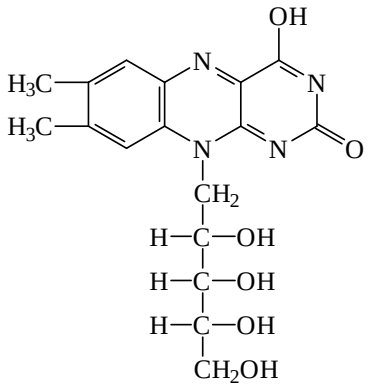
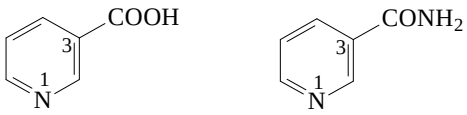


Fig. 11.8. Riboflavin – Vitamin B_2

11.2.3. Niacin – Vitamin B_3

Niacin and its amide, **niacinamide** (Fig. 11.9.) equivalently act as a vitamin. "Niacin" is a generic term describing both vitamins together.



Niacin **Niacinamide**

Fig. 11.9. Niacin and niacinamide - vitamin B_3

Function

The major function of niacin in NAD^+ and $NADP^+$ is **hydrogen transport** in intermediary metabolism. Niacin forms the core of the nucleotide in the coenzyme **NAD^+** (Nicotinamide Adenine Dinucleotide) and its phosphate coenzyme **$NADP^+$** (Fig. 12.8. and 12.9.). NAD^+ (but not $NADP^+$) is essential for energy production, electron transport through hydrogen acceptance (NADH) and donation. NADPH provides the reducing equivalents for reductive biosynthesis reactions.

Deficiency

Niacin can be synthesized from tryptophan, but tryptophan is an essential amino acid that cannot be synthesized in the body. Riboflavin (Vitamin B_2) and pyridoxine (Vitamin B_6) are essential cofactors in synthesizing niacin from tryptophan. The niacin-deficiency disease **pellagra** was first recognized in 1735 by a Spanish physician among people living on corn (maize), a vegetable that is low in both niacin and tryptophan. The main results of pellagra can easily be remembered as "the four D's": diarrhea, dermatitis, dementia, and death.

Most of these enzyme systems function by alternating between the oxidized and reduced state of the coenzymes NAD^+- NADH and $NADP^+$- NADPH. Oxidation-reduction reactions may be anaerobic when pyruvate acts as the hydrogen acceptor (from NADH) and lactate is formed, or the reactions may be coupled to electron transport systems with oxygen as the ultimate hydrogen acceptor; such aerobic reactions occur in respiration. NADH is

involved in the synthesis of high energy phosphate bonds which furnish energy for certain steps in metabolic reactions.

Sources

Niacin is found in most animal and plant tissues. Rich sources are yeast, liver, kidney, heart, and green vegetables. Wheat contains more niacin than corn and the vitamin is also found in milk and egg products.

11.2.4. Vitamin B_5 – pantothenic acid

Pantothenic acid (Fig. 11.10.) is needed to form coenzyme A (CoA) (Fig. 12.16.), and is critical in the metabolism and synthesis of saccharides, proteins and fats. Chemically it is the amide between D-pantoate and beta-alanine.

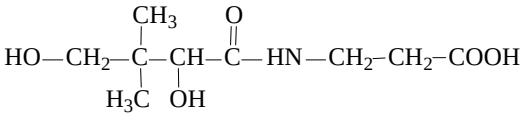


Fig. 11.10. Pantothenic acid – vitamin B_5

Function

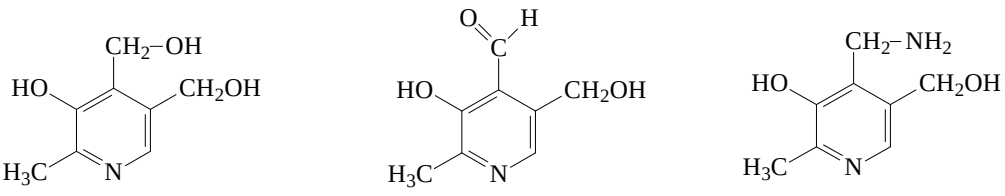
Only the dextrorotatory (D) isomer of pantothenic acid possesses biologic activity. The levorotatory (L) form may antagonize the effects of the dextrorotatory isomer. Pantothenic acid is used in the synthesis of **coenzyme A**. Coenzyme A may act as an **acyl group carrier** to form acetyl-CoA and other related compounds; this is a way to transport carbon atoms within the cell. The transfer of carbon atoms by coenzyme A is important in the biosynthesis of many important compounds such as fatty acids, cholesterol, acetylcholine etc.

Sources

Its name is derived from the Greek *pantothen* (παντόθεν) meaning "from everywhere" and small quantities of pantothenic acid are found in nearly every food, with high amounts in whole grain cereals, eggs, meat. A recent study also suggests that gut bacteria in humans can generate pantothenic acid.

11.2.5. Pyridoxine – Vitamin B_6

Compounds which have vitamin B_6 activity include: pyridoxine (pyridoxol), pyridoxal and pyridoxamine (Fig. 11.11.). Pyridoxine acts as a coenzyme in a number of enzyme systems. **Pyridoxal phosphate** (Fig. 12.17.) is the **coenzyme of decarboxylases**, involved in the decarboxylation of amino acids. It is also the co-factor of the 22 different **transaminases** present in animal tissues. Pyridoxine is sensitive to sunlight, cooking and processing. Cortisone is known to impair the absorption of pyridoxine. Exercising may aid the production of the active form of vitamin B_6.



Pyridoxine (pyridoxol) **Pyridoxal** **Pyridoxamine**

Fig. 11.11. Pyridoxine – Vitamin B_6

Toxicity and symptoms of high intake

Supplementation should be controlled as extreme dosage, such as in excess of 2,000 mg per day, may cause neurological damage. Pyridoxine should be taken together with the entire B group vitamins, and in supplementation the quantity of B_6 should be nearly the same as B_2, as the B_2 is needed to activate the Pyridoxine.

Deficiency of vitamin B_6

Irritability, nervousness and insomnia as well as general weakness, skin changes such as dermatitis and acne as well asthma and allergies might develop when pyridoxine is in short supply. Symptoms may include nails that are ridged, an inflamed tongue as well as changes to your bones - which can include osteoporosis and arthritis. Kidney stones may also appear.

Sources

Good sources of pyridoxine are whole cereals, yeast, egg yolk, liver and glandular tissues.

11.2.6. Biotin – Vitamin H or Vitamin B_7

Biotin (Fig. 11.12.) is a monocarboxylic acid slightly soluble in water and alcohol and insoluble in organic solvents.

Function

Biotin is a **cofactor** responsible for **carbon dioxide transfer** in several **carboxylase** enzymes. It is part of the coenzyme of several carboxylating enzymes fixing CO_2. Biotin is required in several specific carboxylation and decarboxylation reactions, including the carboxylation of pyruvic acid to form oxaloacetic acid.

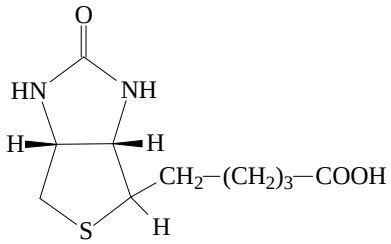


Fig. 11.12. Biotin – vitamin H

Deficiency

Biotin deficiency is a rare metabolic genetic disorder. Biotin deficiency can have a very serious, even fatal, outcome if it is allowed to progress without treatment. Biotin deficiency rarely occurs in healthy individuals, since the daily requirements of biotin are low, many foods contain adequate amounts, intestinal bacteria synthesize small amounts, and the body effectively scavenges and recycles biotin from bodily waste. However, deficiency can be caused by excessive consumption of raw egg-whites over a long period (months to years). Egg-whites contain high levels of avidin, a protein that binds biotin strongly. Once a biotin-avidin complex forms, the bond is essentially irreversible. The biotin-avidin complex is not broken down nor liberated during digestion, and the biotin-avidin complex is lost in the feces. Once cooked, the egg-white avidin becomes denatured and entirely non-toxic.

Initial symptoms of biotin deficiency include: dry skin, dermatitis, fungal infections, rashes, fine and brittle hair, hair loss. If left untreated, neurological symptoms can develop, including: mild depression, changes in mental status, generalized muscular pains.

Sources

Rich sources of biotin are liver, kidney, yeast, milk products, and egg yolks.

11.2.7. Cobalamin, Cyanocobalamin – Vitamin B_{12}

Vitamin B_{12} (cobalamin) (Fig. 11.13.) is an important water-soluble vitamin. It contains cobalt in its active centre. In contrast to other water-soluble vitamins it is not excreted quickly in the urine, but rather accumulates and is stored in the liver, kidney and other body tissues. As a result, a vitamin B_{12} deficiency may not manifest itself until after 5 or 6 years of a diet supplying inadequate amounts. Vitamin B_{12} is composed of a complex tetrapyrrol ring structure (corrin ring) and a cobalt ion in the center.

Function

Methylcobalamin and 5-deoxyadenosylcobalamin are active forms of vitamin B_{12}. Vitamin B_{12} participates in the **synthesis of DNA** and **red blood cells** and is vitally important in maintaining the health of the insulation sheath (**myelin sheath**) that surrounds nerve cells.

Deficiency

It is now clear though, that a vitamin B_{12} deficiency can have serious consequences. A deficiency often manifests itself first in the development of **neurological dysfunction** that is almost indistinguishable from senile dementia and Alzheimer's disease. A low level of vitamin B_{12} has also been associated with asthma, depression, AIDS, multiple sclerosis, tinnitus, diabetic neuropathy and low sperm counts. Some symptoms of a deficiency will include a sore tongue, weakness, fatigue, and weight loss, back pain and apathy. Severe deficiency may result in **pernicious anemia.** Pernicious anaemia is a serious disease characterized by large, immature red blood cells.

Older people may have a vitamin B_{12} deficiency because stomach acidity is low, reducing the body's ability to remove vitamin B_{12} from the protein in meat. Abnormal growth of bacteria in the small intestine may reduce the absorption of vitamin B_{12}. Disorders that impair the absorption of nutrients in the intestine can reduce the absorption of vitamin B_{12}. Liver disorders may interfere with the storage of vitamin B_{12}. Surgery that removes the stomach (where intrinsic factor is produced) or the part of the small intestine where vitamin B_{12} is absorbed can result in a deficiency. A strict vegetarian diet may also cause vitamin B_{12} deficiency because vitamin B_{12} is available only in animal products. Infants who are breastfed by a mother who is a strict vegetarian are at risk of vitamin B_{12} deficiency. Usually, vitamin B_{12} deficiency is due to inadequate absorption.

Absorption of vitamin B_{12}

Unlike most nutrients, absorption of vitamin B_{12} actually begins in the mouth where small amounts of unbound vitamin B_{12} can be absorbed through the mucosa membrane. Food protein bound vitamin B_{12} is digested in the stomach by proteolytic gastric enzymes which require an acid pH. Once the B_{12} is freed from the proteins in food, an intrinsic factor (IF) (a protein synthesized by gastric parietal cells) is secreted in the stomach and in the duodenum binds to free vitamin B_{12} to form a B_{12}-IF complex. If this step fails due to gastric parietal cell atrophy (the problem in pernicious anemia), sufficient B_{12} is not absorbed later on, unless administered orally in relatively massive doses (500 to 1000 µg/day). Vitamin B_{12} must be attached to IF for it to be absorbed, as receptors on the enterocytes in the terminal ileum of the small bowel only recognize the B_{12}-IF complex. In addition, intrinsic factor protects the vitamin from catabolism by intestinal bacteria.

Once the B_{12}-IF complex is recognized by specialized ileal receptors, it is transported into the portal circulation. The vitamin is then transferred to transcobalamin II (TC-II/B_{12}), which serves as the plasma transporter of the vitamin. Genetic deficiencies of this protein are known, also leading to functional B_{12} deficiency.

For the vitamin to serve inside cells, the TC-II/B_{12} complex must bind to a cell receptor, and be endocytosed. The transcobalamin-II is degraded within a lysosome, and free vitamin B_{12} is finally released into the cytoplasm, where it may be transformed into the proper coenzyme, by certain cellular enzymes.

Individuals who lack intrinsic factor have a decreased ability to absorb vitamin B_{12}. Vitamin B_{12} is mainly excreted in the bile, which is produced in the liver and stored in the gallbladder. However, most of the vitamin B_{12} that is secreted in the bile is recycled via enterohepatic circulation. Due to the efficient enterohepatic circulation of vitamin B_{12}, the liver can store several years' worth of vitamin B_{12}.

Sources

The richest dietary sources of vitamin B_{12} are liver, especially lamb's liver, and kidneys. Eggs, cheese and some species of fish also supply small amounts, but vegetables and fruits are very poor sources.

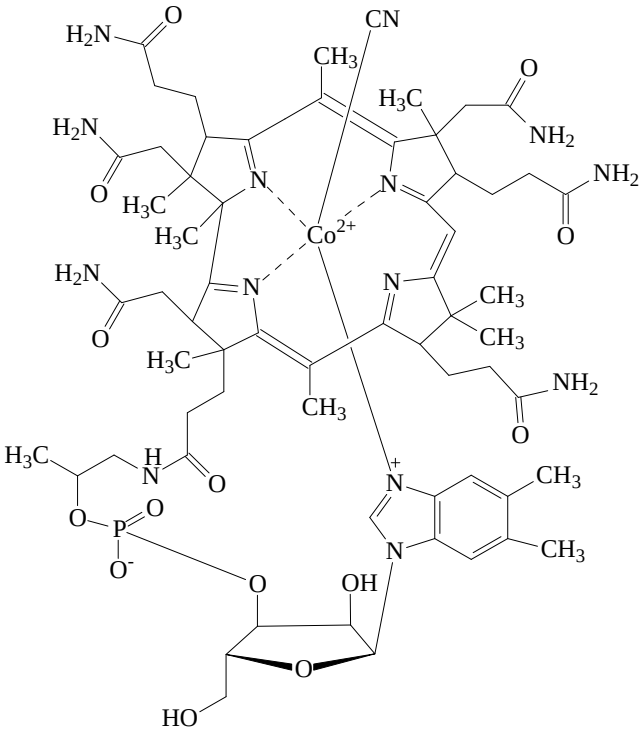


Fig. 11.13. Cobalamin - Vitamin B_{12}

11.2.8. Folic acid – Vitamin B_9 (folinic acid, folacin, pteroylglutamic acid)

Function

- Active forms of folic acid act as coenzymes (e.g. tetrahydrofolic acid) in a number of **single-carbon-transfer reactions**. They help convert vitamin B_{12} to one of its coenzyme forms and help synthesize the DNA required for all rapidly growing cells. Therefore, folate is necessary for the production and maintenance of new cells. This is especially important during periods of rapid cell division and growth such as infancy and pregnancy. Folate is needed to replicate DNA.
- Folic acid (Fig. 11.14.) is essential for the synthesis of **dTMP** (2'-deoxythymidine-5'-phosphate) from dUMP (2'-deoxyuridine-5'-phosphate).
- It is also required for the proper metabolism of the essential amino acid methionine that is found primarily in animal proteins.
- Folic acid is very important for all women who may become pregnant. Adequate folate intake during the periconceptional period, the time just before and just after a woman becomes pregnant, helps protect against a number of congenital malformations including **neural tube defects**. Neural tube defects result in malformations of the spine (spina bifida), skull, and brain (anencephaly). Spina bifida occurs when the fetus' spinal column does not close to protect the spinal cord; this closure should happen within the first few weeks of the pregnancy. Spina bifida causes neurological problems and sometimes, varying levels of mental retardation. The risk of neural tube defects is significantly reduced when supplemental folic acid is consumed in addition to a healthy diet prior to and during the first month following conception.

Deficiency

Folate deficiency hinders DNA synthesis and cell division, affecting most clinically the bone marrow, a site of rapid cell turnover. Large red blood cells called megaloblasts are produced, resulting in **megaloblastic anemia**. A folic acid deficiency has been clearly linked to an elevated level of **homocysteine**, a sulfur-containing amino acid. High homocysteine levels, in turn, have been linked to cardiovascular disease.

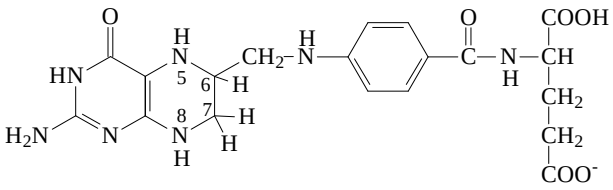


Fig. 11.14. Folic acid

Sources

Foods with folic acid in them include green leafy vegetables, fruits, dried beans, peas, sunflower seeds, liver and nuts**.**

11.2.9. Ascorbic acid – Vitamin C

Vitamin C (Fig. 11.15.) is purely the L-enantiomer of ascorbate; the opposite D-enantiomer has no physiological significance. When L-ascorbate, which is a strong reducing agent carries out its reducing function, it is converted to its oxidized form, L-dehydroascorbate. L-dehydroascorbate can then be reduced back to the active L-ascorbate form in the body by enzymes.

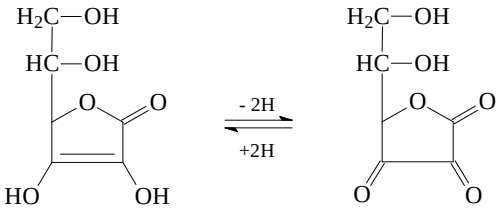


Fig. 11.15. L-Ascorbic acid (Vitamin C – left) and L-Dehydroascorbic acid (right)

Function

In humans, vitamin C is a highly effective **antioxidant**, as well as an **enzyme cofactor** for the biosynthesis of many important biochemicals.

Vitamin C acts as an electron donor for eight different enzymes. Of the eight enzymes, three participate in collagen hydroxylation. Two other vitamin C dependent enzymes are necessary for synthesis of carnitine. Carnitine is essential for the transport of fatty acids into mitochondria for ATP generation. The remaining three vitamin C dependent enzymes have the following functions: one participates in the biosynthesis of norepinephrine from dopamine, one adds amide groups to peptide hormones, greatly increasing their stability, and one modulates tyrosine metabolism.

Biosynthesis

The vast majority of animals and plants are able to synthesize their own vitamin C, through a sequence of four enzyme-driven steps, which convert glucose to vitamin C. Along with the rest of the ape family in which we reside, humans have no capability to synthesize vitamin C.

Deficiency

Scurvy is an avitaminosis resulting from lack of vitamin C, as without this vitamin, the synthesized **collagen** is too unstable to meet its function. Scurvy leads to the formation of liver spots on the skin, spongy gums, and bleeding from all mucous membranes. The spots are most abundant on the thighs and legs, and a person with the ailment looks pale, feels depressed, and is partially immobilized. In advanced scurvy there are open, suppurating

wounds and loss of teeth and, eventually, death. The human body cannot store vitamin C, and so the body soon depletes itself if fresh supplies are not consumed through the digestive system.

Sources

The richest natural sources are fruits and vegetables.

Control questions

1. How are vitamins classified according to their solubility?
2. Excessive bleeding is caused by the lack of which vitamin?
3. Which vitamin requires a specific glycoprotein, so-called intrinsic factor (IF factor) for its absorption?
4. Which vitamins are part of NAD^+, FAD, FMN, biocytin?
5. During the vision cycle vitamin A:
    a) is phosphorylated – dephosphorylated
    b) its side chain is cleaved off
    c) is changing its conformation from *cis* to *trans*
6. Which vitamin increases the absorption of calcium in the gut?
7. What is the active form of vitamin D_3 in humans?
8. Which of the following vitamins are necessary for 1) the activity of *alcohol dehydrogenase* 2) collagen synthesis and 3) transamination of alanine and α-ketoglutaric acid?
    a) ascorbic acid
    b) pyridoxine
    c) niacin
    d) riboflavin
    e) vitamin B_{12}
9. Write the formula of a compound, from which vitamin C is synthesized in majority of vertebrates.
10. Name vitamins with antioxidant activity.

12. ENZYMES AND COENZYMES

Enzymes are **proteins** that catalyze (*i.e.* accelerate) chemical reactions. The enzymes are usually globular proteins. Like all proteins, enzymes are made as long, linear chains of amino acids that fold to produce a three-dimensional product. Individual protein chains may sometimes group together to form a protein complex. However, some RNA have also enzymatic function (ribosymes), they can play a role e.g. in proteosynthesis. As with all catalysts, enzymes are not consumed by the reactions they catalyze, nor do they alter the equilibrium of these reactions. Almost all processes in a biological cell need enzymes in order to occur at significant rates.

In enzymatic reactions, reactants (the molecules at the beginning of the process) are called **substrates (S)**, and the enzyme converts them into different molecules, the **products (P)**.

Enzymes catalyze the forward and backward reactions equally. They **do not alter the equilibrium** itself**,** but only the speed at which it is reached. Usually, in the presence of an enzyme, the reaction runs in the same direction as it would without the enzyme, just more quickly.

Nevertheless, if the equilibrium is greatly displaced in one direction, that is, in a very exergonic reaction, the reaction is effectively irreversible. Under these conditions the enzyme will, in fact, only catalyze the reaction in the thermodynamically allowed direction. Furthermore, enzymes can couple two or more reactions, so that a thermodynamically favorable reaction can be used to "drive" a thermodynamically unfavorable one. For example, the hydrolysis of ATP is often used to drive other chemical reactions.

Enzymes differ from chemical catalysts by following properties:

- Catalysts are inorganic compounds, enzymes are largely organic in nature.
- Enzymes are more effective – rate of enzyme catalyzed reaction is much higher.
- Enzymes are more specific than chemical catalysts. They have much higher specificity towards substrates (reactants) and they form more specific products. Enzymatic reactions have rarely by-products.
- Different factors speed up enzymatic reactions (pH, temperature, concentration of enzyme and substrate…).
- Enzymes require milder reaction conditions – temperatures under 100^{o}C, atmospheric pressure, neutral pH. Chemical catalysts often require higher temperatures and pressure and extreme values of pH.

Active site of enzymes

Enzymes are proteins. However, not the whole protein molecule is catalytically active, only a small portion. Place in which a substrate (S) is converted into a product (P) is called the **active site** of the enzyme. In the active site there are functional groups responsible for binding a substrate (and also a cofactor), so called **binding site** and functional groups participating directly in the chemical conversion of S into a P (formation and cleavage of bonds), so called **catalytic site**. Some enzymes have more active sites (2-4), therefore they can bind several S molecules.

1. Active site is a tridimensional cleft formed by functional groups located in different parts of polypeptide chain. Amino acid residues forming the active site can be located far apart from each other in the enzyme´s primary structure, but in the terciary structure they are located close together.
2. Active site represents only a small portion of the whole protein molecule. Most amino acid residues are not in contact with the S, but serve to form tridimensional structure of the active site.
3. Substrates are bound to the enzymes by weak electrostatic interactions, hydrophobic or hydrogen bonds.
4. Bond of the S to the active site of the enzyme is very specific. Only a minor change in the chemical structure of the S can prevent binding of the S to the active site of the enzyme.

Most enzyme reaction rates are millions of times faster than those of comparable uncatalyzed reactions. In order to do its work, an enzyme must unite with the reactant (Fig. 12.1).

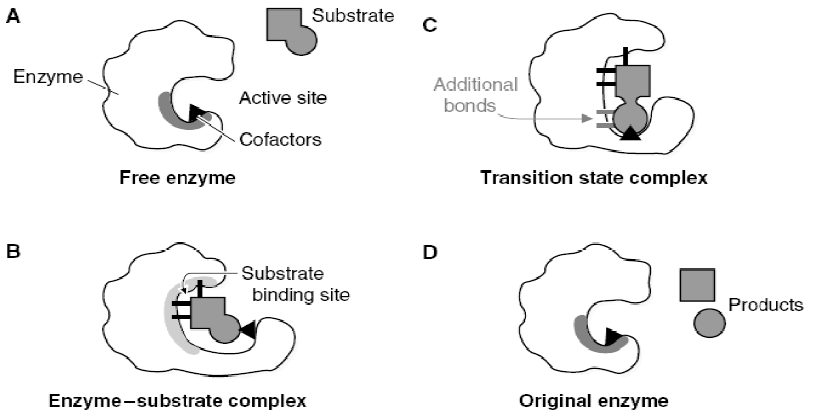


Fig. 12.1. Illustration of enzymatic reaction A.
The enzyme contains an active catalytic site, with a region or domain where the substrate binds. The active site also may contain cofactors, nonprotein components that assist in catalysis. ***B.*** *The substrate forms bonds with amino acid residues in the substrate binding site. Substrate binding induces a conformational change in the active site.* ***C.*** *Functional groups of amino acid residues and cofactors in the active site participate in forming the transition state complex, which is stabilized by additional noncovalent bonds with the enzyme.* ***D.*** *As the products of the reaction dissociate, the enzyme returns to its original conformation.*

Successful binding of enzyme and substrate requires that the two molecules be able to approach each other closely over a fairly broad surface. Thus, there is the analogy that a substrate molecule binds its enzyme like a key in a lock.

12.1. The mechanism of enzyme catalysis

In typical enzyme-catalyzed reactions, substrate and product concentrations are usually hundreds or thousands of times greater than the enzyme concentration. Consequently, each enzyme molecule catalyzes the conversion of many substrates molecules to a product.

Let´s consider a general reaction: **S** (substrate) ⟶ **P** (product)

Spontaneous transition of substrate S into a product P is prevented by an energy barrier. Energy supplied into the system in order to overcome the barrier is called **activation energy**. Enzymes decrease the activation energy of reactions. Activation energy of the enzymatic reaction is lower than activation energy of the direct conversion of S into a P without catalytic action of enzymes. Enzymes bind temporarily to one or more reactants and **lower the amount of activation energy** needed and thus **speed up the reaction.** How does an enzyme lower the activation energy? Often by holding substrate molecules in a position where they react more readily.

All catalysts, including enzymes, function by forming a **transition state**, with the reactants, of lower free energy than would be found in the uncatalyzed reaction (Fig. 12.2). The substrates usually need a large amount of energy to reach the transition state, which then decays into the endproduct. The enzyme stabilizes the transition state, reducing the energy needed to form this species and thus reducing the energy required to form products.

12.1.1. Activated or transition states

To initiate a conversion of substrate to product, some energy must be expended in most cases. Thus the addition of energy, such as heat, to a reaction increases the proportion of molecules of reactants in the activated or transition state, and therefore the conversion of reactants to products proceeds more quickly.

An alternate way to increase the rate of the reaction is to reduce the energy barrier of activation. In so doing, the proportion of molecules that can pass the barrier is greater.

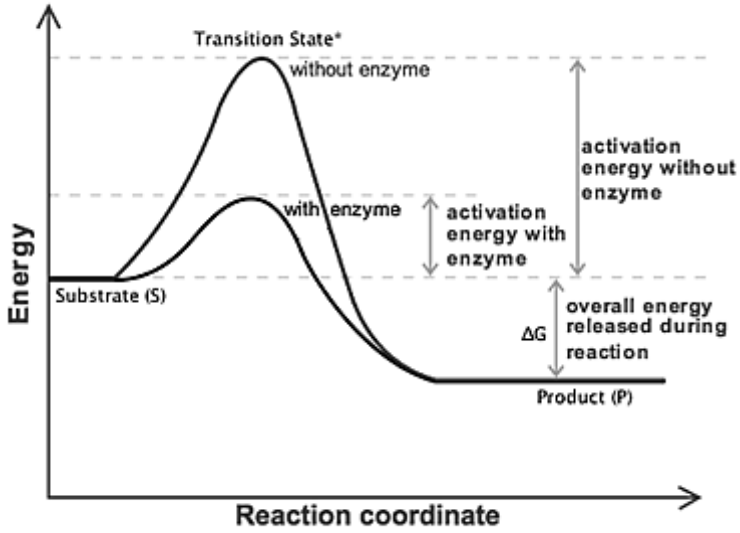


Fig. 12.2. Energetic changes during uncatalyzed and catalyzed reaction

12.1.2. Enzyme-substrate complex

The decrease of the activation barrier in enzyme-catalyzed reactions is achieved by the formation of a complex between the enzyme (E) and substrates. In the simplest case of single-substrate reactions, a reversible reaction may be represented: where ES* represents a **transition state.** Enzymes can store energy from the binding of the substrates and use it later to make catalysis more efficient.

Between the binding of substrate to enzyme, and the reappearance of free enzyme and product, a series of complex events must take place:

Simplified reactions:

$$E + S \leftrightarrow ES \leftrightarrow ES^* \leftrightarrow EP \leftrightarrow E + P$$

- The enzyme (E) and substrate (S) form a reaction intermediate (ES). This intermediate has lower activation energy than the reaction between reactants without a catalyst**.** The intermediate complex that forms, when substrate(s) and enzyme combine, is called the **enzyme substrate (ES) complex**. The formation of an enzyme-substrate complex is the first step in enzymatic catalysis.
- Enzyme substrate (ES) complex must pass to the **transition state (ES*)**
- The transition state complex must advance to an **enzyme product complex (EP)**
- The enzyme product complex (EP) is finally competent to dissociate to **product (P) and free enzyme (E)**.

12.1.3. Quantitative analysis of single-substrate enzyme kinetics

The fundamental theory of enzyme catalysis is based on the classic studies of Michaelis and Menten and of Haldane. The catalytic action of an enzyme on a given substrate can be described by two parameters: K_M (the Michaelis constant), which measures the affinity of an enzyme for its substrate, and v_{max} which is the maximum velocity that can be obtained without increasing the amount of enzyme.

Michaelis constant (K_M) is **the concentration of substrate** at which the reaction rate is **half-maximal ($v_{max}/2$).** The smaller the value of K_M, the more avidly the enzyme can bind the substrate from a dilute solution and the smaller the concentration of substrate needed to reach half-maximal velocity. A small $_{KM\ i}$ndicates high affinity, meaning that the rate v_{max} will approach more quickly. Both K_M and v_{max} are constants of specific values for any enzyme under the conditions of their measurement. They are the appropriate parameters for comparison of enzyme behavior.

The key features of the hyperbolic plot in Fig. 12.3. are marked by points A, B and C. At lower substrate concentrations, such as at points A and B, the lower reaction velocities indicate that at any moment only a

portion of the enzyme molecules are bound to the substrate. In fact, at the substrate concentration denoted by point B, exactly half the enzyme molecules are in an ES complex at any instant and the rate is exactly one half of v_{max}. At substrate concentrations near point A the rate appears to be directly proportional to substrate concentration. At high substrate concentrations the rate of the reaction represented by point C is almost equal to v_{max}, and the difference in rate at nearby concentrations of substrate is almost negligible. If the Michaelis-Menten plot is extrapolated to infinitely high substrate concentrations, the extrapolated rate is equal to v_{max}. The very small differences in reaction velocity at substrate concentrations around point C (near v_{max}) reflect the fact that at these concentrations almost all of the enzyme molecules are bound to substrate, all active sites of the enzyme are saturated by the substrate and the rate is virtually independent of substrate.

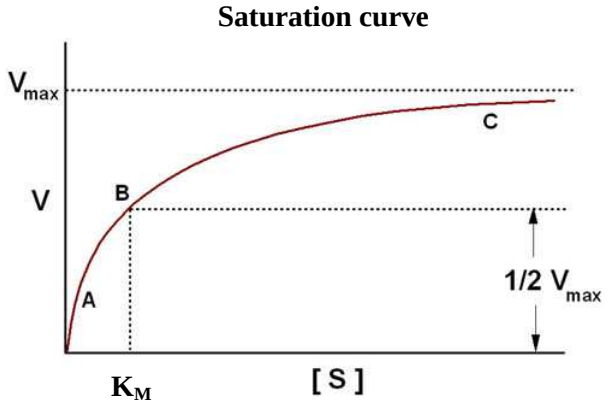


Fig. 12.3. Michaelis – Menten saturation curve. Reaction velocity as a function of substrate concentration

The hyperbola described by a plot of reaction velocities as a function of substrate concentrations is difficult to use. If the reciprocals of the velocities are plotted as a function of reciprocal substrate concentrations, the hyperbola is converted to a straight line. Such linear double-reciprocal plots are far easier to construct and interpret. A transformation of the typical curve shown in Fig. 12.3 is presented in Fig. 12.4. The double-reciprocal plots are frequently called ***Lineweaver-Burk*** plots.

If a few assumptions are made regarding the experimental situation, one can obtain a useful mathematical equation that describes the enzyme kinetics. Assume for the present that:

1. The system involves only a **single** substrate and **irreversible** reactions.
2. Concentration of the substrate is much higher than concentration of the product (**[S] ›› [P])** and **[P]** is negligible under these conditions, there is no intermediate or product inhibition.
3. Enzymes consist of single subunit, there is no allostericity or cooperativity.

The relation between K_M, substrate concentration [S], and reaction velocities (v) is established and written as follows:

Michaelis-Menten equation:

$$v = \frac{[S] \cdot v_{max}}{[S]+K_M}$$

Lineweaver-Burk equation:

$$\frac{1}{v} = \frac{K_M}{v_{max}} \cdot \frac{1}{[S]} + \frac{1}{v_{max}}$$

One can see, that when the velocity is equal to half the maximum velocity (that is, $\mathbf{v} = ½\ \mathbf{v_{max}}$), then [S] is equal to K_M. Both K_M and [S] are expressed in the same units, moles per liter.

In any experimental situation, v_{max} depends on the amount of enzyme present. If an experiment could be performed containing 1 mol of enzyme, the resulting activity would be termed the **turnover number**, which is expressed as moles of substrate converted to product per minute (or per second) per mole of enzyme. In those cases where the enzyme contains more than one active site per molecule, the turnover number is corrected accordingly. One would then use the turnover per mole of active site of the enzyme. Turnover numbers are of value in comparing the same enzyme from different tissues and in comparing different isozymes.

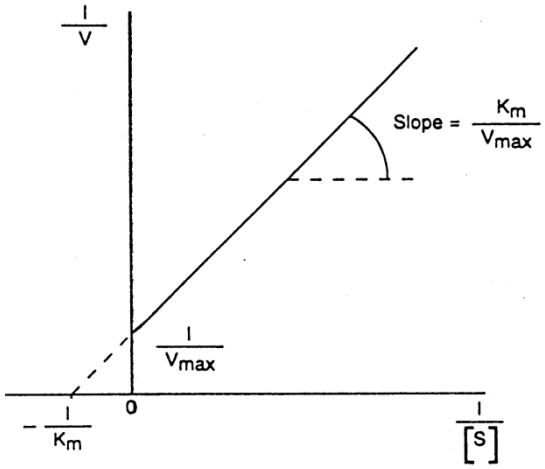


Fig. 12.4. Lineweaver-Burk transformation of Michaelis-Menten curve

Unit of enzyme activity is a **katal,** which is an enzyme activity which changes **one mol of substrate per second** at optimal pH and by enzyme saturated with substrate. In experiments there are used also mkat (10^{-3} kat), μkat (10^{-6} kat), nkat (10^{-9} kat) and so on. **Specific activity** of enzyme is number of **katals per 1 kg** of proteins which is equivalent to number of μkat.mg^{-1} proteins.

In older literature the enzymatic activity was expressed as international unit **U.** It is an enzyme activity which changes **1 micromole of substrate per 1 minute** at standard conditions. Relation between the two units:

1 kat = 6 . 10^7 U **1 U = 16.67 nkat = 16.67 . 10^{-9} kat**

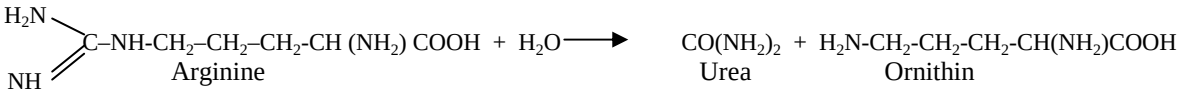
The velocity of enzyme reaction is measured by changed concentration of substrates or products per time unit.

12.1.4. Calculation of enzyme activity

Calculate a) % of inhibition of *arginase* activity by L-lysine (inhibitor) and

b) arginase activity (without the inhibitor) in katals per gram of tissue after 15 min incubation if absorbance of the standard urea solution with concentration 15 mmol.l^{-1} is 0.55, absorbance of the sample of 2%-homogenate without L-lysine is 0.42 and with L-lysine 0.12. The same volumes of the sample and the standard were pipetted.

Arginase is an enzyme catalyzing following reaction:



a) % of inhibition of *arginase* activity by L-lysine

A $_{\text{without lysine}}$ = 0.42.....100 % enzyme activity
A $_{\text{with lysine}}$ = 0.12.....x

x = 0.12 ×100 / 0.42 = **28.5 %** activity
Lysine reduced enzyme activity to 28.5 %.

% of inhibition of enzyme activity: I = 100 – 28.5 = **71.5 %**

Lysine inhibited enzyme activity by 71.5 %.

b) Calculation of enzyme activity in katals/g of tissue

Activity of enzyme in katals (**katal** = **mol/s**) we can calculate from concentration of a product (urea) produced by *arginase* during 1 second of reaction.

$A_{standard}$ = 0.55 ..... $c_{standard}$ = 15 mmol/l
A_{sample} = 0.42 x

x = 0.42 × 15 / 0.55 = 11.45 mmol/l,

or using the formula:

$$C_{sample} = \frac{A_{sample}}{A_{standard}} . C_{standard}$$

We found that during 15 min (900 s) reaction 11.45 mmol of urea per liter was produced. We need to find out how many moles of urea will be produced per 1 second (because kat = mol/**s**).

11.45 mmol/l.....900 s
x.....1 s

katal

x = 11.45 / 900 = 0.0127 mmol/s /l = 0.0127 mkat/l

Our task is to calculate activity of enzyme in kat/**g** of tissue (not in kat/**l)**. Therefore, we need to know how many grams of tissue is present in 1 liter of 2% homogenate.

2% homogenate ..... 2 g of tissue..... 100 ml
20 g of tissue..... 1000 ml = 1 liter
In 1 L of homogenate there are 20 g of tissue.

Activity calculated above - 0.0127 mkat / **l** of homogenate corresponds to how many mkat / **g** of tissue?
0.0127 mkat .....1 liter = 20 g tissue
x..... 1 g

x = **6.4×10^{-7} kat/g tissue = 0.64 µkat/g** tissue
Activity of *arginase* is 0.64 µkat per 1 g of tissue.

12.1.5. Two or more substrate kinetics

Michaelis-Menten kinetics strictly applies only to those enzymes that employ a single substrate. A much larger number of enzymes use more than one substrate at a time. For such multireactant enzymes, the coenzyme (e.g. NAD^+) is a **cosubstrate**. The kinetic analysis of multireactant enzyme systems requires an extension of the fundamental principles established by Michaelis and Menten. Since the enzyme and both substrates are simultaneously required for catalysis to occur, it is appropriate to describe the **ternary mechanism** as a formation of ternary catalytic complex by a symbol ($E \cdot S \cdot NAD^+$). In forming this, two binary complexes are possible, represented by ($E \cdot S$) and ($E \cdot NAD^+$). Following catalytic transformation, ($E \cdot P \cdot NADH$) could equally well produce ($E \cdot P$) and ($E \cdot NADH$); these, in turn, could decompose to liberate free enzyme and the second product.

Kinetic analysis sometimes permits an independent distinction between the two possible sequences of such a reaction mechanism. In the case of *3-phosphoglyceraldehyde dehydrogenase*, the mechanism is known as **ordered** because the addition of NAD^+ needs to precede the addition of the substrate (Fig. 12.5). Other examples are known in which the sequence of addition is not obligatory, but **random.**

3-phosphoglyceraldehyde dehydrogenase can catalyze the following reaction:

Glyceraldehyde-3-phosphate + NAD^+ + Pi $\rightleftharpoons$ 1,3-bisphosphoglycerate + NADH + H^+

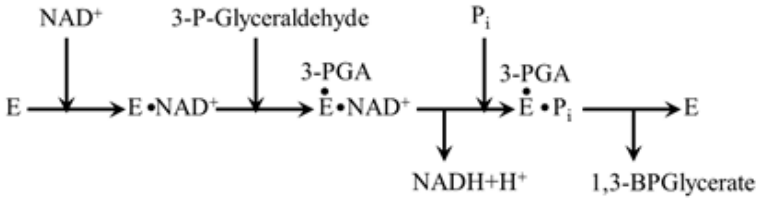


Fig. 12.5. A simplified scheme of the ternary ordered kinetic mechanism of *3-phosphoglyceraldehyde dehydrogenase*
E – enzyme, NAD^+ - nicotinamide adenine dinucleotide, 3-P-glyceraldehyde or 3-PGA – 3-phospho-glyceraldehyde, Pi – phosphate, 1,3-BPGlycerate – 1,3-bisphosphoglycerate

Another pattern for a multireactant enzyme system is known as the "**Ping-Pong" mechanism**. In this case one substrate must be bound and one product released before the second substrate is bound or the second product released (Fig. 12.6.). Typical examples of this pattern are found in the *aminotransferases*.
Reaction catalyzed by *aspartate aminotransferase* by the ping-pong mechanism:

Aspartate + α-ketoglutarate $\rightleftharpoons$ oxaloacetate + glutamate

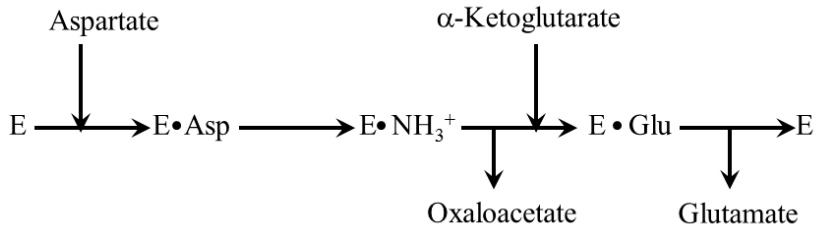


Fig. 12.6. "Ping-Pong" kinetic reaction of *aspartate aminotransferase*
E – enzyme, Asp – aspartate, Glu – glutamate

12.2. Cofactors

Some enzymes such as *trypsin*, or *pepsin* consist of polypeptide chains only. The protein polypeptide portion of the enzyme is called an **apoenzyme**. Other enzymes contain in addition to a protein part also a nonprotein portion called **cofactor**, which is a different low-molecular weight nonprotein compound. Such **enzymes** are called **holoenzymes or complex enzymes** (Fig.12.7).

The cofactors may be **inorganic cofactors** (e.g. metal ions, such as Zn^{2+} or Mg^{2+}) or they may be **organic compounds**. Enzymes that require a metal in their composition are known as **metalloenzymes** if they bind and retain their metal atom(s) under all conditions with very high affinity. Those which have a lower affinity for metal ion, but still require the metal ion for activity, are known as **metal-activated enzymes**.

Based on how tightly **organic** cofactors are bound to an enzyme, they are called **coenzymes** or **prosthetic groups.** Prosthetic groups are low-molecular nonprotein portions of enzymes bound to an enzyme by covalent bonds (e.g. heme, lipoic acid, biotin). Coenzymes (e.g. NAD^+) are connected to a particular enzyme by weak intermolecular interactions only temporarily.

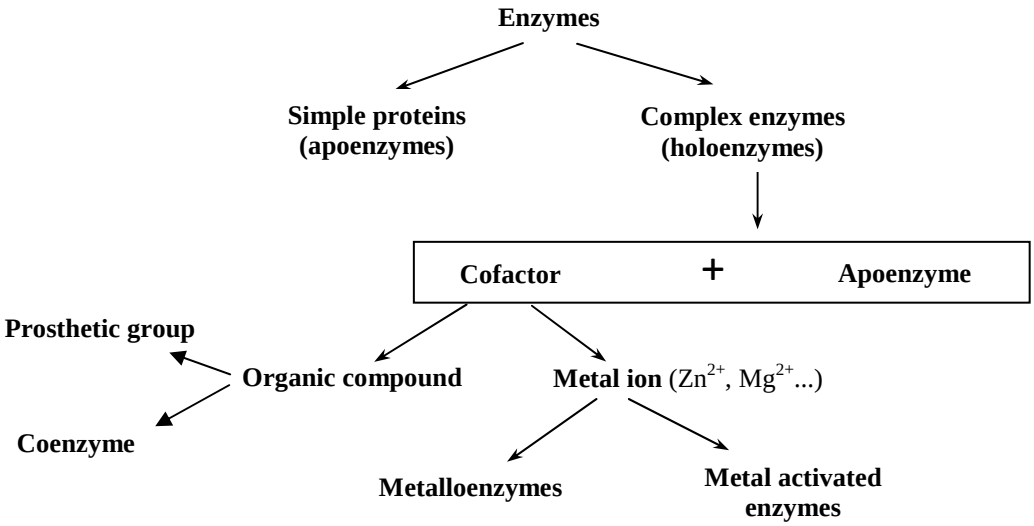


Fig. 12.7. Composition of enzymes

In contrast to substrate for which a given enzyme is specific, coenzymes are common to many different enzymes. For example, about 700 enzymes are known to use NADH as coenzyme. An important group of coenzymes are the B vitamins, which are essential to the action of many enzymes.

Table 12.1 Vitamins which are coenzyme precursors

Vitamin	Coenzyme	Catalyzed reaction	Disease caused by vitamin lack
Thiamin (B_1)	Thiamine diphosphate	Oxidative decarboxylation	Beri-beri
Riboflavin (B_2)	Flavin mononucleotide (FMN) Flavine adenine dinucleotide (FAD)	Oxido-reduction reactions - coenzymes carrying the hydrogen molecule	*
Nicotinamide (B_3)	Nicotinamide adenine dinucleotide (NAD^+) Nicotinamide adenine dinucleotide phosphate ($NADP^+$)	Oxido-reduction reactions - coenzymes carrying the hydrogen anion (hydride ion) H^-	Pellagra
Pantothenic acid (B_5)	Coenzyme A	Acyl group carrier	*
Pyridoxin (B_6)	Pyridoxal phosphate	Transamination reactions Decarboxylation reactions of AA β - elimination reactions (serine dehydratase)	*
Folic acid (B_9)	Tetrahydrofolate	Transport of one carbon groups	Megaloblastic anemia
Biotin (H)	Biocytin	CO_2 fixation	*

* *Rare in humans* AA – amino acids

The functional role of coenzymes is to act as transporters of chemical groups from one reactant to another. The chemical groups carried can be as simple as the hydride ion H^- ($H^+ + 2e^-$) carried by NAD^+ or the mole of hydrogen carried by FAD; or they can be even more complex as the amine ($–NH_2$) carried by pyridoxal phosphate.

Since coenzymes are chemically changed as a consequence of enzyme action, it is often useful to consider coenzymes to be a special class of substrates, or second substrates, which are common to many different holoenzymes. In all cases, the coenzymes donate the carried chemical groups to an acceptor molecule and are thus regenerated to their original form. This regeneration of coenzyme and holoenzyme fulfills the definition of an enzyme as a chemical catalyst, since (unlike the usual substrates, which are used up during the course of a reaction) coenzymes are generally regenerated.

Coenzymes may be classified:

1. **Coenzymes binding hydrogen** (NAD^+, $NADP^+$, FMN, FAD, lipoic acid) **or accepting electrones** (coenzyme Q, porfyrin derivatives)
2. **Coenzymes transferring a group of atoms** (adenosine phosphates, coenzyme A, thiamin diphosphate, pyridoxal phosphates, biocytin, tetrahydrofolate...)

12.2.1. Coenzymes transferring hydrogen or electrones

a) Nicotinamide adenine dinucleotide (Fig. 12.8.), abbreviated **NAD^+**, is a coenzyme found in all living cells. The compound is a dinucleotide, since it consists of two nucleotides joined through their phosphate groups. One nucleotide contains an adenine base and the other nicotinamide (**vitamin B_3**). In metabolism, NAD^+ is involved in **redox** reactions. The coenzyme is, therefore, found in two forms in cells: NAD^+ is an oxidizing agent – it accepts a hydride anion (H^-**)** from other molecules and becomes reduced. This reaction forms NADH, which can then be used as a reducing agent.

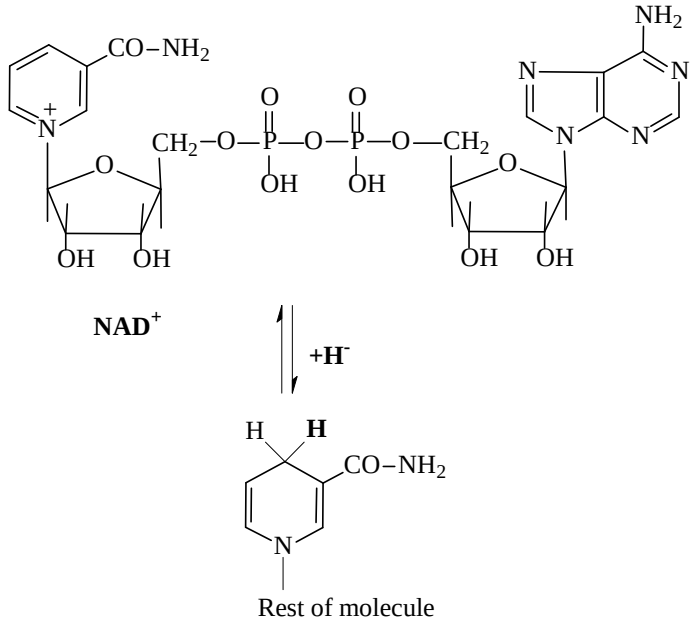


Fig. 12.8. Nicotinamide adenine dinucleotide NAD^+ and its reduced form NADH

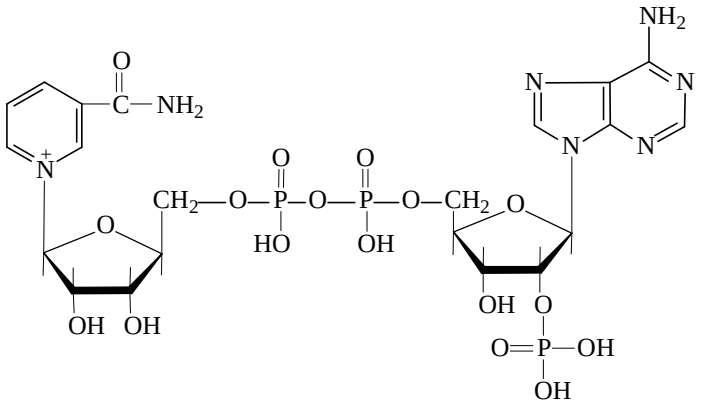
In metabolism, redox reactions (summarized in formula below) involve the removal of two hydrogen atoms from the reactant (RH_2), in the form of a **hydride ion** (**H^-**), and a proton (H^+). The proton is released into solution, while the reactant RH_2 is oxidized to R and NAD^+ reduced to NADH by transfer of the hydride (**H^-**) to the nicotinamide ring (Fig. 12.8).

$$RH_2 + NAD^+ \rightarrow NADH + H^+ + R$$

From the hydride electron pair (H^-), one electron is transferred to the positively charged nitrogen of the nicotinamide ring of NAD^+, and the hydrogen atom is transferred to the C_4 carbon atom opposite this

nitrogen. The reaction is easily reversible, when NADH reduces another molecule and is reoxidized to NAD^+. This means the coenzyme can continuously cycle between the NAD^+ and NADH forms without being consumed.

Some NAD^+ are also converted into nicotinamide adenine dinucleotide phosphates ($NADP^+$) (Fig.12.9). The chemistry of this related coenzyme is similar to that of NAD^+, but it has different roles in metabolism.



Obr.12.9. Nicotinamide adenine dinucleotide phosphate ($NADP^+$)

b) Flavin nucleotides

Flavin adenine dinucleotide (**FAD**) is a redox cofactor involved in several important reactions in metabolism. The molecule consists of a **riboflavin** moiety (**vitamin B_2**) bound to the phosphate group of an **ADP molecule.** The **flavin** group (isoalloxazine ring) is bound to **ribito**l, a sugar alcohol, by a carbon-nitrogen bond, not a glycosidic bond. FAD (Fig. 12.10) can exist in two different redox states FAD and $FADH_2$. FAD can be reduced to $FADH_2$, whereby it accepts two hydrogen atoms (two electrons and two protons).

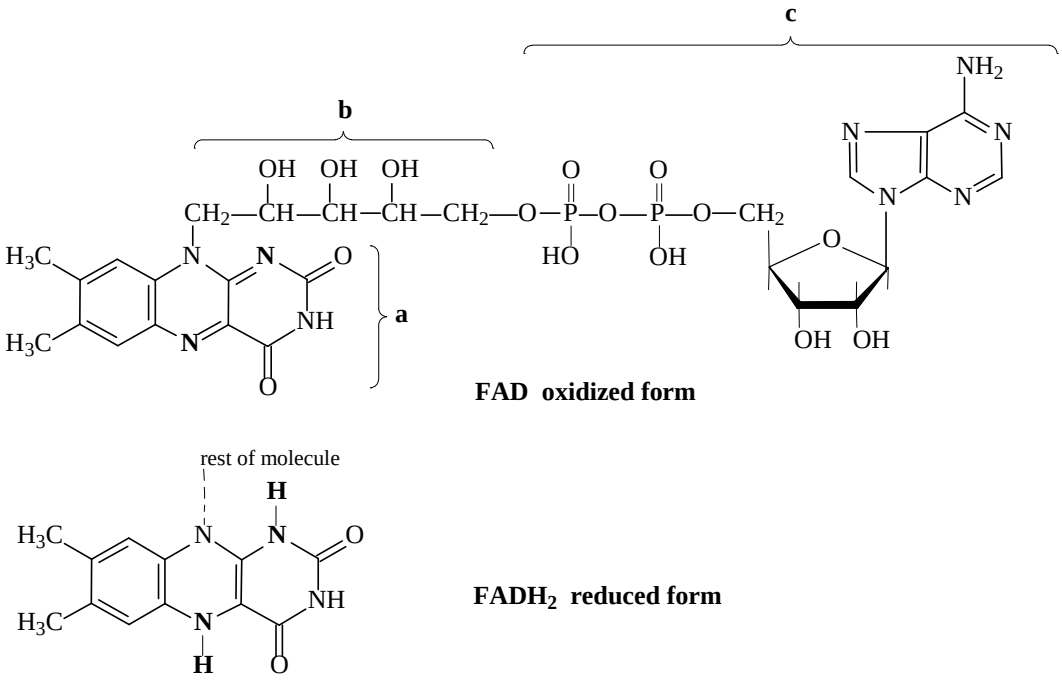


Fig. 12.10. Flavin adenine dinucleotide in oxidized (FAD) and reduced ($FADH_2$) form
***(a = flavin** - isoalloxazine ring; **b** = ribitol; **c** = ADP; **a+b** = riboflavin)*

FAD has an aromatic ring system, whereas $FADH_2$ has not. This means that $FADH_2$ is significantly higher in energy, without the stabilization that aromatic structure provides. $FADH_2$ is an energy-carrying molecule, because, if it is oxidized, it will regain aromaticity and release all the energy represented by this stabilization.

Flavin mononucleotide (FMN) (Fig. 12.11) functions as a prosthetic group of various oxidoreductases. During catalytic cycle, the reversible interconversion of oxidized (FMN), and reduced ($FMNH_2$) forms occurs.

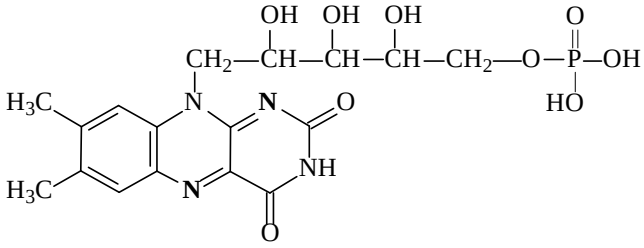


Fig. 12.11. Flavin mononucleotide (FMN)

FAD and FMN in oxidized forms are yellow, however, after accepting two hydrogen atoms, a coloreless leuko form is produced. These color changes are used to monitor the course of reactions catalyzed by flavoproteins.

c) Lipoic acid (Fig. 12.12) is a cyclic disulfide, which is involved in **redox** processes (e.g. it is a coenzyme of *pyruvate dehydrogenase* complex in the oxidative decarboxylation of pyruvate). In addition to hydrogen it transfers also an acetyl group. It is an important **antioxidant** acting in hydrophilic and lipophilic environments *in vitro*. Lipoic acid is a **growth factor** for certain microorganisms.

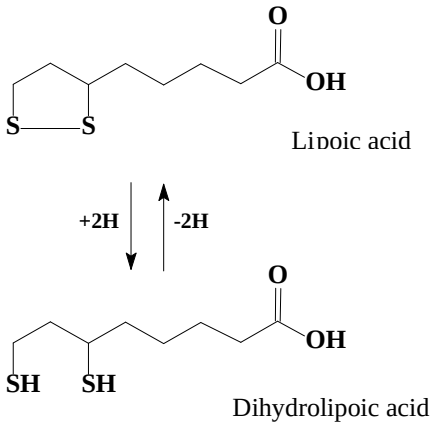


Fig. 12.12. Lipoic acid and dihydrolipoic acid

Owing to the presence of two thiol groups, dihydrolipoic acid is a **chelating agent**. It chelates both intracellular and extracellular mercury in the brain and in the body.

d) Porphyrin derivatives are heterocyclic macrocycles composed of four modified pyrrole subunits interconnected at their α carbon atoms via methine bridges (=CH-). Porphyrins are aromatic. Some iron(II) - containing porphyrins are called hemes (Fig. 12.13). Heme is a cofactor of the protein hemoglobin and it is also a prosthetic group of cytochromes. Heme contains an iron atom embedded in a porphyrin ring system (Fig. 12.13). The Fe is bonded to 4 N atoms of the porphyrin ring. Hemes in the three classes of cytochrome (a, b, c) differ slightly in substituents on the porphyrin ring system. Cytochromes participate in the transport of electrones in the respiratory chain in mitochondria.

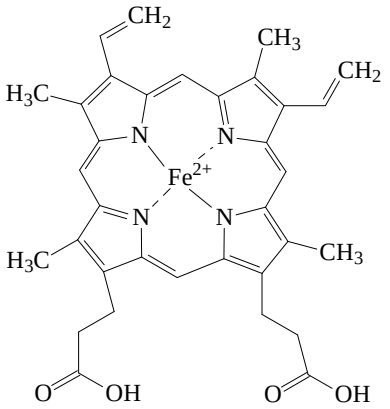


Fig. 12.13 Heme group of hemoglobin. An iron (Fe) atom in the middle is complexed to four interior nitrogen atoms

e) Coenzyme Q (CoQ) **– ubiquinone** is a 1,4-benzoquinone. It is present in most eukaryotic cells, primarily in the mitochondria. It is a component of the electron transport chain and participates in aerobic cellular respiration, generating energy in the form of ATP. There are three redox states of coenzyme Q_{10}: fully oxidized (ubiquinone), semiquinone (ubisemiquinone), and fully reduced (ubiquinol). The CoQ molecule is continually going through an oxidation-reduction cycle. As it accepts electrons, it becomes reduced (hydroquinone, ubiquinol). As it gives up electrons, it becomes oxidized (quinone) (Fig. 12.14). In its reduced form it acts as an **antioxidant.** It inhibits both the initiation and the propagation of lipid and protein oxidation. It also regenerates other antioxidants such as vitamin E. CoQ prevents oxidation of LDL, which may provide benefit in cardiovascular diseases. The various kinds of coenzyme Q can be distinguished by the number of isoprenoid subunits in their side-chains. The most common coenzyme Q in human mitochondria is CoQ_{10}. Q refers to the quinone head and 10 refers to the number of isoprene repeats in the tail.

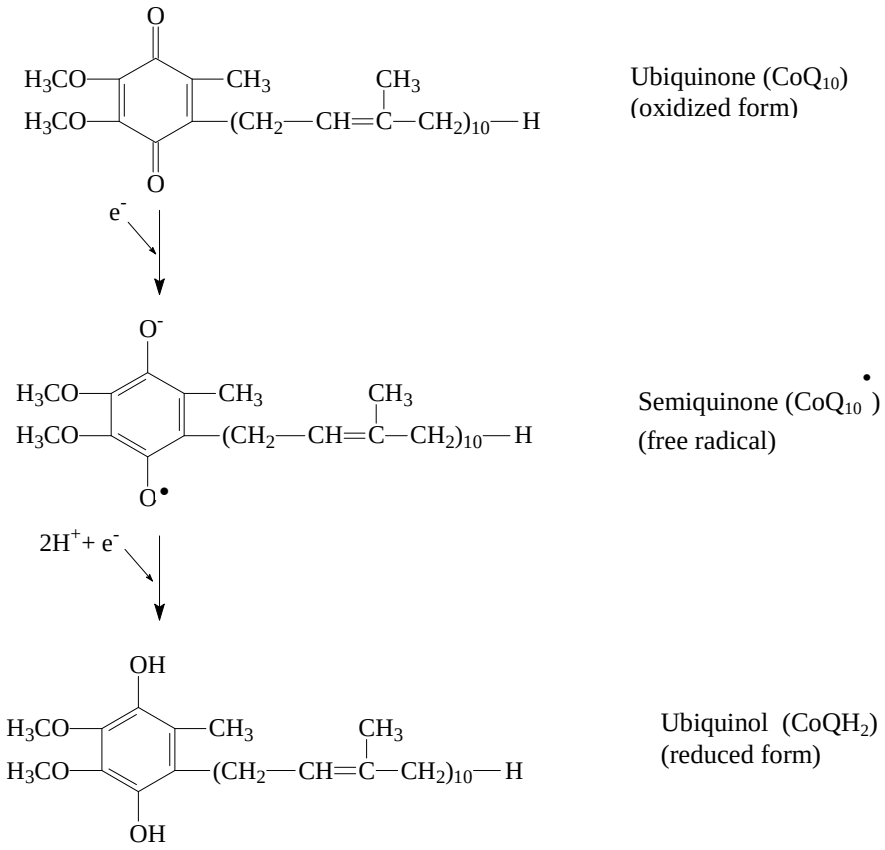


Fig. 12.14 Conversion of ubiquinone to ubiquinol

12.2.2. Coenzymes transferring group of atoms

a) Adenosine phosphates (Fig. 10.7.) are basic donors and acceptors of phosphoric acid in all living organisms. We recognize folowing adenosine phosphates: adenosine monophosphate (AMP), adenosine diphosphate (ADP), adenosine triphosphate (ATP). Different parts of ATP molecule can be transmitted and can activate compounds for reactions.

b) Thiamine diphosphate (pyrophosphate) (Fig. 12.15) is a coenzyme that participates in the transfer of acetaldehyde and glycolaldehyde. Thiamine diphosphate is a derivative of thiamine (vitamin B_1) (Fig. 12.9.). Enzymes with thiamin cofactor catalyze decarboxylation of α-oxoacids (pyruvate, α-oxo-glutarate).

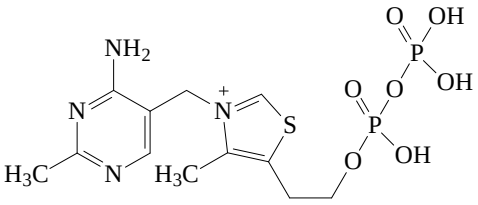


Fig.12.15. Thiamine diphosphate

c) Coenzyme A (Fig. 12.16) Part of its structure is vitamin B_5 - pantothenic acid (vitamin B_5), followed by nucleotide adenosine-3',5'-bisphosphate (ADP) and 2-aminoethanethiol. Transmitted acyls bind by thioester bond to a thiol group of 2-aminoethanethiol.

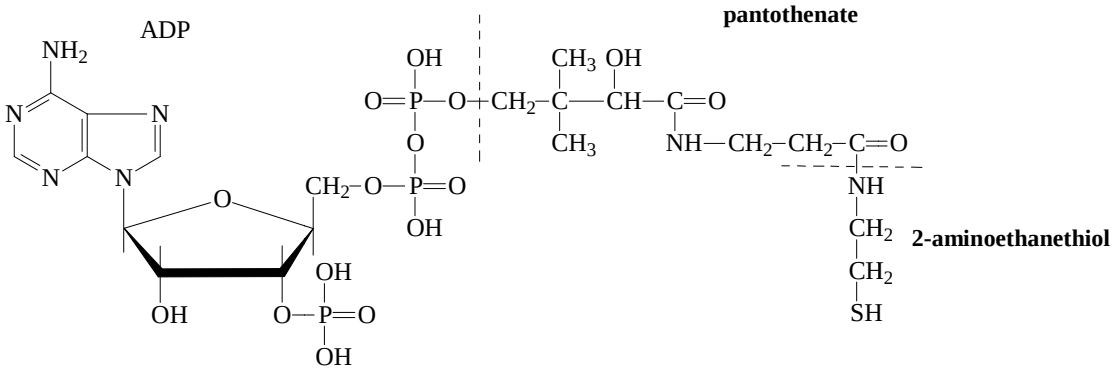
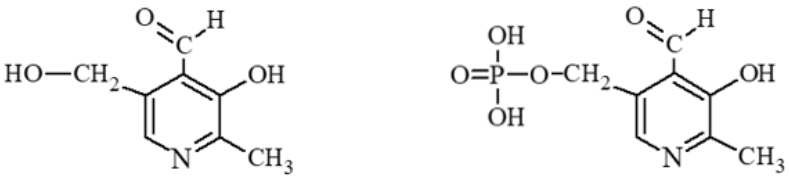


Fig. 12.16. Coenzyme A

d) Pyridoxal phosphate (Fig. 12.17) is a coenzyme of enzymes catalyzing some decarboxylation, transamination and deamination reactions of amino acids. It is the active form of vitamin B_6.



Pyridoxal **Pyridoxal phosphate (PLP)**

Fig. 12.17. Pyridoxal (vitamin B_6) and pyridoxal phosphate coenzyme

e) 5,6,7,8-tetrahydrofolate is a coenzyme obtained by two successive reductions of folic acid (Fig.12.15). Tetrahydrofolate provides transmission of one carbon residues that bind to the N^5 or N^{10}. It is an important cofactor in the synthesis of purine and thymine nucleotides.

f) **Biocytin** is a coenzyme of enzymes catalyzing transfer of carboxylic groups. It is formed by the linkage of biotin (vitamin H) (Fig. 11.12) to lysine residues of the enzyme (carboxylase). In biocytin CO_2 binds to the N^1 to form carboxybiocytin (Fig. 12.18), by which the CO_2 is transferred in carboxylation reactions. Biocytin is important in the metabolism of lipids and saccharides. It participates in maintaining steady blood glucose levels.

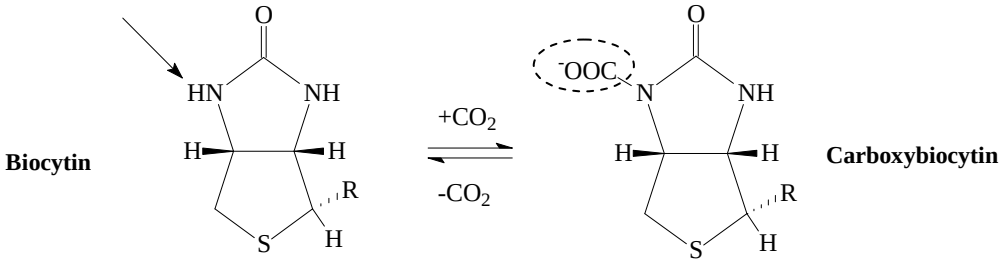


Fig. 12.18. Conversion of biocytin to carboxybiocytin
R – rest of valeric acid bound by amide bond to lysine residue of the enzyme (carboxylase)

12.3. Enzyme specificity

Many inorganic catalysts, for example, charcoal or finely divided platinum, show little specificity toward the substances on which they exert a catalytic effect. Thus, a mere handful of selected catalysts is sufficient for much synthetic organic chemistry work practiced in industry or the laboratory.

Enzymes are usually very specific as to which reactions they catalyze and the substrates that are involved in these reactions. Complementary shape, charge and hydrophilic/hydrophobic characteristics of enzymes and substrates are responsible for this specificity.

Urease, an enzyme that hydrolyzes urea to carbon dioxide and ammonia, has almost an absolute specificity toward urea, and only one other compound that can be split by *urease* is known. Similarly, *catalase* is almost completely specific toward hydrogen peroxide, which it converts into water and oxygen. *Chymotrypsin*, a pancreatic *proteinase*, shows a somewhat lesser specificity toward its substrate. It prefers to cleave peptide bonds in which one participant amino acid has an aromatic ring. It also preferentially attacks peptide bonds in the interior of a peptide chain, but even this requirement is relative. Other proteolytic enzymes show various types of specificity. *Trypsin* hydrolyzes only those peptide bonds to which arginine or lysine contribute the carboxyl group.

We recognize two types of enzyme specificity:

1. Specificity of effect

Cofactor is responsible for this type of specificity. Each enzyme can catalyze only a certain type of a chemical reaction. According to the type of catalyzed reaction we recognize 6 classes of enzymes:

Enzymes are classified into six classes:

1. ***Oxidoreductases*** catalyze a variety of oxidation-reduction reactions and frequently employ coenzymes such as nicotinamide adenine dinucleotide (NAD^+), its phosphate derivative ($NADP^+$), flavin adenine dinucleotide (FAD), or lipoate. Common trivial names include *dehydrogenase, oxidase, peroxidase,* and *reductase.*
2. ***Transferases*** catalyze transfers of groups such as amino, carboxyl, carbonyl, methyl, acyl, glycosyl, or phosphoryl. Kinases catalyze the transfer of phosphoryl groups from adenosine triphosphate (ATP) or other nucleotide triphosphates. Common trivial names include *aminotransferase (transaminase*), *carnitine acyl transferase, and transcarboxylase*
3. ***Hydrolases*** catalyze cleavage of bonds between a carbon and some other atoms by addition of water. Common trivial names include *esterase, peptidase, amylase, phosphatase, urease, pepsin, trypsin,* and *chymotrypsin.*
4. ***Lyases*** catalyze breakage of carbon-carbon, carbon-sulfur, and certain carbon-nitrogen (excluding peptide) bonds. Common trivial names include *decarboxylase, aldolase, synthase, citrate lyase,* and *dehydratase.*

5. ***Isomerases*** convert a molecule from one isomer to another. Isomerases can either facilitate intramolecular rearrangements in which bonds are broken and formed or they can catalyze conformational changes. Trivial names include *epimerase*, *racemase*, and *mutase*.
6. ***Ligases*** catalyze the formation of bonds between carbon and oxygen, sulfur, nitrogen, and other atoms. The energy required for bond formation is derived from the hydrolysis of **ATP**; the term *synthetase* is reserved for this group. Trivial names include *thiokinase* and *carboxylase*.

2. Substrate specificity

It is the ability of a certain enzyme to catalyze the conversion of a particular substrate. We recognize enzymes **strictly specific, less specific and nonspecific.** Strictly specific enzymes are generally specific for a particular steric configuration (optical isomer) of a substrate. Enzymes that attack D saccharide will not attack the corresponding L isomer. Less specific enzymes are e.g. lipases – they convert several different compounds with the same type of functional group. Nonspecific enzymes (phosphatases, esterases) catalyze breaking of the same type of bond.

12.4. Models of enzyme action

There are several models of how enzymes work: the lock-and-key model and the induced fit model.

a) Lock and key hypothesis

This is the simplest model to represent how an enzyme works (Fischer model). The substrate simply fits into the active site (like a key into a lock) to form a reaction intermediate (Fig. 12. 19).

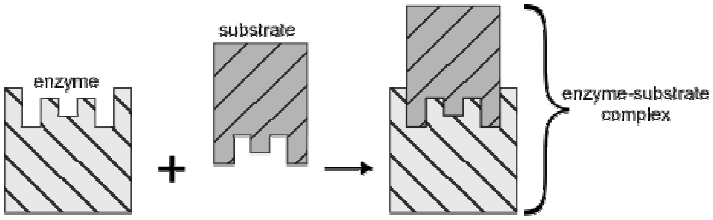


Fig. 12.19. Lock and key model

b) Induced fit hypothesis

In 1958 Daniel Koshland suggested a modification to the lock and key model. In this model the enzyme molecule changes shape as the substrate molecules gets close. The change in shape is 'induced' by the approaching substrate molecule. This more sophisticated model relies on the fact that molecules are flexible because single covalent bonds are free to rotate.

12.5. Types of enzyme catalysis

We recognize three types of enzyme catalysis:

1. **Acid/base catalysis** depends on the dissociated residues of amino acids (mainly His, Tyr, Glu, Asp, Lys), which may provide or accept protons from substrate.
2. **Covalent catalysis** involves the substrate forming a transient covalent bond with residues in the enzyme active site. The active site of an enzyme contains a reactive nucleophilic/electrophilic group that attacks the substrate through covalent forces. The covalent bond must, at a later stage in the reaction, be broken to regenerate the enzyme. This mechanism is found in enzymes such as proteases like chymotrypsin and trypsin, where an acyl-enzyme intermediate is formed.
   According to the type of a reactive group in the enzyme active site we recognize:
   a) **electrophilic catalysis**
   b) **nucleophilic catalysis**
   During electrophilic or nucleophilic enzymatic reactions, the ability of some atoms or groups of atoms to accept or give off electrons is used leading to the transfer of electrons in the molecule of a substrate and resulting in the formation or breakdown of the covalent bond in the reaction.

3. **Metal ion catalysis** involves metal ions (Zn^{2+}, Mn^{2+}, Mg^{2+} and so on) used in catalytic site of enzymes that allow the formation of nucleophilles or electrophilles and help the reaction occur in a faster pace.
   We recognize two classes of enzymes (see chapter 12.2):
   - metalloenzymes
   - metal-activated enzymes

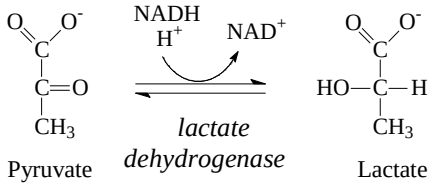
   Metal ions participate in catalysis in three ways:
   1) bind and properly orient the substrate for the reaction
   2) mediate the redox reaction
   3) electrostatically stabilize negative charges on the substrate

In reality, most enzyme mechanisms involve a combination of several different types of catalysis.

12.6. Isozymes

As enzymes have a more or less broad range of substrate specificity, it follows that a given substrate may be acted on by a number of different enzymes, each of which uses the same substrate(s) and produces the same product(s). These types of enzyme are in many species, including humans. The individual members of a set of enzymes sharing such characteristics are known as **isozymes**. **Isozymes** (also known as **isoenzymes**) **are enzymes that differ in amino acid sequence but catalyze the same chemical reaction** and exhibit differing degrees of efficiency. They might be distinguished by their optimal pH, kinetic properties or immunologically. *Lactate dehydrogenase* and *malate dehydrogenase* have been thoroughly studied as examples of isozymes.

Lactate dehydrogenase (**LD** or **LDH**, EC 1.1.1.27) is *oxidoreductase* catalyzing the reversible interconversion of pyruvate and lactate:



LDH has five isoenzymes numbered 1-5. Each isoenzyme is a tetramer consisting of two different subunits, M and H (Fig. 12.20). The two subunits, differing in amino acid content and sequence, can be combined into tetramers in five ways. The possible combinations can be separated by electrophoresis. If one subunit type is identified as "M" (the major form found in muscle or liver) and the second as “H” (the major form found in heart), the tetramers could have the compositions M_4, M_3H, M_2H_2, MH_3, or H_4. These can be separated by electrophoresis; those with an increasing content of the H subunit have an increasingly larger negative charge, whereas the M_4 isozyme has a slightly positive net charge.

In humans the content of several isozymes differs in heart and liver, and this difference is used in diagnostic differentiation of diseases of the liver and myocardium. In both disease states LDH leaks out of the damaged cells, and its concentration in blood serum increases.

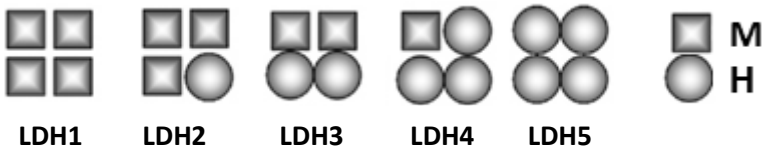


Fig. 12.20. *Lactate dehydrogenase* tetramer - 5 isozymes

12. 7. Mechanisms of regulation of enzymatic activity

Catalytic activity of enzymes may be regulated by many ways:

1) **Without the change in the quantity of enzyme molecules** - changing of enzyme activity by its increase (activation) or decrease (inhibition). Reaction rates may be altered by various parameters:

   A. **Physico-chemical conditions**
      a) substrate concentration
      b) pH
      c) temperature
      d) ionic strength
      e) redox potential

   B. **Presence of activators / inhibitors** – different compounds can cause increase (activation) or decrease (inhibition) of enzyme activity. We recognize following types of specific enzyme inhibition:
      a) competitive
      b) noncompetitive
      c) uncompetitive

   C. **Allosteric regulation of enzyme activity**
      a) Cooperative model
      b) Sequential model

   D. **Regulation by modification of enzyme molecule**
      a) Limited proteolysis
      b) Covalent modification
      c) by metal ions

   E. **Compartmentalization of enzymes**

2) **By the change of quantity of enzyme molecules**

   A. **Induction and repression** of enzyme production
   B. **Regulated degradation of proteins**

12.7.1. Regulation of enzyme activity without change in the quantity of enzyme molecules

12.7.1.1. Regulation of enzyme activity by physico-chemical conditions

a) Effect of substrate concentration on enzyme activity

The relation between the increased concentration of substrate and the velocity of enzyme activity is described in chapter 12.1.3 (Fig. 12.3).

b) Effect of pH on enzyme activity

Enzymes are amphoteric molecules containing a large number of acid and basic groups, mainly situated on their surface. The charges on these groups will vary, according to their acid dissociation constants, with the pH of their environment. This will affect the total net charge of the enzymes and the distribution of charge on their exterior surfaces, in addition to the reactivity of the catalytically active groups. These effects are especially important in the neighbourhood of the active sites. Taken together, the changes in charges with pH affect the activity, structural stability and solubility of the enzyme. Each enzyme has its specific pH (**pH optimum**) at which the activity of the enzyme is highest. The pH optima (Fig. 12.22) vary widely; *pepsin* which exists in the acid environment of the stomach, has a pH optimum at about 1.5, whereas *arginase*, an enzyme that cleaves the amino acid arginine, has its optimum at 9.7. However, most enzymes have optima that fall between pH 4 and 8 (Tab. 12.2).

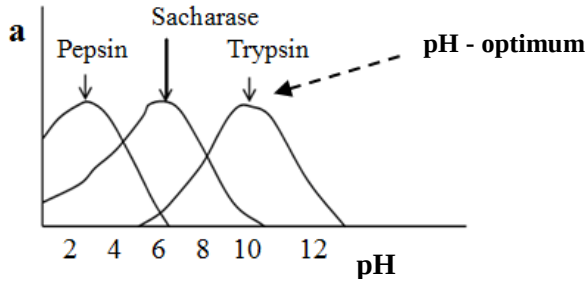


Fig. 12.22. Effect of pH on enzyme activity

Some enzymes show a wide tolerance for pH changes, but others work well only in a narrow range. If any enzyme is exposed to extreme values of pH, it is denatured. The sensitivity of enzymes to altered pH is one reason why regulation of body pH is so closely controlled and why departures from normal may involve serious consequences.

There will be a pH, characteristic of each enzyme, at which the net charge on the molecule is zero. This is called the **isoelectric point** (pI), at which the enzyme generally has minimum solubility in aqueous solutions.

Tab. 12.2. Optimal pH values for activity of selected enzymes

Enzyme	pH
Amylase from saliva	6.8
Amylase from pancreatic liquid	6.8
Saccharase from digestive liquid	6.2
Saccharase from yeast	5.0
Lipase from stomach liquid	5-6
Lipase from pancreatic liquid	7-8
Pepsin	1.5-2.5
Cathepsin D	3.0-3.5
Urease	7.2-7.5
Catalase	7.0

c) Effet of temperature t on enzyme activity

As the temperature increases the kinetic energy of the substrate and enzyme molecules also increases (the kinetic energy curve) (Fig. 12.23.). Therefore, there will be more collisions of the substrate with the enzyme active site leading to the increase in the rate of reaction. As the temperature increases enzyme stability decreases (the enzyme stability curve) (Fig. 12.23.). The kinetic energy of the enzyme atoms increases causing vibrations in the enzyme molecule that lead to the hydrogen bonds to breaking and shape changes in the active site. The optimal temperature is at the highest rate of the reaction. It is a compromise between decreasing enzyme stability and kinetic energy of the reactants. Once an optimum temperature is reached, any further increase in temperature causes changes in enzyme conformation. The substrate may then not fit properly onto the changed enzyme surface. Therefore the rate of reaction decreases. At some higher temperature above optimum, we reach a point where the protein denatures, the conformation is altered irreversibly and the polypeptide chain cannot refold. At this point the enzyme is completely inactivated. Fig.12.23 depicts that changes in activity above or below the optimum temperature are not always symmetric.

d) Effect of ionic strength on enzyme activity

Concentration of salts influences enzyme activity because the salts affect the hydration of proteins and consequently their solubility and shape of molecules. Solubility of proteins at low ionic strengths increases with

the concentration of salt (so-called salting in). Increasing salt concentration increases the solubility. At very high ionic strengths charges of protein molecules are shaded, leading to the existence of very weak electrostatic interactions between protein molecules, and thus solubility is reduced. This phenomenon is called salting out.

e) Effect of redox potential

Oxido-reduction potential (redox potential) is a measure of the ability of agents to bind or release electrons, i.e. it is a measure of the strength of oxidative or reducing agent. The more positive redox potential, the greater is the substance afinity to electrons and its tendency to be reduced.
Redox potential can affect:

- status of some oxidizable groups (especially-SH)
- the layout of the whole molecule of the enzyme
- binding of substrate (formation of crosslinks -SS-).

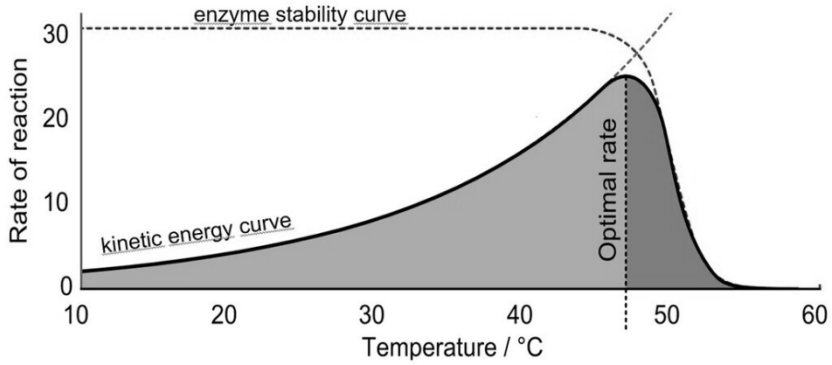
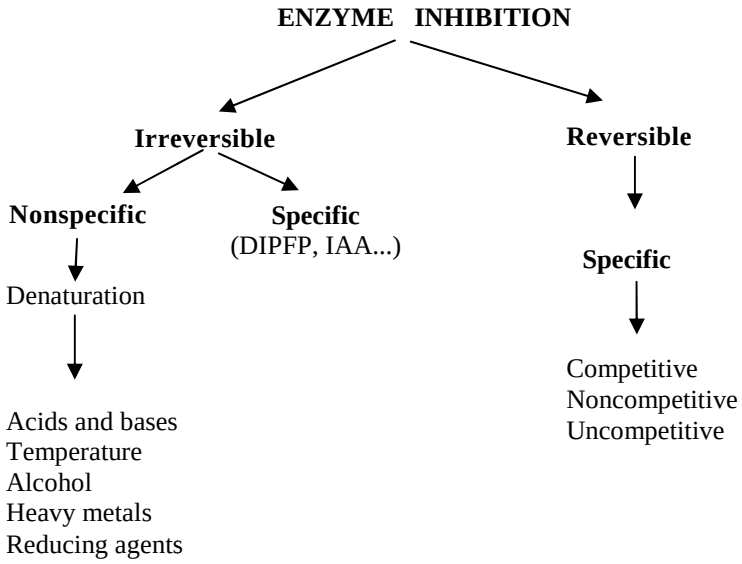


Fig. 12.23. Effect of temperature on enzyme activity

12.7.1.2. Enzyme inhibition

Inhibitors slow down or completely stop the enzymatic reaction. They can react with either an important component of protein molecules (e.g. copper or iron in the prosthetic group of oxidases) or with reactive groups of the enzyme protein (e.g. formaldehyde binds to free amino groups changing spatial structure of apoenzyme). Types of enzyme inhibition are shown in the following diagram (DIPFP – diisopropyl fluorophosphate, IAA - iodoacetamide).



Inhibitions can be:

1) Irreversible - enzyme activity cannot be recovered

2) Reversible - inhibitor can be removed (e.g. by dialysis) and activity can be restored.

Recovery from reversible inhibition depends on the removal of the inhibitor from the system, whereas recovery from irreversible inhibition requires the synthesis of fresh enzyme.

12.7.1.2.1. Irreversible inhibition

Irreversible inhibitors may be **specific,** they inhibit only one class of enzymes and do not inactivate all proteins as for example extreme values of pH and temperature that cause denaturation of all proteins (i.e**. nonspecific inhibition**). Specific irreversible inhibitors can cause covalent modification of enzyme structure and thereby inactivate the enzyme. They may bind at the active site, or at a different site. The net result is the same - irreversible loss of enzyme activity. Some irreversible inhibitors are important drugs. Penicillin acts by covalently modifying the enzyme *transpeptidase*, thereby preventing the synthesis of bacterial cell walls and thus killing the bacteria. Aspirin acts by covalently modifying the enzyme *cyclooxygenase*, reducing the synthesis of inflammatory signals.

Irreversible inhibitors can be divided into three categories: group-specific reagents, substrate analogs and suicide inhibitors.

Group-specific reagents react with specific groups of amino acids. Two examples of group-specific reagents are diisopropyl fluorophosphate (DIPFP; Figure 12.24) binding to the -OH group in the active site and iodoacetamide (Figure 12.25) reacting with the –SH group in the active site of the enzyme.

Diisopropyl fluorophosphate (DIPFP) (Fig. 12.24) is the organophosphate compound that is used to prepare the nerve gas sarin and other organophosphate toxins, such as various insecticides. DIPFP reacts with the hydroxyl group of serine in the active site of *acetylcholine esterase* preventing the enzyme to degrade the neurotransmitter acetylcholine. Inactivation of acetylcholine esterase produces violent spasms of the pulmonary system and interferes with normal neuromuscular and cardiac function. Similar agents are employed as insecticides in agriculture and sometimes may be severely or fatally toxic to humans. DIPFP inhibits many other enzymes, which have a serine (-OH group) in the active center.

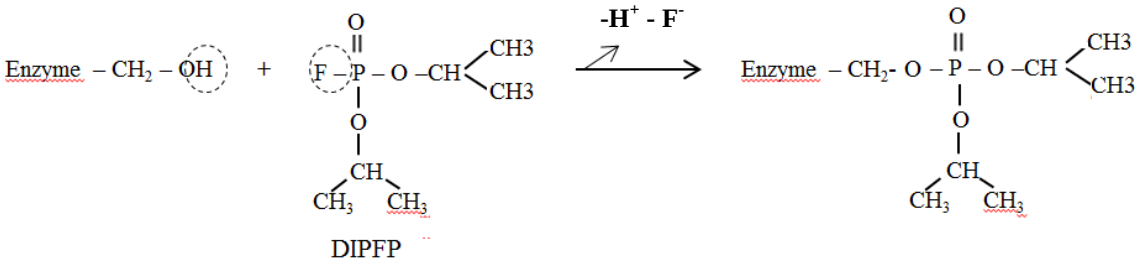


Fig. 12.24. Irreversible inhibition of enzyme activity by diisopropyl fluorophosphate (DIPFP)

Iodoacetamide (IAA) covalently binds to -SH group of cysteine, so the enzyme cannot form disulfide bonds (Fig. 12.25.). Iodoacetamide is very toxic. It acts as a human carcinogen and can cause reproductive damage.

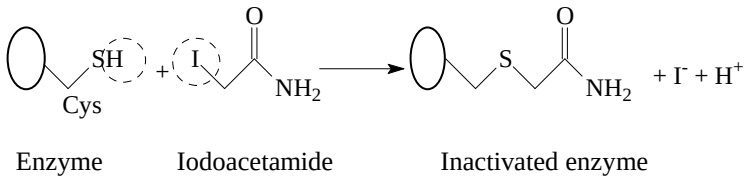


Figure. 12.25. Iodoacetamide binding to the enzyme-SH groups

Substrate analogs are molecules that are structurally similar to the substrate and covalently modify active site residues. They are thus more specific for the enzyme active site than are group-specific reagents.

Suicide inhibitors are modified substrates that provide the most specific means to modify an enzyme active site. The inhibitor binds to the enzyme as a substrate and is initially processed by the normal catalytic mechanism.

The mechanism of catalysis then generates a chemically reactive intermediate that inactivates the enzyme through covalent modification.

Most of the human poisonings are essentially caused by inhibition of some enzymes. E.g. cyanide is a classic example of an irreversible inhibitor. It binds covalently to the mitochondrial enzyme *cytochrome oxidase* and inhibits electron transfer on oxygen resulting in respiratory arest and the loss of energy production. Irreversible inhibitors are generally considered to be poisons and are not suitable for therapeutic purposes.

Toxicity of **heavy metal ions** is caused by strong binding of metal ions (such as mercury, lead, aluminium or iron) to a functional group of the enzyme. Heavy metals are relatively nonspecific for the enzyme to inhibit. E.g. mercury binds to sulfhydryl groups (-SH) in the active center of enzymes. Lead manifests its toxicity by replacing normal metal in enzyme structure.

12.7.1.2.2. Reversible inhibition

Reversible inhibitors bind to the enzyme by noncovalent interactions such as hydrogen bonds, hydrophobic interactions and ionic bonds. Multiple weak bonds between the inhibitor and the active site create a strong and specific binding. Some reversible inhibitors bind to the enzyme so tightly that they are essentially irreversible. Examples of such an inhibitor is allopurinol or methotrexate.

According to the kinetic characteristics and a place on the surface of enzyme molecules to which inhibitor is bound we classify inhibitors to competitive, non-competitive and uncompetitive.

12.7.1.2.2.1. Competitive inhibition

Competitive inhibitor is structurally similar to the substrate. Competitive inhibitors bind reversibly with the enzyme in competition with the substrate. When the inhibitor, [I], is bound to the enzyme, the normal substrate cannot form the [ES] active complex, and thus less enzyme is available for catalysis. A sufficient concentration of the substrate will overwhelm the inhibition, and the v_{max} will be the same as with no inhibitor present. At concentrations in which substrate and inhibitor are more comparable, the K_M for the substrate is lower than K_M^I for the inhibitor. This can be seen in kinetic analysis, expressed as a Michaelis – Menten and Lineweaver-Burk plots (Fig. 12.26.), in which the slopes change (Figure B) but the intercepts ($1/v_{max}$ on the 1/v axis) remain the same.

Competitive inhibitors **do not alter the v_{max}** but **they increase K_M**.

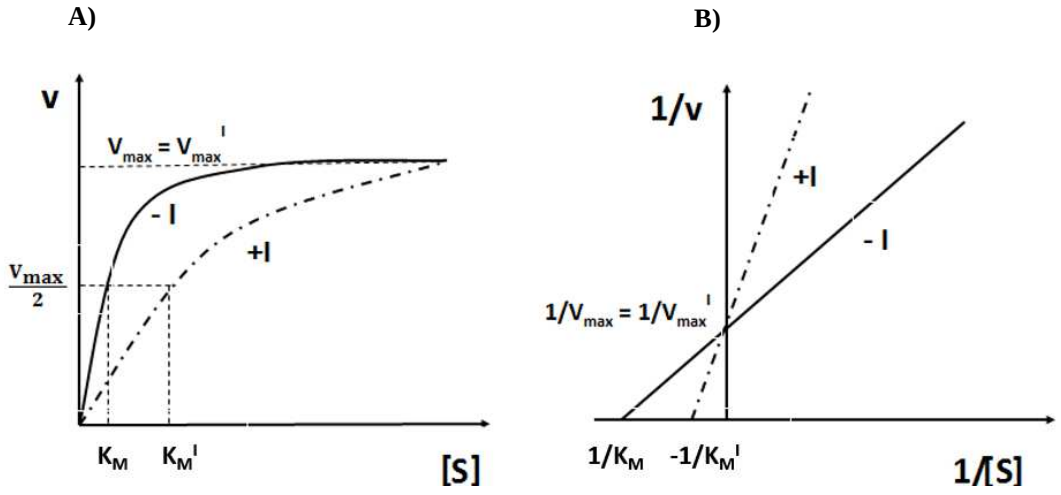
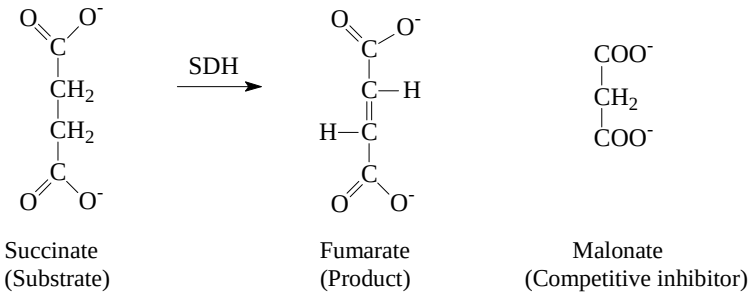


Fig. 12.26. Competitive inhibition depicted by Michaelis and Menten (A) and Lineweaver-Burk (B) plots in the reaction without inhibitor (solid line) and with a competitive inhibitor (dashed line).
v is the reaction rate, K_M is the Michaelis constant, K_M^I is the Michaelis constant of inhibited reaction, v_{max}^I is the maximal reaction rate of inhibited reaction and [S] is the substrate concentration

An example of a competitive inhibition is the mitochondrial enzyme *succinate dehydrogenase* (SDH) catalyzing the following reaction:



Succinate (Substrate) | Fumarate (Product) | Malonate (Competitive inhibitor)

Malonate, structurally similar to succinate, is a competitive inhibitor of SDH. It can bind to the enzyme, but can not be dehydrogenated. Its effect can be abolished by sufficiently high concentration of succinate.

Competitive inhibition is also used in the treatment of cancer by chemotherapeutic agents, and as a first aid during **methanol** poisoning (Fig. 12.27). Toxicity of methanol is caused by its metabolites rather then by methanol itself (methanol causes CNS depression). Methanol is oxidized in the liver by *alcohol dehydrogenase* to formaldehyde and by *aldehyde dehydrogenase* to formic acid (Fig. 12.27). Formaldehyde is a very strong neurotoxin. It can precipitate proteins and nucleic acids . Formic acid can decrease pH resulting in acidosis. The early signs of methanol poisoning include drowsiness and drunkenness. After 8 to 36 hours also headaches, dizziness, coma or convulsions can appear. The most typical symptom of poisoning is blurred vision, retinal damage and blindness.

Ethanol acts in humans as an antidote to methanol and ethylene glycol poisoning (Fig. 12.27.). Methanol and ethylene glycol are metabolized at lower speed compared to ethanol. In addition, ethanol has much higher affinity for *alcohol dehydrogenase* than methanol or ethylene glycol, so it is the preferred substrate. This allows ethanol administered as an antidote in methanol and and ethylene glycol intoxications to slow down the metabolism of methanol and ethylene glycol and thus significantly reduce the biochemical and clinical effects.

Ethylene glycol is in the industry also known as Fridex. It is an alcohol with two -OH groups (diol), a chemical compound widely used in antifreeze mixtures for automobiles. Ethylene glycol is toxic after ingestion. Because of its sweet taste, sometimes children and animals ingest a large dose of this mixture. Poisoning is manifested by the following symptoms: vomiting, metabolic acidosis, cardiovascular disorders and eventually acute renal failure. The cause of toxicity is not the ethylene glycol itself but its metabolites. The most important metabolites causing neurotoxicity and nephrotoxicity are glycolic acid, glyoxalic and oxalic acids (Fig. 12.27.) which reduce pH leading to acidosis.

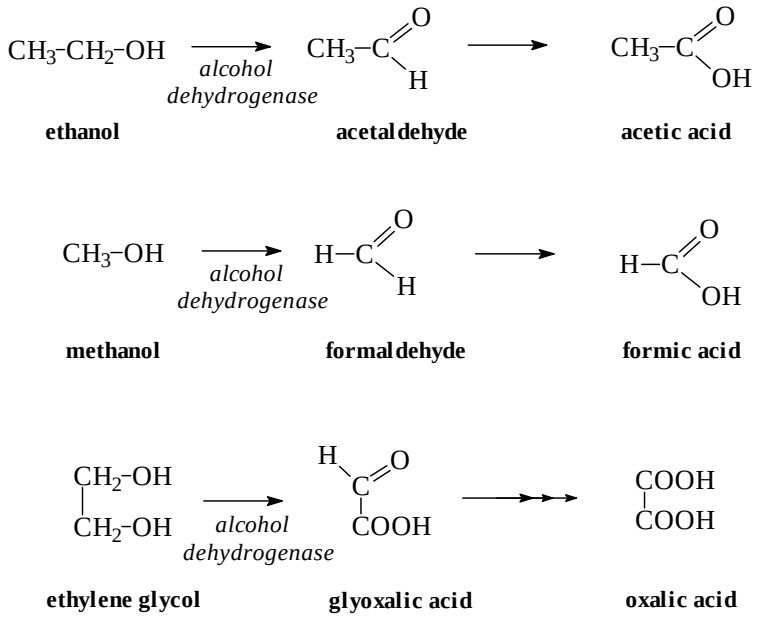


Fig. 12. 27. Ethanol as a competitive inhibitor in the toxicity of methanol and ethylene glycol

Allopurinol (Fig. 12.28) is used in medical praxis in the treatment of gout, the disease caused by the accumulation of uric acid salt crystals in the joints and joint fluid, especially in the ankle and toe. Allopurinol is a structural analogue of the natural purine base, hypoxanthine. It is a competitive inhibitor of *xanthine oxidase*, the enzyme responsible for the conversion of hypoxanthine to xanthine and of xanthine to uric acid, the end product of purine metabolism in man. Allopurinol is metabolized to the corresponding xanthine analogue, oxypurinol (alloxanthine), which also is an inhibitor of *xanthine oxidase*. Alloxanthine remains bound in the active center of enzyme and prevents the second reaction step.

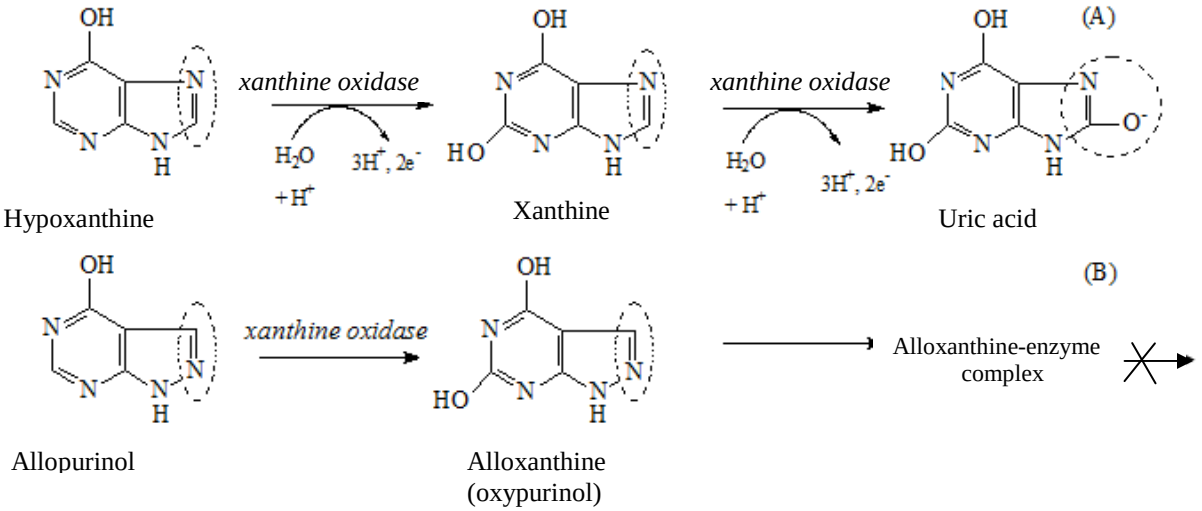


Fig. 12.28. Reactions of oxidation of hypoxanthine to uric acid by the enzyme *xanthine oxidase* (A). Allopurinol, structurally similar to hypoxanthine, is oxidized by *xanthine oxidase* to alloxanthine, which remains bound in the active center of the enzyme inhibiting its activity (B).

12.7.1.2.2.2. Noncompetitive inhibition

Noncompetitive inhibitors bind to the enzyme outside the active center, they are structurally distinct from substrates. Noncompetitive inhibitors **reduce v_{max} without a change in the K_M** for the substrate (Fig. 12.29). This type of inhibition is rare but can occur in multimeric enzymes.

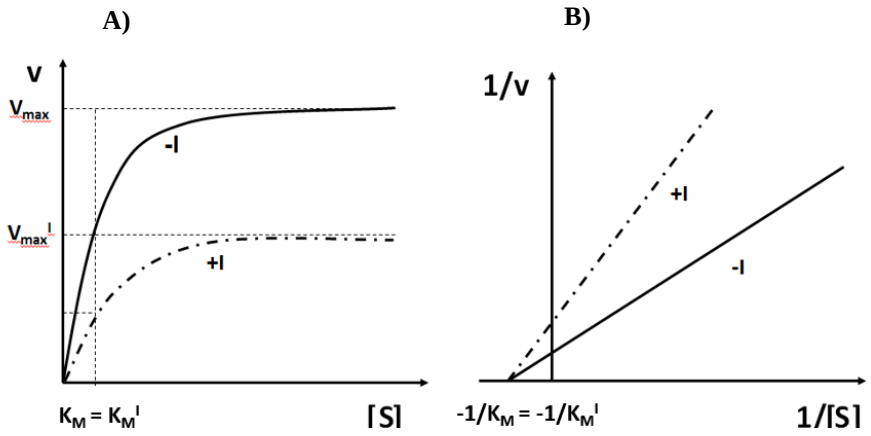


Figure. 12.29. Noncompetitive inhibition depicted by Michaelis and Menten (A) and Lineweaver-Burk (B) plots in the reaction without inhibitor (solid line) and with a noncompetitive inhibitor (dashed line).
v is the reaction rate, K_M is the Michaelis constant, $K_M{}^I$ is the Michaelis constant of inhibited reaction, $v_{max}{}^I$ is the maximal reaction rate of inhibited reaction and [S] is the substrate concentration

12.7.1.2.2.3. Uncompetitive inhibition

Uncompetitive inhibitors can bind only to the complex enzyme-substrate and not to the free enzyme resulting in the nonactive ternary complex enzyme-substrate-inhibitor. In this case **both v_{max} and K_M are changed**. The Lineweaver-Burk plot (Fig. 12.30) shows parallel lines at the different inhibitor concentrations.

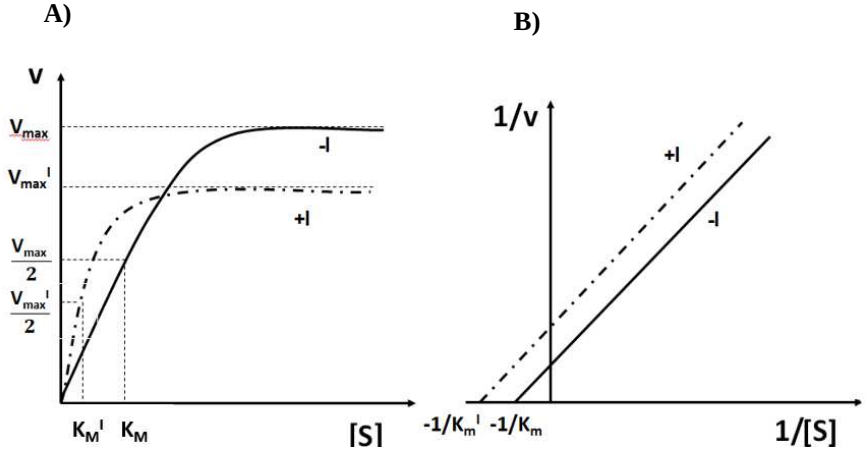


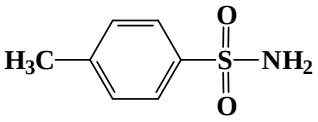
Fig. 12.30. Uncompetitive inhibition depicted by Michaelis and Menten (A) and Lineweaver-Burk (B) plots in the reaction without inhibitor (solid line) and with a noncompetitive inhibitor (dashed line).
v is the reaction rate, K_M is the Michaelis constant, $K_M{}^I$ is the Michaelis constant of inhibited reaction, $v_{max}{}^I$ is the maximal reaction rate of inhibited reaction and [S] is the substrate concentration

Tab. 12.3. The single-substrate reactions, the three types of inhibition are summarized

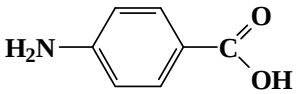
Type of inhibition	V_{max}	K_M
Competitive	Unchanged	Increased
Noncompetitive	Decreased	Unchanged
Uncompetitive	Decreased	Decreased

Coenzyme analogues as drugs

Enzymes can also be inhibited by affecting associated coenzymes or prosthetic groups. This is of considerable importance in designing chemotherapeutic agents. One of the earliest antibiotic drugs was p-toluenesulfonamide, an analogue of p-aminobenzoic acid, structures of which follow:

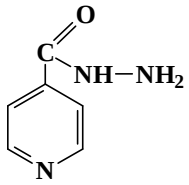


p-Toluene sulfonamide

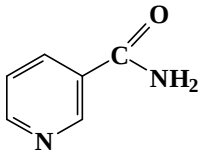


p-Aminobenzoic acid

Certain microorganisms produce folic acid, which contains a p-aminobenzoylacid residue in its structure. Toluene sulfonamide interferes with microbial synthesis of folic acid. Since folic acid is an essential coenzyme involved in the biosynthesis of purines and thymine, sulfonamides inhibit growth of the pathogenic organisms. On the basis of these observations a large series of substituted sulfonamides was produced, some of which are still used in clinical practice.



Isoniazid



Nicotinamide

Similarly, biosynthesis of pyridine nucleotide coenzymes requires incorporation of a nicotinamide moiety. An analogue of nicotinamide is the drug known as isoniazid, shown above. It interferes with the biosynthesis of nicotinamide coenzymes and is particularly useful in slowing growth of the organisms that cause human tuberculosis.

Unfortunately, many pathogenic organisms have become resistant to one or more of these drugs, so the search for new antibiotic agents continues.

12.7.1.3. Allosteric regulation of enzyme activity

In addition to **single subunit** enzymes (non-regulatory enzymes), there is a group of **allosteric** enzymes. Allosteric enzymes (regulatory) are enzymes that change their conformational ensemble upon binding of an effector, which results in an apparent change in binding affinity at a different ligand binding site. Allosteric enzymes need not be oligomers as previously thought.

Some allosteric enzymes are composed of subunits of identical or closely related peptide chains. They can bind more than one molecule of substrate. **Allosteric effectors** are substances (inhibitors and activators), which by binding to allosteric sites of the enzyme, induce its conformational change, and thus the activity of the enzyme. One or more of the functional sites on these enzymes may be *catalytic* (C), whereas one or more other sites may be *regulatory* (R) and not identical with the catalytic or active sites. In some instances R and C sites are on different subunits; in other instances the allosteric and catalytic sites are located on the same subunit. The transition between an active and inactive state of the enzyme can be induced by binding allosteric effectors to an allosteric center: binding of an activator (as well as a substrate) turns allosteric conformation from an inactive to

an active state of enzyme. Binding of an inhibitor turns allosteric conformation from an active to an inactive state.

When the reaction velocity of an allosteric enzyme is plotted as a function of substrate concentration, a sigmoid rather than a hyperboloid curve is obtained, as shown in Fig. 12.31. Allosteric enzymes do not follow the kinetics of the Michaelis-Menten model.

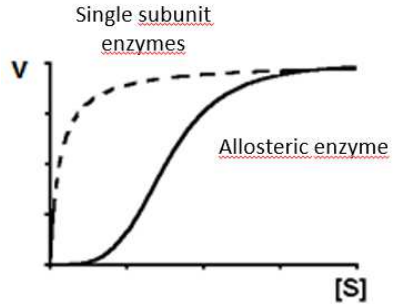


Fig. 12.31. Dependence of enzyme reaction rates (v) on the concentration of substrate ([S]) for an allosteric (solid line) and single subunit (dashed line) enzymes

With increasing concentration of substrate, the substrate binds to the enzyme and causes a conformational change in the enzyme active site so that other substrate molecules can bind more easily. There is a balance between active and inactive conformations of the enzyme. The amount of active and inactive enzyme depends on the relative concentrations of substrates and inhibitors. The sigmoidal curve represents the relation between the velocity of the enzyme and concentration of the substrate when the enzyme has either more sites for binding the substrate or more subunits with only one site for substrate binding.

The shapes of the allosteric curves are changed considerably by altering the concentration of either positive or negative effectors, as indicated by the plus and minus marks (Fig.12.32). Decreasing the amount of negative effector or increasing the amount of positive effector produces a response equivalent to lowering the K_M of the substrate.

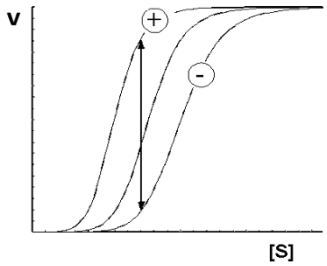


Fig. 12.32. Reaction kinetic curve of the allosteric enzyme without any allosteric effector present (line in the middle), with allosteric activator (line with (+) mark) and with allosteric inhibitor (line with (-) mark).

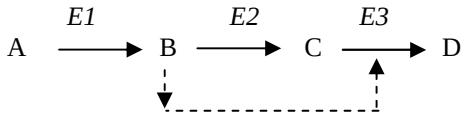
Summary of **allosteric enzymes** properties

Allosteric enzymes:

1. are enzymes that do not obey Michaelis-Menten kinetics
2. display sigmoidal plots of the reaction velocity (v) versus substrate concentration [S]
3. the binding of substrate to one active site can affect the properties of other active sites in the same enzyme molecule
4. their activity may be altered by regulatory molecules that are reversibly bound to specific sites other than the catalytic sites

Allosteric and active sites may not be on the same subunit. Several enzymes in addition to catalytic subunits with active sites contain also regulatory subunits binding loosely or tightly to the catalytic subunits. Inhibitors or activators can bind to the regulatory subunits causing conformational change of the enzyme molecule. If with increasing concentration of the substrate the shape of the curve of the reaction rate dependence on the substrate concentration is not hyperbolic but sigmoidal (Fig. 12.31), we can assume that this is an allosteric enzyme.

Allosteric enzymes are an important part of the so-called regulation of the multienzyme systems in metabolic pathways. Types of enzyme regulation in metabolic pathways include **feed-forward activation** and **feedback inhibition.** In feed-forward activation, an early metabolite (e.g. B) activates an enzyme further down the metabolic pathway (e.g. *E3*).



In feed-back inhibition, an excess of product inhibits the regulatory enzyme at an earlier step. E.g. in successive reactions (Fig. 12.33) transforming a substrate A to a product P, the product becomes a regulator of activity of one of the first enzymes in the pathway. E_1 enzyme (Fig. 12.33) has often allosteric properties and the product P acts as an allosteric inhibitor. In this way the product P regulates its own production. The mechanism of this regulation is referred to as a **feed-back control**. This type of inhibition occurs in biological systems very often. This mechanism effectively regulates the synthesis of intermediates according to the need of the cell and maintains a stable internal environment of living organisms.

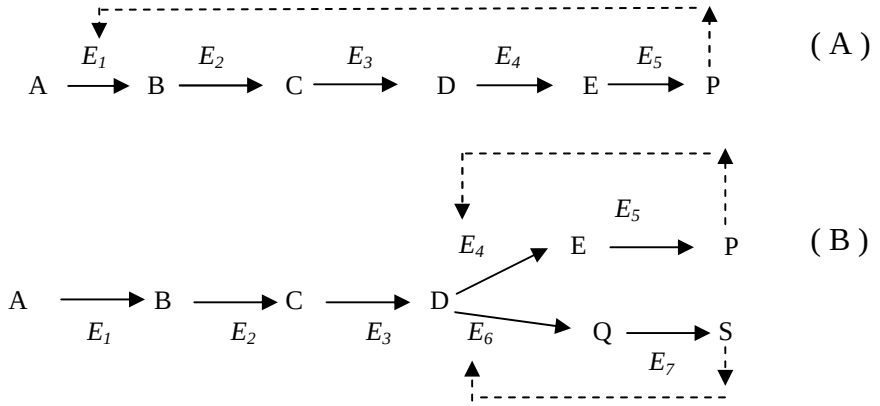


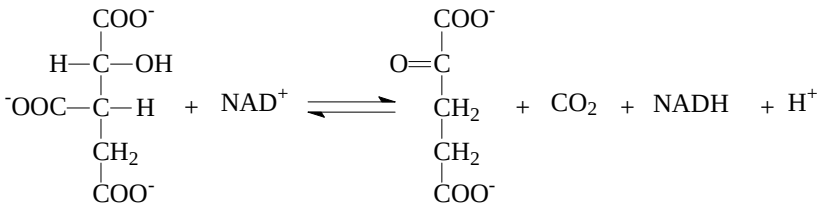
Fig. 12.33. Feed-back regulation

(A) *Reactant A is converted to a product P through several intermediate products (B, C, D, E). Production of these intermediates is catalyzed by enzymes E_1, E_2, E_3, E_4, E_5. Final product P can inhibit one of the first enzymes (E_1) of the metabolic pathway (feed back regulation).*

(B) *Chain metabolic pathway. Products of each branch (P, S) can regulate activity of branching enzymes E_4 a E_6 by a feed-back control.*

Regulatory enzymes control different metabolic pathways; they are located at the beginning of the metabolic pathways, at the junctions of different metabolic pathways or in the slowest reactions. Allosteric (noncovalent) regulation may permit fine-tuning of metabolic pathways. Several types of regulation may occur in a single regulatory enzyme.

Isocitrate dehydrogenase **(**ICDH) is an example of an allosteric enzyme. It is a tetramer and all four of its peptide chains are required for its activity. ICDH catalyzes the following reaction:



Isocitrate α-Ketoglutarate

NAD^+ and **ADP** are **positive** allosteric effectors, whereas NADH and ATP are negative allosteric effectors.

NAD^+ is a required coenzyme, ADP increases the affinity of the enzyme for NAD^+, and *vice versa*. **Citrate** is also a positive effector. The corresponding but opposite effects are noted with the **negative** allosteric agents **ATP** and **NADH**.

Thus this is an example of an enzyme that is controlled by at least three distinct allosteric effectors, one of which is related closely to isocitrate in the Krebs cycle, and one of which happens to be a required coenzyme. A schematic representation of these effectors and the ways in which they alter the activity of *isocitrate dehydrogenase* is shown in Fig. 12.34.

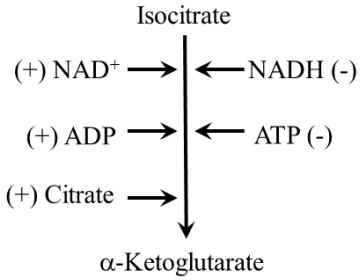


Fig. 12.34. Allosteric effectors of isocitrate dehydrogenase

There are two theories explaining activation of allosteric enzymes:

1) Cooperative (symmetric) model
2) Sequential model

Cooperative (symmetric) model was proposed by Jacques Monod, Jeffries Wyman and Jean-Pierre Changeux (MWC model) and the sequential model by Koshland, Nemethy and Filmer. Both models predict that enzyme subunits exist in one of two conformations: inactive - tensed T or active - relaxed R, and that relaxed subunits bind substrate more readily than subunits in the inactive state. The two conceptions are different in the mechanism of conversion of one form to the other.

12.7.1.3.1. Cooperative symmetric model

In 1965 Monod has assumed that the mechanism of changing conformation from inactive T (tensed) to active R (relaxed) form underlies the principle "all or nothing" (Fig.12.35). It means that conformation change of one subunite induces changes of all other subunits. Activation of this mechanism is triggered by the relevant effector. When the effector is the own substrate which has the higher affinity to the R-form, the relation between the velocity and the concentration of substrate is represented by the sigmoidal curve (Fig. 12.32.). During interaction of the substrate with the enzyme and with attached effector in the allosteric site of R-form, the movement of equation state from the T to R form is stimulated, and the change from sigmoidal to hyperbolic curve is possible. Different situation occurs during interaction of a negative effector bound in the allosteric site of the monomers in the T-form by which the equation moves to the T-form, which has low affinity to the substrate. As a consenquence, the maximum velocity of enzymatic reaction can be reached with the higher concentration of the substrate.

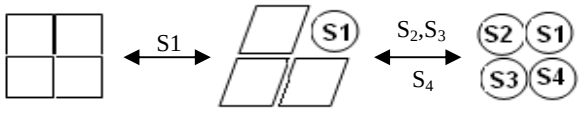


Fig. 12.35. Cooperative (symmetric) model of allosteric regulation

Square represents the T conformation (inactive) of the enzyme subunit, circle represents the enzyme subunit in the R conformation (active) after binding to the substrate (S1 to S4). After binding the first substrate molecule to one subunit of the enzyme, all other subunits undergo conformational changes so that the next substrate molecule can bind very easily.

12.7.1.3.2. Sequential model

Cooperative model was modified by Koshland in 1966 and was named a **sequential model** (Fig.12.36). The sequential model suggests that the subunits are linked in such a way that the conformational change in one subunit causes gradual changes in other subunits. Thus, all subunits are not in the same conformation. Binding of substrate to one subunit changes conformation of only the neighboring subunit and thus facilitates the binding of another substrate molecule.

The positive effectors accelerate the change from the low affinity to high affinity subunits, on the contrary, the negative effectors this process retard.

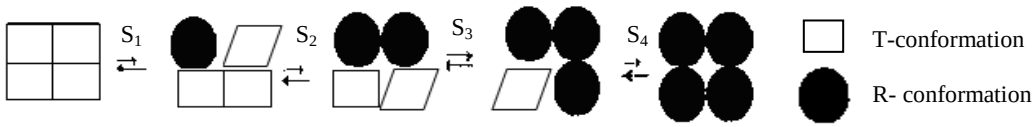


Fig. 12.36. Sequential model - *square represents the T conformation (inactive) of the enzyme subunits, the circle the enzyme subunit in the R conformation (active) after binding to the substrate (S1 to S4).*

Allosteric (regulatory) enzymes represent the most important autoregulatory mechanism of the cells. Any metabolic pathway in the cell allows regulation of a certain key enzyme, which is allosteric and mainly localized at the beginning of the whole cascade reactions. The end product of the metabolic pathway is usually a negative effector of the key enzyme of that metabolic pathway.

The major features of allosteric control are summarized below.

Allosteric enzymes:

- are usually composed of more than one polypeptide chain
- may contain two separate functional centers, one of which is catalytic and the other regulatory
- may be subject to either positive or negative control by one or more factors

12.7.1.4. Regulation of enzyme activity by modification of enzyme molecule

Regulation of enzyme activity by modification of the enzyme molecules can occur in several ways:

1) limited proteolysis
2) covalent modifications
3) exposure to metal ions

12.7.1.4.1. Limited proteolysis

Limited proteolysis is a proteolytic cleavage of the inactive form of the enzyme called proenzyme (**proprotein**) or **zymogen**, to the active form, by cleaving off a small portion of proenzyme molecule that acts as an inhibitory segment. This will enable the substrate to approach the enzyme´s active site. Enzymes performing limited proteolysis are *proteinases* (*proteases*) or *peptidases* that can selectively cleave peptide bonds. Proteolytic enzymes are active in a number of biochemical reactions. Proteolysis is an irreversible process.
Significant portions of some enzymes can be removed without loss of activity. An inactive protein can be converted to an active enzyme by cutting off a portion of the peptide chain. Typical examples are the digestive proteinases, *pepsin*, *trypsin*, and *chymotrypsin*, each of which is produced and stored as an inactive *proenzyme*, or a *zymogen*. When the zymogen pepsinogen is released into the gastric juice, it loses a peptide fragment (44 amino acids “AA") in the acid gastric environment and is converted to active *pepsin*. Proteosynthesis of inactive proenzyme molecules is very important because endogenous cellular proteins could be degraded by the production of active forms of enzymes.

E.g. digestive enzymes *pepsin*, *trypsin* and *chymotrypsin* (Fig. 12.37) are produced and stored as pepsinogen, trypsinogen and chymotrypsinogen in an inactive form. They are transported to the digestive tract, where they are activated. This process prevents the enzymes to digest the pancreas or other tissues before they get to the point of operation. Digestive *proteinase* zymogen - chymotrypsinogen is activated by *trypsin* (Fig. 12.37). Trypsinogen in turn is activated by *enterokinase* in *trypsin*, which is regulated by hormones.

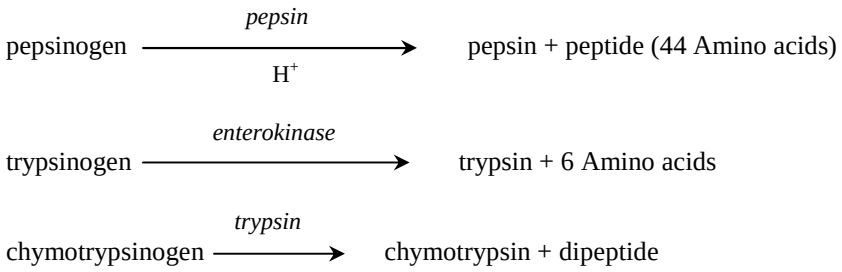


Fig. 12.37. Activation of proenzymes by cutting off a portion of the peptide chain

Activation of zymogens by a scission of a peptide fragment is not limited only to proteolytic enzymes. Also the conversion of the prohormone proinsulin to insulin uses the same mechanism of activation. Sometimes a sequential activation of enzymes occurs; one enzyme is activated, which activates the second one, then the third one resulting in a cascade of activations such as that found in blood clotting (for example conversion of prothrombin to thrombin).

Proteases are involved in digesting long protein chains into short fragments, splitting the peptide bonds that link amino acid residues. Some of them can detach the terminal amino acids from the protein chain (***exopeptidases***, such as *aminopeptidases, carboxypeptidase A*); the others attack internal peptide bonds of a protein (***endopeptidases***, such as *trypsin, chymotrypsin, pepsin, papain, elastase*).

Proteases are divided into six major groups according to the character of their catalytic active site and conditions of action:

a) ***serine proteases*** are examples of enzymes with serine residue in the active center. The serine *proteases* contain hydroxyl group of serine as a catalytic group. They include the digestive enzymes *trypsin, chymotrypsin, elastase* and *thrombin.* They differ in their specificity. E.g. *chymotrypsin* cleaves peptide bonds formed with an aromatic amino acid residues whose carbonyl carbon is part of a peptide bond. *Trypsin* cleaves peptide bonds formed with positively charged lysine and arginine. *Elastase* is not as specific as the previous two digestive enzymes, it cleaves the peptide bonds formed of the small, neutral residues. Serine *proteases* are sensitive to inhibition by organic fluorophosphates such as diisopropyl fluorophosphate (Fig. 12.24).

b) ***aspartate proteases*** have pH optimum in the acidic range, and their catalytic functions involve two aspartic acid residues. These proteases exhibit diverse functions, e.g. digestion (*pepsin* and *chymosin*), protein degradation in lysosomes (*cathepsin D* and *E*), the regulation of blood pressure (*renin* - aspartate protease that is necessary for the production of angiotensin, a hormone stimulating smooth muscle contractions and reducing excretion of salts and fluids). Aspartate proteases cleave peptide bonds between two hydrophobic amino acid residues.

c) ***metalloproteases*** are any protease enzymes whose catalytic mechanism involves a metal. Most *metalloproteases* require zinc, but some use cobalt. The metal ion is coordinated to the protein via three ligands. The fourth coordination position is taken up by a labile water molecule. The *metalloproteases* include *carboxypeptidase A, a* variety of *matrix metalloproteinases* and *lysosomal proteases*.

d) ***cysteine proteases*** use as catalytic group thiol -SH group of cysteine residue. They include plant enzymes *papain, bromelain,* microbes (streptococcal *protease*) and animal enzymes (*cathepsin B*).

e) ***threonine proteases*** are a family of proteolytic enzymes harbouring a threonine residue within the active site. The prototype members of this class of enzymes are the catalytic subunits of the proteasome.

f) ***glutamate protease*** contain a glutamic acid residue within the active site.

Alternatively, *proteases* may be classified by the optimal pH in which they are active:

- ***Acid proteases*** **-** exhibit maximum activity and stability in acid conditions (pH 2.0–5.0) and are inactivated at pH values above 6.0. Acid proteases have a low isoelectric point and are low in basic amino acids
- ***Neutral proteases***
- ***Basic proteases*** (or alkaline proteases)

12.7.1.4.2. Regulation of enzyme activity by covalent modifications

An important means of enzyme regulation involves covalent modification not related to proteolysis. Most frequently the modification involves phosphorylation or dephosphorylation, glycosylation or nucleotide binding (adenylation, uridylation). Reversible covalent modification is a major mechanism for rapid regulation of enzyme activity, as opposed to enzyme induction, which is a slow mechanism regulating the concentration and enzyme activity (in term of hours, days or weeks).

Activation of enzymes by their phosphorylation/dephosphorylation

This type of activation is a very important regulatory mechanism of metabolic processes. It is estimated that one-third of the proteins in the human proteome are substrates for phosphorylation at some point. Phosphorylation or dephosphorylation are mediated by enzymes known as *protein kinases* and *protein phosphatases*, respectively. *Protein kinases* attach phosphate groups to serine, threonine, and tyrosine residues of the substrate molecules. *Protein phosphatases* can detach phosphate groups from substrates (Fig. 12.38). Substrates for *kinase* activity are diverse and include lipids, sacharides, nucleotides and proteins. The cycle of phosphorylation and dephosphorylation can be very rapid, making the activity of the protein exquisitely sensitive to regulation in this way.

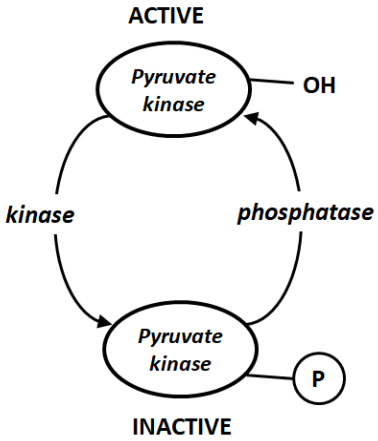


Fig. 12.38. Regulation of *pyruvate kinase* by phosphorylation
The phosphorylated enzyme is inactive and dephosphorylated is active.

There are several ways in which phosphorylation-induced change can happen:

- The phosphate group may prevent binding of a substrate or ligand. Being strongly negatively charged, the phosphate may disrupt electrostatic interactions between an enzyme and its substrate. Alternatively, it may block substrate-binding by steric hindrance.
- Phosphorylation may cause a dramatic change in the conformation of the enzyme

Phosphorylation / dephosphorylation is stimulated mainly by hormones after their bindig to the cell receptors. The information brought to the cell by the hormone may be mediated by e.g. the cyclic adenosine-3´,5´-monophosphate (cAMP). This nucleotide is a second messenger activating phosphorylation / dephosphorylation of some specific proteins mainly enzymes. It is very interesting that phosphorylation of enzymes could have an opposite effect; some enzymes are activated after their phosphorylation (for example *glycogen phosphorylase)* but some enzymes lose their activity (for example *glycogen synthase* or complex *pyruvate dehydrogenase*). Another group of enzymes is activated when they are dephosphorylated.

E.g. in response to hormon glucagon, phosphorylation of several enzymes occurs, including *glycogen synthase* (synthesizing glycogen) and *glycogen phosphorylase* (cleaves glycogen), which help regulate blood glucose levels (Fig. 12.39). *Glycogen phosphorylase* is active when phosphorylated, the phosphorylation of *glycogen synthase* leads to inactivation.

Secretion of glucagon (from α-cells of pancreas) is stimulated by **hypoglycemia** (reduced glucose levels in blood). If blood glucose levels fall down (fasting, exercise), glucagon is secreted and stimulates glycogenolysis (breakdown of glycogen to glucose-1-phosphate by *glycogen phosphorylase*) and gluconeogenesis. Glucagon

binds to its membrane receptors in the liver, activates *adenylate cyclase*, which cleaves ATP to cAMP (Fig. 12.40). Increased levels of cAMP lead to activation of *protein kinase A* (PKA), which phosphorylates various proteins. The result is activation (phosphorylation) of cascade of phosphorylating enzymes including *glycogen phosphorylase*, which breaks down glycogen into glucose, thus increasing blood glucose levels.

Enzyme synthesizing glycogen (*glycogen synthase*) is inactive when phosphorylated due to the secreted glucagon.

During **hyperglycemia** (increased glucose levels in blood) insulin is released from pancreatic β-cells. Insulin produced in response to high blood glucose triggers another signaling cascade leading to dephosphorylation, and thus activation of *glycogen synthase*, resulting in the synthesis of glycogen from glucose.

Activation of *protein phosphatase* leads to dephosphorylation of enzymes, thereby inactivates enzymes that were activated by phosphorylation (*glycogen phosphorylase*) and activate the enzymes that were inactive after phosphorylation (*glycogen synthase*). Hyperglycemia will result in glycogen synthesis in the liver. Thus insulin antagonizes the effects of glucagon-induced cascade.

Thus, in glucose metabolism, phosphorylation reciprocally activates glycogen breakdown and inhibits glycogen synthesis, so that the control mechanism of covalent modification acts to regulate energy flux through two otherwise competing systems.

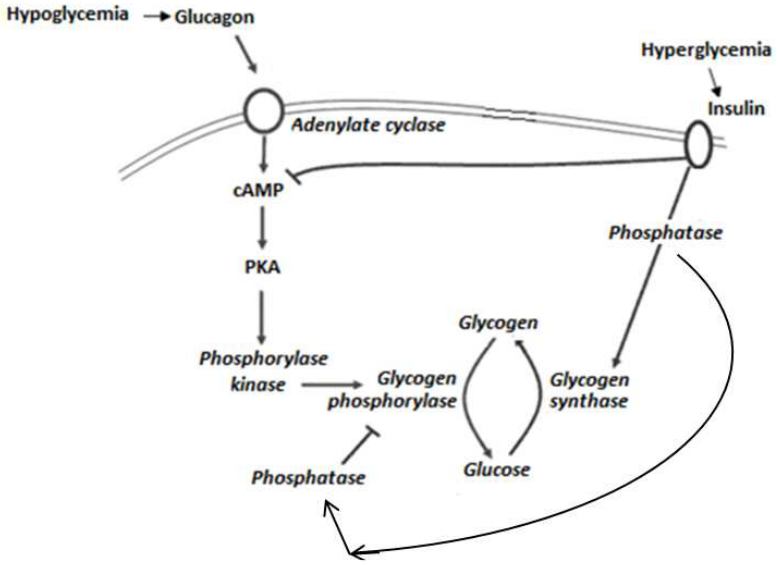


Fig. 12.39. Antagonistic effects of glucagon and insulin in maintaining glycemia

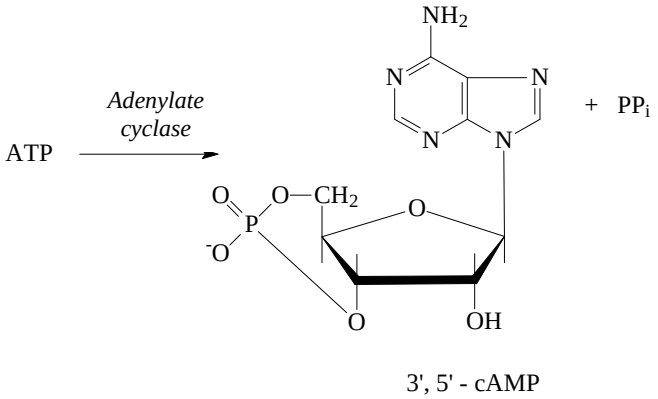


Fig. 12.40. Conversion of ATP to cAMP by *adenylate cyclase*

A similar reaction exists whereby guanosine triphosphate (GTP) is converted to cyclic guanosine monophosphate (cGMP).

12.7.1.4.3. Activation of enzymes by effect of metal ions

Metal ions are very important for catalytic function of some enzymes. Metalloenzymes can be divided into two groups:

1. **Metalloenzymes,** where the **metal ions are strongly bound to their molecules,** a metal ion is a component of their molecule. Participation of the metal ions in the catalytic activity has been prooved. When the ions are inhibited, enzyme activity is decreased or completely lost. In *oxidoreductases* there are iron, copper, manganese, some other enzymes contain zinc or iron-porfyrine complex. There are enzymes containing a trace element, for example *glutathione peroxidase* contains selenium which is very important for some types of enzymatic reactions.
2. **Enzymes activated with metal ions**, where the **metal ions are not integrated strongly in the enzyme** molecule, but their presence is necessary for catalytic activity of enzymes. In addition to the first group of metal ions there are also bivalent ions such as calcium, magnesium as well as monovalent ions such as natrium, kalium but also cobalt and some trace elements.

There has been much knowledge about participation of the metal ions in the mechanism of enzyme activity. Metal ions can exert their activity by many ways. Metal ions:

- directly participate in the catalysis. Iron ion directly participates in the transport of electrons by some *oxidoreductases*
- are bound to substrates and provide the bond to the active site of enzyme. This type occurs in many *phosphohydrolases*, where the Mg^{2+} is bound to the phosphate ester before it binds to the active site of enzyme
- are bound to some ionized groups of enzyme proteins and can change their conformation. This activity of metal ions can increase or decrease the velocity of enzymatic reaction.

12.7.2. Regulation of enzyme activity by change in the quantity of enzyme molecules

12.7.2.1. Enzyme induction and repression

Enzyme **synthesis** (transcription and translation of enzyme genes) can be **increased** or **decreased** in response to the changing environment of a cell. This method of gene regulation is called **induction** and **repression**. Induction involves *de novo* synthesis of enzyme molecules. These changes are quantitative (increase or decrease in the number of generated enzyme molecules). Synthesis of enzymes in contrast to covalent modification is a relatively slow mechanism to regulate the concentration and activity (in term of hours, days or weeks).

Cellular enzymes are either constitutive or inducible. **Constitutive enzymes** are present at virtually a constant concentration during the life of a cell. They are continuously synthesized because their role in maintaining cell processes or structure is indispensable. **Inducible enzymes** are produced ("turned on") in cells in response to a particular substrate; they are produced only when needed. In the process of induction, the substrate, or a compound structurally similar to the substrate, evokes formation of the enzyme and is called an **inducer**. Conversely, if the concentration of the substrate for a longer period is reduced, there is a reduction or complete suppression of gene expression. These enzymes must be able to respond quickly to changing conditions.

The processes of gene regulation have been well studied, particularly in microorganisms, where a series of inductive enzymes were discovered. E.g. *E. coli* growing in the absence of the disaccharide lactose, does not contain any enzymes for utilization of this saccharide. However, within a few minutes in a medium with lactose, the bacterium begins to synthesize the enzymes necessary for its processing.

Advances in molecular biology have contributed to a better understanding of the repression of various enzymes by exogenous substances. Cosiderable attention has been devoted to inhibitory effects of many antibiotics on gene expression as well as on post-transcriptional processes synthesizing proteins including enzymes. To the transcription inhibitors belong actinomycin and rifampicin (inhibitor of synthesis of *DNA-dependent RNA polymerase*) and the translation inhibitors include other known antibiotics (chloramphenicol, streptomycin, tetracycline, etc.).

12.7.2.2. Regulation of enzyme activity by regulated degradation

Enzyme levels are determined by the rate of their synthesis and degradation. Degradation is regulated by intracellular *proteases,* which hydrolyze peptide bonds and release peptides that are subsequently cleaved by *peptidases.* ***Endopeptidases*** cleave internal bonds and produce shorter peptides. ***Exopeptidases*** cleave off free amino acids from amino-terminus (N-terminus) and carboxy-terminus (C-terminus) of peptides and proteins.

There are two major pathways mediating the degradation of proteins in eukaryotic cells:

- ATP independent metabolic pathway - in **lysosomes** - in which extracellular proteins associated with cell membrane and intracellular proteins with a long half-life are degraded.
- ATP - and ubiquitin-dependent metabolic pathway - in **proteasomes** – for degradation of proteins with a short half-life.

1. **Lysosomes** are cell organelles containing various hydrolytic enzymes that degrade proteins and other substances taken by endocytosis. Lysosomes are non-specific. They can be described as the stomach of the cell. They are also used for the digestion of macromolecules from phagocytosis (ingestion of other dying cells or larger extracellular material, like foreign invading microbes), etc.
2. **Proteasome** is present in many copies in each cell. It is a cellular organelle which main function is degradation of unneeded or damaged proteins by proteolysis using the *proteases.* Proteasomes are a major mechanism by which cells regulate the concentration of endogenous proteins. Proteins mainly degraded by proteasomes are transcription factors, cyclins (they must be degraded in order to prepare the cell for the next step of the cell cycle), proteins encoded by viruses or other intracellular pathogens, proteins that are incorrectly folded. Proteins destined for degradation are marked by a small polypeptide (76 amino acids) called **ubiquitin**. Once the protein is labeled by ubiquitin, it is a signal for *ligase* to attach another ubiquitin. This will lead to a formation of a polyubiquitinated chain (with at least 4 units of ubiquitin) which binds to the protein destined for degradation.

   Proteasomes are very large protein complexes with *protease* activity. Apart from lysosomes, they are the major proteolytic apparatus of cells. It is estimated that 80% of proteins destined for proteolysis are degraded in proteasomes. Proteasomes are found in the nucleus and cytoplasm of eukaryotic and prokaryotic cells. Proteasome is composed of two functional protein complexes. The first part – the **core particle** (the body of the proteasome) (Fig. 12.41) with the sedimentation constant of 20S, has the catalytic activity and the shape of the barrel. It contains several different *proteases* with their active sites facing inside the barrel. The body of the barrel is sealed on both sides by caps which are formed from protein complexes with the sedimentation constant of 19S, also known as **regulatory particles**. The regulatory particles are involved in detection of polyubiquitinylated protein and its pulling into the cavity of the proteasome (Fig. 12.41). In the proteasomes the protein is unfolded and the ubiquitin units are cleaved off. Protein is broken down into short oligopeptides, peptides with 7-8 amino acid residues, which can be further degraded into amino acids and used in the synthesis of new proteins.

   It has been shown that the ubiquitin-proteasome system has also other functions: it participates in the recognition of antigen structures, it plays an important role in regulating the cell cycle, promotes cell proliferation and plays an important role in apoptosis. The disturbances in the ubiquitin-proteasome system occur in neurological disorders associated with the accumulation of protein complexes (Alzheimer's, Huntington's, Parkinson's diseases and others).

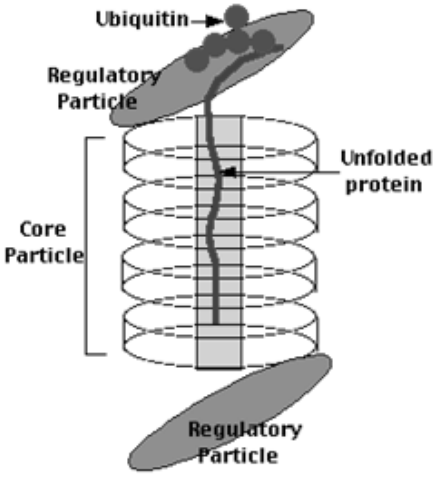


Fig. 12.41. Proteasome and its subunits – *protein from the regulatory particle plays an important role in recognition of the substrate destined for degradation in the proteasome. Polyubiquitinylated chain binds to the receptor for ubiquitin located on the regulatory particle.*

12.8. Enzyme nomenclature

Initially, there were no rules for naming enzymes. Enzymes were called by their **trivial names**, usually ending with suffix -in. Some of them we use even today (*pepsin, trypsin*). Later, the name of the substrate or the type of catalyzed reactions plus the suffix **-ase** was selected for the enzyme names:

Substrate + **-ase** (*lip**ase**, prote**ase***)
Type of a catalyzed reaction + **-ase** (*oxid**ase**, hydrol**ase**, transamin**ase***)

In order to establish reasonable rules for the naming of a rapidly growing number of enzymes, International Union of Biochemistry and Molecular Biology (IUBMB) adopted rules for the **systematic functional classification** and nomenclature of enzymes. Enzymes are classified according to the nature of chemical reactions they catalyze.

There are six main classes of reactions that enzymes catalyze, that are further divided into subclasses and subsubclasses. Each enzyme is designated by two names (recommended and systematic name) and four numbers (Enzyme Commision number). Its recommended name is convenient for routine use and it is rather trivial nomenclature. Its systematic name is used for precise identification of the enzyme. It consists of the name of the substrate plus the type of reaction according to the major classification group with the suffix **-ase**. The Enzyme Commission number (EC number) is a numerical classification scheme for enzymes, based on the chemical reactions they catalyze. EC numbers do not specify enzymes, but enzyme-catalyzed reactions. If different enzymes (for instance from different organisms) catalyze the same reaction, then they receive the same EC number. In the systematic name figures indicate the class of the enzyme (we distinguish six classes of enzymes), subclass, subsubclass and the sequence number in the subsubclass.

E.g. **recommended name:** *carboxypeptidase A*
systematic name: *peptidyl-L-aminoacid hydrolase*
classification number EC: EC 3.4.17.1.

Number 3 indicates the main class of enzymes (*hydrolases*), the second number 4 indicates the subclass (peptide bonds), the third number 17 is a number of another subgroup (metalocarboxypeptidases, *carboxypeptidase A* binds Zn^{2+}, necessary for its catalytic activity) and the fourth number is the sequence number of the enzyme in the subgroup 17.

Similarly,
recommended name: *alcohol dehydrogenase*
systematic name: *alcohol: NAD^+ - oxidoreductase*
classification number EC: EC 1.1.1.1.

Tab. 12.4. The trivial and international classification of some enzymes

Number	Trivial names	International classification
EC 1.1.2.3	*Lactate dehydrogenase*	*S-lactate:ferricytochrome and 2-oxidoreductase*
EC 1.1.3.22	*Xanthine oxidase*	*Xanthine:O_2-oxidoreductase*
EC 1.11.1.6	*Catalase*	*H_2O_2:H_2O_2-oxidoreductase*
EC 1.15.1.1	*Superoxide dismutase*	*Superoxide:superoxide reductase*
EC.2.6.1.1	*Aspartate transaminase*	*L-aspartate: oxoglutarate-aminotransferase*
EC 3.1.3.1	*Alkaline phosphatase*	*Orthophosphate monoester phosphohydrolase*
EC 4.6.1.1	*Adenylyl cyclase*	*ATP-diphospate lyase*
EC 5.1.3.11	*Celobiosa epimerase*	*Celobiose-2-epimerase*
EC 6.1.1.7	*Alanyl-t-RNA-ligase*	*L-alanin:$tRNA^{Ala}$-ligase*

12.9. Enzymes in medicine

Enzymes with their huge specific effects represent ideal agents for therapy. Their protein nature restricts their wider therapeutic use in the body and is a base of their antigen character, low stability in the body and also the difficulty to obtain them in sufficient quantity and purity. Therefore, enzymes have been used as medicaments only locally for a long time. Enzyme analysis is an integral part of modern clinical and biochemical investigation methods. Determination of an enzyme activities is used as an indicator of the condition of the human body. Concentrations, and thus the activities of many enzymes in plasma increase during tissue damage. Many enzymes and isoenzymes are specific for a particular tissue, elevated enzyme activities may indicate which tissue is damaged. Reduced enzyme activities in turn may indicate congenital metabolic defects.

Enzymes are used as a therapeutic agent in human and veterinary medicine. In addition to free enzyme preparations there are introduced also immobilized enzymes (e.g. in the form of pills). E.g. digestive enzymes are administered to patients with their deficiencies in the form of pills, *trypsin* is used to clean wounds from pus. Immobilized enzymes may also be part of a device known as an artificial kidney, which is used in patients with impaired renal function to eliminate urea and other waste products from the human body.

Table 12.5 lists some of the enzymes that have already been employed on a clinical basis, together with the types of disease states for which the assays are used.

In addition to measurement of the total enzyme activity, the isozyme pattern of certain enzymes is also used. The detection and quantitation of isozymes are of growing importance in differential diagnosis.

A number of enzymes are assayed (measured) during myocardial infarction in order to diagnose the severity of the heart-attack. Dead heart muscle cells spill their enzyme contents into the serum. Thus, the level of *aspartate aminotransferase* (AST) in the serum rises rapidly after a heart attack. Together with AST, *lactate dehydrogenase* (LDH) and *creatine phosphokinase* (CK) levels are monitored. In infectious hepatitis, the *alanine aminotransferase* (ALT) level in the serum can rise to ten times normal. There is also a concurrent increase in AST activity in the serum.

In some cases, the administration of an enzyme is a part of therapy. After duodenal or stomach ulcer operations, patients are advised to take pills containing digestive enzymes that are in short supply in the stomach after surgery.

Tab. 12.5. A brief list of enzyme assays known or presumably useful in diagnosis or treatment of a disease

Enzyme	Organ or disease of interest
Commonly assayed	
Acid phosphatase	Prostatic carcinoma
Alkaline phosphatase	Liver, bone disease
Amylase	Pancreatic disease
Aspartate aminotransferase	Liver, heart disease
Alanine aminotransferase	Liver, heart disease
Lactate dehydrogenase	Liver, heart, red blood cells
Creatine kinase	Heart, muscle, brain
Less commonly assayed	
Ceruloplasmin	liver, brain, Wilson´s disease
Aldolase	Muscle, heart
Trypsin	Pancreas, intestine
Glucose 6-phosphate dehydrogenase	Red blood cells (genetic defect)
γ-Glutamyl transpeptidase	Liver disease
Ornithine transcarbamylase	Liver disease
Pseudocholinesterase	Liver (poisonings, organophosphates)
Pepsin	Stomach
Hexose 1-phosphate-uridyl transferase	Galactosemia (genetic defect)
Lipoprotein lipase	Hyperlipoproteinemia
Elastase	Pancreas
Plasmin	Blood-clotting disease

12.9.1. Origin and role of enzymes

Enzymes located in the plasma can be divided into two groups:

1) Secretory enzymes are released into the environment and can be divided into:

- ***Functional plasma enzymes*** - their role is to catalyze reactions in the bloodstream. They include e.g.enzyme complexes involved in blood clotting. Some of these enzymes are produced in the liver, therefore their activities in the plasma decrease during liver damage.
- ***Functional gastrointestinal enzymes*** - catalyze reactions within the gastrointestinal tract, allowing digestion and absorption of food components. This includes e.g. pancreatic enzymes (*amylase, lipase, trypsin*). The obstacles in the way by which these enzymes get into the digestive tract or the damage of the cells in which they are formed, are the causes of the changed activities of these enzymes in serum.

2) Cellular enzymes represent a large group of enzymes that play a role in the metabolism of cells and into the bloodstream they get after disintegration or damage of these cells. Their activities significantly increase during damage of organs of their origin. This includes for example *transaminases, creatine kinase, glutamate dehydrogenase*. A small portion of these enzymes are released into the bloodstream under physiological conditions.

Control questions

1. Which of the substrates S_1, S_2, S_3 or S_4 is the most specific to an enzyme, when K_{M1} = 100 µmol/l, K_{M2} = 2 mmol/l, K_{M3} = 70 µmol/l and K_{M4} = 320 µmol/l?
2. How much product is produced after 10 min reaction catalyzed by an enzyme with activity of 100 µkat?
3. Succinate dehydrogenase (SDH) catalyzes a conversion of succinate to fumarate. SDH has K_M = 5 µmol/l and v_{max} = 50 µmol/min/mg protein for succinate. At which substrate concentration the reaction velocity reaches v = 25 µmol/min/mg protein?
4. How are tagged (labeled) proteins destined for degradation in the proteasome? Describe the proteasome structure and function.
5. Calculate *xanthine oxidase* activity in katal/g of tissue. After 20 min incubation absorbance of the standard solution of uric acid is 0.6 and absorbance of the sample of 3% homogenate is 0.4. Concentration of the standard solution is 5 mmol/l. The same volume of sample and standard solutions were pipetted.
6. Calculate % of inhibition of INT reduction and activity of *superoxide dismutase* in suspension of leukocytes when absorbance of the reaction mixture of the control sample (without SOD) is 0.3 and absorbance of the reaction mixture with the sample (containing SOD) is 0.21. SOD activity express in units U.
7. Calculate *saccharase* activity in katals per g of proteins when absorbance of the standard glucose solution with concentration of 2 mmol/l after 15 min reaction is 0.45 and absorbance of the sample containing 3 g of proteins per liter of reaction mixture is 0.66. The same volume of sample and standard solutions were pipetted.
8. Explain mechanisms of multisubstrate reactions. Give an example to each mechanism.
9. Describe two theories on binding the substrate to an enzyme.
10. How are coenzymes classified? Give examples.
11. How are proteolytic enzymes classified? What is their significance?

13. OXIDANTS, ANTIOXIDANTS AND OXIDATIVE (REDOX) STRESS

The existence of life depends on oxygen and passes away in its absence much faster than in the absence of some other required substances. Aerobic life processes require oxygen for controlled oxidation of carbon-containing molecules accompanied with release of energy. However, although oxygen is essential for life, under certain circumstances, it can also be very harmful. Its toxic effect is based on the ability to react with other surrounding compounds, while oxygen itself may undergo the one-electron reduction to form the superoxide anion radical, and during other subsequent reactions it can form so-called reactive metabolites (RM). Reactive metabolites can be produced in many reactions and systems during physiological as well as pathological processes.

Tab. 13.1. Examples of processes or sites of free radicals production

Internal sources of free radical formation	External sources of free radical formation
Phagocytes	Cigarette smoke
Mitochondria	Environmental pollution
Peroxisomes	Radiation
Ischemic-reperfusion conditions	Chemotherapeutics
Reactions catalyzed by *xanthine oxidase*	Ultraviolet light
Reactions of the arachidonic acid cascade	Some medicines, pesticides, anesthetics, organic solvents
Reactions involving transition metal ions	Ozone
Inflammation	
Extreme exercise	

13.1. Free radicals and reactive metabolites

Free radicals are atoms, molecules or their fragments with one or more unpaired electrons capable for a short time of independent existence. They are either electroneutral or they have an anionic or cationic character. The simplest radical is a hydrogen atom with only one unpaired electron. In addition, we recognize **free radicals derived from oxygen, nitrogen or various organic compounds**.
Free radicals are mostly very reactive substances which can pair their unpaired electron with an electron taken from other compounds, thus causing their oxidation. Therefore they are called **oxidants**. From free radicals other very reactive metabolites can be formed (Fig. 13.1.). Often they can be even more reactive and toxic than their maternal molecules.

From a chemical viewpoint free radicals may be formed by following types of reactions:

a) **homolytical** cleavage of covalent bond due to radiation, ultraviolet or ionizing radiation, visible light in the presence of a photosensor, the thermal degradation of organic materials, etc.:

$$A : B \longrightarrow A^{\bullet} + {}^{\bullet}B$$

b) oxidation of the compound A by loosing an electron, while radical $R^{\bullet}$ is formed:

$$A^{\bullet} + R \longrightarrow A + R^{\bullet}$$

c) reduction of the compound B by accepting an electron resulting in production of $B^{\bullet}$ radical:

$$B + e^{-} \longrightarrow B^{\bullet}$$

In biological systems, in particular, radicals are produced by electron transfer according to the reactions of b) and c) types.

Free radicals (FR) can be derived from:
a) oxygen,
b) nitrogen,
c) organic compounds.

Free radicals derived from oxygen include **superoxide anion radical** (shortly **superoxide**) ($O_2^{\bullet -}$) or derived from nitrogen ($NO^{\bullet}$) (**nitroxide**, shortly NO) can form many derivatives of radical form (**hydroxyl radical**, $HO^{\bullet}$**, nitrogen dioxide**, $NO_2^{\bullet}$), or non-radical form (**hydrogen peroxide** H_2O_2, **hypochlorous acid** HOCl, **singlet oxygen** $^1O_2$ and **ozone** O_3). Common name for all these reactive compounds is the "reactive metabolites" (RM) or "reactive oxygen/nitrogene species" (ROS/RNS).

13.1.1. Reactive oxygen species

Reactive oxygen species ROS is a collective term that includes both oxygen radicals and certain non-radicals (e.g. HOCl, HOBr, O_3, ONOO, $^1O_2$, and H_2O_2) that are oxidizing agents and/or are easily converted into radicals. All oxygen radicals are ROS, but not all ROS are oxygen radicals.

Superoxide *in vivo* occurs as a product of physiological processes (in neutrophils during phagocytosis), or as a chemical "accident" caused by auto-oxidation reactions and "transition" of electrons from the electron transport chain in mitochondria to oxygen. Superoxide is formed in neutrophils by the enzyme *NADPH-oxidase* from NADPH and oxygen and this reaction is involved in antibacterial processes in phagocytic cells.

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

In addition, superoxide produced in proper amount is an useful metabolite with important role as a signaling molecule in processes such as e.g. cell division, and even can serve as a terminator of lipid peroxidation.

On the other hand, in reaction catalyzed by *xanthine oxidase* superoxide is formed in higher concentration in cells with ischemic-reperfusion conditions (for example in muscle cells during extreme exercise), which is an example of negative effect of superoxide production.

$$\text{xanthine} + O_2 \xrightarrow{xanthine\ oxidase} \text{uric acid} + (H_2O_2) + O_2^{\bullet -}$$

Superoxide is not very reactive, but some biological targets are sensitive to its molecule. It is generally accepted that in the body damage particularly non-protonized form ($O_2^{\bullet -}$) plays a role, although protonized form ($HO_2^{\bullet}$) is more reactive and causes a DNA damage. The superoxide can react in aqueous environment either as a reductant (e.g. reaction with cytochrome c) or as an oxidant exerting its toxic properties which are enhanced in the presence of transition metal ions. Superoxide toxicity is based in triggering the series of subsequent reactions generating free radicals and other reactive metabolites (e.g. a hydroxyl radical, hydrogen peroxide) (Fig. 13.1.).

Hydrogen peroxide is the product of the two-electron reduction of molecular oxygen or dismutation of superoxide radical. Dismutation may be catalyzed by *superoxide dismutase*.

H_2O_2 is a weak oxidizing agent but it can oxidize any thiol (–SH) groups of proteins. The oxidation of specific thiol groups of proteins can initiate intracellular metabolic physiological and pathophysiological processes. The cause of the toxic effect of hydrogen peroxide is the hydroxyl radical formation which is generated by **Haber-Weiss** reaction:

$$O_2^{\bullet -} + H_2O_2 \longrightarrow O_2 + {}^{\bullet}OH + {}^{-}OH$$

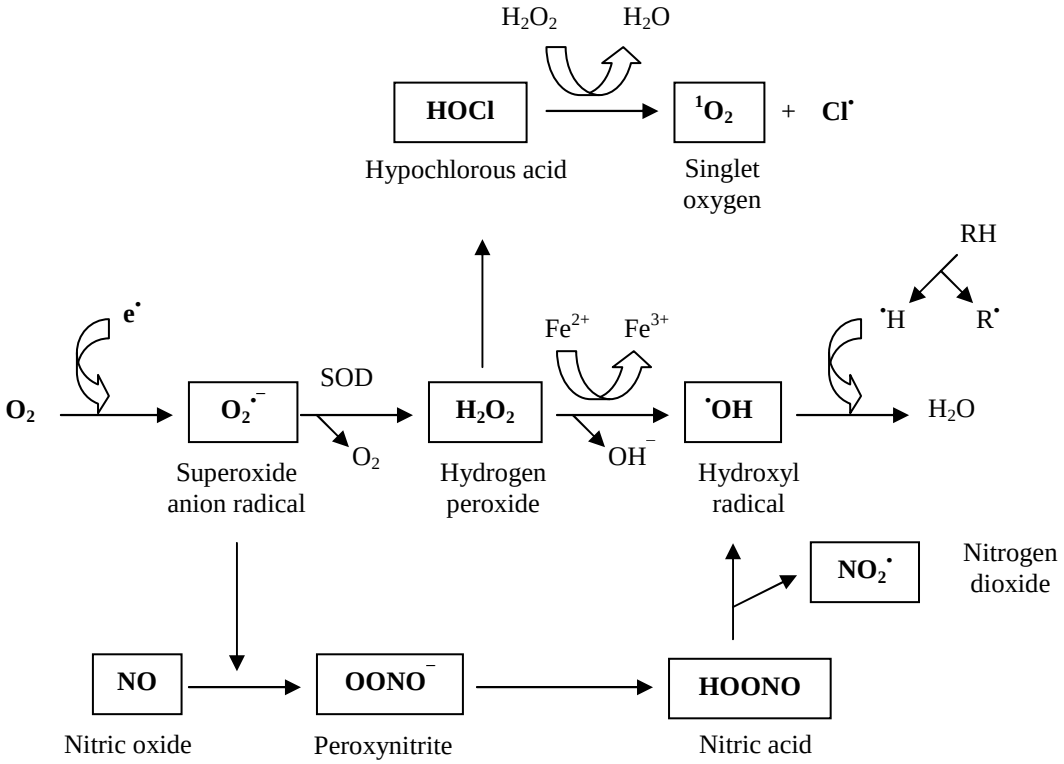


Fig. 13.1. Mutual relations among reactive metabolites

Production of $^{\bullet}OH$ in this reaction proceeds very slowly as long as this reaction is not catalyzed by transition metal ions. While transition metal ions (M^{n+}) such as Cu^{2+} and Fe^{3+} catalyze this reaction, it proceeds much more rapidly according to the following scheme:

$$O_2^{\bullet-} + M^{n+} \longrightarrow O_2 + M^{(n-1)+}$$

$$M^{(n-1)+} + H_2O_2 \longrightarrow M^{n+} + {}^{\bullet}OH + {}^{-}OH$$

The second reaction represents so called **Fenton reaction** where hydrogen peroxide reacts directly with metal ions, leading to formation of a harmful hydroxyl radical, e.g. reaction with ferrous ion:

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

Large amount of radicals can be produced in the body not only during general metabolism (ubiquinol radical during the electron transfer in the respiratory chain, the ascorbate radical during ascorbic acid detoxification reactions, etc.), but also by effects of the negative factors and exogenous compounds (xenobiotics).

The most reactive radical in the organism is **hydroxyl radical**. It reacts with each neighbouring molecule. Its toxicity is manifested mainly in the place of its production. Hydroxyl radical initiates peroxidation of cell membrane lipids as well as free unsaturated fatty acids and can damage the proteins and nucleic acids. There is not known any endogenous antioxidant yet which would eliminate it.

Singlet oxygen is an excited form of triplet oxygen. It may be produced in the reaction of superoxide with the hydroxyl radical:

$$O_2^{\bullet-} + {}^{\bullet}OH + H^{+} \longrightarrow H_2O + {}^{1}O_2$$

It reacts mainly with the unsaturated molecules in sites with double bonds, thus participating in the peroxidation of lipids. The singlet oxygen is formed also in the reaction of hypochlorite with hydrogen peroxide, which is important in leukocytes, in the *myeloperoxidase* microbicidal system:

$$OCl^- + H_2O_2 \longrightarrow H_2O + {}^1O_2 + Cl^-$$

Singlet oxygen is important due to its chemical reactivity. It belongs to active oxidants of unsaturated fatty acids in lipids, of proteins, amino acids, cholesterol, and other biologically active substrates, which is the base of its cytotoxicity.

ROS can react very rapidly with biologically important molecules such as lipids, proteins and nucleic acids causing their *primary damage*. During these reactions, different products can be formed, such as e.g. aldehydes, causing the *secondary damage* to cells and organs.

In spite of their negative effects, ROS have maintained their positive role in the living nature since the period of life genesis through whole millenniums till now. There are number of processes or systems where ROS have irreplaceable role in the function of many biological phenomena, such as phagocytosis, reproductive processes or in some special cases e.g. in signaling pathways.

13.1.2. Reactive nitrogen species

From nitrogen, both radical and nonradical reactive metabolites (RMN) are derived such as **nitric oxide**, shortly **nitroxide** ($NO^\bullet$), **nitrogen dioxide** ($NOO^\bullet$) and **peroxynitrite** ($ONOO^-$).

Nitroxide belongs to the fundamental signals taking part in regulation of intercellular communication and cellular functions and is synthesized by many cells from arginine. Synthesis of $NO^\bullet$ was proven in macrophages, endothelial cells, Kupffer cells and hepatocytes, in small and frontal brain, in the epithelial cells of kidney, adrenal glands and in the heart.

$NO^\bullet$ is unstable compound, which quickly reacts with hemoglobin, myoglobin, oxygen or superoxide. In non-enzymatic reactions in aqueous media $NO^\bullet$ can react with oxygen generating other nitrogen oxides, nitrites and nitrates:

$$2\,NO^\bullet + O_2 \longrightarrow 2\,NO_2$$

$$2\,NO_2 \longrightarrow N_2O_4 \xrightarrow{H_2O} NO_2^- + NO_3^- + 2H^+$$

$$NO^\bullet + NO_2 \longrightarrow N_2O_3 \xrightarrow{H_2O} 2\,NO_2^- + 2H^+$$

The subsequent reactions of these compounds with secondary amines result in the formation of carcinogenic nitrosamines.

Nitrates and their intermediates may also participate in the nitration reaction (Fig. 13.1.). Since $NO^\bullet$ contains an unpaired electron it can rapidly react with superoxide to form peroxynitrite anion:

$$NO^\bullet + O_2^{\bullet-} \longrightarrow ONOO^-$$

Peroxynitrous acid, which is formed by protonation of peroxynitrite anion decomposes rapidly and produces reactive hydroxyl radical:

$$ONOOH \longrightarrow {}^\bullet OH + NO_2 \longrightarrow NO_3^- + H^+$$

These reactions accomplish connection between reactive oxygen species (in macrophages and neutrophils they are produced by *NADPH oxidase*) and reactive metabolites of nitrogen. The result is the formation of the most effective microbicidal and cytotoxic substance of professional phagocytes – hydroxyl radical.

13.1.3. Radicals derived from organic compounds

There are many organic substances, which can have a free unpaired electron. The unpaired electron can be associated with a number of atoms. For example, in the presence of transition metal ions the thiols are oxidized to the alkyl thiol radical ($RS^\bullet$). In the presence of oxygen it can form alkyl thiol peroxyl radical ($RSO_2^\bullet$), but also oxidize other substances to form other radicals. Radicals possessing an unpaired electron on the carbon atom are formed in the environment as well as in many biological systems. For example, during the metabolism of carbon tetrachloride in the liver microsomes there is formed a trichloromethyl peroxyl radical ($^\bullet O_2CCl_3$) in the reaction of trichloromethyl radical with oxygen. Also radicals with unpaired electron on the nitrogen atom are known, e.g. phenyldiazenyl radical is formed during oxidation of phenylhydrazine in erythrocytes. Phenyldiazenyl

radical is considered to be the most detrimental of above mentioned radicals since it denatures hemoglobin and stimulates lipid peroxidation, which may cause hemolysis of erythrocytes.

There are much more radicals of organic compounds (e.g. alkoxyl radical $RO^{\bullet}$, peroxyl radical $ROO^{\bullet}$). Some of them are of physiological importance, but others can trigger free radical reactions and damage biologically important molecules.

$$ROOH + Fe^{2+} \longrightarrow RO^{\bullet} + Fe^{3+} + {}^{-}OH$$

$$ROOH + Fe^{3+} \longrightarrow ROO^{\bullet} + Fe^{2+} + H^{+}$$

The formed e.g. lipid radicals can branch oxidative damage to lipids and cell membranes.

13.2. Antioxidant defence systems

From the biological point of view, **antioxidants** are compounds which at low concentration prevent oxidative damage to molecules by oxidants. Products of the reaction between oxidant and antioxidant should not be toxic and should not branch the radical reaction.

Formation and effect of FR at physiological conditions is under control of various defence systems in order to avoid FR activity at the wrong place and damage to the body´s own important biomolecules. Defence systems reduce toxicity of RM through:

- mechanisms preventing FR formation
- systems eliminating already formed ROS (so-called antioxidants),
- repair systems eliminating oxidatively damaged molecules.

Prevention of FR formation can be e.g. the elimination of free metal ions (Fe and Cu) by different chelators (e.g. ferritin, albumin, deferoxamine), or inhibition of enzymes catalyzing radicals formation (e.g. allopurinol inhibits *xanthine oxidase* catalyzing formation of the superoxide).

Detoxification of already formed ROS (e.g. superoxide, peroxyl and alkoxyl radicals, hydrogen peroxide, etc.) is provided by antioxidant enzyme systems and low molecular weight antioxidants. They protect the cell from oxidative damage to important biomolecules, and prevent initiation of branching chain reactions of free radicals. These defence systems are called **antioxidants**. According to the mechanism of FR elimination antioxidants can be divided into following groups:

1. **Scavengers** – e.g. enzyme *superoxide dismutase* (*SOD*) scavenges $O_2^{\bullet -}$ and converts it into non-radical molecules – molecular oxygen O_2 and paradoxically another oxidant hydrogen peroxide H_2O_2. Hydrogen peroxide is decomposed under physiological conditions by other antioxidant enzymes *catalase* or *glutathione peroxidase* (*GPx*).
2. **Catchers** (trappers) – e.g. vitamin E traps $^{\bullet}OH$ and converts it into a relatively stable radical
3. **Quenchers** – e.g. carotene quenching singlet oxygen.

Non-enzymatic antioxidants are of great importance in antioxidant protection against oxidative damage. They can more easily pass into cells and be distributed in all compartments of the body.

The **repair mechanisms** (removal of damaged molecules from the body) represent the second line of defence and are applied when antioxidants fail. These systems also include the enzymes which are capable to reduce the oxidized compounds and restore their function, for example *glutathione reductase, dehydroascorbate reductase* or *methemoglobin reductase*. In addition, the proteolytic systems are responsible for the degradation of denatured, potentially toxic proteins and peptides. The *lipases* (e.g. *phospholipase* A_2) can remove oxidatively damaged fatty acids and prevent their participation in the development of chain reactions. Other enzymes of the repair system are those repairing oxidative damage to DNA.

Antioxidants have a various structure and according to the size of their molecule they can be classified to the **high-molecular weight** and **low-molecular weight** compounds. More accurate classification is present in Tab. 13.2.

Tab. 13.2 Examples of natural antioxidants, their distribution and the main detoxifying effect

Antioxidants	Detoxifying effect to
Enzyme antioxidants	
Superoxide dismutase	Superoxide
Catalase	Hydrogen peroxide
Glutathione peroxidase	Hydrogen peroxide, organic peroxides
Peroxidases	peroxides
Nonenzymatic high-molecular weight antioxidants	
Ceruloplasmin	Superoxide, oxidation of Fe^{2+}, inactivation of Cu^{2+} a Cu^{+}
Albumin	Hydroperoxides of fatty acids
Transferrin	Chelator of Fe^{3+}
Low-molecular weight hydrophilic antioxidants	
Glutathione	$^{\bullet}OH$ a $^1O_2$
Ascorbic acid	$O_2^{\bullet-}$, $^{\bullet}OH$, $^1O_2$, organic radicals
Uric acid	$O_2^{\bullet-}$, $^{\bullet}OH$ (*in vitro*), $^1O_2$, chelator of metal ions
Thiols (cysteine)	$O_2^{\bullet-}$, $^1O_2$
Bilirubin (bound to proteins)	Peroxyl radical
Low-molecular weight lipophilic antioxidants	
Tocoferols	$^{\bullet}OH$, $ROO^{\bullet}$
Carotenoids	$^{\bullet}OH$, $^1O_2$
Ubiquinol $CoQH_2$	Peroxyl radical

13.2.1. High-molecular weight antioxidants

High-molecular weight antioxidants include e.g. the enzyme *superoxide dismutase* (SOD), *catalase*, *glutathione peroxidase* or nonenzymatic proteinaceous antioxidants, e.g. transferrin and albumin. Enzyme antioxidants are important especially in intracellular space.

Cu/Zn Superoxide dismutase (SOD) specifically catalyzes dismutation of the superoxide radical to non-radical molecules O_2 and H_2O_2.

The general mechanism of superoxide dismutation catalyzed by *superoxide dismutase* can be expressed by following equations. It is characterized by the redox cycle of copper atom $Cu^{2+}/Cu^{+}/Cu^{2+}$ etc.

$$\text{Enzyme-Cu}^{2+} + O_2^{\bullet-} \longrightarrow \text{Enzyme-Cu}^{+} + O_2$$

$$\text{Enzyme-Cu}^{+} + O_2^{\bullet-} \xrightarrow{2H^{+}} \text{Enzyme-Cu}^{2+} + H_2O_2$$

In the human organism there are present three SOD isoforms. Cu/Zn-SOD present in the cytoplasm of eukaryotic cells, EC-Cu/Zn-SOD present in extracellular space and Mn-SOD present in mitochondria. They differ in the type of a metal in the active site, in the number of subunits, in the amino acid composition of the apoenzyme, in the sensitivity to inhibitors, etc.

Decreased activities of *superoxide dismutases* are associated with the hypothesis of cancerogenesis based on the loss of control of cell division and differentiation.

Catalase catalyzes decomposition of H_2O_2 to water and oxygen. The majority of aerobic organisms excluding some bacteria and algae, contain ***catalase*** in peroxisomes.

$$H_2O_2 + H_2O_2 \longrightarrow 2\,H_2O + O_2$$

The enzyme ***glutathione peroxidase*** **(GPx)** occurs in two forms: selenium-independent and selenium-dependent GPx, differing in the number of subunits, in the selenium bond at the active centre and in the catalytic mechanism.

Selenium-independent GPx (glutathione-S-transferase, GST) catalyzes detoxification of various xenobiotics. Selenium atom with oxidative number (II) which is present in the enzyme molecule, does not participate in the catalytic mechanism.

Selenium-dependent glutathione-peroxidase (GPx) is composed of four subunits, while each subunit contains one selenium atom in the active center bound in the modified amino acid selenocysteine. The significance of these selenoenzymes is based on elimination of peroxides – potential substrates for Fenton-type reaction.

Glutathione peroxidase cooperates with the tripeptide glutathione (GSH) present in cells at relatively high (millimole) concentration. The substrate for GPx reaction is H_2O_2 or an organic peroxide. *Glutathione peroxidase* decomposes peroxides to water or alcohol and at the same time it oxidizes GSH to GSSG.

$$2\ GSH + H_2O_2 \rightarrow GSSG + 2\ H_2O$$

$$2\ GSH + ROOH \rightarrow GSSG + ROH + H_2O$$

The ratio GSH:GSSG is 100:1. In cells, the enzyme *glutathione reductase* (GR) occurs catalyzing GSSG reduction to GSH. Cofactor of GR is NADPH produced in the pentose cycle by *glucose-6-phosphate dehydrogenase.*

$$GSSG + NADPH + H^+ \rightarrow 2\ GSH + NADP^+$$

13.2.2. Low-molecular weight hydrophilic and lipophilic antioxidants

Low-molecular weight antioxidants include e.g. hydrophilic vitamin C, glutathione, uric acid in some situations or lipophilic antioxidants such as vitamin E or coenzyme Q.

Natural flavonoids (e.g. catechin, quercetin) or other phenolic (e.g. ferulic acid, caffeic acid) or polyphenolic compounds (e.g. resveratrol) also contribute to antioxidant capacity of the organism, getting in the organism by food as natural constituents of fruits and vegetables and exerting significant antioxidative ability.

From experimental studies as well as clinical practice an increasing body of evidence suggests that antioxidants need not play always a positive role. This fact has to be kept in mind especially during therapeutic administration of these compounds.

The most effective low molecular weight lipid antioxidants include α-tocopherols (vitamin E). **Vitamin E** (see Fig. 11.5.) is a term used for a group of compounds composed of the mixture of eight derivatives exerting the activity of α-tocopherol.

The main function of vitamin E is its antioxidative ability, for which phenolic group of tocol is predominantly responsible. Vitamin E is able to cease radical chain reactions (e.g. by the reaction with peroxyl radical $LOO^{\bullet}$) or to trap directly oxygen radicals (e.g. hydroxyl radical $HO^{\bullet}$). During these reactions tocopheryl radical ($E^{\bullet}$) is formed. It may react with another peroxyl radical and create a new product (EOOL), or be reduced by ascorbate or ubiquinol to the active α-tocopherol.

Vitamin E acts synergistically with vitamin C (Fig. 13.2.). This is possible due to the location of vitamin E in the membrane where its chroman ring with the hydroxyl group is oriented into the hydrophilic part of the membrane where at the border line of the two phases it can come into contact with ascorbic acid which can regenerate vitamin E. In this reaction ascorbate radical ($C^{\bullet}$) is formed, which could be further oxidized to dehydroascorbate. Regeneration of dehydroascorbate proceeds in reaction catalyzed by *dehydroascorbate reductase*.

Orientation of nonpolar phytol moiety of vitamin E inward the membrane is of great physiological importance since the contact of vitamin E with coenzyme Q10 ($CoQH_2$) is possible, which also can regenerate vitamin E in the membrane:

$$E^{\bullet} + CoQH_2 \longrightarrow CoQH^{\bullet} + E$$

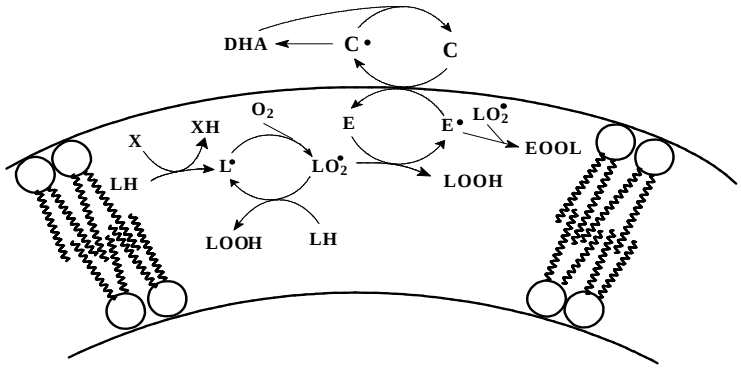


Fig. 13.2. Regeneration of tocopheryl radical by ascorbate

E – tocopherol, E• – tocopheryl radical, C – ascorbate, C• – ascorbate radical, DHA – dehydroascorbate, LH – lipid, LOOH – lipoperoxide, X – oxidant, $LO_2^{\bullet}$ – lipoperoxyl radical

Ascorbic acid has multiple antioxidant properties. It is an important antioxidant in human plasma, even though it is usually consumed more quickly than the other antioxidants. The antioxidant capacity of ascorbate is related to the rate of its reaction with a number of FR (mainly peroxyl radicals), and also to the fact that semidehydroascorbate radical is poorly reactive. Under the *in vivo* conditions semidehydroascorbate radical is reduced back to the ascorbate by the action of enzyme systems:

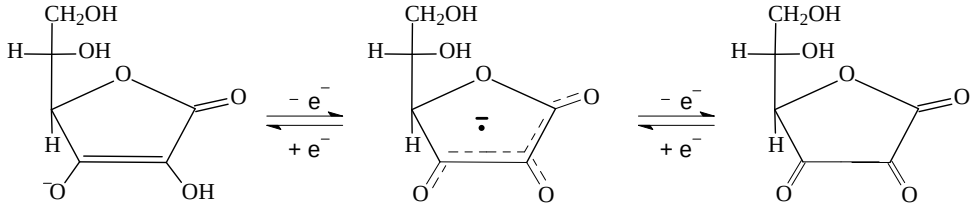
a) *NADH-semidehydroascorbate reductase* catalyzed reaction:

$$\text{semidehydroascorbate} + \text{NADH} \longrightarrow \text{ascorbate} + \text{NAD}^+$$

b) *dehydroascorbate reductase* catalyzed reaction:

$$\text{semidehydroascorbate} + 2\,\text{GSH} \longrightarrow \text{ascorbate} + \text{GSSG}$$

Antioxidative properties of **the ascorbic acid** are shown in reaction (Fig. 13.3.), where e^- can be from $O_2^{\bullet -}$, $HO_2^{\bullet}$, $ROO^{\bullet}$, $RO^{\bullet}$, $GS^{\bullet}$ and $^{\bullet}OH$.



Ascorbate — Ascorbate Free Radical — Dehydroascorbate

Fig. 13.3. Antioxidative properties of the ascorbic acid

Ascorbic acid is also able to recycle other important antioxidants from their radical forms, e.g. α-tocopherol or glutathione.

The antioxidative effect of ascorbic acid is strictly confined by the presence of ions of transition metals. In their presence ascorbate behaves prooxidatively through the „Fenton" type reaction. In the presence of ascorbic acid, metal ions in so-called „catalytically effective form" can catalyze formation of toxic reactive metabolites, such as $^{\bullet}OH$ radical. Moreover, the prooxidative effect of ascorbic acid *in vivo* is associated with autooxidation of ascorbate to dehydroascorbate, thus changing the redox state of cells leading to changed expression of some genes.

Glutathione plays a significant role in protection of the organism against oxidative damage for several reasons: (i) It is a cofactor of some enzymes participating in detoxification mechanisms of oxidative stress, as e.g. *glutathione peroxidase, glutathione transferase, dehydroascorbate reductase*. (ii) GSH is a direct trapper of $^{\bullet}OH$ radical and $^1O_2$, it detoxifies H_2O_2 and lipoperoxides during catalytic action of *glutathione peroxidase*. (iii) Glutathione can reduce the tocopheryl radical directly or indirectly during the reduction of semidehydroascorbate to ascorbate regenerating these important antioxidants back to their active form. Oxidized glutathione is regenerated by *glutathione reductase* (GR) cooperating with NADPH which is produced in the pentose cycle of glucose degradation.

Carotenoids are pigments of plant or microbial origin (see Chapter 11.1.1.). Carotenoids can react with singlet oxygen and return the molecule of the excited oxygen into the basic energetic state. Carotenoids can also directly trap free radicals. Of the biologically important natural carotenoids, the most efficient quencher is lycopene. Vitamin A exerts only a negligible antioxidative ability.

Coenzyme Q (CoQ) is associated with metabolism of free radicals: with their formation, e.g. in mitochondria (prooxidative properties), as well as with their elimination (antioxidative properties).

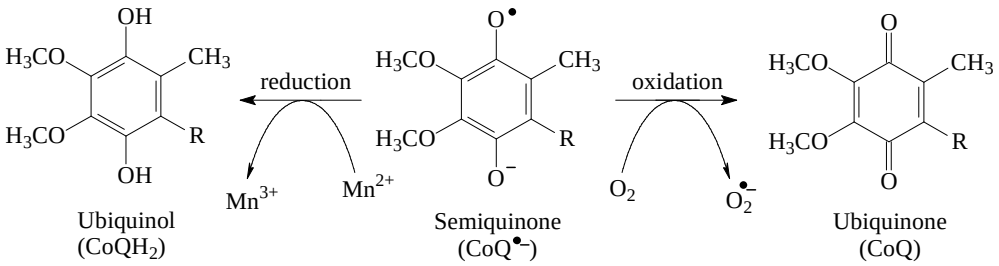


Fig. 13.4. Two possible directions of the semiquinone change
R (in human mitochondria) = (–CH₂–CH=C(CH₃)–CH₂–)₁₀H

The antioxidative ability of coenzyme Q can be exerted also through regeneration of vitamin E due to the neighbouring location of both compounds in the membrane lipid bilayer as is mentioned above.

Bilirubin is a linear tetra pyrrole formed *in vivo* by the oxidative splitting of the heme. Bilirubin plays antioxidant role in newborns at the first days after birth, when other antioxidant systems are not fully working. In healthy adults bilirubin occurs in plasma only at micromolar concentration and is therefore without any physiological importance as an antioxidant.

Uric acid was originally considered a catabolic product of degradation of purine metabolites. Uric acid is present in human plasma at high concentration (0.12 – 0.45 mmol.l^{-1}) and is a significant quencher of $^1O_2$, trapper of the hydroxyl radical and chelator of transition metals. At physiological conditions uric acid stabilizes ascorbate in human serum which is ascribed to its chelating ability.

Flavonoids are phenolic compounds spread in the plant kingdom. They include more than 4 000 different derivatives and their list constantly increases. Formation of so many derivatives is possible due to the substitution of hydrogen atoms at different sites of the basic structures by hydroxyl, methoxyl and other groups.

They are effective in both hydrophilic and in lipophilic environments. Antioxidant properties are conditioned by phenolic –OH groups present in their molecules. Consumption of flavonoid-rich food is associated with a lower incidence of coronary heart disease, myocardial infarction, cancer, neurodegenerative diseases, psychical diseases and other chronic diseases. Dietary flavonoids have been suggested to exert health benefits through antioxidant and other biomodulating mechanisms. However, the antioxidative ability of flavonoids cannot be exerted *in vivo* because their absorption from food is low. The plasma level of flavonoids is an order of magnitude lower than the level of other antioxidants in plasma, such as vitamin C, E and uric acid (see Tab. 13.3.). Moreover, the half-life of flavonoids in plasma is short because after ingestion they are metabolized to other derivatives than original substances present in food.

The positive effect of flavonoids on the organism is manifested in several respects. In addition to the secondary effect of the antioxidative ability, e.g. through stimulation of antioxidative enzymes such as SOD and GPx, they have also a vasodilating, anti-thrombotic, anti-inflammatory and anti-apoptotic effects as well as anti-mutagenic ability.

13.2.3. The profile of antioxidants in human plasma

Antioxidant status of blood plasma consists of:

1. antioxidant enzymes (EC SOD, GPx, GR and *catalase*) - are present in very low quantities
2. proteins without catalytic activity (ceruloplasmin, transferrin, lactoferrin, haptoglobin, hemopexin, albumin)
3. low-molecular weight antioxidants.

Tab. 13.3. The most important low-molecular weight antioxidants in human plasma

Antioxidants	Concentration [μmol/l]
Water-soluble	
Uric acid	200 – 400
Ascorbic acid	30 – 150
Bilirubin	5 – 20
Reduced glutathione	< 2
Lipid-soluble	
α-tocoferol	15 – 40
Ubiquinol	0.4 – 1.0
Lycophene	0.5 – 1.0
β-caroten	0.3 – 0.6
Lutein	0.1 – 0.3
Zeaxantin	0.1 – 0.2
α-caroten	0.05 – 0.1

The antioxidant capacity of plasma is different depending on age. With age the proportion of urate increases (20 – 30 %). In children, ascorbic acid predominates, in adults it represents approximately 15 % of plasma antioxidant capacity. Currently the testing of plasma antioxidant capacity is not considered to be clinically relevant since it may not always reflect the antioxidative state in cells and organs.

Based on growing information from experimental and clinical practice on the effect of antioxidants, it has been shown that they may not always have a positive role. These facts should be kept in mind, especially in unreasonable and thoughtless therapeutic use of these substances.

Generally it can be summarized, that **the antioxidant in one system under certain circumstances, may not be as effective as antioxidant under different circumstances in all other systems.**

13.3. Oxidative stress and toxic effect of free radicals

When some of the protective systems of the organism against free radical toxicity fail, action of free radicals becomes uncontrolled, leading to the damage to molecules, cells and organs and potentially to the death of the organism.

The consequence of the negative effect of FR and RM is called **oxidative stress**. From today´s point of view, ***oxidative stress*** can be defined as ***an imbalance between production and elimination of reactive metabolites of oxygen and nitrogen, in favour of their production, leading to potential damage***. During oxidative stress, biologically important molecules and cells can be damaged and this can be significant in the pathogenesis of many diseases. Fig. 13.5. shows associations between cell damage and oxidative stress.

The term „oxidative stress“ is not quite correct term also from chemical point of view. Oxidation never occurs alone but only in association with reduction. Oxidation and reduction are shortly called the redox reactions. Therefore „oxidative stress“ should be correctly named „**redox stress“**.

As it was already mentioned, oxidative stress is characterized by an imbalance between oxidants and antioxidants. Since the oxidants are reduced during the oxidation of other molecules and the antioxidants are oxidized, reactions of oxidants and antioxidants are associated with electron transfer. Therefore each cell is characterized by a specific concentration of the electrons (redox state) stored in many cellular components. The **redox state** of cells is maintained in a relatively narrow range, similarly as the pH value is maintained. Under pathological conditions, the redox state of the cell changes to lower or higher values. State of redox equilibrium plays an important role for example in the etiology of cancer in which the cells are preferably multiplied or killed

according to their redox state (the ratio oxidant / antioxidant), which has a big importance in the treatment of tumors.

Oxidative stress is characterized by indicators (markers) of oxidative stress. These markers are typical products, which are formed during oxidative damage to biologically important molecules. They are for example damaged nitrogen bases in DNA, products of oxidative damage to proteins and lipoperoxidation.

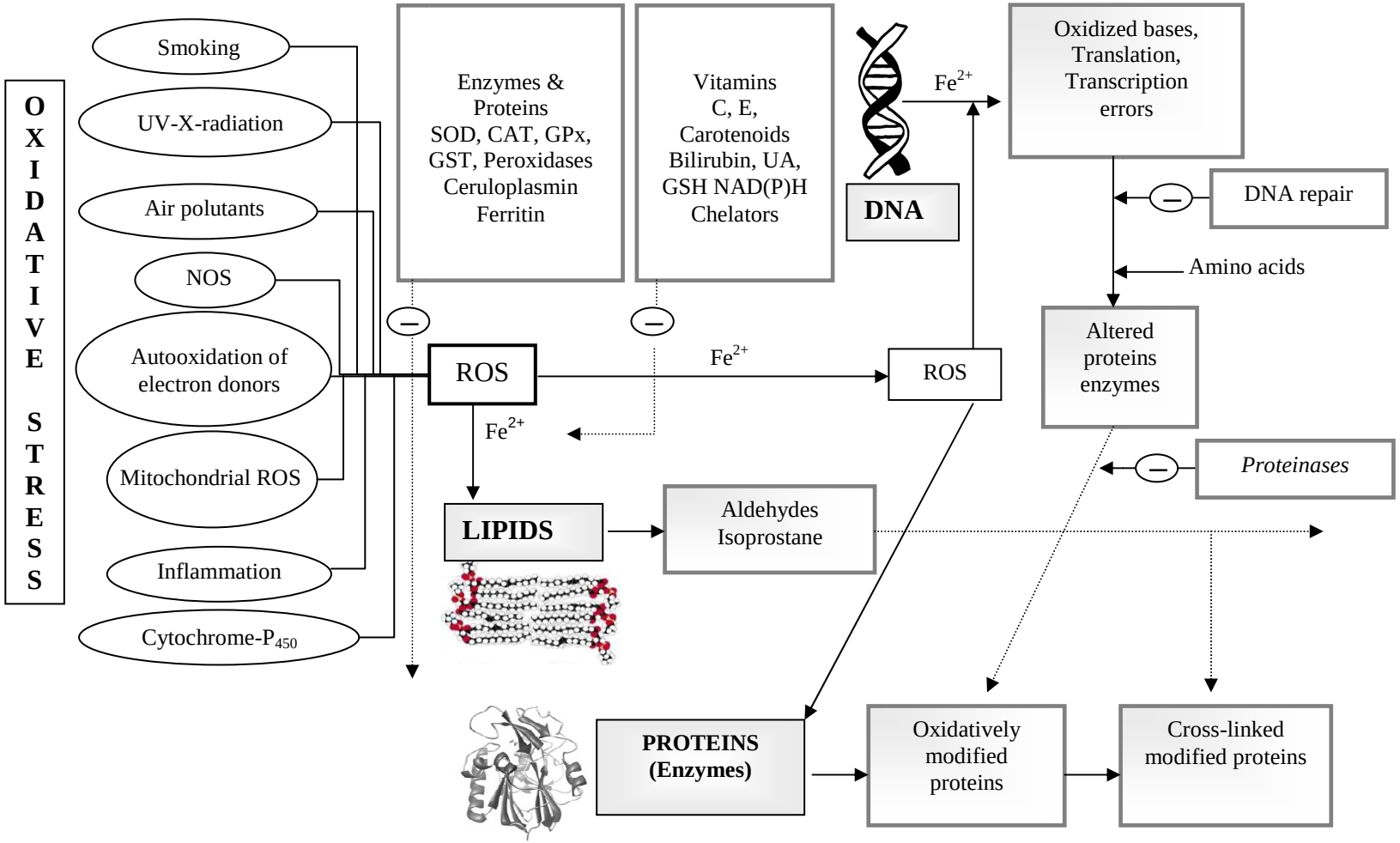


Fig. 13.5. Oxidative stress and its impact on a cell

ROS – reactive oxygen/nitrogen species and reactive metabolites, NOS – nitroxide synthase, SOD – superoxide dismutase, CAT – catalase, GPx – glutathione peroxidase, UA – uric acid, GSH – glutathione in reduced form

13.3.1. Oxidative damage to biological membranes

Free radicals and reactive metabolites oxidatively damage biological membranes through lipoperoxidation. The most endangered are polyunsaturated fatty acids (PUFA), that are an important part of phospholipids.

Susceptibility of PUFA to oxidative damage depends on the number of double bonds in the chain. The PUFAs with two and more double bonds are more prone to oxidative damage than unsaturated fatty acids with only one double bond. For example oleic acid with one double bond (18:1) is almost undamaged by lipoperoxidation.

Membrane lipid peroxidation is initiated by the action of any factor that has the ability to remove a hydrogen atom from the methylene group ($–CH_2–$) of an unsaturated lipid (LH) leading to formation of lipid radical ($L^{\bullet}$). In the mechanism of PUFA peroxidation, the following phases can be discerned: initiation, propagation, branching, and termination. Peroxidation of lipids is illustratively shown in Figure 13.6.

Lipoperoxidation could be initiated by the hydroxyl radical ($^{\bullet}OH$), peroxyl ($ROO^{\bullet}$) and alkoxyl ($RO^{\bullet}$) radicals. It is followed by rearrangement of double bonds in the molecule of lipid radical and the lipid conjugated diene radicals ($L^{\bullet}$) are formed. Reaction of $L^{\bullet}$ with molecular oxygen yields to the formation of $LO^{\bullet}$ or $LOO^{\bullet}$ radicals, that are able to react with another molecule of lipid (LH) and a new lipid radical ($L^{\bullet}$) is formed with simultaneous formation of lipoperoxide (hydroperoxide, LOOH). This is the second step of lipoperoxidation named *propagation*. It is followed by the *secondary initiation* that is initiated by transition metal ions (Fe^{2+}, Cu^{+}) and hydrolipoperoxides, when new different radicals are formed (Fig. 13.6.). *Branching* of the chain reaction can be brought about by homolytic cleavage of O–H and O–O bonds in the LOOH molecule. The radicals $LO^{\bullet}$, $LOO^{\bullet}$, and $^{\bullet}OH$ are formed, which can enter the reactions with new lipid molecules. *Termination* of reactions occurs by mutual recombination of lipid radicals, or by their reaction with inhibitors of radical reactions (antioxidants, scavengers).

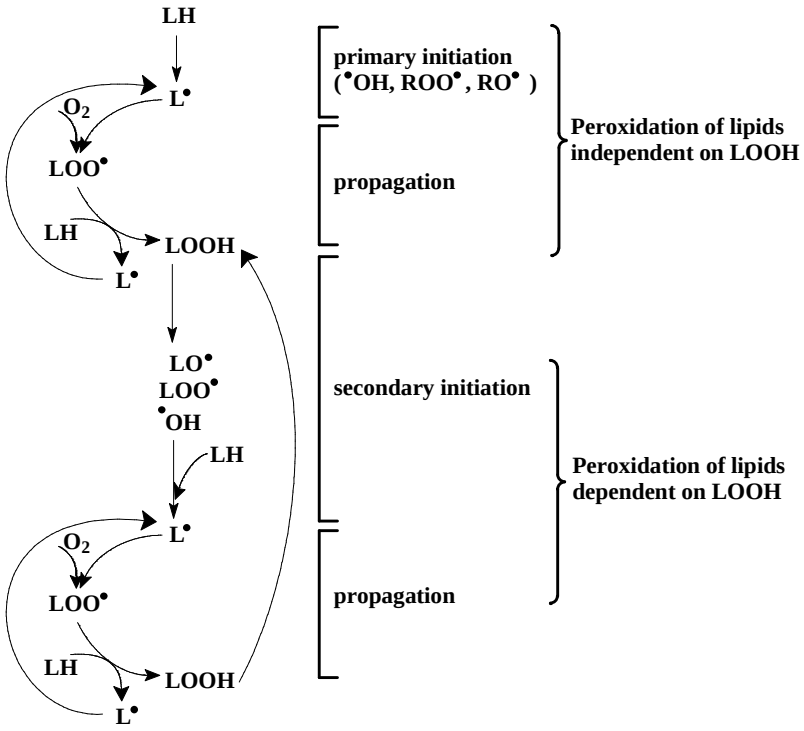


Fig. 13.6. Scheme of lipoperoxidation

LH – lipid, $L^{\bullet}$ - lipid radical, $LO^{\bullet}$ - alkoxyl radical, $LOO^{\bullet}$ - peroxyl radical, LOOH – hydrolipoperoxide

Lipid hydroperoxides are primary products of lipoperoxidation. These compounds are unstable particularly in the presence of transition metal ions, namely of Cu and Fe ions. LOOH are degraded into secondary products, such as aldehydes, ketones, carboxy compounds, dienes, ethane, pentane. These products are used as markers of lipoperoxidation. **Malondialdehyde** (MDA, $HOC–CH_2–COH$) is one of the most abundant secondary products. However, MDA can be formed as an oxidative product also from other molecules such as saccharides and DNA. MDA is able to react with $–NH_2$ groups of lipids (e.g., of phosphatidylethanolamine) and proteins, forming the

polymeric end product of lipidperoxidation – **lipofuscin**, which is called the "aging pigment". Lipid peroxides and their secondary products get into serum. Elevated levels of these markers usually indicate the damage to cell membranes or tissues. In concordance with this, levels of lipid peroxidation products can be indicators of the seriousness of the disease.

Lipoperoxidation causes structural degradation of biological membranes and has a negative effect on their basic physical-chemical properties, receptors and enzymes associated with membranes, and transporting functions of membrane. Functional changes of membrane components can be caused not only by the changes in physical-chemical interactions protein − lipid, but also by chemical reactions with both, radical and non-radical products of lipid peroxidation.

13.3.2. Oxidative damage to proteins

Oxidative damage to proteins is not so well examined as lipoperoxidation. The reason is the large numbers of different proteins and amino acid units present in proteins and peptides, which can be substrates for oxidants. ROS modify amino acid units in proteins leading to the change of conformation of proteins as well as to the change of their biological function. The changed side chains of amino acids can react with each other or with other compounds (for example with aldehydes or hydroxyaldehydes) and cross-linked bonds are formed, and the native basis of them can be completely changed.

Carbonyl groups of proteins are used for characterization of oxidative damage to proteins after reaction of oxidants (hydroxyl radicals) with side chains of amino acids (for example lysine or proline units). The protein carbonyls are the primary products of protein damage and are able to damage other molecules. There are known two mechanisms of their effect – oxidation and reduction. Oxidative action of carbonyl groups results in formation of hydroperoxides of proteins. For the reduction of these modified proteins in the cell important reductants are depleted, such as ascorbic acid or glutathione. In the second mechanism, damaged protein acts as a reductant, it reduces metal ions to pro-oxidative form ($Fe^{3+} \rightarrow Fe^{2+}$). In both cases, it tends to increase oxidative stress in cells.

At higher concentrations of NO radical (nitroxide) this reacts with superoxide to form peroxynitrite anion $ONOO^-$ (nitroperoxide) (Fig. 13.1.). During this reaction nitrotyrosine is formed in reaction of $ONOO^-$ with tyrosine residues. Nitrotyrosine is considered to be a marker of protein nitration damage.

13.3.3. DNA damage by ROS

Nucleic acids are very prone to oxidative stress. Oxidative damage to DNA could proceed by different ways: ionized irradiation, photooxidation, by peroxides through reactions catalyzed by ions of transition metals, by hydroxyl radical, as well as by other oxidative reagents (for example cytostatics, some antitumor antibiotics or chemical *nucleases* – redox-active coordination compounds), which are able to break up DNA oxidatively. DNA damage can be carried out on purine or pyrimidine heterocyclic base or saccharide unit. Oxidation of nitrogen bases can be followed by mutagenesis. Oxidative damage to deoxyribose can induce cleavage of one or both chains of double helix named „breaks“.

Particularly hydroxyl radical is responsible for nitrogen bases and deoxyribose damage. It is able to eliminate hydrogen atom from deoxyribose, as well as it can be added to nitrogen bases. There were identified more than 20 oxidatively modified purines and pyrimidines. The basic product of oxidative DNA damage, **8-oxo-2-deoxyguanine (8-oxo-dGua)** or nucleoside **8-oxo-2-deoxyguanosine** (**8-oxo-dGuo**) (Fig. 13.7.) and their lactic forms (**8-hydroxyguanine and 8-hydroxyguanosine)** are generally used markers of oxidative damage to DNA.

Superoxide and hydrogen peroxide probably do not take part in cleavage of DNA. They can act secondary as mediators of hydroxyl radical production.

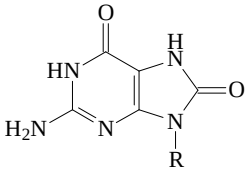


Fig. 13.7. 8-oxo-deoxyguanosine *(R – deoxyribose residue)*

The processes of oxidative DNA damage in healthy cells of the body are highly toxic because they are the basis of mutations and may result in death of the cells and the organism. However, if the mechanism of DNA cleavage could be used in cancer cells to stop their uncontrolled division, it would be a success of free radicals chemistry.

A lot of nitrogen base modifications result in the mutations or in the blocking of replication.

The ions of transition metals can markedly participate in oxidative DNA damage. Oxidative damage in the presence of transition metals ions can be caused by two mechanisms:

a) ***indirect effect, when ions of transient metals*** are catalysts of $^{\bullet}OH$ production from H_2O_2,

b) ***direct effect, when oxo-complexes of transition metals*** with DNA are formed. These metal oxo-complexes are characterized by the large oxidative strength.

13.4. The positive effect of reactive oxygen species

Under the physiological conditions, there is needed a certain level of ROS to the proper functioning of certain systems. The moderate oxidative stress can be beneficial, therefore, a complete suppression of RM production could be toxic.

The positive role of the FR has been associated for example with reproduction processes, phagocytosis of microbes after the invasion to the body, or with some biochemical reactions such as hydroxylation, carboxylation or peroxidation reactions or reduction of ribonucleotides.

It is now believed that the ROS play an important biomodulatory role in the regulation of intercellular and intracellular transmission of information, by affecting signaling pathways. In this case different cells respond to the action of the FR and RM differently, depending on the type and level of FR and the RM, on the level and type of antioxidants, and on the time and site of action of oxidants. Depending on these conditions, the cells may react differently – by increased proliferation, cell cycle change, aging of the cells, activation of apoptosis and necrosis, by activation or inhibition of gene expression of antioxidant enzymes or repair system. Changing of the redox state of the cell activates or inhibits the activity of signal molecules of different signaling pathways, and thereby affects the "fate" of cells.

13.5. Free radicals and "free-radical" diseases

For the healthy functioning of the body it is essential to maintain a balance between production and scavenging of free radicals. The causes of the impairment of this balance towards oxidative reactions could be caused by:

- increased concentration of promoters and free radical initiators,
- increased production of free oxygen radicals,
- decreased activity of protective enzyme systems,
- lack of natural antioxidants.

Pathological state is caused mostly by combination of several, if not all causes. The consequence of that imbalance is the gradual degradation of cellular structures that induces local functional changes in tissues, organs and through in the whole organism. The relationships between the free radicals and their metabolites, antioxidants and consequent damage to biologically important molecules are illustrated in Fig. 13.8.

Resulting changes may become evident after prolonged exposure of organism to oxidative stress. The group of diseases, in pathology of which the free radicals play a role, is referred to as "free radical diseases". Factors that determine development of these diseases can be divided into four groups:

1. **Genetic factors** – e.g. Fanconi anemia and Bloom syndrome, in which there is genetically determined lack of the body protection against oxygen radicals or Down's syndrome with increased activity of the antioxidant enzyme Cu/Zn SOD.
2. **Combination of genetic factors and the effect of environment** – *Lupus erythematosus*, in which increased sensitivity to DNA damage by free radicals was observed.
3. **Environmental factors** – e.g. malignant diseases and atherosclerosis (increased concentration of initiators and promoters of free radicals).
4. **Disorders of the metabolic systems** – e.g. ischemic-reperfusion disorders of heart, brain, intestinal tract, etc.

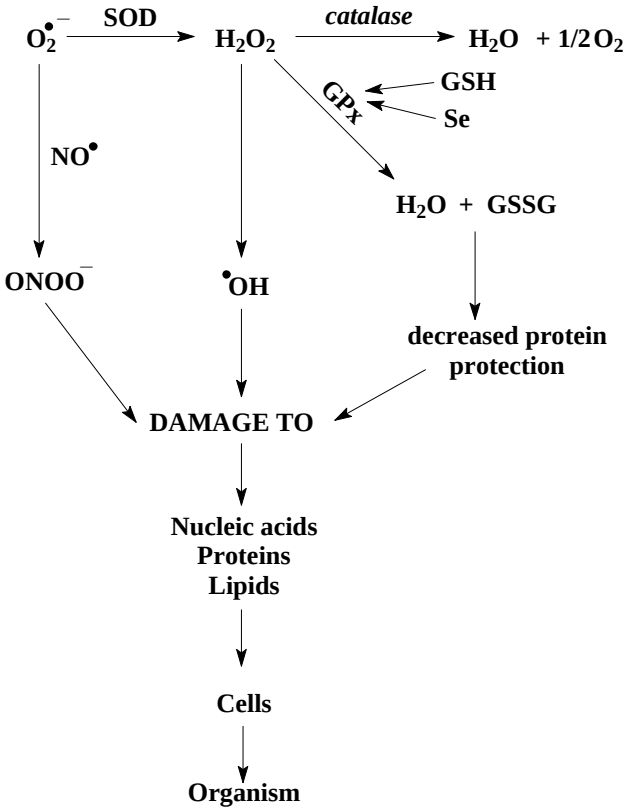


Fig. 13.8. The relationship between free radicals, their metabolites, antioxidants and the damage to biologically important compounds

Tab. 13.4. Some diseases associated with free radicals

Inflammatory processes	Ischemic reperfusion disorders
Immunological diseases	Carcinogenesis
Neurological diseases	Mutagenesis
Atherosclerosis	Liver disease
Alzheimer's disease	Gastrointestinal diseases
Osteoarthritis	Mental disease
Senile cataract	Psoriasis
Parkinson's disease	Aging
Diabetes mellitus	

Control questions:

1. Define the term "free radical". Give three examples.
2. Give examples of reactive oxygen species (radicals and non-radicals).
3. Give an example of enzymatic reaction producing the superoxide in the organism.
4. Write the Fenton reaction.
5. Write the Haber - Weiss's reaction.
6. What is the product of reaction of superoxide with nitroxide? Write this reaction and describe its importance.
7. Define the term "antioxidant" from a biological point of view.
8. Name high-molecular weight antioxidants.
9. Name low-molecular weight antioxidants.
10. Write reaction of superoxide elimination by SOD.
11. Write the reaction characterizing the antioxidant ability of *catalase.*
12. Write the reaction characterizing *glutathione peroxidase* antioxidant ability.
13. Explain the importance of cooperation between vitamin C and vitamin E.
14. Define the oxidative stress.
15. Which compound is used as a marker of oxidative damage to lipids? Write the formula.
16. What is a marker of oxidative damage to DNA? Write the formula.
17. What do you think? Are free radicals "just bad" and antioxidants a “just good"?

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