MASARYK UNIVERSITY

Faculty of Medicine

ESSENTIALS OF

MEDICAL CHEMISTRY AND BIOCHEMISTRY

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Content

The text has been created and designed for one-semester English program of General medicine and Dentistry studies in the Faculty of Medicine, Masaryk University, Brno.

The selection of topics emphasizes the biochemical processes and the metabolic conversions of compounds occurring in the human body. The text describes the most important biomolecules in the human tissues (saccharides, lipids, amino acids, proteins, nucleic acids), however, it also comprises essential facts from general chemistry (energy, equilibrium, kinetics, acids and bases, redox reactions, solutions, etc.), summarizes biologically important elements (macroelements, microelements), and treats main groups of organic compounds (alcohols, aldehydes, amines, carboxylic acids, heterocycles, etc.).

Some special topics are also included like colloidal solutions, dispersions, osmotic pressure, Latin nomenclature, steroids, terpenes, glycoproteins, vitamins, selected toxic substances.

Some structural formulas, indicated by a side dotted line, are presented just to illustrate the issue; they are not demanded in testing.

The authors believe that *Essentials of Medical Chemistry and Biochemistry* will become a very useful and friendly tool for students to acquire primary knowledge for the following study of Biochemistry I and Biochemistry II.

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1 Basic Chemical Definitions

Elementary Particles of Matter, Substances, Elements, Compounds

A **substance** is a specific kind of matter that exhibits stable physical characteristics at definite conditions. All substances contain atoms.

An **atom** is the smallest electrically neutral particle that still keeps the chemical characteristics (quality) of the corresponding element. It consists of a positively charged nucleus and negatively charged electron shell. The arrangement of electrons in the shell influences the chemical characteristics of an atom.

A **molecule** is the smallest part of a pure substance that still keeps the chemical characteristics of this substance. It is electroneutral, characterised by a specific composition (nature and number of the present atoms) and structure (their mutual arrangement in space, kind of bonds). Di- or polyatomic molecules of one type of chemical element have isoatomic composition (they contain one kind of atom e.g. H_2, O_2, N_2, Cl_2). Molecules of compounds have heteroatomic composition, e.g. HCl, H_2O, etc. In some substances (e.g. solid substances with crystal structure), the individual molecules are not identifiable. In this case, we formally appoint the smallest part of the substance (the smallest numerical ratio of atoms), the composition of which still describes the composition of the whole substance, and we call it **formula unit** (e.g. a pair of ions Na^+ and F^- in sodium fluoride, carbon atom in diamond, Si atom and two O atoms in silicon dioxide, etc.). From this formula unit (the repeating part) the name of the substance is derived.

According to the composition, chemically pure substances and mixtures (homogeneous, colloid, and heterogeneous) are distinguished. In nature, substances usually occur in the form of mixtures.

A chemically pure substance (chemical individual) consists of the same particles with stable characteristic properties (boiling point and melting point, density, refractive index, specific rotation). We distinguish two groups of chemically pure substances – elements and compounds.

An element is a chemically pure substance consisting of atoms with the same proton/atomic number. If the element is formed by atoms with the same mass number, it is called a **nuclide**. Atoms of the given element can be non-combined (noble gases), form molecules (gaseous dioxygen O_2), or they are combined altogether to form more complex structures (e.g. covalent bonds – diamond, metal bonds in metals, covalent bonds and van der Waals forces – graphite).

A compound is a chemically pure substance consisting of two or more different atoms. The atoms can be arranged in isolated molecules (gaseous carbon dioxide) or they can form more complex structures (crystalline structures – sodium chloride).

A mixture (dispersion system) consists of several different, chemically pure substances. The properties of mixtures are not stable; they depend on the composition of the mixture. A mixture can be homogeneous (formed by only one phase – air, solutions), colloid (e.g. egg white in water) or heterogeneous (more phases physically separated from each other – water with sand).

Expressing the Amount of Substance and Their Structural Elements

The amount of any substance (chemical element, compound, mixture) can be expressed in several ways: mass (m), volume (V) or the number of basic structural particles, atoms, ions, molecules (N).

Atomic and Molar Mass

Mass (m) is an additive quantity that does not depend on temperature and pressure. The mass of the structural particles can be expressed in common mass units (kg, g). The **absolute mass of an atom** (the value of its resting mass) is very small, about 10^{-27}–10^{-25} kg. Thus, the absolute quantities are impractical for everyday use. Mass can be more conveniently expressed by the comparative standard (object with a mass similar to the mass of these particles). This standard was stated during the last correction in 1961 to be the atom of a carbon nuclide ^{12}C. Exactly 1/12 of the rest mass of this atom $m_0(^{12}C)$ was determined as an accessory unit. It is the <u>u</u>nified **atomic mass unit** (symbol **u**); in biochemistry and molecular biology, it is more often called **dalton** (symbol **Da**). The value of its mass is called the **atomic mass constant** (symbol m_u).

$$m_u = 1/12\ m_0(^{12}C) = 1.660\ 54 \cdot 10^{-27}\,\text{kg} = 1\,\text{u} = 1\,\text{Da}$$

The absolute atomic masses are not as important from the chemical point of view as the ratio (the relative amount), under which the atoms react with each other. Therefore, we most frequently use the relative (proportional) expression of mass (related to 1/12 of the mass of the carbon nuclide ^{12}C).

The relative atomic mass (A_r) is a dimensionless number representing how many times the mass of a given atom (X) is higher than the atomic mass constant m_u. It is given by the ratio between the (absolute) mass of one atom $m(X)$ in kg to the atomic mass constant m_u in kg.

For the given element X it is accepted that:

$$A_r(X) = \frac{m(X)}{m_u}$$

The relative molecular mass (M_r) is the sum of the relative atomic masses of all atoms present in the molecule. It is equal to the ratio of the molecular mass $m(XY)$ in kg to the atomic mass constant m_u in kg.

Volume

Volume is typically used in case of liquids or gases. It is an additive quantity only in case of ideal systems, in gaseous states, where individual particles do not affect each other. The effect of temperature and pressure on the volume is very significant. **Avogadro's law** is of great importance for calculations of ideal gas volume: at the same pressure and temperature, equal volumes of different gases contain the same number of particles. In simplified calculations, the gaseous substances will be assumed to be ideal gas.

Substance Amount

The substance amount (n) is the quantity related to the number of particles (N). If we use one molecule as the unit for quantity of a substance, we would deal with very large numbers. Therefore, in chemistry, one **mole** is used as the basic unit of the substance amount. It contains exactly the same number of

elementary entities (molecules, atoms, ions, electrons) as there are atoms in exactly 12 g of the carbon nuclide ^{12}C. This number is expressed by the **Avogadro constant $N_A = 6.022 \cdot 10^{23}$ mol^{-1}**. The substance amount (n) is then calculated as $n = N / N_A$. The mole is one of the seven SI base units. When the mole is used, the kind of elementary entities must always be specified.

Molar Quantities

Quantities related to the mole are **molar** (mass, volume, charge) quantities.

The molar mass (M) is the (absolute) mass of $6.022 \cdot 10^{23}$ basic particles, e.g. 1 mole of the given substance expressed in **g mol^{-1}**. It can be calculated by multiplying the absolute mass of an atom or molecule by the Avogadro constant:

$$M = m_x N_A$$

where m_x is a mass of one atom or molecule.

Because the atomic mass can be expressed as the product of the relative atomic mass and atomic mass unit, it follows that $M = A_r \cdot u \cdot N_A$, which after substitution gives:

$$M = A_r \cdot 1.66 \cdot 10^{-27} \cdot 6.022 \cdot 10^{23} \text{ kg mol}^{-1} = A_r \cdot 1.66 \cdot 10^{-24} \cdot 6.022 \cdot 10^{23} \text{ g mol}^{-1}$$

Because the product $1.66 \cdot 10^{-24} \cdot 6.022 \cdot 10^{23}$ is approximately equal to one, it implies that the mass of one mole of substance **M is numerically equal to the relative atomic or molecular mass**, **but it is expressed in grams**.

If we know the mass of a substance containing a certain number of moles of this substance, the molar mass is expressed by the equation:

$$M = \frac{m}{n}$$

The molar volume of a gas (V_M) is the volume of one mole of an ideal gas under standard conditions[1]. It is equal to **22.4 l mol^{-1}** at 0°C and 101.3 kPa. It is calculated by dividing the volume of a gas (V) by the quantity of a substance (n):

$$V_M = \frac{V}{n}$$

The molar charge is the electric charge of one mole of charged particles with one elementary charge and it is the product of the elementary charge and the Avogadro constant N_A. **The elementary charge** is the smallest possible charge of a particle (the absolute quantity of the charge of an electron or proton) and it is equal to **$1.602 \cdot 10^{-19}$ C** (coulombs). The charge of one mole of particles with one elementary charge is then the **Faraday constant $F = 1.602 \cdot 10^{-19}$ C $\cdot 6.022 \cdot 10^{23} = $ 96 485 C mol^{-1}**.

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[1]Standard conditions for temperature and pressure have been established by many institutions, e.g. since 1982 IUPAC has established a temperature of 0°C and a pressure of 100 kPa (1 bar) – the corresponding V_M is 22.7 l mol^{-1}.

2 Chemical Bonds

Except noble gases, all compounds are composed of atoms that are held by chemical bonds forming the molecules or crystalline structures. When a chemical bond is formed, the electrons are redistributed to form a new, energetically more convenient arrangement. The strength of the chemical bond is measured by the energy needed to its cleavage, which equals to the energy released when this bond is formed. It is called bond energy and is given in kJ mol^{-1}. The basic types of chemical bond are **covalent** and **ionic**.

Covalent Bond

The covalent bond is characteristic for **non-metal** elements. The bond energy of the covalent bonds is usually in the range of 200–1,000 kJ mol^{-1}. The lengths of covalent bonds are between 0.07–0.12 nm.

The principle of the covalent bond is the sharing of one or more electron pairs between two atoms. When these atoms approach so close that two of their orbitals, each containing one electron, are overlapped, the bond is created (an exception is the donor-acceptor bond, see later). Effective overlapping (and the formation of a bond) occurs when the sharing of atoms creates a stable and energetically more favourable electron configuration, usually corresponding with the electron configuration of the nearest noble gas. The sharing of one electron pair between two atoms creates a **simple** bond. Overlapping occurs on the join line of the nuclei of the reacting atoms where the highest electron density is also located. This bond is called **σ (sigma) bond**. A higher number of shared electron pairs between the two atoms implicate the formation of a **multiple** covalent bond. The multiple covalent bonds consist of one **σ** (sigma) bond and one or two **π** (pi) bonds. In the case of these bonds, the highest electron density is above and under the symmetry plane of the orbitals, which lies on the join line of the nuclei. Multiple bonds are typical for carbon organic compounds. Multiple covalent bonds are stronger than single bonds, but the bond energy of a double bond is not equal to the doubled energy of the single bond, etc.

Polarity of Bonds and Molecules

If the covalent bond binds two equal atoms, e.g. in the diatomic molecules of gases (N_2, O_2, F_2), the shared electrons are distributed equally between both atoms; such a bond is called **nonpolar**. **A polar bond** is formed when two different atoms are bonded and the shared electron pair is distributed unequally between them. How unequally the electrons are shared depends on the relative abilities of the two different atoms to attract electrons. One atom then acquires a partial negative charge ($-\delta$) and the other partial positive charge ($+\delta$). The polarity of the individual bonds influences the total polarity of the molecule, i.e. the distribution of the positive and negative charge in the whole molecule. The measure of polarity is the dipole moment. In diatomic compounds, the dipole moment of the bond is equal to the dipole moment of the molecule. The polarity of polyatomic molecules depends on their molecular geometry; the dipole moment is given by the vector sum of dipole moments of the individual bonds. It means that a molecule can be **nonpolar**, even if it contains **polar** covalent bonds (e.g. CO_2).

Valence of Elements

Each element has a characteristic valence that determines the number of covalent bonds it can form. The valence of an element is limited by the number of valence atomic orbitals. Atoms of hydrogen, which contain only one valence orbital, can form only one covalent bond. Atoms of carbon (and also nitrogen) are maximally tetravalent as they contain four valence atom orbitals.

Coordinate Covalent Bond

Aside from the typical covalent bond there can also exist a special **coordinate covalent bond** (donor-acceptor or dative bond). In this type of covalent bond, both electrons are provided to a bond by the same atom. The **donor atom** has a **free electron pair**, i.e. a pair of electrons still not involved in any bond, and the **acceptor atom** has a **free valence orbital** and accepts an electron pair for sharing in the bond. The coordinate covalent bond is mostly found in the complex compounds. The acceptor of the electron pair is usually a cation or atom of a transition metal; it is referred to as the **central atom**. Donors of the electron pairs (molecules, anions) are called **ligands**. The number of ligands bonded to the central atom gives the **coordination number**. The most frequently occurring compounds have coordination numbers 6 or 4.

Ionic Bond

The ionic bond is the extreme form of the polar covalent bond. The shared electrons are pulled into the vicinity of the more electronegative atom, so that two ions are created. The cation and anion with opposite charges are attracted by electrostatic forces. An approximate definition states that the ionic bond predominates between atoms with the difference of electronegativities greater than 1.7. At the difference of electronegativities ≥ 2.0 the bond is typically ionic, nevertheless the ionic character never reaches 100%. Besides the typical binary salts (NaCl, KF), the ionic bond occurs in other salts of inorganic and organic acids, which are formed by cations and anions such as NH_4^+, NO_3^-, SO_4^{2-}. In these particles, e.g. $NH_4^+NO_3^-$, the character of the bond is ionic.

Intermolecular Forces

Intermolecular forces are attractive forces **between molecules** (or between the parts of a macromolecule) that influence the consistence of substances. The energy of these interactions is much lower than the energy of the covalent bonds and the ionic interactions in crystal lattices – therefore they are defined as **weak** non-bonding interactions or **non-covalent bonds.** The strength of the intermolecular forces determines, e.g. the state of the given substance and affects its solubility. Their biological importance lies in maintaining the secondary, tertiary or quaternary structure of biopolymers, the stability of supramolecular structures such as biomembranes, and in specific biological interactions (binding a substrate to an enzyme, an antibody to an antigen, a hormone to a receptor).

Hydrogen Bonds

Hydrogen bonds are the strongest intermolecular forces. They are found in substances in which the hydrogen atom is bonded to a strongly electronegative atom – nitrogen, oxygen, or fluorine. The elec-

tronegative atom strongly attracts the shared electron pair; the small hydrogen atom has little electron density around it.

Under these circumstances, the hydrogen atom carries a partial positive charge and is able to form a weak bond with a free electron pair of the electronegative atom of the other molecule. In formulas it is marked by punctuation: X-H$\cdots$Y. The bond energy is greater than in the case of other non-covalent interactions, it reaches up to several tens of kJ mol^{-1}. The presence of the hydrogen bonds in a certain system is distinctly displayed by the increase of intermolecular attractive forces.
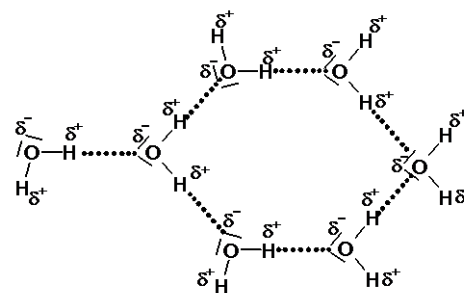

Hydrogen bonds between water molecules

Hydrogen bonds have special importance for the properties of water. Because oxygen in H_2O has two lone pairs of electrons and two covalently bonded hydrogen atoms, each water molecule is able to form hydrogen bonds with up to four other molecules at the same time. Thus aggregates (clusters) with a different number of molecules and with circular or spatial arrangement are formed. They have tetrahedral arrangement in ice – each molecule binds four others and six-member rings are formed. The crystal lattice is relatively loose (lower density of ice, increase of volume).

The hydrogen bonds can be **intermolecular** and **intramolecular**. Large numbers of hydrogen bonds are formed between C=O and -NH- groups of peptide chains. They play an important role in the formation of the secondary structures (α−helix, β−structure). The formation of hydrogen bridges between the purine and pyrimidine bases is the principle of the replication, transcription, and translation of the genetic code.
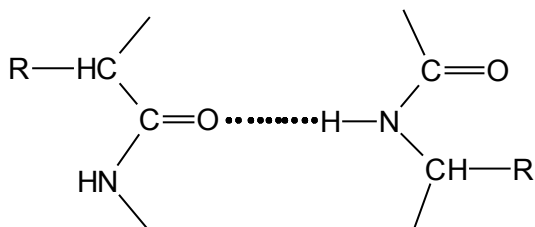
Intramolecular H-bond between peptide chains
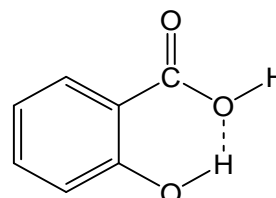

Intramolecular bridge in sulfosalicylic acid

van der Waals Forces

These forces include electrostatic and dispersion interactions.

The principle of **electrostatic interactions** is attractive coulombic forces between the positive and negative electrical charge. The electrical charge can be either whole in ions, or partial in dipoles.

Ion-ion interactions. These interactions include the formation of ion pairs (salt bridges) in solutions. For example, ion pairs are formed between amino acid side chains (-COO$^-\cdots^+$NH$_3$-) in proteins. This interaction is important mainly for the association of subunits participating in the quaternary structure of proteins.

Ion-dipole or dipole-dipole interactions. They are weaker than the previous interactions. In polar substances, they are responsible for their lower volatility in comparison with the analogical non-polar compounds. They cause the solvation of ions in polar solvents. Intensity of these interactions depends directly on the charge and indirectly on the size of ion. For example, the smaller Na^+ ion exhibits stronger interaction with water molecules than the bigger Cl^- anion. They participate in the stabilisation of the tertiary structure of proteins. They contribute to the solubility of polar substances in polar solvents.

| Dipole-dipole interactions | Ion-dipole interactions | Cation-dipole interaction |
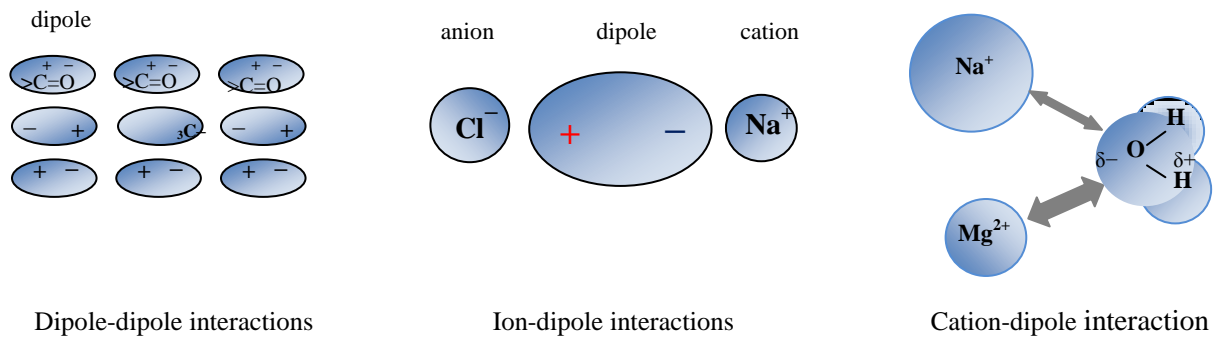
Dipole-induced dipole interaction. In non-polar molecules, especially if they contain easily polarisable structures, a weak dipole moment is induced in the electric field of ions or in the close vicinity of the permanent dipoles. Formation of this dipole moment results in corresponding electrostatic attractive forces.

Dispersion forces (London forces) occur in nonpolar substances (hydrocarbons, gaseous elements). Dispersion forces are the result of momentary shifts in the symmetry of the electron cloud in the molecule. As soon as a slight positive charge is produced at one end of the molecule, it induces a slight negative charge in one end of the molecule next to it – induced dipole. The bond energy induced by the van der Waals forces is weaker (by two or three orders) than the energy of the covalent bonds.
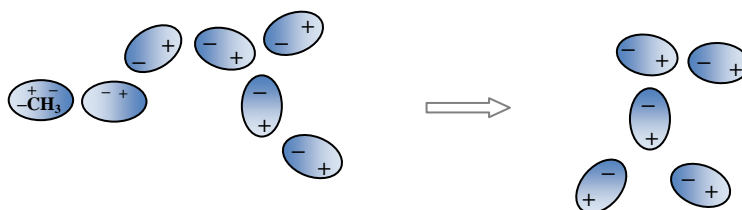
Time-limited interactions of temporary induced dipoles

Hydrophobic interactions arise between the hydrophobic (non-polar) molecules in an aqueous environment. If you add some oil to water and stir it mechanically, water molecules surrounding oil drops are organised, attracted to each other by H-bonds. The order of water molecules increases, the entropy of the system (water + oil) decreases. After some time, oil drops spontaneously combine to form a larger one. At this moment, water molecules are again free and less organised with higher entropy.

The bond energy of the individual interactions is negligible, but if they occur in large quantities, they reach considerable strength. This type of interaction is important in the structure of biomolecules, e.g. in the stabilisation of biological membranes and the tertiary structure of proteins.

3 Energetics of Chemical Reactions

The energetics of chemical reactions is often considered from two principle points of view. The first aspect describes the change of total energy of a system during the chemical reaction; the second aspect examines the spontaneity of chemical processes.

Basic Definitions

A chemical system is a portion of the universe where a chemical reaction takes place. The system can be simple (e.g. a reagent vessel with the reacting components) or complex (cell, organism, part of the biosphere). Each system is limited, i.e. it is unambiguously separated from its surroundings. **An isolated system** exchanges neither matter nor energy with its surroundings. **A closed system** exchanges only energy with its surroundings. **An open system** exchanges both energy and matter. Biological systems are mostly open, the exchange of energy and substances with the surroundings proceeds constantly in them.

State quantities determine the state of the system in each moment. The basic state quantities include pressure p, volume V and temperature T.

Internal Energy

At the given conditions, each closed system has a certain value of the **internal energy U**. The absolute value of U, which can include all possible forms of energy, is not generally known for the individual systems. However, U is the state quantity and during the transition of a system from one state into another we can measure its change $\Delta U = U_2 - U_1$.

The change of the internal energy of a system involves heat Q released or absorbed by a system during the reaction, as well as work done *on* or *by* the system: $\Delta U = Q + \Delta w$.

The equation above is the mathematical expression of the **First law of thermodynamics**.

Heat (Q) reflects energy that increases the disordered (chaotic) movement of particles and changes temperature of the system and its surroundings. The most common form of **work (w)** in chemical reactions is mechanical volume work. This is work that the system performs or receives at the expense of volume changes at given constant pressure. Significant volume changes can only occur in reactions proceeding in a gaseous state or during reactions in which gaseous components arise or disappear.

Enthalpy

To characterise processes taking place at constant pressure we use the **enthalpy (H)** state quantity, which expresses the heat content of the system. The change of enthalpy ΔH equals to heat absorbed or released during the reaction proceeding at the constant pressure:

$$\Delta H = H_2 - H_1 = Q_p$$

If none of the reacting substances is in the gaseous state, the volume changes during this reaction can be neglected. In this case, the change of internal energy is practically equal to the change of the heat content ($\Delta H \approx \Delta U$).

According to the value of the reaction heat we distinguish **exothermic** reactions if the heat is released ($\Delta H < 0$), and **endothermic** reactions ($\Delta H > 0$) if the heat is absorbed.

Each reaction can be expressed as a series of processes, during which the bonds between atoms in the reactants are at first destroyed and the new bonds in the products are then formed. To cleave the original bonds we have to add a certain quantity of energy, which is proportional to their strength. During the formation of new bonds, the energy is released. The value of ΔH expresses the difference between the bond energy of products and reactants. Its negative value states that the products are energetically more favourable (their bonds are stronger) than the reactants.

The values of the heats of reaction for different reactions, related to the standard state of the reactants and temperature 298 K, are known. For certain reaction types, the heat of reaction has special names, e.g. the heat of formation, combustion, dissociation, solution, etc.

The enthalpy H is a state quantity; its change is given only by the difference between the enthalpy of a system in the initial and the final state. Therefore, ΔH does not depend on the path taken between the two states. This principle is included in two thermochemical laws (Lavoisier-Laplace's and Hess's – see textbook of Biophysics).

Spontaneity of Chemical Processes. Entropy

The values of enthalpy inform us about reaction heat, but not about the fact whether the reaction occurs spontaneously. **Spontaneous** processes do not need external intervention such as added energy to happen. For example, gas spontaneously expands from a higher pressure site to a lower pressure site, dissolved substances diffuse from the region with higher concentration into the region with lower concentration, iron is oxidised to its oxides. Spontaneous reactions proceed until the system reaches its highest stability, i.e. equilibrium. The direction of the spontaneous reaction can be reversed only when energy from the external environment is supplied.

What are the driving forces of the spontaneous chemical and physical processes? Firstly, it is the above-mentioned tendency to minimalize the internal energy of a system. However, we can see in practice that some endothermic processes can also be **spontaneous**. This is because another stability criterion exists, and this is the tendency to achieve the lowest value of order of the system at the given temperature as possible. It means that organised structures generally tend to convert into the state with a higher degree of randomness or disorder. The increase of disorder in the system occurs, for example, during the transformation of the solid state into the liquid or gaseous state, during the decomposition of complex molecules to simple molecules, during the transformation of the crystalline form into the amorphous form, during the decrease of the amount of electric charges in particles or at least their delocalisation, etc.

The measure of the disorder of the system is described by the state quantity called **entropy S**. The more the system is disordered, the greater the probability of its existence and the higher its entropy. The change in entropy reflects the loss of some part of thermal energy into the surroundings. It is expressed as the ratio between the thermal content of a system and the absolute temperature at which the process is proceeding. For reversible processes $\Delta S = Q/T$ and for irreversible processes $\Delta S > Q/T$.

In closed systems, exothermic ($\Delta H < 0$) and reversible processes are spontaneous at a given temperature and pressure ($Q \approx \Delta H$), if the entropy of the surroundings is equivalently increased ($\Delta S_{surroundings} \approx -\Delta H$).

In open systems, the total change of entropy includes the sum of change of the entropy of the system and the change of entropy of the environment, which surrounds the system:

$$\Delta S = \Delta S_{system} + \Delta S_{surroundings}$$

Gibbs Energy

Gibbs energy (free enthalpy), $G = H - TS$, involves the effect of both criterions, i.e. entropy and enthalpy on the spontaneity, for processes in closed systems at constant temperature and pressure. The change of Gibbs energy ΔG is measured as the difference between the Gibbs energy of a system in thermodynamic equilibrium (G_2) and its initial state (G_1). Supposing, that the change of concentration caused by the reaction is minimal, it is valid:

$$\Delta G = G_2 - G_1 = \Delta H - T\Delta S$$

where ΔH is the change of enthalpy and ΔS the change of entropy between the final and initial state.

The equation expresses the **Second law of thermodynamics**. ΔG equates to the maximal energy that the system can use to do non-volume work. The expression $T\Delta S$ represents the minimal part of the total energy that cannot be converted for any useful work, but only as heat.

ΔG is thus the criterion of spontaneity of the reactions taking place at constant temperature and pressure. For a spontaneous process ΔG has negative value; for a non-spontaneous process $\Delta G > 0$. The processes with negative ΔG are called **exergonic**, processes with $\Delta G > 0$ are **endergonic**. Each spontaneous reaction proceeds until the available energy decreases. When the equilibrium is reached, ΔG decreases to zero and the reaction stops.

A negative value of ΔG of the reaction proceeding in the given direction is essential for its <u>spontaneous</u> course. However, it is important to remember that Gibbs energy relationships are not in any way indicators of kinetic stability or instability. ΔG does not directly influence the speed of the reaction course. Even systems with a highly negative value of ΔG can be very stable and their reactions proceed in measurable speeds only in the presence of catalysts.

Standard Gibbs Energy Change

The standard state Gibbs energy change $\Delta G°$ of the given chemical reaction can be calculated from the tabulated values of $\Delta H°$ and $\Delta S°$:
$\Delta G° = \Delta H° - T\Delta S°$.

This equation expresses the change ΔG of the chemical reaction, when all the components of the system (reactants and products) in its initial state have activity (concentration) $1\,mol/L$. They reach the state of thermodynamic equilibrium, where the activities (concentrations) of reactants and products correspond with the value of the equilibrium constant. The data are tabulated for the standard temperature, pressure, and pH = 0. Biochemical reactions proceed in a diluted solution with nearly neutral pH. Therefore, in biochemistry – unlike in physical chemistry – we define the standard state of biological organisms at pH = 7. The changes of the Gibbs energy and equilibrium constant at the defined conditions are marked with a superscript sign ('), i.e. $\Delta G'$, $\Delta G°'$ and K'.

Relation between $\Delta G°$ and ΔG

The ΔG of the systems, whose initial concentrations are different from l mol/L at the start of the reaction, is derived from the equation:

$$\Delta G = \Delta G° + RT \ln Q_r$$

where R is the gas constant, T absolute temperature, and Q_r reaction quotient which expresses the actual (non-equilibrium) concentration of the reactants.

For the Gibbs energy of a general reaction $aA + bB \rightleftarrows cC + dD$, it is valid that:

$$\Delta G = \Delta G° + RT \ln \frac{[C]_i^c \, [D]_i^d}{[A]_i^a \, [B]_i^b}$$

where $[A]_i$, $[B]_i$, $[C]_i$, $[D]_i$ are **initial or instantaneous (non-equilibrium)** concentrations of the reactants. If instantaneous concentrations of reactants and products are l mol/L, then the logarithm equals zero, and $\Delta G = \Delta G°$.

Whether the reaction in the closed system will proceed spontaneously in the given direction, depends only on ΔG, not on $\Delta G°$. However, it is obvious that reactions with strongly negative $\Delta G°$ will be practically irreversible, while reactions with significantly positive $\Delta G°$ will not proceed in the given direction. Reactions with a not distinctly high positive or negative value of $\Delta G°$ can proceed in the both directions depending on the concentration of the substances in the reaction system.

The above-mentioned relation also indicates the relation between $\Delta G°$ and the equilibrium constant K. If the system reaches equilibrium state, the concentrations of [A], [B], [C], and [D] become equilibrium concentrations and $Q = K$. At the same time, ΔG decreases to zero. Then it is valid that:

$$0 = \Delta G° + RT \ln K \quad \text{and} \quad \Delta G° = -RT \ln K.$$

**Energetic Coupling of the Reactions – Macroergic Compounds**

Endergonic reactions cannot proceed spontaneously, but their course can be facilitated by the **coupling** with any exergonic reaction with a high negative value of ΔG. This principle is used in living systems. The cells of living organisms contain organic compounds which are able to "capture and store" energy released during metabolic exergonic processes. During endergonic processes they can release this energy by decomposing their molecules. These substances are called **macroergic** (compounds with high-energy content) and serve as a sort of "energy storage" of the cells.

The definition "compounds with a macroergic bond" presented in older textbooks should no longer be used in this context. The energy released during decomposition of high-energy molecules does not come from the cleavage of one particular bond, but it is the result of the rearrangement of bonds and electrons in the whole molecule.

The most frequent energy carriers in cells are organic derivatives of phosphoric acid (phosphate). The most common example is adenosine triphosphate (ATP), a nucleotide composed of adenine, ribose, and three phosphates. It is formed during the endergonic reaction of adenosine diphosphate (ADP) with inorganic phosphate (P_i).

$$ADP + P_i + energy \rightarrow ATP + H_2O \qquad \Delta G°' = 30.5 \text{ kJ mol}^{-1}$$

During the cleavage of ATP the energy stored in the compound is released, and it can be used in a coupled reaction or other process.

$$ATP + H_2O \rightarrow ADP + P_i + energy \qquad \Delta G^{\circ\prime} = -30.5 \text{ kJ mol}^{-1}$$
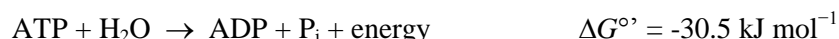
The coupling in biochemical reactions is realised with the help of enzymes and their cofactors through a common intermediate. Another way of coupling involves the synthesis of a high-energy compound with simultaneous decomposition of another macroergic compound. The mutual coupling of endergonic and exergonic processes is the common principle of the energy utilisation in living systems. The energy of exergonic processes, which are not connected with endergonic processes, is released in the form of heat.

The role of ATP for the energetic metabolism is demonstrated by the fact that in the human body about 140 moles (~ 70 kg) of ATP are daily produced and decomposed. Besides ATP, the other purine and pyrimidine nucleoside triphosphates (GTP, CTP, UTP, etc.) can function as macroergic compounds. However, they are not as universal as ATP and they are used only in some specific reactions. Moreover, cells also contain other high-energy content metabolites. These are especially compounds with phosphoester, phosphoanhydride, phosphoamide, and thioester bonds. Examples of such compounds are presented in the table below.

Selected high-energy compounds[a]

Compound	$\Delta G^{\circ\prime}$ (kJ mol^{-1})	Commentary
Phosphoenolpyruvate	−61.9	enol ester of phosphoric acid, the intermediate of glycolysis
Carbamoyl phosphate	−51.5	mixed anhydride (acylphosphate), substrate for urea synthesis
1,3-Bisphosphoglycerate	−50.2	mixed anhydride (acylphosphate), the intermediate of glycolysis
Creatine phosphate	−43.1	substituted amide of phosphoric acid, energy source in the muscles
ATP → AMP + PP$_i$[b]	−32.2	double anhydride of phosphoric acid, PP$_i$ is also a macroergic compound
ATP → ADP + P$_i$[c]	−30.5	double anhydride of phosphoric acid, universal energy source
Acetyl-coenzyme A	−31.4	thioester, important intermediate in metabolism of nutrients

[a] Values of $\Delta G^{\circ\prime}$ strongly depend on conditions (ionic strength, Mg^{2+} concentration) and measurement precision.
[b] PP$_i$ diphosphate (H$_2$P$_2$O$_7^{2-}$/HP$_2$O$_7^{3-}$).
[c] P$_i$ inorganic phosphate (H$_2$PO$_4^-$/HPO$_4^{2-}$).

The table shows that some of the compounds have more negative values of $\Delta G^{\circ\prime}$ in comparison with ATP. These substances can be used to supplement the levels of ATP in the organism during its high consumption.

The typical example is creatine phosphate for the supplementation of ATP during muscle work, when the reaction proceeds: creatine phosphate + ADP $\rightleftarrows$ creatine + ATP.

This reaction covers the energetic need during the first seconds of muscle work. On the other hand, during rest, if the content of ATP is sufficient, the reaction proceeds in the opposite direction and creates the reserve of highly energetic creatine phosphate.

The most important way of ATP formation in the organism is its synthesis by oxidative phosphorylation, which takes place in connection with the respiration chain in the mitochondria.

4 Chemical Kinetics

The part of chemistry that is concerned with the rates of reactions is called **chemical kinetics**. Basic relations are derived from the collision model of the reaction rate, which is extended by the hypothesis of the formation of an activated complex. According to this theory only those particles, which collide and have sufficient energy content, can react with each other. When the particles advance towards each other, the initial bonds of the reactants are gradually weakened and new bonds are created. An unstable transition complex is formed – **activated complex**. To transform the complex to products, a distinct energy level should be reached, so called **activation energy**.

The Rate of Chemical Reaction

The speed, with which the reactants are transformed to products during chemical reaction, is called the rate of a chemical reaction. The rate of a chemical reaction can be defined according to the decrease of reactant concentrations or the increase of product concentrations per unit time. Since the reactants are consuming as the products are appearing, the rate of loss of a reactant is, by convention, designated as being negative whilst the rate of appearance of a product is positive:

In the common equation $$a\text{A} + b\text{B} \rightleftarrows c\text{C} + d\text{D}$$
the rate is expressed by the relation:

$$v = -\frac{1}{a}\frac{d[\text{A}]}{dt} = -\frac{1}{b}\frac{d[\text{B}]}{dt} = \frac{1}{c}\frac{d[\text{C}]}{dt} = \frac{1}{d}\frac{d[\text{D}]}{dt}$$

where d/dt expresses the change of quantity in time.

The rate of a chemical reaction is the time decrease of the molarity of a reactant or the time increase of the molarity of a product, divided by the stoichiometric coefficient of the substance in the reaction equation.

As the concentrations of reactants change continually during reaction, the reaction rate changes too. Thus, the ratio $v = dc_x/dt$ defines the **instantaneous** reaction rate, expressed as the change of concentration of substance X in infinitesimal time period dt. Besides this, we describe the **average** reaction rate, i.e. the decrease or increase of the concentration of X in definite time interval Δt:

$$v = \frac{\Delta[\text{X}]}{\Delta t}$$

As the instantaneous reaction rate is proportional to the immediate concentration of the reactants, which always decreases during the reaction, the instantaneous rate of the reaction decreases too.

The **initial rate** v_o is a velocity measured just at the start of the reaction. The above-mentioned facts imply that it is the highest value of the reaction rate in the course of the reaction at the given concentration of reactants. Its value is not influenced by the decrease of reactants during the reaction. Determination of the initial reaction rate is often used to define the kinetic parameters of the reactions.

Kinetic Equation and Reaction Order

The reaction rate is mostly determined experimentally by observing the change of concentration of the given substance in the reaction mixture during reaction. The results are plotted into the graph of the dependence of the concentration on time, which is described as the kinetic curve. The instantaneous reaction rate at time t is determined as the slope of the tangent to this curve at the given time.
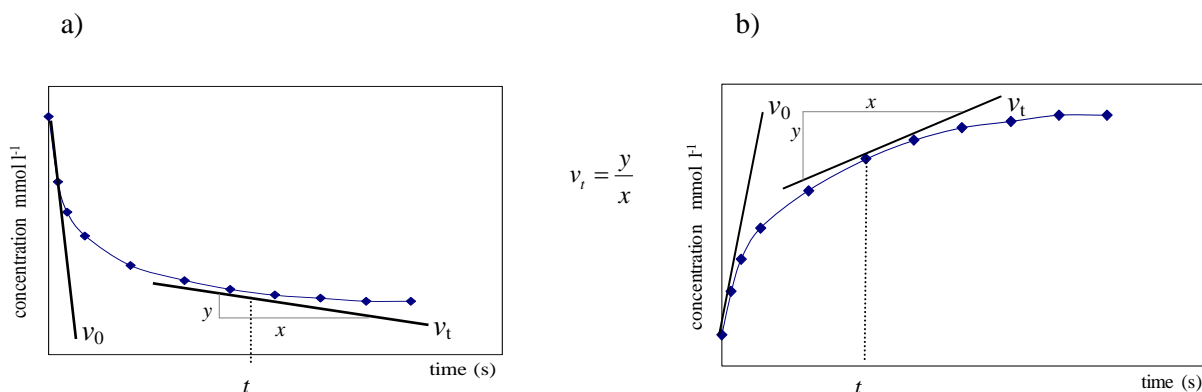
a)   b)

$$v_t = \frac{y}{x}$$

Figure: Determination of initial rate (v_o) and rate at time t (v_t) in the kinetic curve of reactant (a) and product (b)

In practice, it is not always necessary to measure the actual concentration of the studied substance. Instead, we usually measure the change of some of its physical properties, which is dependent on concentration, e.g. absorbance, optical rotation, partial pressure, or radioactivity.

The dependence of the reaction rate on the instantaneous concentration of reactants determined from the experimental data is expressed in the **kinetic equation**. The common formulation of the equation is

$$v = k\,[A]^\alpha\,[B]^\beta$$

Values α and β are so called partial reaction orders; in general they are not identical to coefficients a and b in the stoichiometric equation of the reaction and are only obtained from the experimental measurements. The sum of stoichiometric coefficients $\alpha + \beta$ is defined as **the reaction order**.

If the kinetic equation is $v = k\,[A]$, the reaction is of **the 1st order**, the reaction described by kinetic equation $v = k\,[A]\,[B]$, is the reaction of **the 2nd order,** etc. The reaction order need not be expressed as an entire value, for example, for a reaction with the kinetic equation expressed as $v = k\,[A]^{0.5}\,[B]$, the reaction order will be 1.5.

The term **reaction molecularity** is used to describe the course of chemical reactions. It is the number of particles that have to collide to cause the reaction. The most probable and the most frequent is the collision of two particles, which refers to the bimolecular reaction (molecularity is 2). The molecularity and the reaction order often reach the same values, but they should not be mixed – the term molecularity refers to the reaction mechanism, whereas the reaction order is the empirical value obtained from the experimental data.

The constant k in the kinetic equation is the **rate constant**. The value of rate constant is characteristic of a specific reaction. It defines the rate of the reaction at the unitary concentrations of the reactants and at given conditions. It depends on temperature and activation energy as it is given by the Arrhenius equation:

$$k = A\,e^{-E_\mathrm{A}/RT}$$

where A is the collision factor, E_A the activation energy.

The value of exponent E_A/RT is decisive for the thermal dependence of k (and thus the rate of the whole reaction). If the temperature rises, the value of this exponent decreases and the reaction rate increases.

The influence of temperature on the reaction rate is explained by the collision theory. When the temperature rises, the energy of all reactants increases and it does not depend on the fact if the reactions are exothermic or endothermic. This leads to an increase of the number of effective collisions between the particles and thus the increase of the reaction rate. However, the increase of temperature is limited by the value at which the reacting molecules are no longer stable. This is important mainly in case of reactions proceeding in the biological systems, when the increased temperature leads to the denaturation of proteins or the decomposition of substances.

The Reaction Half-Life

The half-life $t_{1/2}$ of a reaction is the time needed to decrease the concentration of a reactant to half of its initial concentration in the course of the reaction. Mathematically it can be proved, that the half-life of the 1st order reaction is independent on the initial concentration of the given substance, whereas in case of the zero and 2nd order reactions it is concentration dependent.
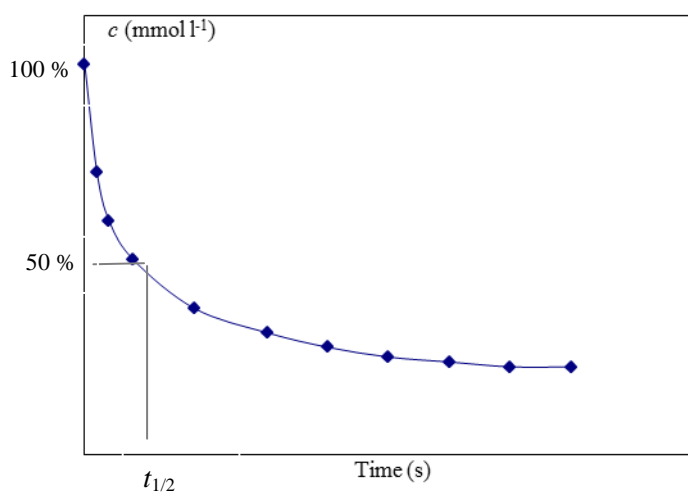
Figure: The half-life in a kinetic curve

Biological or elimination half-life of drugs, metabolites, or natural substances in the organism is defined as a time needed to decrease the level of a substance in the organism to half of its initial concentration. The knowledge of the value of the biological half-life is important for the prescription of the drug dosage.

Zero Order Reactions

These reactions are special types of processes in which the reaction rate is independent on the concentration of the reactant(s) for a certain time period from the start, given special conditions, and the reactions proceed in a closed system. This means that the rate is controlled by something other than collisions that involve reactants. In biochemistry, these are usually the enzymatically catalysed processes with high substrate concentrations, where the enzyme is fully saturated with the substrate. They also include the transport systems across the cell membranes or other saturable processes. The kinetic

equation is written as $v = k\,[A]^0 = k$. The rate of the process is constant; it is independent on the high concentration of the reactant A until its concentration decreases up to the point it becomes decisive. The reaction rate then follows the kinetics of the first or higher order. The kinetic curve has the shape of a line.

First-Order Reactions

Reactions following 1st order kinetics are rare. They are described by the general equation $A \to P$. Typically, they involve dissociations (e.g. $N_2O_3 \to NO_2 + NO$), radioactive decay ($^3_1H \to {}^3_2He + \beta^-$), isomerisation reactions in organic chemistry, and enzymatic one-substrate reactions proceeding at low concentrations of the substrate. Many reactions of the higher order can be arranged in such way that they follow the kinetics of the 1st order.

The kinetic equation of the 1st order reaction is written as $v = k\,[A]$, which means that the actual rate of the reaction is always directly proportional to the concentration of the reactant A. In the same time period, the constant amount of A is consumed, so that the concentration of A and the reaction rate exponentially decrease with time.

The kinetic curve has the shape of an exponential line. The instantaneous rate at time t is determined as the slope of the tangent at the given time.

Second-Order Reactions

These are the most common type of reactions. The general equation is:

$$A + B \to products \qquad or \qquad 2\,A \to products$$
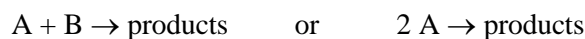
The kinetic equation for both above-mentioned types is $v = k\,[A]\,[B]$ and $v = k\,[A]^2$. The reaction rate is proportional to the concentration of both reactants or to the square of the concentration of reactant A. If the concentration of one of the reactants is sufficiently high, so that its decrease will not influence the reaction rate, the reaction will follow the kinetics of the 1st order.
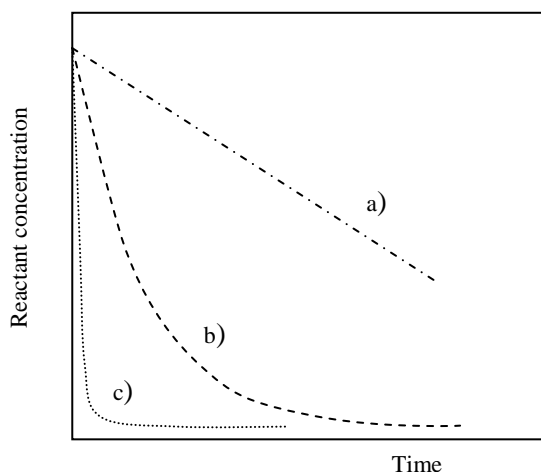
Figure: Kinetic curves for the reactions:
a) 0 order   b) 1st order   c) 2nd order

Factors Affecting the Reaction Rate

The effect of temperature on the reaction rate is described in the aforementioned Arrhenius equation. The effect of pressure is observed only in case of reactions proceeding in the gaseous state. In this case the increase of pressure causes the increase of the reaction rate, because it leads to the decrease of volume and thus to the increase of the concentration of the reactants.

The rate of reactions with at least one of the reactants in the solid state depends also on the size of the reacting particles, the smaller the particles, the higher the speed. This is attributed to an increase in the surface area of the particle and hence an increase in

the number of collisions between the reacting species. The surface area of one mole of small particles of a compound has a larger surface area than one mole of large particles of the same compound.

In addition, in heterogeneous systems the reaction rate depends on more factors, such as, e.g., the speed of the removal of products and the supply of reactants.

Catalysts

Catalysts are substances which change the rate of a chemical reaction, but can be recovered chemically unchanged at the end of the reaction. Positive catalysts (activators) increase the rate of the reaction; inhibitors (negative catalysts) decrease the reaction rate. They work in minute concentrations and their effect is usually specific, i.e. a certain catalyst has a catalytic function only in a certain reaction. Catalysts do not change the yield of the reaction; they only change the time taken to obtain this yield.

The mechanism of the catalytic function of the catalysts is complex and not always known. In the majority of examples the positive catalysts decrease the energetic barrier, which has to be overcome in the course of the reaction, in other examples it is supposed, that the effect of catalysts increases the number of effective collisions between reactants (the value of the frequency factor A in the Arrhenius equation increases). Generally, we can say that catalysts change the sequence of reactions or the character of the intermediates during the transformation of reactants into products. They do not change the amount of products present once a reaction is complete or equilibrium is reached.
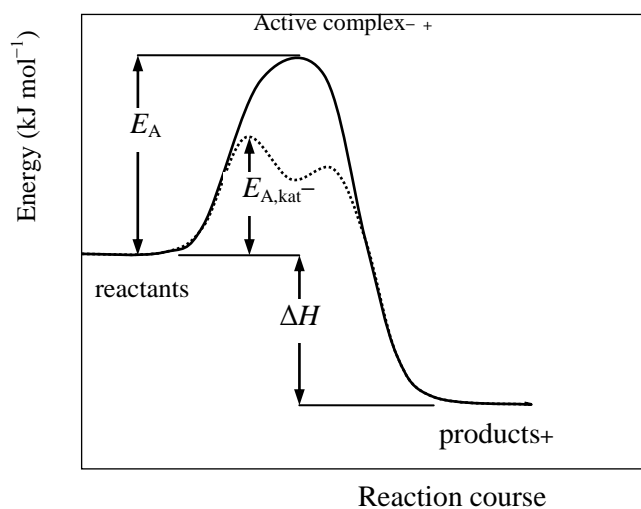
Figure: Energetic diagram of exothermic reaction non-catalysed (——) and catalysed (···)

Enzymatic Catalysis

In biochemical processes, highly effective catalysts are enzymes. These are proteins often working together with low molecular substances called cofactors. The three dimensional structure of an enzyme contains a so-called catalytic (active) site created by the specific arrangement of amino acid side chains. The catalytic site specifically binds the reactant (substrate) and changes it into a transient complex of enzyme-substrate.

A one-substrate enzymatic reaction can be expressed by the equation:

$$E + S \rightleftharpoons ES \xrightarrow{\ k_2\ } E + P$$
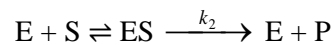
where E enzyme, S substrate, ES enzyme-substrate complex, P product.

The rate of enzymatic reaction is defined as an increase in the concentration of product (P) in time or as the rate at which enzyme-substrate complex (ES) is decomposed into product(s):

$$v = \frac{\Delta[P]}{\Delta t} = k_2[ES]$$

The rate of enzymatic reaction depends on both the substrate concentration and the amount of enzyme, and its capability to catalyse the chemical conversion. **Molecular activity of enzyme** (turnover number) is defined as the number of substrate molecules that one saturated enzyme molecule can convert to product per second.

At very low concentrations of the substrate, the enzyme reaction rate is directly proportional to the concentration of the substrate, i.e. the reaction kinetics is of the first order with respect to substrate concentration. With increasing concentration of the substrate in solution, the rate of the enzyme reaction further increases (but simultaneously the reaction order decreases) until all catalytic enzyme sites are occupied ("saturated") by substrate, the rate of enzyme reaction is zero order kinetics with respect to the substrate. From this moment, the speed of enzymatic reaction does not depend on the substrate concentration ([ES] gets maximal value), but only on the speed of enzyme-substrate (ES) conversion into product(s).
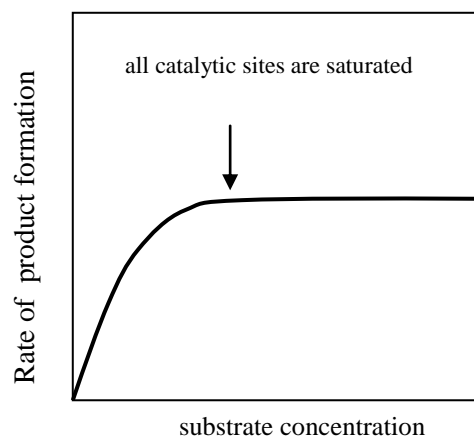
Figure: The dependence of the initial rate of enzyme reaction on the substrate concentration

5 Chemical Equilibrium

Chemical reactions proceed until they reach a dynamic chemical equilibrium in the closed system, i.e. until such state of the system in which its composition does not change. Even if the chemical processes are still going on in the system, their net effects are mutually cancelled.

Equilibrium State, Equilibrium Constant

The equilibrium state of a closed system is, after the completion of the chemical reaction, quantitatively described by the equilibrium constant K. The value of this constant is always the same for the given reaction and temperature (and in case of systems composed of gaseous components the given pressure).

For the general reaction: $a\text{A} + b\text{B} \leftrightarrows c\text{C} + d\text{D}$

which is started by the mixing of substance A and B, it is valid:

- At the start of the reaction, the formation of products C and D will proceed with a rate $v_1 = k_1 [\text{A}]^a [\text{B}]^b$. The rate v_1 will be maximal at the beginning, with the decreasing amount of reactants it will decrease.
- The formed products will be converted reversely to reactants with the rate $v_2 = k_2 [\text{C}]^c [\text{D}]^d$. The rate v_2 will increase with the increasing concentration of products.
- After some time, the rate of product formation v_1 equals the rate of product conversion to reactants v_2; equilibrium is established in the system.

Therefore, at equilibrium, at constant temperature: $k_1 [\text{A}]^a [\text{B}]^b = k_2 [\text{C}]^c [\text{D}]^d$ and from this:

$$K_c = \frac{k_1}{k_2} = \frac{[\text{C}]^c [\text{D}]^d}{[\text{A}]^a [\text{B}]^b}$$

The presented equation is the **law of mass action** or the Guldberg-Waage law: the product of the concentrations of reaction products divided by the product of the reactant concentration, each concentration term raised to stoichiometric coefficients for the respective species, is the constant at equilibrium.

K_c is the concentration equilibrium constant which does not take into account the difference between concentrations and activities. The real state is more precisely expressed by the thermodynamic equilibrium constant K_a derived from the activities of the reacting substances. The equilibrium constant K described in the text below is actually K_c or K_p (in case of gases).

According to the convention, K is a dimension-less number. The value of the equilibrium constant implies the state of the reaction system in the equilibrium:

- If $K > 1$, products prevail in the equilibrium reaction mixture.
- If $K < 1$, reactants prevail at equilibrium.
- Reactions with values of K near 1, are called typically reversible reactions.
- If the value of K is extremely high, it means that practically all reactants changed to products at equilibrium – these reactions are sometimes regarded as irreversible.
- On the other hand, if K is very low, the reaction almost does not proceed in the given direction. Thus, the reaction going on in the opposite direction is then preferred.

Reaction Quotient

The value of equilibrium constant K and the known actual (initial) concentrations of substances in the closed reaction system can indicate the direction of reaction. In such case, it is convenient to calculate the **reaction quotient** Q_r (see Chapter 3). It is calculated from the same equation like that used for K, however, we use the **non-equilibrium** (actual or initial) concentrations of the components in the reaction mixture instead of the equilibrium concentrations:

$$Q_r = \frac{[C]_i^c \, [D]_i^d}{[A]_i^a \, [B]_i^b}$$

where $[A]_i$, $[B]_i$, $[C]_i$, $[D]_i$ are instantaneous concentrations of substances.

The value of Q indicates what change will occur in order to reach equilibrium.

If $Q < K$, there are less products than there should be at equilibrium and the reaction will proceed from left to right ($A + B \rightarrow C + D$) to establish equilibrium (until $Q = K$).

If $Q > K$, the concentration of products is higher than that at equilibrium and the reaction will proceed from right to left ($A + B \leftarrow C + D$).

Factors Affecting the Equilibrium State

The equilibrium state of a system can be disturbed by stresses applied to the system such as changes in concentration and temperature. Some reactions occurring in a gaseous state may be affected by a change of pressure. The disturbance of the equilibrium state in any of the above-mentioned examples starts processes that tend to relieve the stress and establish a new equilibrium state (Le Chatelier principle).

· **Concentration**

The increase or decrease of concentrated reactants or products (at constant temperature and pressure) causes the change of the reaction rates v_1 and v_2, but the rate constants k_1 and k_2, and thus also the equilibrium constant do not change. The reaction proceeds in one or the opposite direction until equilibrium **with changed equilibrium concentrations, but an unchanged equilibrium constant.** The effect of the change of concentration of the reactant or product at equilibrium can be derived from the relation:

$$K_c = \frac{k_1}{k_2} = \frac{[C]^c [D]^d}{[A]^a [B]^b}$$

For example, if increasing the concentration of reactant A in the reaction mixture, the product [A] [B] will increase too. To maintain the constant value of K, the concentration of products [C] and [D] has to also increase. Thus, a portion of reactants A and B reacts to form products C and D.

· **Change of Temperature**

An increase in temperature favours whichever reaction requires heat (endothermic reaction) and a decrease in temperature favours the reaction that releases heat (exothermic reaction). The value of equilibrium constant K varies with changes in temperature.

· **Change of Pressure**

The change of pressure causes significant change in equilibrium only when the number of moles of gaseous products and reactants differ. An increase in pressure shifts equilibrium in the direction that produces the smaller number of molecules in the gaseous phase. The value of the equilibrium constant remains unchanged.

Factors influencing the formation of a new equilibrium and the equilibrium constant in the exothermic reaction ($\Delta H < 0$) in which substance A dissolved in an aqueous solution reacts with gas B to form gas C with stoichiometry:

A(aq) + 2 B(g) $\rightleftarrows$ C(g) + heat
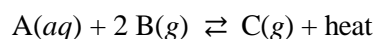
is shown in the following table:

Factors supporting the equilibrium shift to the right (formation of product C)	Change in K
Increasing the concentration of reactants A and B	no
Decreasing the concentration of product C	no
Increasing the pressure	no
Cooling the reaction mixture	yes

Applying opposite events, such as temperature increase or pressure reduction, the reverse reaction will be favoured.

Steady State

The true equilibrium states can be realised only in closed systems. However, the living organisms behave as open systems, i.e. they exchange both energy and matter with their surroundings and apparently equilibrium states, which are established in these systems, are of different character. The individual metabolic pathways are kept in a steady state by the fact that the activities of enzymes ensure the constant rate of transformation of substrates and thus create the stationary concentrations of intermediate products. If the system accepts and produces the same amount of matter and energy at the given time, the concentration of substances inside the system does not change and the **steady (stationary) state** is created**.**

6 Liquid Dispersions

Dispersion systems contain particles of one or more substances dispersed in the continuous (dispersing) phase that forms the major part of the system. Liquid dispersion systems have great importance for processes in living systems. The three types of dispersion systems are distinguished according to the size of dispersed particles.

Analytical dispersions (true solutions). The dispersed particles (small molecules, atoms, ions) are smaller than 1 nm. These are solutions of low molecular compounds, e.g. NaCl, glucose. Dispersed particles can only be proven in the systems by analytical methods.

Colloidal dispersions. The dispersed particles are smaller than the mean wavelength of near ultrared light (~ 1,000 nm) and greater than 1 nm. The dispersed particles do not settle spontaneously; they do not cross the semi-permeable membranes and are visible only in an ultramicroscope or an electron microscope. The colloidal dispersions are solutions of low molecular substances which aggregate into larger complexes, so called micelles or solutions of macromolecules ($M_r > 5,000$).

Coarse dispersion. The dispersed particles are greater than 1,000 nm, they are visible in the common microscope and they settle spontaneously. They include suspensions and emulsions.

The above-mentioned dispersion systems are not strictly distinguished; there is a smooth transition among them. Each system has characteristic physicochemical properties.

The selected properties of dispersion systems

Feature	True solution[a]	Colloidal solution	Coarse dispersion
Typical example	glucose in water	blood plasma	soil in water
Size of particles	< 1 nm[b]	1–1,000 nm[c]	> 1,000 nm
Visibility of particles	no	electron microscope	optical microscope
Sedimentation of particles	no	in ultracentrifugation	yes
Diffusion	quick	slow	not significant
Colligative[d] properties	significant	mildly significant	not significant
Transparency	yes	no (opalescence)	no (turbidity)
Proof of particles	analytical methods	electron microscope	optical microscope

[a] Analytical dispersion. [b] Ions, small molecules. [c] Macromolecules (e.g. proteins).
[d] Properties of the solution which depend on the amount of dissolved particles, but not on their properties.

The term analytical (true) solution (often only solution) belongs to the homogenous (one-phase) dispersion system. The dispersed phase is a dissolved substance (solute) and the dispersing phase is the solvent. The solute can be a gas, liquid, or solid. We are used to dealing with liquid solutions, where the solvent is a liquid, most often water (aqueous solutions).

7 Solubility of Substances

Particles of a dissolving substance are dispersed in the solvent during dissolution. In this process the forces which hold particles in the arranged solid/liquid state are interrupted and the dissolved particles are solvated. For example, during the dissolution of a solid substance, the molecules of a solvent with the help of attractive forces and the thermal movement "drag up" the molecules and ions of the dissolving substance from its crystalline structure and surround them with its oppositely charged groups – they solvate them. The dissolution is accompanied by **heat of dissolution,** which is absorbed by the solution from its surroundings (the solution is cooled down) or is released by the solution (the solution warms up). When the solid particles are dissolving, the solution usually cools down (endothermic process). Sometimes the amount of the captured heat is so large that the freshly prepared solutions are used as **freezing mixtures**, e.g. mixing of crushed ice (0°C) and NaCl in the ratio 3:1 cools the mixture down to −21°C.

Forces acting between solvent molecules and solute particles depend on their chemical properties. In case of non-polar substances or solvents these are van der Waals forces, in case of more polar substances we can find electrostatic interactions or hydrogen bonds. Ionic compounds are easily dissolved in polar solvents (water) where the electrostatic ion-dipole interactions take place. During dissolution, polar substances dissociate in polar solvents (water) – see Chapter 14.

Analytical solutions are formed by the spontaneous dissolving of one substance in the other. The ability of a substance to be dissolved in a given solvent (**the solubility of substances**) depends on the chemical characteristics of solute and solvent, on temperature, in gases also on pressure. Substances of certain polarity easily dissolve in solvents with similar polarity (*similia similibus solvuntur*, like dissolves like), e.g. polar substances in water. Therefore, we divide solvents according to their polarity into polar and non-polar. The quantitative measure of the solvent's polarity is the dipole moment of its molecules.

The typical polar solvent is water; non-polar solvents include hexane, benzene, diethyl ether, tetrachloromethane. Both types of solvents dissolve substances that which contain a polar and non-polar part (e.g. hydroxyl-derivatives, carboxylic acids, amines) in their molecule. In case of higher alcohols, amines and carboxylic acids, where the non-polar part prevails, solubility in non-polar solvents increases and in polar solvents decreases. Organic compounds containing –OH or –NH_2 groups are dissolved in solvents that can form hydrogen bonds with their molecules, e.g. in water, alcohols.

The amount of dissolved substance is in thermodynamic balance with the un-dissolved substance in the pure state. Solubility is the maximal amount of a substance which dissolves in a given amount of solvent at the given temperature to form a **saturated solution** (e.g. grams of a substance in 100 grams of solvent). The solubility of poorly soluble substances is given by the **solubility product** (see Chapter 17). Solubility of the solid substances depends on the type of solvent and on temperature; the solubility of gases also depends on pressure.

Solubility of solid substances depends on their heat of dissolution and temperature. Solubility of substances, which absorb heat when dissolved in the given solvent, increases with the growing temperature. On the other hand, in case of substances which release heat, solubility in the given solvent decreases with the growing temperature. Solubility of substances with very small heat changes during dissolution (e.g. NaCl) does not strictly change with the temperature.

8 Quantitative Composition of Solutions

The quantitative composition of solutions can be expressed in many different ways, see the table below.

The different types of concentrations (substance B)

Quantity	Symbol	Definition[a]	Units
Molarity	c_B	n_B/V	mol l^{-1}, mmol l^{-1}
Mass concentration	ρ_B	m_B/V	g l^{-1}, mg l^{-1}
Mass fraction	w_B	m_B/m	kg kg^{-1}, g g^{-1}
Volume fraction	φ_B	V_B/V	l l^{-1}, ml ml^{-1}
Molality	c_{mB}	n_B/m_r	mol kg^{-1}

[a] V volume of the solution, m mass of the solution, m_r mass of the solvent.

Molarity defines the substance amount n_B of the solute B in the given volume of the solution (V). The molarity of B is specified by the symbol c_B, $c(B)$, or [B].

$$c_B = \frac{n_B}{V}$$

We usually express the molar concentration in mol l^{-1} (mmol l^{-1}, µmol l^{-1}). In British texts, the unit mol l^{-1} is specified by the symbol M, thus e.g. the solution NaOH with concentration 0.5 mol l^{-1} can be expressed as 0.5 M NaOH.

Molarity depends on the temperature as the volume of a solution changes with temperature. The molar concentration of aqueous solutions decreases with the increasing temperature. Solutions of equal molarity contain the same number of molecules and ions of solute in the same volume.

In clinical biochemistry, the distribution of ions in the body fluids (ionograms) is expressed by the **chemical equivalents**. A chemical equivalent is such molar quantity that is equivalent to 1 mole of protons and electrons. For example, the concentration of Ca^{2+} in serum is 2.5 mmol l^{-1}, which is related to 5 mmol of chemical equivalents in 1 litre. It is because 2.5 mmol of Ca^{2+} could be formally substituted by 5 mmol of protons. Similarly, 1 mol of SO_4^{2-} is adequate to 2 moles of chemical equivalents, l mol of PO_4^{3-} to 3 moles of chemical equivalents, etc.

The molality defines the substance amount of the solute (n_B) in the given mass of the solvent (m_r). The unit of molality is mol kg^{-1}. Molality does not depend on temperature, because the mass of the solvent is not influenced by the temperature (see Chapter 10).

$$c_{mB} = \frac{n_B}{m_r}$$

The mass concentration defines the mass of the solute B (m_B) in the given volume of the solution (V). The mass concentration is mostly used in a case when M_r is unknown, e.g. to express the total concentration of proteins in blood serum. The mass concentration in solutions is usually related to 1 litre, e.g.

the amount of ions in mineral waters is given in mg l^{-1}, the highest acceptable concentration of toxic substances in the wastewaters in µg l^{-1}. The concentration in gaseous mixtures is referred to 1 m^3.

$$\rho_B = \frac{m_B}{V}$$

Density (ρ) defines the mass of a substance (e.g. solution) divided by its volume, $\rho = m/V$.

The mass fraction (w_B) is defined as the ratio of the mass of the solute B (m_B) to the total mass of the solution (m). The mass of solute and solution is put in the same units. The composition of solutions expressed by the mass fraction is not dependent on temperature.

$$w_B = 100\,\frac{m_B}{m} \quad \% \,(w/w)$$

The mass fraction is usually expressed in **mass per cents**. It is the mass of a substance in grams, which are dissolved in 100 g of the solution (mass %).

$$w_B = 100\,\frac{m_B}{m} \quad \% \,(w/w)$$

Low concentrations in the sample can be expressed in **per mills** (e.g. one thousandth of the total mass, e.g. the mass of a component in grams in 1 kg of the solution), 1‰ = 0.1%:

$$w_B = 10^3\,\frac{m_B}{m} \quad \‰\,(w/w) \qquad\qquad w_B = 10^6\,\frac{m_B}{m} \quad ppm\,(w/w)$$

Very low concentrations (mg of a substance in 1 kg of the solution) are expressed in **ppm** (parts per million, i.e. one millionth of the total amount). This method is usually used to evaluate the degree of pollution of, e.g. the atmosphere, waters.

The volume fraction (φ_B) is the ratio of the volume of the solute to the total volume of the solution. It typically expresses the composition of liquids and gases.

$$\varphi_B = \frac{V_B}{V} \qquad\qquad \varphi_B = 100\,\frac{V_B}{V} \quad \% \,(v/v)$$

The volume of component B (V_B) and the total volume of a mixture (V) must be measured under the same conditions (temperature, pressure) and they have to be put in the same units into the relation. The volume fraction can be expressed in per cents (vol. %) or in ppm (v/v).

If the composition of a solution is expressed in the form of a fraction and no symbol of the corresponding quantity is presented (i.e. w or φ) or values are not accompanied by corresponding symbols w/w, w/v, v/v) and the dissolved substance is in the pure state solid, we talk about the mass fraction/per cent. On the other hand, if the dissolved substance is in pure state liquid / gaseous, we talk about volume fraction / per cents. For example, 12% alcohol in wine means, that in 1 litre of wine we can find 120 ml of ethanol, thus the volume per cents. In contrast, 0.9% of sodium chloride solution is given in mass per cents.

9 Colloidal Solutions

Colloidal systems exhibit characteristic properties resulting mainly from the size of dispersed particles. The colloidal particles penetrate through ordinary filters, but not the semipermeable membranes, visible in an ultramicroscope, that scatter the passing light and exhibit only minimal colligative properties. The colloidal particles often carry an electric charge on their surfaces that is of the same sign and prevents their aggregation (the stability of the colloidal systems is increased by the mutual repulsion of the equally charged particles). Addition of electrolytes can result in the coagulation of colloidal particles, because the charge on their surface can be neutralised by the effect of the oppositely charged ions.

Types of Colloidal Dispersion Systems

According to the number of phases the colloidal dispersion systems are divided into **heterogeneous** dispersions (properties are similar to coarse dispersions) and **homogenous** dispersions (colloidal solutions – properties are similar to true solutions).

According to the affinity of dispersed particles to the dispersing medium, we distinguish lyophobic and lyophilic colloidal dispersions. As the most common dispersing medium is water, we use the expressions hydrophobic and hydrophilic colloids. Additionally, the hydrophilic colloids are further divided according to the character of dispersed particles into micellar and molecular dispersions.

Hydrophilic colloidal solutions are created by the spontaneous dissolution of solid substances in water. They are similar to true solutions in the way of their formation and stability. Dispersed particles are surrounded by the molecules of solvent (they are solvated) and thus stabilised. The solid phase obtained by the evaporation of the solvent can be transformed into the colloidal solution again by the addition of the pure solvent – these colloids are reversible. If the dispersed particles are macromolecules, we speak of **molecular colloids**, e.g. the solutions of proteins, nucleic acids or polysaccharides. If the dispersed particles are aggregates of low molecular substances (micelles), which are created by the spontaneous reversible association of molecules, we speak of **micellar colloids**. The stability of the micellar colloids is much lower compared to molecular colloids, because micelles are only stabilised by weak van der Waals forces.

Structure and Stability of Molecular Colloids

Macromolecular substances, e.g. proteins, create aqueous colloidal solutions, if they contain polar or ionisable groups in their structure. The typical colloidal solution is blood plasma, which contains dissolved proteins (albumin and many other). The solubility of macromolecules in water depends on the ratio of hydrophilic and hydrophobic groups, and their spatial distribution. For example, in case of proteins it is the side chain of individual amino acids. The macromolecule is arranged in such way in the solution that its hydrophobic parts, which do not have the tendency to be hydrated, are oriented to the centre of the colloidal particle, whereas the polar groups are exposed on the surface of the particle and they interact with water. The presence of polar or ionisable groups on the surface of the colloidal particles triggers their solvation. They are covered by an electric bilayer from the ions of some electrolytes neutralising the charges of the colloidal particle.

The inner layer of the electric bilayer is associated with the surface of the particle. It follows the movements of the particle; it is called the adsorption layer.

The outer layer contains mostly ions of the opposite charge than those in the inner layer, the ions are orientated more freely (diffuse layer). This layer creates an intermediate phase between the colloidal particle and dispersion environment.
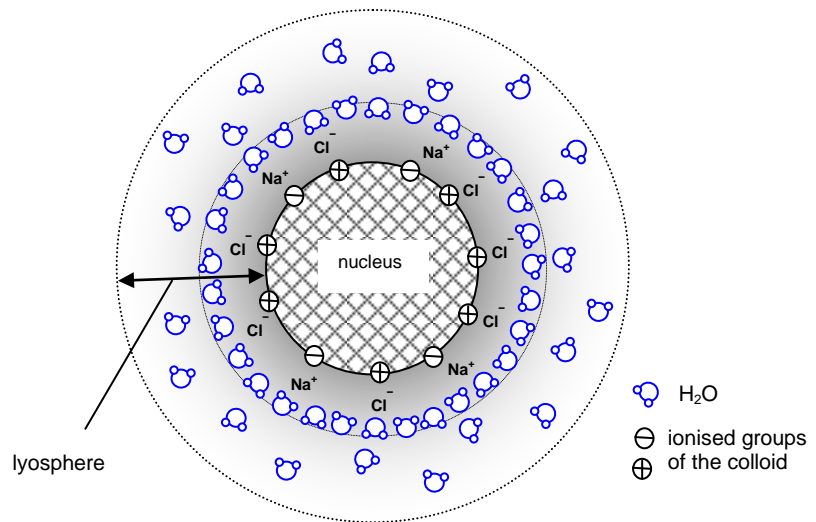
Colloidal solution stability depends on the electric charge on the surface of colloidal particles, which prevents the charged particles from aggregation, and on the solvation. If the stabilising factors are removed, **coagulation** follows and the precipitate is formed.

Stability of colloidal solutions is influenced by the presence of electrolytes. Slight addition of an electrolyte increases the stability of colloidal solution (**salting in effect**); at a certain concentration of the neutral electrolyte, the colloid has the highest solubility. When the salt concentration is increased, some solvent molecules are attracted by the salt ions, which decrease the number of solvent molecules interacting with the charged part of the colloid. As a result of the competition between the added salt ions and the other dissolved solutes for water molecules, the solute-solute interaction is stronger than the solvent-solute interaction; the colloid coagulates by forming hydrophobic interaction with each other to stabilise themselves. This process is known as **salting out**. The technique of salting out is used to separate a range of macromolecules, especially proteins.

For example, the proteins of blood plasma can be separated by the precipitation with the saturated ammonium sulfate solution; solutions of ammonium sulfate with different ionic strength are used for so-called fractional salting out of proteins. The salting out effect of an electrolyte is connected with its hydration, which depends on the charge and size of the ion. Salting out is usually a reversible process, the coagulum can be turned into the solution again by dilution.

Dehydration of the colloidal particle can also be achieved by the addition of organic solvents with high affinity to water, e.g. ethanol and acetone. At temperatures near 0°C, this process is reversible.

The charge of the amphoteric colloidal particles, e.g. proteins, depends on the ionisation of the polar groups, which is influenced by pH. At pH of the isoelectric point, the total charge of particles is zero, and their stability is lowest, the increase or decrease of pH increases the charge of particles and thus the stability of the colloidal solution.

Colloidal solution stability also depends on its concentration. More concentrated solutions of molecular colloids are less stable and more easily coagulate. **Coacervation** is the separation of the colloidal solution into two liquid phases, which usually differ in the concentration of macromolecule.
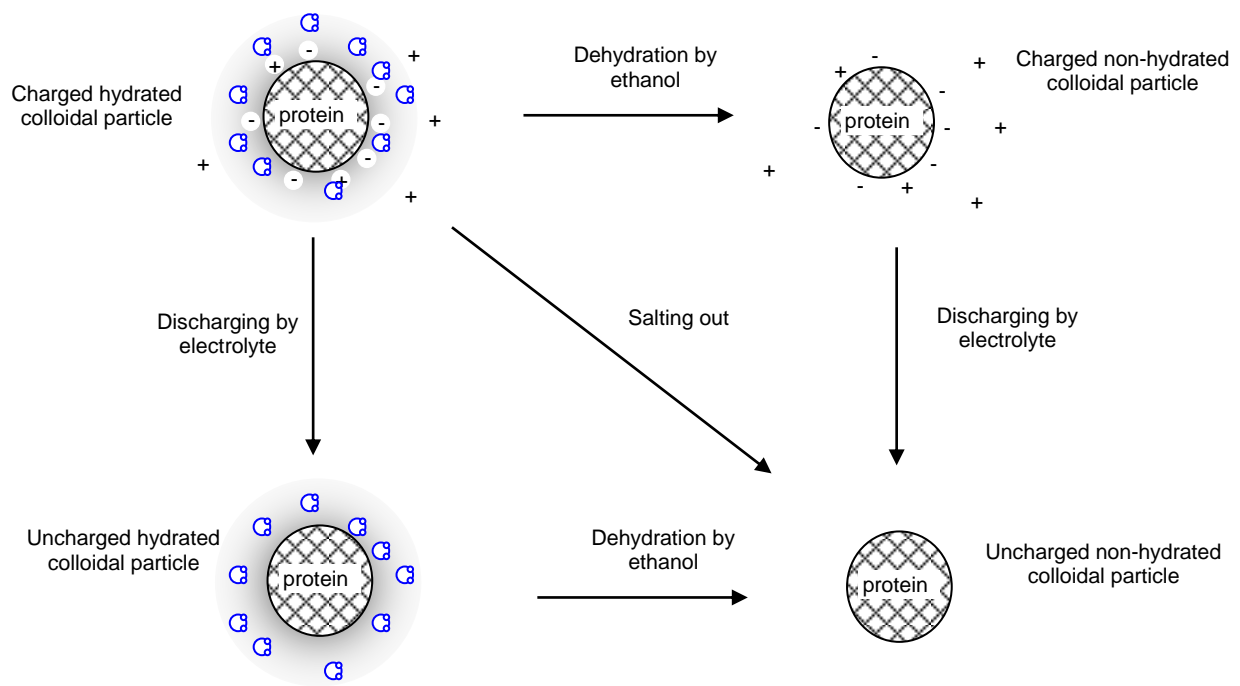
Figure: The coagulation of molecular colloids

Gels

Under certain conditions, macromolecular hydrophilic particles can form gels. Gels are colloidal dispersions, where the solute creates a three-dimensional network in which liquid is trapped thus limiting the movements of the whole system. Gels are of a jellylike nature, they exhibit certain mechanical properties of solid substances as well as the typical properties of liquids. Gels are formed by the thickening or cooling of the sufficiently concentrated solutions. For example, cooling of about 2% hot agar solution (polysaccharide of seaweed),

gelatine (denatured collagen), or starch (polysaccharide) creates the corresponding gel. Gels can also be formed through swelling – a solid polymer absorbs solvent, the network structure is formed with the slight increase of volume.
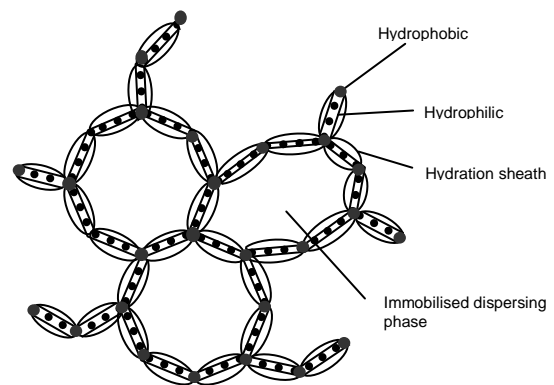
Many gels change properties with time; they are aging. The spontaneous shrinking of a gel with expulsion of liquid is called syneresis. If the gel is dried up, we obtain **xerogel**.

The gel structures of biopolymers are used in a range of fields. The agar gels are used in microbiology to prepare cultivating media and in immunology; they form the matrix for the diffusion methods. The starch gel serves as the carrier for the electrophoretic methods. Food products with the gel base include jellies, puddings, and jams. Moreover, some medical and cosmetic preparations are in the form of gel. In the organism, fibrin gel is formed in plasma during blood coagulation.

Properties of Colloidal Solutions

Optical properties. Colloidal particles have linear dimensions often comparable to the wavelength of the visible light. If the refraction index is different from that of the solvent, the light is dispersed on the surface of these particles – they scatter light and the system is opaque. Very small colloidal particles primarily disperse light with the shorter wavelength, i.e. blue and violet (blue colour of the sky, bluish coloration of diluted milk).

The light scattering is used to determine the concentration of colloidal dispersions. **Nephelometry** measures the decrease of the intensity of light scattered at a given angle (usually 70° or 75°), **turbidimetry** measures the reduction in the intensity of the light beam. Nephelometric and turbidimetric methods can be used not only to determine the concentration, but also to determine the relative molecular mass, size, and shape of the colloidal particles.
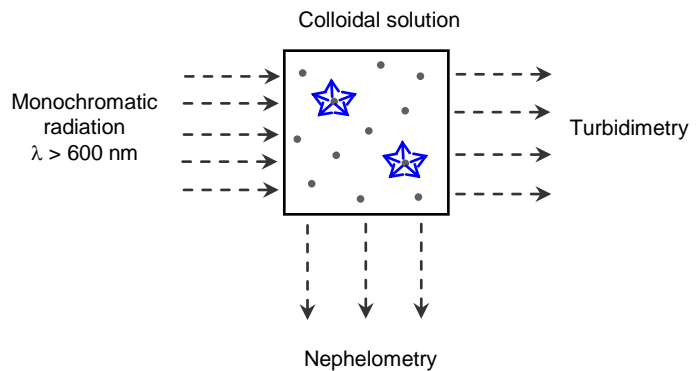
Figure: Principle of light scattering methods

Dialysis

Colloidal particles can be separated from smaller particles. For this purpose, we use **dialysis** – diffusion of small molecules and ions from the colloidal solution across **the dialysing membrane** (e.g. cellophane) into the pure solvent. The dialysing membrane only transmits the molecules of solvent and other small molecules, macromolecular substances do not pass the dialysing membrane because of their size and low speed of diffusion. This principle is used to purify blood in an artificial kidney, where the patient's blood is purified from the low molecular substances, especially urea which passes to the dialysing solution.
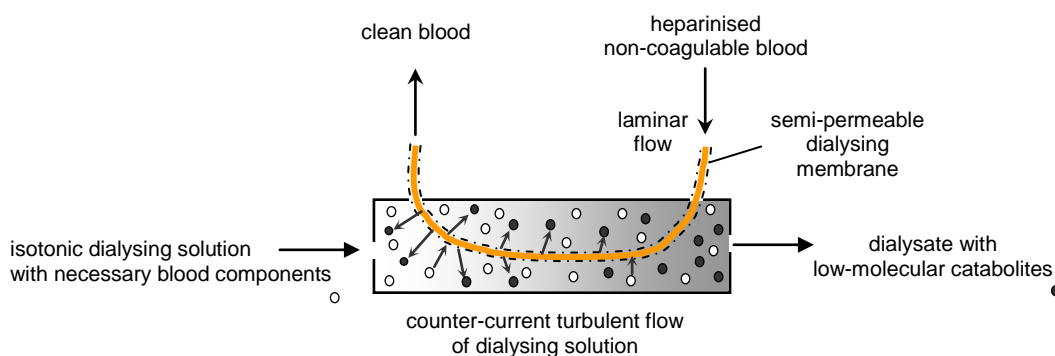
Figure: Principle of artificial kidney

10 Osmotic Pressure

Diluted solutions exhibit **colligative properties** that depend only on the number (quantity) of solute particles, not on its chemical quality. The colligative characteristics include:

- the lowering of vapour pressure over solutions of non-volatile substances, which is presented by the elevation of the boiling point

- depression of the freezing point (1 mole of solute decreases the freezing point of an aqueous solution by $-1.86°C$)

- the osmotic pressure.

The method based on the measurement of the boiling point elevation is **ebullioscopy**, measurement of the freezing point depression is **cryoscopy**, and the measurement of osmotic pressure is **osmometry**.

Any of the colligative properties can be used to determine the molar masses of non-volatile dissolved substances, mainly macromolecular substances. The relations for colligative properties were derived from the molality, because its value does not depend on the temperature and it states the constant ratio between the number of molecules of the solute and the solvent. In case of diluted aqueous solutions, when the density approximates to 1 kg dm^{-3} the molar concentration can be used instead of molality.

Osmotic pressure

If we separate the solution of a substance from the pure solvent (water) by the semipermeable membrane, which only allows the passage of solvent molecules and not solute molecules, then the molecules of solvent will penetrate into the solution. The solution becomes gradually diluted and increases its volume. This spontaneous dilution of the solution by the penetration of solvent molecules through the semipermeable membrane is called **osmosis**. **Osmotic pressure** (Π) is defined as the external pressure exactly sufficient to oppose osmosis and stop it.
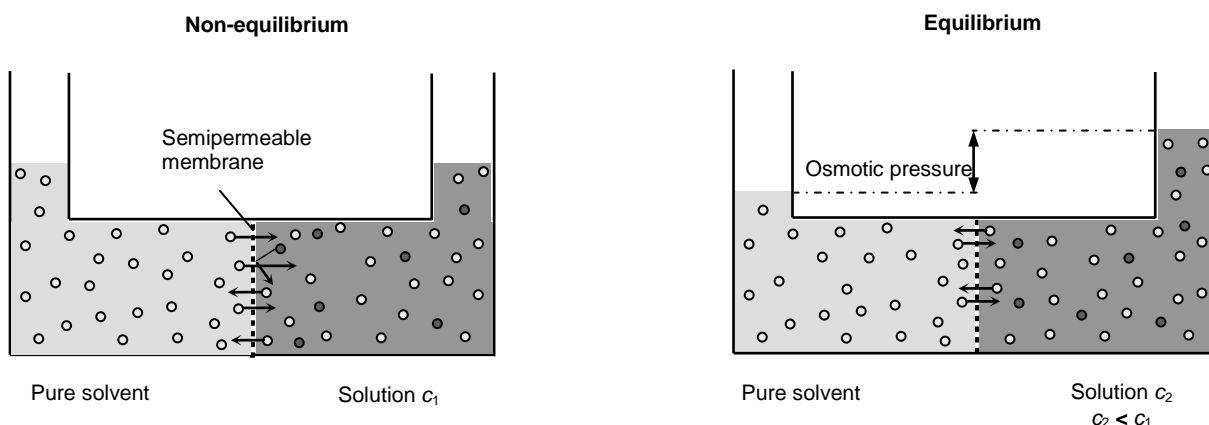
Figure: Principle of osmosis

Osmosis can be reversed by applying a pressure greater than the osmotic pressure. This process is called **reverse osmosis**. The principle of reverse osmosis is used for the preparation of drinking water (desalination of saltwater) or highly pure water and for the purification of extremely contaminated wastewaters.

The approximate expression for **osmotic pressure** (kPa) in dilute solution is as follows:

$$\Pi = i\,c\,R\,T$$

where i is the number of particles resulting from the dissociation of one molecule of the solute, c is the molarity of the solution (mol l^{-1}), R is the ideal gas constant (8.314 J mol^{-1} K^{-1}) and T is the absolute temperature (K).

If the solute is a non-electrolyte, $i = 1$.

If the solute is ionised, then in the highly diluted (ideal) solutions, each ion is osmotically active. For a strong electrolyte $i = 2, 3, \ldots N$, where N is the number of ions produced by dissociation of one molecule. For a weak electrolyte the value of i depends on the degree of dissociation. If there is an association of particles of solute in a solution, then $i < 1$, e.g. during the formation of dimers $i = 0.5$.

Expressions for the osmotic pressure are derived for the ideal solutions; in real solutions we have to take into account the decrease of activity of particles caused by ionic interactions.

In practice, to evaluate the osmotic pressure we use two ways of expressing the concentration of the osmotically active particles: osmolarity and osmolality.

Osmolarity (SI unit mmol l^{-1}) corresponds to $i\,c$ from the expression for the calculation of osmotic pressure. It is only an approximate value; it does not consider the activity coefficients of ions. It can be easily calculated from the molarity of the main solutes in the solution. It is always greater than the real property of the solution (osmolality). For example, NaCl solution with osmolarity of 308 mmol l^{-1} is isotonic with blood plasma, whose osmolality is about 285 mmol kg^{-1} H$_2$O.

Osmolality (mmol kg^{-1} H$_2$O) is a thermodynamically more exact quantity for osmotic properties of solutions, independent on the temperature. It is determined by the measurement of the freezing point depression or by the direct measurement of osmotic pressure. The quantity determined in this way includes the differences between real and ideal solutions (it reflects the real activity of solutes). The osmolality of blood serum in healthy people is between 275–295 mmol kg^{-1} H$_2$O.

In clinical practice, the osmolality of blood plasma is estimated from the molarity (mmol l^{-1}) of the main plasma components. Many empirical relations have been proposed, for example:

blood plasma osmolality (mmol kg^{-1} H$_2$O) $\approx$ 2 [Na$^+$] + [glucose] + [urea]

blood plasma osmolality (mmol kg^{-1} H$_2$O) $\approx$ 1.86 [Na$^+$] + [glucose] + [urea] + 9.

These calculations are also carried out in case we know the value of osmolality from the experimental measurements. A significant difference between the approximate calculation and the measured value (**osmolar gap**) implies the presence of the larger amount of low molecular non-ionised substances, which are not normally present in plasma, e.g. alcohol, acetone, ethylene glycol. The osmolar gap thus indicates the concentration of the toxic substance in plasma if there is no method for their direct determination available.

Molecules of blood plasma proteins, especially albumin, moderately contribute (~ 0.5 %) to the total osmotic pressure of blood plasma. The capillary wall, which separates plasma from the interstitial fluid (ISF), behaves in rough approximation like a semi-permeable membrane (impermeable for proteins, permeable for water and low molecular substances). The concentration of proteins is therefore higher in blood plasma than in ISF. The capillary wall is thus exposed to **colloidal-osmotic (oncotic)** pressure, which is about 3 kPa. The oncotic pressure is highly important for the transport of water and low molecular compounds (catabolites) from the interstice into capillaries. The decrease of protein concentration in the blood decreases oncotic pressure, resulting in fluid accumulation in the interstice of peripheral tissues and formation of oedemas.

Biological membranes are not semipermeable in the right sense of the word, because they are relatively permeable for both the solvent and many dissolved low molecular weight substances, e.g. urea and alcohol. These compounds do not contribute to the osmolality (**effective osmolality, tonicity**), which is responsible for the movement of water across cell membranes.

If we consider effective osmotic pressure on the biological membrane, which is given by the concentration of solutes not penetrating across membrane, we distinguish three types of solutions. If the solutions on both membrane sides have the same effective osmotic pressure, they are **isotonic**, the solutions with a lower concentration of non-penetrating particles are **hypotonic**, and the solutions with a higher concentration are **hypertonic**. Water passes across biological membrane always from the hypotonic environment to the hypertonic compartment until effective osmotic pressures (tonicity) on both sides of the membrane are equal.

The **effective osmotic pressure** is very important for the movement of water between the various compartments of the body. It is worth noting, e.g. active reabsorption of ions and nutrients from the intestinal lumen, or water absorption from the proximal tubule into the blood in the kidney, or therapeutically administered mannitol, which is not absorbed by the kidney, acts as an effective diuretic.

Isotonic solutions with biological fluids contain solutes not escaping from solutions into the cells (mostly electrolyte solutions). In medicine, it refers to solutions with the same osmotic pressure as the blood plasma, e.g. the **saline solution** containing 154 mM NaCl. In this sense, isotonic solutions containing only substances easily penetrating across cell membranes (e.g. urea) or substances used as a source of energy (e.g. glucose) cannot be used, because these solutions gradually become hypotonic in the presence of cells.

The effective osmolality (tonicity) of blood plasma is estimated from the empirical relationship:

Effective osmolality of plasma (mmol $kg^{-1}H_2O$) $\approx$ 2 $[Na^+]$ + [glucose].

The relation is valid if there are no other exogenous osmotically active substances present, for example mannitol for therapeutic purposes.

11 Coarse Dispersions

The stability of coarse dispersions depends, similarly to lyophobic colloids, on the electric charge of dispersed particles, which prevents their aggregation. They are usually precipitated irreversibly with a small amount of electrolyte. The stability of the coarse dispersion can be increased by the addition of surfactants. The dispersion of light on the particles causes turbidity, observed in any direction.

Suspensions are coarse dispersions formed by dispersing solid particles (poorly soluble) in the liquid medium. A concentrated suspension is a **paste**. Dispersed particles easily settle. The addition of a stabilising agent (protective colloids) can decrease the speed of sedimentation. In the biological systems, the stabilisation of insoluble salts by proteins is important (e.g. calcium hydrogen phosphate in milk). **Blood** is the suspension of blood cells in plasma, which forms the dispersing medium. Some **drugs** are applied in the form of suspension, e.g. suspension of aluminium and magnesium hydroxide in water for reduction of gastric acidity. **Toothpastes** are also examples of suspensions, used every day.

Emulsions are coarse dispersions formed from two mutually immiscible liquids, where one liquid is dispersed in the form of small droplets in the other one. According to the polarity of phases we recognise emulsions of two types: *o/w* (oil in water) or *w/o* (water in oil); generally, *w* is used for the more polar phase, *o* for the less polar phase. In the first example, the polar liquid creates a continuous dispersion medium and non-polar liquid is dispersed. In *w/o* emulsions, the phases are reversed. Because of large difference between the refraction indexes of both liquids, the emulsions are opaque.

The formation and stability of emulsions can be increased by the addition of **emulsifying agents** (**emulsifiers**). The emulsifiers usually decrease the surface tension on the interface between the polar and non-polar liquid, thus they act as surface-active substances (surfactants).

Some electrolytes (thiocyanates, iodides) can influence the surface potential; some molecular colloids (proteins, gelatine, Arabic gum) increase the viscosity of the dispersing medium; both decrease the thermal motion of droplets and stabilising emulsions.

Many natural emulsions are known, e.g. milk (type *o/w*, emulsifier casein), butter (type *w/o*), rubber latex, etc. The emulsions are formed in the intestines of animals, where the fats ingested in food are emulsified in order to be effectively digested (emulsifiers are the salts of bile acids and fatty acid ions formed by lipolysis). Some drugs are applied in the form of emulsions, especially in the form of ointments. Ointments are emulsions of either type *o/w* (hydrophilic, acting on the surface, they can be washed up by water) or the type *w/o* (fatty, semisolid, incorporated drugs penetrate deeper into the skin). Cosmetic preparations, like hydrating ointments and skin lotions, are emulsions of *o/w*. On the other hand, the protective ointments used to treat the dry cracked skin are of *w/o* type.

Examples of different emulsion types and their composition

Emulsion	Type	Polar phase	Non-polar phase	Emulsifier(s)
Milk	*o/w*	water (88%)	milk fat (4%)	casein, phospholipids
Butter	*w/o*	water (20%)	milk fat (80%)	casein, phospholipids
Margarines	*w/o*	water (20–40%)	vegetable fat (60–80%)	mono/diacylglycerols, lecithin

12 Adsorption and Adsorbents

When two different phases of matter are in contact (solid/liquid, liquid/gas), they share a surface called an **interface**. On a surface, molecules have neighbours of the same type only on one side. Thus, surface molecules are different from those in the bulk.

Bulk molecules have neighbours in all directions. Forces acting on the molecules in the bulk are equal in all directions (and thus the resulting force is zero), whereas molecules, which are on the surface, are affected by the intermolecular forces asymmetrically (only from the inside of the phase, molecules are attracted to the centre of the phase).

Adsorption is the surface phenomenon. Matter is extracted from one phase and concentrated at the surface of a second phase. The processes can occur at an interface between any two phases, such as, liquid-liquid, gas-liquid, or liquid-solid interfaces. A substance, which is able to concentrate another substance on its surface, is an **adsorbent**. Adsorption is reversible; the opposite process is called **desorption**.

Figure: Forces acting on the interface

Solid adsorbents are chemically inert substances with a large specific surface (10–1,000 m^2g^{-1}). They contain very gently dispersed particles or they are porous. They are capable of binding large quantities of other substances from the solution or gas, with which they are in contact, on their surface. The amount and kind of adsorbed substances depends on temperature (inversely), on the structure of adsorbent (mainly on polarity and the specific surface), and on the structure of substance being adsorbed. The interaction between the adsorbent and substance adsorbed is based on non-covalent interactions.

We distinguish two main types of adsorbents: polar and non-polar.

Polar adsorbents mainly bind polar substances on their surface. Therefore, they can be used for the adsorption of polar substances dissolved in non-polar solvents. Water is polar to such extent that it takes the majority of the surface of the polar adsorbent and deactivates it. Therefore, some polar adsorbents are used as drying agents (e.g. removing water from the organic solvents, removing moisture from the air). Amongst the most common polar adsorbents, we can find silica gel (dried gel of silicic acid), aluminium oxide, and a range of modified silicates and aluminosilicates.

Non-polar adsorbents adsorb non-polar substances from the more polar medium (e.g. from aqueous solutions). Solvents with lower polarity like, e.g. alcohols decrease their adsorption ability. The known non-polar adsorbent is charcoal (*carbo adsorbens*). It is an elementary carbon in the graphite form, prepared by the carbonisation of wood or sawdust.

The adsorption principle is used in many fields. Some adsorbents are used in medicine. Charcoal and hydrated aluminium-magnesium silicate (Smecta[®]) are used as effective intestinal adsorbents in case of acute and chronic diarrhoea of different origin. The adsorption coal is also applied in case of poisoning, especially drug poisoning.

A very important application of adsorption is the adsorption chromatography (see Practicals).

13 Surfactants

A **surfactant** is an organic substance that can cumulate even in low concentration at the interface of phases (liquid/liquid or liquid/gas) and decrease the surface tension of liquid. Molecules of the surfactant have a common structural feature: a long non-polar chain (tail) and a very polar, often ionised group (head).

non-polar part (tail)   polar part (head)
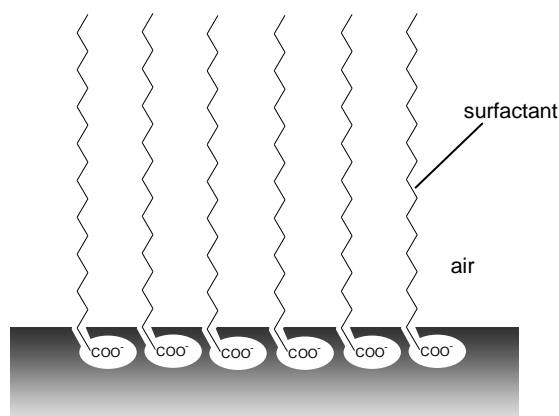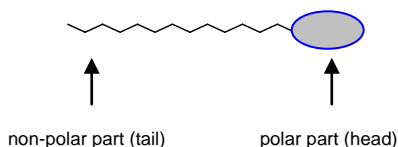
Figure: Surfactant (soap) on the surface of water

Surfactant molecules are arranged in such way that the non-polar parts of the molecule are directed towards the non-polar phase, whereas the polar parts immerse in the polar phase.

For example, when a small amount of surfactant (e.g. soap) is added to water, its molecules are adsorbed on the interface of phases (surface of water) to form a monomolecular layer. If the concentration of the surfactant in the solution gets higher, the surface of the liquid becomes fully saturated with the surfactant and the concentration of free molecules inside the phase will increase. After exceeding the critical micellar concentration, the molecules of a surfactant will form spherical aggregates of colloidal dimensions, called **micelles**. In a micelle, in aqueous solution, the surfactant molecules will be oriented with their non-polar ends towards the centre and polar ends facing out. As a result, a colloidal solution will be created.

Surfactants exhibit several practically useful effects:

Solubilising effects. When the concentration of the surfactant is so high that a sufficient amount of micelles is created in the aqueous solution, a limited amount of the dispersed non-polar phase can be picked up and incorporated into the interior of the micelle. The non-polar phase may include, e.g. particles of grease, oil, or other types of "dirt" insoluble in water. Cleansing and washing agents act on this principle. The system maintains the properties of the col-

Figure: Micelle in aqueous solution

loidal solution at the same time. When the increasing size of the micelles exceeds the dimensions of the colloidal particles, the colloidal solution is transformed into emulsion.

Emulsifying effects. Surfactants can also concentrate on the interface of two immiscible liquids (e.g. water/oil, water/paraffin). Coarse emulsions created by shaking water and oil are unstable. When a surfactant is added into a water-oil mixture, and shaken, a stabilised emulsion is created.

Cytotoxic effect. Some surfactants invade the cell membranes and destroy, e.g. bacteria. They are used for the disinfection of hands, surfaces, etc.

Structural Types of Surfactants

According to the character of the polar part of the molecule, we distinguish ionic and non-ionic surfactants. Ionic surfactants are then divided into anionic and cationic.

Anionic surfactants are the most common surfactants. **Soap** (sodium salt of C_{12}–C_{18} fatty acids) is the oldest surfactant. It is the main component of toilet bar soaps and some washing powders. A big disadvantage of soaps is the fact that in hard water they create insoluble magnesium and calcium salts. Toilet soaps also contain other substances improving their utility properties.

sodium stearate (sodium soap)
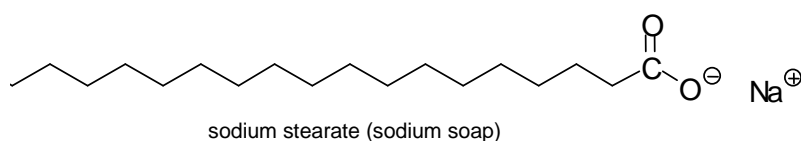
Fatty acids are also released in the small intestine during the hydrolysis of dietary fats by the action of pancreatic lipase. Under alkaline pH in the intestine lumen, they are partially dissociated into anions that contribute to emulsifying fat. For proper lipid digestion in the small intestine, bile acids are needed, which also belong to the group of anionic surfactants.

glycocholate

bile acid pictogram

non-polar steroid skeleton

polar groups
(−OH and −COO⁻)
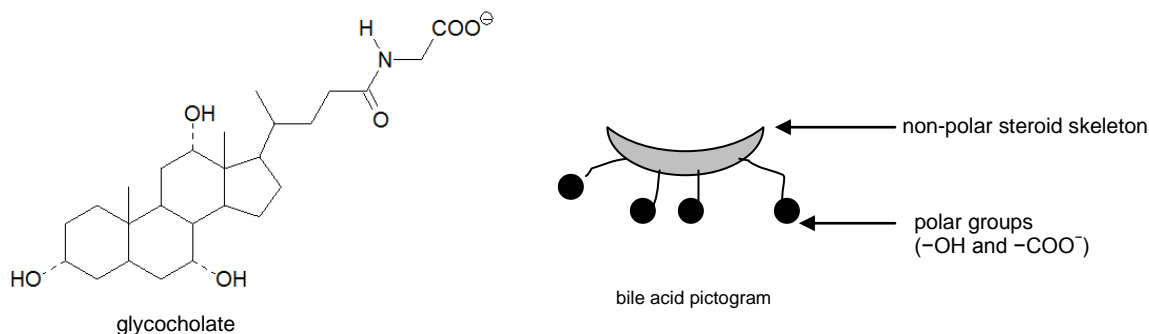
Synthetic anionic surfactants include sodium alkyl sulfates ($R-O-SO_3^-$ Na^+) and sodium alkane sulfonates ($R-SO_3^-$ Na^+) with C_{12}–C_{18} alkyl group.

sodium alkyl sulfate
(sodium octadecyl sulfate)

sodium alkane sulfonate
(sodium octadecane sulfonate)
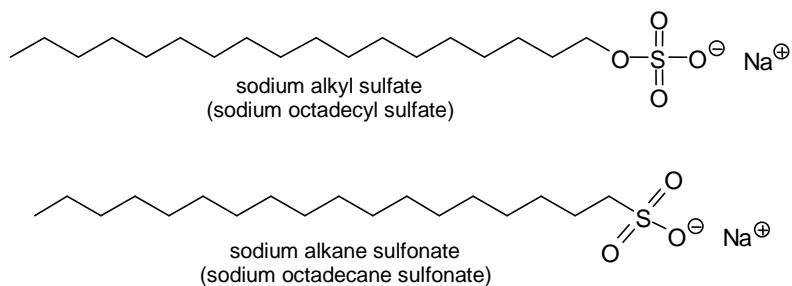
Cationic surfactants include the quaternary ammonium salts. These surfactants are the main component of fabric softeners and antistatic preparations for washing, while in cosmetics they are used in hair conditioners. They also have microbicidal properties, some of which are used as antiseptics acting especially against gram-positive bacteria. Two very common examples of antiseptics are:

- benzyldodecyldimethylammonium bromide (Ajatin),

- trimethyl[1-(ethoxycarbonyl)pentadecyl]ammonium bromide (Septonex).

Cationic surfactants cannot be combined with anionic ones as they destroy their antiseptic and emulsifying effects by formation of insoluble aggregates.

Ajatin
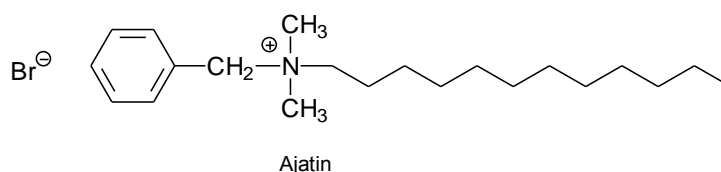
Amphoteric surfactants contain in the polar part of the molecule both a positive and negative charge. From the natural substances, they include soluble proteins and phospholipids (phosphatidyl cholines, phosphatidyl serines, sphingomyelins). Synthetic amphoteric surfactants are the derivatives of betaine and are widely used in shampoos, liquid soaps, and similar products.

phosphatidyl choline (lecithin)
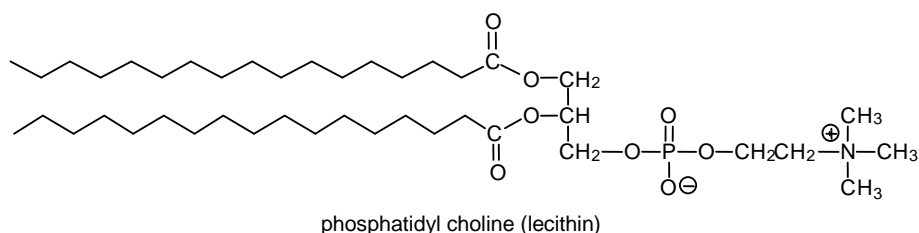
Non-ionic surfactants do not carry a charge. They include, e.g. esters or ethers of polyethylene glycol, or alkylpolyglycosides, higher alcohols, monoacylglycerols, from natural saponins substances (glycosidic surfactants contained in plants).

polyethylene glycol ether
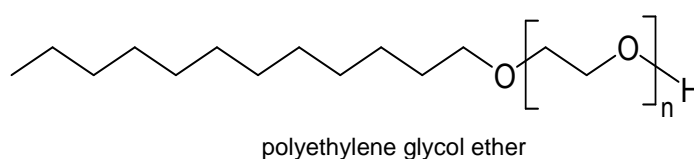
Types of surfactants

Surfactant	Polar part of the molecule	Examples
Anionic	anion (+ accompanying cation)	soaps, alkyl sulfates, alkane sulfonates, bile acids
Cationic	cation (+ accompanying anion)	quaternary ammonium salts
Amphoteric	anion + cation	phospholipids, proteins, synthetic betaines
Non-ionic	non-ionic group	fatty alcohols, monoacylglycerols, polyethylene glycols

14 Electrolytes

Electrolytes are substances which dissociate to ions during interaction with molecules of a polar solvent and are capable of conducting an electric current in the solution. Electrolytes include molecules of ionic character (e.g. NaCl, KOH) or substances with a very polar covalent bond (e.g. HCl, HNO_3, CH_3COOH). Ions liberated by dissociation are surrounded by solvent molecules which form a solvation shell. If the solvent is water, we speak about the **hydration shell**.

Depending upon the degree of ionisation, the electrolytes are divided into two types: **weak** electrolytes and **strong electrolytes**. Weak electrolytes are dissociated only partly and equilibrium is established between non-ionised molecules and ionic species:

$$BA\ (s) + H_2O \rightarrow BA\ (aq) \rightleftarrows B^+(aq)\ +\ A^-(aq)$$
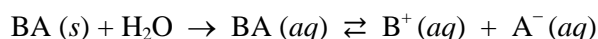
Strong electrolytes are nearly completely dissociated in their solutions containing only hydrated cations and anions. **Non-electrolytes** do not dissociate; only molecules are present in their solutions.

Weak electrolytes include weak inorganic acids and bases, most organic acids, and weak organic bases such as amines and nitrogen heterocycles. Solutions of weak electrolytes, after the equilibrium has been established, are quantitatively described by the dissociation constant.

Dissociation constant K_D for a weak electrolyte BA, whose dissociation was described above, is derived from the equilibrium constant K_c:

$$K_c = \frac{[B^+][A^-]}{[BA][H_2O]} \qquad \Rightarrow \qquad K_D = \frac{[B^+][A^-]}{[BA]}$$

K_D can be considered as a constant only in extremely diluted solutions which are close to ideal solutions. In more concentrated solutions ($c \geq 1$ mmol l^{-1}) the intermolecular distances are so small that the influence of interionic electrostatic forces is greater and the value of K_D decreases.

Strong Electrolytes

Strong electrolytes include strong acids and strong hydroxides, and the majority of salts. Only few salts make an exception and behave as weak electrolytes. An example is calcium citrate, which is soluble, but in water almost a non-dissociating complex compound. This property is used for the binding of calcium in the preparation of non-coagulable blood.

In extremely diluted solutions of strong electrolytes, their ions are in a sufficient distance from each other and their charges are not mutually influenced. Because of electrostatic ion-ion interactions in the solution, the more concentrated solutions of strong electrolytes behave differently from an ideal state. Due to electrostatic forces the ions are less mobile and the solution behaves as if there were a lower number of ions. These differences are expressed by correction coefficients. One of them is the mean **activity coefficient γ** which can be experimentally determined for the given pair of ions and concentration. The product of the activity coefficient and concentration then gives the **activity of ions a_i**:

$$a_i = \gamma_i c_i$$

The activity coefficient γ_i depends on the concentrations and charges of all kinds of ions present in the solution and its value is also influenced by adding an indifferent electrolyte into the solution. The activity coefficients of solutions with a very low concentration of electrolytes are almost equal to one

and decrease with the increasing concentration. The unit of activity is the same as that for concentration, to which it is related (mol l^{-1} or mol kg^{-1} of H_2O).

Mean activity coefficients for aqueous solutions of NaCl (25°C)

Molality (mol/kg of H_2O)	0.01	0.1	1
Mean activity coefficient γ	0.902	0.778	0.657

Ionic Strength

A number of properties of the solutions containing ions are affected by the electrostatic interactions between charges carried by ions. A very useful function including the effect of charges is the **ionic strength I**. It cannot be measured directly as it is calculated from the concentration and charge of individual ions. The ionic strength of the diluted solutions can be calculated from the equation:

$$I = \frac{1}{2}(c_1 z_1^2 + c_2 z_2^2 + ...) = \frac{1}{2}\sum c_i z_i^2$$

where c is the concentration in mol l^{-1} and z the charge of the given ion. The table below represents the values of ionic strength of three different salts with the same concentrations. It is evident that ionic strength depends not only on concentration but also on the charges of ions.

Salt	Concentration (mol/l)	Ionic strength (mol/l)
NaCl	0.1	0.1
$CaCl_2$	0.1	0.3
$MgSO_4$	0.1	0.4

The ionic strength determines the values of activity coefficients and in this way it affects all quantities that depend on activities of ions, for example pH, rate constant, equilibrium and dissociation constant, product of solubility, changes of Gibbs energy, etc. The ionic strength also significantly influences the properties of ionised macromolecules with amphoteric groups like proteins and nucleic acids. Therefore in experiments whose result may be affected by ionic interactions, the medium ionic strength must be precisely defined.

The ionic strength of blood plasma has an almost constant value of 160 mmol/kg of H_2O. The principal components affecting it are the main ions Na^+, Cl^-, HCO_3^-, and also ionised proteins (polyanions). The cytoplasmic ionic strength is mainly affected by ions K^+, Mg^{2+}, organic phosphates, HCO_3^-, SO_4^{2-}, and ionised proteins.

15 Acid-Base Reactions

Acid-base reactions occur between acids and bases and include the transfer of proton (H^+).

The Definitions

According to the Brønsted-Lowry theory, an acid is a proton donor, a base is a proton acceptor. When H^+ is released from the acid, its conjugate base is formed. When a base accepts a proton, it is transformed into the conjugate acid. Acid and its conjugate base or the base and its conjugate acid form a **conjugate pair**.

H^+ protons are not able to exist independently, therefore they are not free in the solution, but only in the hydrated form as H_3O^+ or $H(H_2O)_n^+$. However, to simplify equations and calculations, these ions are often described as hydrogen ions by the symbol H^+.

The ability of an acid to release H^+ is exhibited only in the presence of a base which is able to accept this proton. On the other hand, the properties of a base are displayed only in an acidic environment. The conjugate pair cannot exist separately, but always in the combination with another conjugate pair. Generally, each acid-base reaction can be described as the combination of two conjugate pairs.
In aqueous solutions, the second conjugate pair is derived from water that behaves as an amphiprotic solvent. **Amphiprotic** substances have the properties of both acids and bases.

Example: $NH_3 + H_2O \rightleftarrows NH_4^+ + OH^-$	$H_2CO_3 + H_2O \rightleftarrows HCO_3^- + H_3O^+$
The ammonium cation is the conjugate acid to NH_3 and H_2O is an acid (its conjugate base is OH^-).	Water behaves as a base (oxonium cation is its conjugate acid).

In terms of the Brønsted-Lowry theory, the character of solvent has an important function. Besides water, other solvents can also be used (e.g. liquid ammonia, anhydrous acetic acid) at which the acid-base properties of a substance can change. A substance can behave as an acid in one solvent and as a base in another. The choice of the solvent can influence the dissociation of substances: acetic acid behaves as a base in perchloric acid; in liquid ammonia acetic acid becomes a strong acid. The Brønsted theory not only explains the behaviour of substances in different solvents, but it primarily gives an overall view of the range of processes (hydrolysis of salts, neutralisation reactions, buffer systems), which are needed for understanding the behaviour of biological systems.
A more general view on acid-base reactions is given by the **Lewis theory**, which connects the acidic or basic character of substances with their electron structure. Lewis acids accept an electron pair; Lewis bases donate an electron pair. The Lewis theory is usually applied to explain the mechanism of organic reactions.

Autoprotolysis of Water
Water (chemically pure) belongs to very weak electrolytes (very small electric conductivity indicates the weak dissociation of water molecules into ions). Water molecules have an amphiprotic character – they can release or accept protons. The transfer of one proton can also proceed between two molecules of water. This process is called **self-ionisation (autoprotolysis)**:

$$2\,H_2O \rightleftarrows H_3O^+ + OH^-$$

and characterised by the equilibrium constant K_c:

$$K_c = \frac{[H_3O^+][OH^-]}{[H_2O]^2}$$

The equilibrium of the reaction lies well to the left side ($K_c = 10^{-16}$). The concentration of non-dissociated water molecules can be considered as constant and practically equal to the total concentration of water (55.5 mol l^{-1}). We can include the concentration of water into K_c and we get a new constant called **the ionic product of water** $K_w = K_c[H_2O]^2 = [H_3O^+][OH^-]$.

At 25°C, $K_w = 10^{-14}$ mol^2 l^{-2}, with increasing temperature K_w rises (at 37°C, $K_w = 2.5 \times 10^{-14}$ mol^2 l^{-2}). In chemically pure water, at 25°C, $[H_3O^+] = [OH^-] = 10^{-7}$ mol l^{-1}.

The pH Scale

The solution, in which the concentrations of H^+ and OH^- ions are equal, $[H^+] = [OH^-] = 10^{-7}$ mol l^{-1}, is described as **neutral**. Solutions, in which $[H^+]$ is greater than 10^{-7} mol l^{-1}, are **acidic** and solutions with $[H^+]$ lower than 10^{-7} mol l^{-1} are regarded as **basic**.

The acidity of the environment is evaluated according to the concentration of hydrogen ions H^+. With regard to the wide range of H^+ concentrations, it is convenient to express them in the logarithmic scale. Therefore, the **pH** was introduced, defined as the negative decimal logarithm of the activity of hydrogen ions. The activity of hydrogen ions in diluted solutions can be substituted by the concentration of H^+:

$$pH = -\log a_{H^+} \approx -\log [H^+]$$

Similarly we can define the pOH quantity for hydroxide ions, i.e. $pOH = -\log [OH^-]$. The pH and pOH quantities are in relation given by the ion product of water in the logarithmic form:

$$\log 10^{-14} = \log [H^+] + \log [OH^-]$$

and after conversion:

$$14 = pH + pOH$$

In neutral solutions, when $[H^+] = [OH^-]$, pH = 7. Acidic solutions have pH < 7 (with the increasing acidity the pH decreases) and in basic solutions pH > 7 (with the increasing basicity of the solutions, $[H^+]$ decreases and the pH increases). From the known concentration of the hydrogen (or hydroxide) ions, we can calculate pH. On the other hand, from the given pH we can calculate the concentration of H^+ using the relation $[H^+] = 10^{-pH}$.

Strong and Weak Acids

Strong acids belong to the group of strong electrolytes. In aqueous solutions, they are almost completely dissociated and the resulting anions do not react with water and are not able to bind H^+. Anions of strong acids are weak conjugate bases; they do not participate in acid-base reactions. They are sometimes called "strong" anions in clinical biochemistry, which indicates that they are derived from strong acids; in fact, they are **spectator ions**. The examples are Cl^- and SO_4^{2-} ions in the blood serum.

Binary strong acids include only hydrochloric, hydrobromic and hydroiodic acid. Oxoacids involve mainly sulfuric acid, nitric acid, chloric, and perchloric acid. Strong acids include also some less common oxoacids with a higher number of oxygen atoms, generally those that have the difference between the number of O and H atoms ≥ 2 in the stoichiometric formula. Organic acids are, in general, weak acids; examples of strong organic acids are alkane sulfonic and alkyl sulfuric acids.

Strong acids	HCl, HBr, HI, H_2SO_4, HNO_3, $HClO_3$, $HClO_4$, CF_3COOH, CCl_3COOH, $R\text{-}SO_3H$, $R\text{-}O\text{-}SO_3H$

Weak acids belong to weak electrolytes. During dissolution in water the equilibrium is established between non-dissociated molecules and resulting ions, which is characterised by the dissociation constant K_A or pK_A. For a weak acid of the HA type (e.g. acetic acid) it is valid:

$$HA + H_2O \rightleftarrows A^- + H_3O^+$$
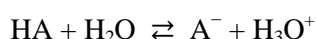
$$K_A = \frac{[A^-][H^+]}{[HA]} \quad \Rightarrow \quad pK_A = -\log K_A$$

The anion of a weak acid (A^-) is a strong conjugate base; it participates in acid-base reactions (see hydrolysis below). The weaker the acid, the higher the affinity of the anion to H^+ and thus the stronger the conjugate base. Acids with pK_A of 1–3 are called medium strength acids. Weak acids include almost all carboxylic and other organic acids and the remaining inorganic acids.

The values of pK_A of selected weak acids in water at 25°C

Acid	pK_A	Acid	pK_A
HOOC−COOH	1.25; 4.29	H_2CO_3	6.35; 10.33
H_3PO_4	2.16; 7.20; 12.29	H_2S	7.07; 12.20
HCOOH	3.75	HCN	9.21
Ascorbic	4.17; 11.57	C_6H_5−OH	9.98
CH_3COOH	4.76	Stearic	10.15

Strong and Weak Bases

Strong bases belong to the group of strong electrolytes. They include metal hydroxides of the first and second main subgroup (except beryllium) and tetraalkylammonium hydroxides. In water, they completely dissociate and liberate the hydroxide OH^- ion, which is the principle of their basicity. Cations do not undergo acid-base reactions. In clinical biochemistry, the cations of strong hydroxides are called "strong" cations (Na^+, K^+, Ca^{2+}, Mg^{2+}).

Strong hydroxides	NaOH, KOH, $Mg(OH)_2$, $Ca(OH)_2$, $Ba(OH)_2$, $(NR_4)^+OH^-$

Weak bases are weak electrolytes. The equilibrium in the solution of a weak base B (e.g. NH_3) is characterised by the protonation constant K_B (or pK_B):

$$B + H_2O \rightleftarrows BH^+ + OH^-$$

$$K_B = \frac{[BH^+][OH^-]}{[B]} \qquad \Rightarrow \qquad pK_B = -\log K_B$$
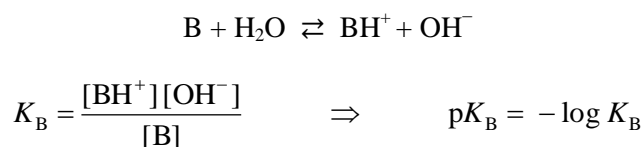
Alternatively, the dissociation constant of conjugate acid K_A (or pK_A) can be used:

$$BH^+ + H_2O \rightleftarrows B + H_3O^+$$

$$K_A = \frac{[B][H^+]}{[BH^+]} \qquad \Rightarrow \qquad pK_A = -\log K_A$$
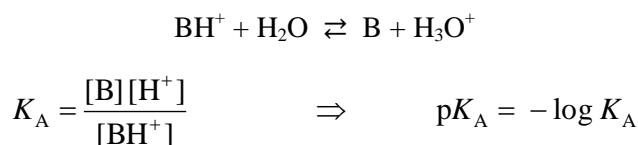
Comparing the expressions for K_B and K_A we can derive their relationship:

$$K_B K_A = K_w \qquad pK_B + pK_A = 14$$

Weak bases include ammonia, amines and other nitrogen compounds (including most heterocycles) in which nitrogen has a free electron pair for binding proton. Weak bases include also weak hydroxides, e.g. $Be(OH)_2$, $Al(OH)_3$, and hydroxides of transient metals like $Cu(OH)_2$, $Fe(OH)_3$. They are mostly insoluble in water. They are weak electrolytes, and therefore they only partially dissociate.

Values of pK_B of selected weak bases in water at 25°C

Base	pK_B	Base	pK_B
PO_4^{3-}	1.71	Imidazole	6.90
Pyrrolidine	2.70	HCO_3^-	7.65
Methylamine	3.36	Pyridine	8.82
CO_3^{2-}	3.67	CH_3COO^-	9.24
NH_3	4.75	Caffeine	13.40

Calculating pH

To calculate the pH of an aqueous solution we need to know the concentration of H^+ ion in moles per litre. In all calculations, we have to distinguish between a strong or weak acid (base).

Strong acids and hydroxides 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, $[H^+]$ is directly equal to the total concentration of acid (c_A). Similarly, in monobasic hydroxides, the concentration $[OH^-]$ equals the total concentration of hydroxide (c_B). We can then calculate the **pH of a strong acid (hydroxide) solution** from the expressions:

$$pH = -\log c_A \qquad\qquad pH = 14 + \log c_B$$

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.

The given expressions for the calculation of pH are valid for the range of concentrations from 10^{-3} to $5 \cdot 10^{-7}$ mol l^{-1}. If c_A or $c_B > 10^{-3}$ mol l^{-1}, we have to consider the activity of ions instead of concentration for more precise calculations. For quick pH calculations we can use the concentrations bearing in mind that for $c > 10^{-3}$ mol l^{-1} the calculated values are only approximate. On the other hand, in case of concentrations lower than $5 \cdot 10^{-7}$ mol l^{-1} the H^+ ions from water self-ionisation have to be involved.

Weak acids and bases only partially dissociate in solutions, therefore we have to use their dissociation constants in pH calculations:

$$K_A = \frac{[A^-][H^+]}{[HA]} \qquad\qquad K_B = \frac{[BH^+][OH^-]}{[B]}$$

In not very diluted solutions (if dissociated < 10% of acid molecules), the concentration of non-dissociated acid molecules [HA] can be taken as approximately equal to the total (analytical) concentration of acid c_A, thus $[HA] \approx c_A$. Concentration of dissociated ions (H^+ and A^-) is equal, $[H^+] = [A^-]$. Then we can modify the expression for K_A:

$$K_A = \frac{[H^+]^2}{c_A} \quad \Rightarrow \quad [H^+] = \sqrt{K_A c_A}$$

Analogically, we can derive the expression for a weak base. After the modification of K_B, we get:

$$[OH^-] = \sqrt{K_B c_B}$$

Hydrolysis of Salts

Solutions of salts usually behave as strong electrolytes; during dissolution in water they completely dissociate to ions. Resulting ions may react with water and the pH of a solution changes. Whether such reaction occurs or not depends on the salt origin. Anions of strong acids and cations of strong hydroxides do not react with water; dissociated ions (spectator ions) will be only hydrated.

Anions of weak acids and cations of weak bases react with water to form corresponding weak acids and bases, respectively, until the equilibrium given by K_A and K_B, respectively, is established. This reaction is defined as the **hydrolysis of salts**.

Both processes mentioned above (dissociation of salts and hydrolysis of ions) must be distinguished from each other. At first salt dissociates when dissolved in water, after which dissociated ions can be hydrolysed. Salts are products of the neutralisation reaction between an acid and a base. They can be divided into four types:

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

Salt dissociates to a cation and anion in aqueous solution, for example:

$$CH_3COONa \rightarrow Na^+ + CH_3COO^-.$$
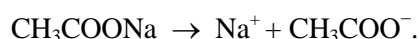
The cation of this salt (Na^+) comes from the strong hydroxide, does not react with water, and is found in hydrated form in the solution. The anion of this salt (CH_3COO^-) originates from the weak acid, and therefore it is the subject of hydrolysis, reacts with water to form acetic acid until the equilibrium is established:

$$CH_3COO^- + H_2O \rightleftarrows CH_3COOH + OH^-.$$
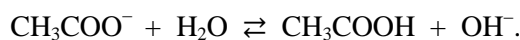
The solution of this salt has a **slightly alkaline reaction** due to some excess of OH^- ions by the hydrolysis of the anion of weak acid.

B. Salts of weak bases and strong acids

e.g. NH_4Cl, $(NH_4)_2SO_4$, NH_4NO_3, $(CH_3)_3NHCl$, C_5H_5NHCl (pyridinium chloride), $CuSO_4$, $FeCl_3$

There are two groups of salts in this category. One group possesses an ammonium cation or another protonated nitrogen base, the second group are the salts derived from weak hydroxides. The initial process is dissociation.

In the first group, e.g.

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

The anion of this salt (Cl^-) originates from the strong acid, it does not react with water, and it is present in the hydrated form in the solution. The cation of this salt undergoes hydrolysis and produces a **slightly acidic reaction**:

$$NH_4^+ + H_2O \rightleftarrows NH_3 + H_3O^+$$
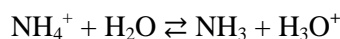
Salts of the second type contain a cation derived from weak hydroxides, e.g.

$$FeCl_3 \rightarrow Fe^{3+} + 3\ Cl^-$$
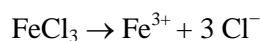
The anion (Cl^-) comes from the strong acid, it does not react with water, and occurs in the hydrated form in the solution. The iron(III) cation makes a hexaaquacomplex: $Fe^{3+} + 6\ H_2O \rightleftarrows [Fe(H_2O)_6]^{3+}$.

Such aquacation (originating from weak hydroxide) acts as a Brønsted acid, releases one H^+ from one coordinated water molecule:

$$[Fe(H_2O)_6]^{3+} + H_2O \rightleftarrows [Fe(H_2O)_5OH]^{2+} + H_3O^+$$
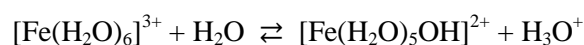
The solution of this salt has a **slightly acidic reaction**. An excess of H_3O^+ ions comes from the hydrolysis of complex aquacation.

C. Salts of weak acids and weak bases

e.g. CH_3COONH_4, NH_4NO_2, $CuNO_2$, aluminium lactate

After the salt is dissociated in water to ions, they are both independent subjects of hydrolysis, both hydroxide (OH^-) and oxonium (H_3O^+) ions arise.

The pH value of this salt solution depends on the component which is relatively stronger. If the acid HA is stronger than the base B, then $pK_A < pK_B$ and pH < 7, the solution has an acidic reaction. And *vice versa*, if the base is stronger, then $pK_A > pK_B$ and pH > 7, the solution has a basic reaction. If the

dissociation constants of both components are nearly equal as, for example, in case of ammonium acetate ($pK_A \approx pK_B$), the pH of the hydrolysed salt solution remains neutral.

D. Salts of strong acids and strong hydroxides

e.g. $NaCl$, Na_2SO_4, $Ca(NO_3)_2$, KI

The salts of strong acids and strong hydroxides are completely dissociated in aqueous solutions. Cations and anions **do not hydrolyse;** they are found in hydrated forms in solutions. The pH value of these solutions remains **neutral**.

E. Hydrogen salts

e.g. $NaHS$, $NaHCO_3$, $Ca(H_2PO_4)_2$, K_2HPO_4

Hydrogen salts are formed by the partial neutralisation of polyprotic acids. Anions of polyprotic acids have an amphiprotic character. Part of these anions (HA^-) hydrolyse like the conjugate base of a weak acid H_2A, with the dissociation constant K_{A1}:

$$HA^- + H_2O \rightleftarrows H_2A + OH^-$$
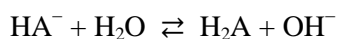
At the same time, another portion of anions (HA^-) acts as a weak acid with the constant K_{A2}:

$$HA^- + H_2O \rightleftarrows A^{2-} + H_3O^+$$
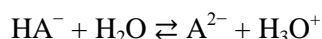
For the calculation of pH we can derive the relation which implies that the resulting pH depends on the dissociation constants and not on the salt concentration:

$$pH = \tfrac{1}{2}\, pK_{A1} + \tfrac{1}{2}\, pK_{A2}$$

The relation is very useful; we can estimate the pH of hydrogen salt solutions from the values of pK_A.

Let us consider the pH of the solution of $NaHCO_3$,

$pK_{A1}(H_2CO_3) = 6.35$; $pK_{A2}(HCO_3^-) = 10.30$;

the approximate pH value $= \tfrac{1}{2}\, pK_{A1} + \tfrac{1}{2}\, pK_{A2} \Rightarrow pH = \tfrac{1}{2}\,(6.35 + 10.30) = 8.33$

The summary on salt hydrolysis is presented in the table below.

Salt origin	Hydrolysis occurs	Hydrolysing 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 and anion	≈ 7
Hydrogen salt, diprotic acid*	yes	anion	$\tfrac{1}{2}\,(pK_{A1} + pK_{A2})$
Dihydrogen salt, triprotic acid*	yes	anion	$\tfrac{1}{2}\,(pK_{A1} + pK_{A2})$
Hydrogen salt, triprotic acid*	yes	anion	$\tfrac{1}{2}\,(pK_{A2} + pK_{A3})$

* With a non-hydrolysable cation.

16 Buffers

Buffers are solutions of weak acids and their salts (conjugate bases) or weak bases and their salts (conjugate acids). Buffer solutions can also be composed of the mixtures of salts of polyprotic acids. Both conjugate species have to be in comparable (or the same) concentrations. Buffers have the ability to precisely set and keep the pH value of a solution. Buffer solutions are resistant to the pH changes caused by the addition of an acid or a base.

A simple example is a buffer composed of a weak acid and its salt (e.g. acetic acid and sodium acetate = acetate buffer). In the solution of such buffer, we find the dissociated and non-dissociated molecules of weak acid and ions of the salt. The anion of the salt is the same as the anion of the acid. The equilibrium in a buffer solution is described by the dissociation constant of weak acid:

$$K_A = \frac{[H^+]\,[A^-]}{[HA]}$$

The condition for effective functioning of a buffer is that the amounts of acid and conjugate base are approximately equal. At these conditions the dissociation of a weak acid is supressed due to the presence of a common ion effect (Le Chatelier principle)

The behaviour of a buffer after a strong acid (base) is added can be described in the following way:

· If H^+ ions are added into a buffer, they react with an A^- anion to form a weak acid HA ($H^+ + A^- \to$ HA). A new equilibrium is established in the solution, and since the value of K_A has to remain constant, the ratio of both buffer components (HA, A^-) changes.

· Similarly, if OH^- ions are added, they react with HA ($OH^- + HA \to H_2O + A^-$), and a new equilibrium is established. The value of K_A is constant; the ratio of components changes.

· Thus, in both examples, the buffer solution pH does not change distinctly after the addition of a limited amount of strong acid or base.

A buffer composed of a weak (nitrogen) base and its salt behaves in a similar way. If H^+ ions are added into the solution, they react with the weak base. After the addition of OH^-, hydroxide ions react with the cations of salt to form a free weak base. In both examples, new equilibriums are established, the ratio of buffer components changes, while the pH remains almost the same.

The pH of Buffers

Calculation of the pH of a buffer composed of a weak acid and its salt is derived from the expression for K_A. With certain approximations, we can replace the equilibrium concentration of the non-dissociated HA molecules by the total concentration of the acid c_A, $[HA] \to c_A$, and the equilibrium concentration of the anion A^- can be expressed by the total concentration of the fully dissociated salt c_S, $[A^-] \to c_S$. For the concentration of $[H^+]$ it is valid that:

$$[H^+] = K_A\,\frac{[HA]}{[A^-]} \qquad \text{and after modification} \qquad [H^+] = K_A\,\frac{c_A}{c_S}$$

In the logarithmic form, we get the expression known as the **Henderson-Hasselbalch equation:**

$$pH = pK_A + \log\frac{c_S}{c_A}$$

A similar expression can be derived for the buffer prepared from a weak base and its salt:

$$pH = 14 - pK_B - \log \frac{c_S}{c_B}$$

If the buffer components are described in terms of the Brønsted theory (weak acid and its conjugate base or weak base and its conjugate acid), it is obvious that each buffer generally consists of an acidic and a basic component. We can then write the Henderson-Hasselbalch equation in the general form:

$$pH = pK_A + \log \frac{c_B}{c_A}$$

where c_A is the concentration of buffer acid, c_B the concentration of buffer base, pK_A the dissociation constant of buffer acid.

To calculate the pH of buffers formed from the solutions of polyprotic acids we also use the Henderson-Hasselbalch equation. The typical example is a hydrogen phosphate buffer composed of a mixture of $H_2PO_4^-$ and HPO_4^{2-}. We use $[H_2PO_4^-]$ instead of c_A (concentration of buffer acid) and $[HPO_4^{2-}]$ instead of c_B (concentration of buffer base). pK_A is the dissociation constant of $H_2PO_4^-$.

From the general form of the Henderson-Hasselbalch equation, we can make the following conclusions:

· The pH of a buffer depends on the logarithm of the ratio of buffer components (acid and base) and not on their absolute values.

· If the concentrations of both buffer components are equal, $c_B = c_A$, the pH of the buffer equals the dissociation constant K_A, $pH = pK_A$.

· The pH of the buffer does not change with the dilution.

The Henderson-Hasselbalch equation also has another application; we can derive a degree of ionisation of weak acid (base) at the given pH.
For example, a weak acid HA with $pK_A = 4$ is at $pH = 1$ almost non-dissociated ($[A^-]/[HA] \sim 1/1000$); at $pH = 4$, the concentration of the protonated form is the same as that of an anion ($[A^-]/[HA] = 1/1$); at $pH = 7$, the acid will be almost fully dissociated ($[A^-]/[HA] \sim 1000/1$).

Optimal pH Range of the Buffer
The optimal pH range for effective functioning of a buffer is given by its composition. Simple buffers, composed of two components, function best when the concentration of acidic and basic components are the same. It was derived above that it occurs at $pH = pK_A$. A simple buffer is still effective at pH in the range pH from $(pK_A - 1)$ to $(pK_A + 1)$, which correlates with ratio c_B/c_A from 1/10 to 10/1. Outside that range, the concentration of either the buffer acid or buffer base is too small to effectively resist the effect of added hydroxide ions or protons, respectively.
To make a buffer you must first select a buffer acid whose pK_A is close to the pH you want for the solution. It is necessary to realise that, e.g. $pH = 7$ cannot be effectively kept by using a buffer whose pK_A lies outside the range 6–8.

The ability of a buffer to suppress the changes of pH is expressed by the **buffer capacity β**:

$$\beta = \frac{\Delta n_{\mathrm{H^+, OH^-}}}{V\,\Delta \mathrm{pH}}$$

It describes the effectiveness of the buffer. It is the molar amount of H^+ or OH^- which causes the change $\Delta pH \pm 1$ in one litre of buffer. Practically, we often evaluate the molar quantity of the H^+ or OH^- ions, which cause the change $\Delta pH \pm 0.1$ in one litre of buffer. The buffer capacity reaches its maximum when $pH = pK_A$. When the buffer is diluted, its capacity decreases, but the pH does not change.

To prepare the buffer, you can calculate the concentration of both components according to the Henderson-Hasselbalch equation and mix the solutions. It is also possible to make a buffer by the "titration method". It means that we prepare a solution of one component of the conjugate acid/base pair and the pH will then be adjusted by the addition of a strong base or strong acid. If buffering is needed in a broad pH range, universal buffers prepared from mixtures of weak acids are used (e.g. the Britton-Robinson buffer). In biochemical research, we often use buffers based on amphions, which must fulfil not only the general requirements (good buffer capacity, easy preparation) but also some special demands like no interference with biological processes, membranes, isotonicity, non-toxicity, etc.

Examples of common buffers used in laboratories

Buffer	Buffer base	Buffer acid	pH range
Hydrogen phosphate	Na_2HPO_4	NaH_2PO_4	5.6–8.1
Acetate	CH_3COONa	CH_3COOH	3.6–5.6
Borate	$Na_2B_4O_7$	H_3BO_3	7.1–9.2
Citrate-phosphate	Na_2HPO_4	Citric acid.	2.2–8.0

Titration Curves of Weak Acids and Bases

The graphic expressions of the Henderson-Hasselbalch equation are **titration curves** of weak acids or bases (their horizontal part). A simple example is the titration of acetic acid by sodium hydroxide. Titration follows the neutralisation: $CH_3COOH + NaOH \rightarrow CH_3COONa + H_2O$.
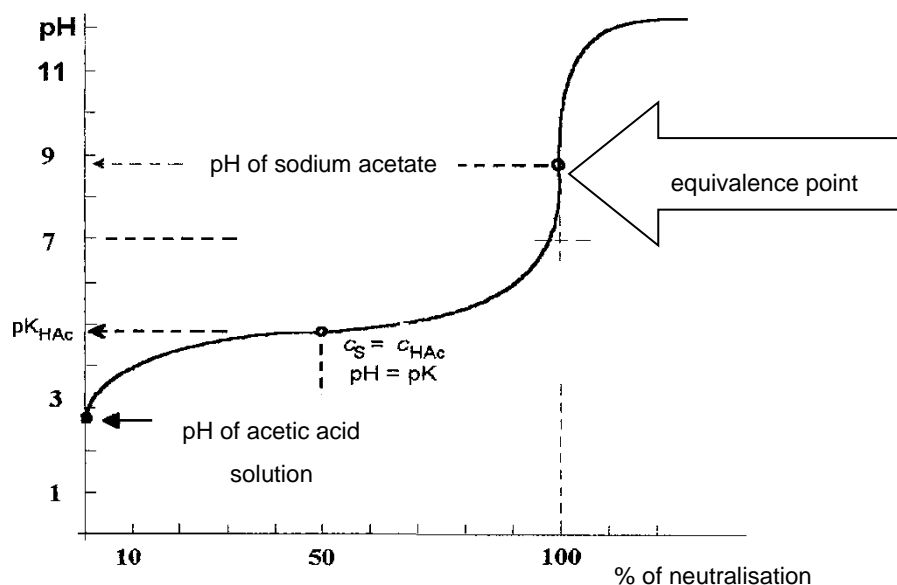
The titration curve expresses the dependence of pH on the amount of added titration agent. At the beginning of titration, only acetic acid is present. In the course of titration, the amount of sodium acetate increases and the solution becomes the mixture of acetic acid and sodium acetate in different ratios until the equivalence point, when the acetic acid is completely neutralised and only the aqueous solution of sodium acetate remains in the reaction system. When the equivalence point is overcome, the amount of hydroxide anions rapidly increases in the solution and the pH of the solution will change rapidly.

The titration curve has **three different phases**. The initial and final phase is characterised by the steep increase of pH. The flat middle part, where pH changes only slightly in quite a large range of the added hydroxide, is the buffer region. The solution contains the mixture of weak acid and its salt and resists the addition of strong hydroxide without distinct changes in pH.

The pH of the solution can be calculated from the Henderson-Hasselbalch equation during the course of titration (except the start and the equivalence point). If the concentration of acetic acid (HAc) and sodium acetate (Ac^-) is the same, pH equals pK_A(HAc). This situation occurs if 50% of the acetic acid is neutralised.

A mixture of weak acid and its conjugate base is an effective buffer, if their concentrations are very close. The course of the curve proves that in the region of the half-neutralisation and its nearest region, when the concentrations of both buffer components are very similar, the change of pH caused by the addition of strong hydroxide is effectively suppressed.

Buffer Systems in the Organism

Several different buffers are involved to keep the constant pH of body fluids. Each of them is characterised by its own Henderson-Hasselbalch equation. In different compartments, they function with varied relevance cooperating with each other. Addition or removal of H^+ is eliminated by different buffer systems proportionally to their capacities. Most important buffer systems are hydrogen carbonate, proteins, and hydrogen phosphate.

The **hydrogen carbonate** (**bicarbonate**) system is the main buffer of extracellular fluids, making up more than half of the buffer capacity of blood. It consists of HCO_3^- and H_2CO_3. The Henderson-Hasselbalch equation also includes physically dissolved CO_2, which is in equilibrium with carbonic acid:

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

Therefore, the **effective concentration** of carbonic acid $[H_2CO_3 + CO_2]$ comprising carbon dioxide is used instead of $[H_2CO_3]$, and **apparent dissociation constant** K_{Aef} is used instead of true K_A. At the ionic strength of blood plasma and 37°C, $K_{Aef} = 7.9 \times 10^{-7}$ and $pK_{Aef} = 6.10$.

$$pH = pK_{Aef} + \log \frac{[HCO_3^-]}{[CO_2 + H_2CO_3]}$$

The effective concentration is directly dependent on the partial pressure of CO_2 in the blood (pCO_2) and on the solubility of CO_2 in blood (s – solubility coefficient). A partial pressure is the pressure of CO_2 in the closed space above liquid (blood), which is in thermodynamic equilibrium with dissolved

CO_2 and H_2CO_3. Thus, the effective molar concentration $[CO_2 + H_2CO_3]$ in $mmol\ l^{-1}$ can be expressed as the product of $pCO_2 \cdot s$, where the solubility coefficient s has at 37°C the value of 0.23 (for pCO_2 in kPa). The Henderson-Hasselbalch equation for the hydrogen carbonate buffer in blood is then:

$$pH = 6.1 + \log \frac{[HCO_3^-]}{pCO_2 \cdot 0.23}$$

The concentration of HCO_3^- is put in $mmol\ l^{-1}$ when the solubility coefficient 0.23 is used. The high effectiveness of this buffer is given by the possibility to regulate the amount of exhaled CO_2.

Proteins. The buffer effect of proteins is caused by their amphoteric character (similarly to amino acids). The ionisable groups of side chains are able to react as weak acids or weak bases. In the physiological pH range (~ 7.4) the imidazole groups of histidine play the most important role ($pK_A = 6.0$).

The values of pK_A of the side chains of amino acids

Amino acid	Group in a side chain	pK_A	Commentary
Aspartate	β-carboxyl (−COOH)	3.9	at pH 7.4 complete dissociation to –COO⁻
Glutamate	γ-carboxyl (−COOH)	4.3	at pH 7.4 complete dissociation to –COO⁻
Histidine	imidazolium	6.0	acidic component of protein buffers
Cysteine	sulfonyl (−SH)	8.3	at physiological pH no reaction
Tyrosine	phenolic hydroxyl (−OH)	10.1	at physiological pH no reaction
Lysine	ε-ammonium ($-NH_3^+$)	10.5	at pH 7.4 is positively charged
Arginine	guanidinium $-NH(NH_2)C=NH_2^+$	12.5	at pH 7.4 is positively charged

The most important protein buffer system in whole blood is hemoglobin/oxyhemoglobin, which makes approximately ⅓ of its buffer capacity. Oxyhemoglobin ($pK_A = 6.2$) is a stronger acid than hemoglobin ($pK_A = 7.8$). Therefore, during oxygenation of hemoglobin (Hb) in the lungs the produced oxyhemoglobin (HbO_2) releases some protons. On the other hand, in the tissues, oxyhemoglobin is transformed into hemoglobin, which behaves as the acceptor of protons.

$$HHb \rightleftarrows Hb^- + H^+ \qquad pK_A \approx 7.8$$
$$HHbO_2 \rightleftarrows HbO_2^- + H^+ \qquad pK_A \approx 6.2$$
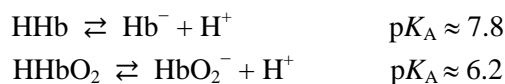
Proteins also participate in the maintenance of pH in blood plasma (mainly albumin) and intracellular fluids (ICF).

Hydrogen phosphates. In the physiological range of pH the hydrogen phosphate buffer composed of HPO_4^{2-} and $H_2PO_4^-$ is important. At the ionic strength of blood plasma, the pK_A of dihydrogen phosphate is 6.8. Hydrogen phosphate buffer works mainly in urine and intracellular fluids.

Buffer systems in whole blood

Buffer system	Abundance	Buffer base	Buffer acid	pK_A
Hydrogen carbonate	50%	HCO_3^-	$H_2CO_3 + CO_2$	6.1
Proteins[a]	45%	Protein-His	Protein-His-H$^+$	6.0–8.0[b]
Hydrogen phosphate	5%	HPO_4^{2-}	$H_2PO_4^-$	6.8

[a] In blood plasma mainly albumin, in erythrocytes hemoglobin. [b] Depends on the type of protein.

Maintaining Constant pH in the Human Body

The maintenance of constant pH is necessary for normal courses of vital functions. It is one of the main priorities of the regulation mechanisms in the organism. Most biological processes proceed at an approximate pH of 7, ranging from 6 to 9. Due to enzymatic catalysis, biochemical processes are distinctly dependent on the environmental pH and even small deviations from the stable values can result in their slowing down or stopping. Therefore, the evaluation of acid-base equilibrium is an important factor for the examination of body condition. The pH of blood is the most important indicator; the reference range is 7.36–7.44. At the same time, we measure other quantities in blood – pCO$_2$, pO$_2$, and the concentration of hemoglobin. The other parameters are then calculated.

Disorders of acid-base balance may be caused by a range of reasons, e.g. the formation of ketone bodies in diabetes, formation of lactate during hypoxia, loss of HCl during vomiting, increased excretion of HCO_3^- in renal malfunction. Lower blood pH is acidemia (the body condition – acidosis), the increase of blood pH above the limit is called alkalemia (the state is alkalosis).

Influence of pH on Dissociation of Weak Acids

The relationship between the concentration of the acidic and basic component of a weak electrolyte is important for the explanation of acid-base properties of weak electrolytes. For example, depending on the environmental pH, weak acids (such as lactic, acetoacetic, β-hydroxybutyric) are present as either acids in their non-dissociated forms at low pH or as anions at higher pH.

Let us consider lactic acid (pK_A = 3.86) that is very common in blood plasma and is also used in infusions. As it follows from the Henderson-Haselbalch equation, at pH = 3.86, exactly 50% of the acid is dissociated, the ratio c_B/c_A = 1. At pH 7.4, the ratio between the concentration of lactate (c_B) and non-dissociated lactic acid (c_A) is > 1000/1 and lactic acid is dissociated from more than 99.9%. Within the pH of blood, lactic acid and the other acids with pK_A < 4.5 do not behave as acids; they are almost fully dissociated. The anions of such acids do not have tendency binding protons and do not have any buffering effects. They are therefore included among strong ("spectator") anions in the blood plasma together with the anions of strong inorganic acids, chlorides (Cl$^-$) and sulfates (SO$_4^{2-}$).

Many drugs also have a character of weak acids (hydroxy acids, barbiturates, sulfonamides, some vitamins) or weak bases (alkaloids, derivatives of phenothiazine). The relation between their pK_A value and environmental pH significantly affects their solubility, absorption, transport, and pharmacological action. Effects of drugs can be modified by substitutions that can trigger a change of their acid-base properties.

17 Precipitation Reactions. Solubility Product

Most salts are totally dissociated to ions during dissolution in water (see Chapter 14). The amount of dissolved salt and thus the amount of ions released into the solution is limited by the solubility of the given salt. If adding a poorly soluble salt to water, after a certain amount some salt remains undissolved. The solution is **saturated** and the heterogeneous equilibrium between a solid phase (*s*) and dissolved, dissociated, and hydrated (*aq*) ions is established:

$$B_nA_m(s) \rightleftarrows n\ B^{m+}(aq) + m\ A^{n-}(aq)$$
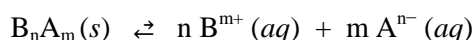
where $B^{m+}(aq)$ and $A^{n-}(aq)$ are ions in an aqueous solution. The equilibrium can be characterised by the equilibrium constant:

$$K = \frac{[B^{m+}]^n [A^{n-}]^m}{[B_nA_m]}$$

where $[B^{m+}]$, $[A^{n-}]$ are the concentrations of ions in the solution (activity coefficients equal 1 because of strongly diluted solutions), $[B_nA_m]$ is the concentration of the substance in the solid phase. As the amount of undissolved salt does not influence the concentration of ions in the saturated solution, $[B_nA_m]$ can be included in the constant K. The new constant is called **solubility product K_s**:

$$K_s = [B^{m+}]^n [A^{n-}]^m$$

Example. The dissociation and heterogeneous equilibrium of silver chloride and calcium phosphate:

$AgCl(s) \rightleftarrows Ag^+ + Cl^-$ $Ca_3(PO_4)_2(s) \rightleftarrows 3\ Ca^{2+} + 2\ PO_4^{3-}$
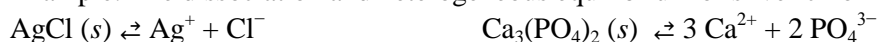
and relations for the solubility products are:

$K_s(AgCl) = [Ag^+][Cl^-]$ $K_s(Ca_3(PO_4)_2) = [Ca^{2+}]^3 [PO_4^{3-}]^2$

The solubility product gives the maximal value that can be reached by the product of ion concentrations in a solution at a specified temperature. If the product of concentrations is $> K_S$, a precipitate is separated out from the solution. Using tabulated data of K_S we can calculate the amount of salt that can be maximally dissolved in the given volume of solvent.

Example. The solubility product of barium sulfate $K_s(BaSO_4, 25\ °C) = 1.4 \times 10^{-10}$. Using this value we can derive what amount of $BaSO_4$ can be maximally dissolved in 1 litre of water and what is the resulting concentration of toxic Ba^{2+} ions in the saturated solution:

$BaSO_4(s) \rightleftarrows Ba^{2+} + SO_4^{2-}$ $K_s = [Ba^{2+}][SO_4^{2-}]$
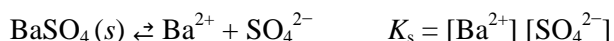
The saturated solution contains the same concentrations of Ba^{2+} and SO_4^{2-}, therefore:

$[Ba^{2+}] = [SO_4^{2-}] = \sqrt{1.4 \cdot 10^{-10}} = 1.18 \times 10^{-5}$ mol/l.

In 1 litre of water, at 25°C, we can maximally dissolve 1.18×10^{-5} mol $BaSO_4$. The concentration of toxic Ba^{2+} ions in the saturated solution is 1.18×10^{-5} mol/l; mass concentration is 1.62 mg/l.

The consequences of limited solubility of some salts are **precipitating reactions**. They occur when ions of poorly soluble salt are encountered in the solution.

Example. If we add a small amount of $CaCl_2$ solution to the solution of Na_3PO_4, a white precipitate of $Ca_3(PO_4)_2$ is formed. Why does it happen?

Both salts are strong electrolytes, fully dissociated in the solution. Thus, there are Na^+, PO_4^{3-}, Ca^{2+}, Cl^- ions in the solution. $Ca_3(PO_4)_2$ belongs to slightly soluble compounds ($K_s = 2.8 \times 10^{-30}$), and therefore when only a small amount of Ca^{2+} and PO_4^{3-} ions meet, a precipitate is immediately formed. On the other hand, NaCl is a very soluble salt; precipitate is not formed, and Na^+ and Cl^- remain in the solution. The precipitation reaction can be described by the overall equation:

$2\ Na_3PO_4\ (aq) + 3\ CaCl_2\ (aq)\ \rightarrow\ Ca_3(PO_4)_2\downarrow + 6\ NaCl\ (aq)$
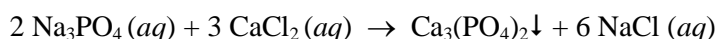
Since all the salts are dissociated: $6\ Na^+ + 2\ PO_4^{3-} + 3\ Ca^{2+} + 6\ Cl^-\ \rightarrow\ Ca_3(PO_4)_2\downarrow + 6\ Na^+ + 6\ Cl^-$

The best way is the net ionic equation: $3\ Ca^{2+} + 2\ PO_4^{3-}\ \rightarrow\ Ca_3(PO_4)_2\downarrow$

The formation of precipitate can be influenced by changing the concentration of one of the ions of poorly soluble salt. For example, if we add sodium oxalate to the saturated solution of calcium oxalate, we will increase the concentration of oxalate ions. To keep the constant value of K_s the corresponding concentration of Ca^{2+} in the solution must be decreased (Le Chatelier principle). It is done by precipitating a further amount of calcium oxalate out from the solution.

Similar processes are important in the organism during the formation of insoluble compounds. If we intake increased amounts of oxalic acid in our food (rhubarb, sorrel, spinach, or ascorbic acid metabolised to oxalate), the concentration of oxalate ions in urine can increase in such way that the product of concentration of Ca^{2+} and oxalate ions will exceed the solubility product. Urinary stones composed of insoluble calcium oxalate start to form, although the concentration of calcium ions is not increased. On the other hand, these stones can also be formed when the level of oxalates in urine is normal, but the level of Ca^{2+} is increased from metabolic reasons or high intake.

The solubility of salts of polyprotic acids may depend on the pH of a solution. Very important is the solubility of phosphates. Phosphoric acid can form three types of salts. Dihydrogen phosphates are all highly soluble in water, while soluble hydrogen phosphates and phosphates include just the salts of alkali metals and ammonium salts (see Table below).

Example. If we add an acid to insoluble $Ca_3(PO_4)_2$, the PO_4^{3-} ions are gradually converted to HPO_4^{2-} and $H_2PO_4^-$, and the precipitate starts to dissolve. The process can be described by the ionic equations:

$Ca_3(PO_4)_2\downarrow + 2\ H^+\ \rightarrow\ 2\ CaHPO_4\downarrow + Ca^{2+}$

$CaHPO_4\downarrow + H^+\ \rightarrow\ H_2PO_4^- + Ca^{2+}$
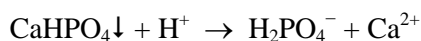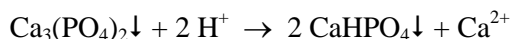
The limited solubility of calcium phosphate is important for the formation of bones and teeth. Their main component is the mineral **biological apatite** (hydroxylapatite) $Ca_5(PO_4)_3OH$, usually contaminated by other ions. The change of pH of blood or saliva can influence the deposition or release of calcium in these tissues. For example, long-term acidosis, which causes the shift in balance between

the highly insoluble phosphate to the more soluble hydrogen phosphate, can result in the increase of Ca^{2+} in plasma (elevated calcaemia).

Intestinal absorption of calcium and magnesium may be reduced by the concurrent intake of phosphates (e.g. drinking *Coca-Cola*) or oxalates (some vegetables).

The tables below present a summary of solubility of most common salts and the solubility products of selected poorly soluble salts.

The solubility of ionic compounds – a simplified survey

Anion	Commentary
Nitrates (NO_3^-)	all salts soluble
Hydrogen carbonates (HCO_3^-)	all salts soluble
Carbonates (CO_3^{2-})	most salts insoluble (exceptions Na^+, K^+, NH_4^+); insoluble carbonates form soluble hydrogen carbonates by acidification
Dihydrogen phosphates ($H_2PO_4^-$)	all salts soluble
Hydrogen phosphates (HPO_4^{2-})	most salts insoluble (exceptions Na^+, K^+, NH_4^+)
Phosphates (PO_4^{3-})	most salts insoluble (exceptions Na^+, K^+, NH_4^+); insoluble phosphates form more soluble hydrogen phosphates and quite soluble dihydrogen phosphates by acidification
Sulfates (SO_4^{2-})	most salts soluble, insoluble sulfates of Ca^{2+}, Ba^{2+}, Sr^{2+}, Pb^{2+}
Chlorides (Cl^-)	most salts soluble, insoluble chlorides of Ag^+, Hg_2^{2+}, Pb^{2+}
Oxalates ($C_2O_4^{2-}$)	poorly soluble calcium oxalate

Solubility products of some poorly soluble salts at 25°C

Salt	K_s	Commentary
$CaSO_4 \cdot 2H_2O$	3.1×10^{-5}	$CaSO_4 \cdot \frac{1}{2}H_2O$ – plaster
$CaHPO_4$	2.3×10^{-7}	formed in alkaline urine
$CaCO_3$	3.4×10^{-9}	limestone, chalk, marble, stalagmites, etc.
$CaC_2O_4 \cdot H_2O$	2.3×10^{-9}	preparation of non-coagulable blood
$BaSO_4$	1.1×10^{-10}	contrast agent in X-ray diagnostic imaging
$Ca_3(PO_4)_2$	2.8×10^{-30}	apatite
$Ca_5(PO_4)_3OH$	3.7×10^{-58}	hydroxylapatite $K_s = [Ca^{2+}]^5[PO_4^{3-}]^3[OH^-]$
$Ca_5(PO_4)_3F$	3.2×10^{-61}	fluorapatite

18 Complex-Forming Reactions

Complexes (coordination compounds) contain coordinate covalent bonds between a central metal atom or cation and molecules or ions referred to as ligands. Complexes may be charged or neutral, depending upon the combining particles. The charge on a complex is the sum of the charges of the constituent parts. Formulas of complexes are written into square brackets.

Hexacyanoferrate(II) anion $[Fe(CN)_6]^{4-}$
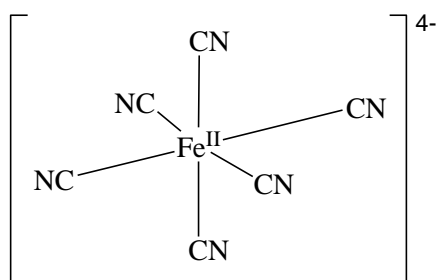
Tetraamminecopper(II) cation $[Cu(NH_3)_4]^{2+}$
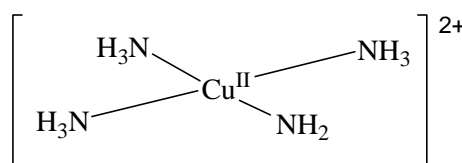
The Nomenclature of Coordination Compounds

The name expresses the number and the names of ligands as well as the name of the central atom. The number of ligands is given in Greek numerals, the ligand names are international (e.g. aqua for H_2O, ammine for NH_3), the anionic ligand names end with *–o* (e.g. Cl^- chloro, CN^- cyano, OH^- hydroxo). The name of the central metal atom or ion followed by its oxidation state in parentheses is given after the ligand names; when a complex ion has a negative charge, the ending *–ate* is added to the name of the central metal element.

· Neutral complex molecules:	$[Pt(NH_3)_2Cl_2]$	diamminedichloroplatinum(II)
	$[CuCl_2(CH_3NH_2)_2]$	dichloro-*bis*(methylamine)copper(II)
· Salts with complex cations:	$[Ag(NH_3)_2]Cl$	diamminesilver(I) chloride
	$[Co(H_2O)_3(NH_3)_3]Cl_3$	triamminetriaquacobalt(III) chloride
· Salts with complex anions:	$Na_2[Fe(CN)_5NO]$	sodium pentacyanonitrosylferrate(III)
	$K_2[PtCl_6]$	potassium hexachloroplatinate(IV)

Chelation. Some organic ligands contain two or more donor atoms with unshared electron pairs in the same ion or molecule (bidentate, resp. polydentate ligands). These compounds are called **chelating agents** because their donor atoms can coordinate to the same metal ion and form a special type of complex with one or more rings called **chelate rings**.

A typical chelating agent is the ethylenediaminetetraacetic acid (EDTA).
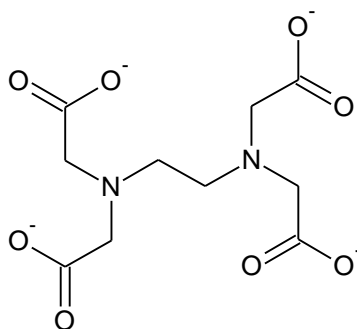
Structural formula of EDTA^{4-}

Chelation ring formation greatly increases the stability of complexes. Chelation also alters the properties of the metal ions bound in complexes so that such ions cannot be proven by the typical analytical reactions. A biologically important chelating agent is citrate.

Examples and Applications of Complex Compounds

Natural coordination compounds are important in many aspects.

Heme, the component of hemoglobin that transports oxygen in the blood, is the complex (chelate) of porphyrin and ferrous ion. **Chlorophyll** is the complex of porphyrin and magnesium ion. **Cobalamin (vitamin B$_{12}$)** is the complex of corrin and cobalt(III) ion.

Various transient metals are complexed with proteins in metalloenzymes. The catalytic activities of some other enzymes depend on coordinative binding of alkaline earth metals. In the body fluids, some metal ions (e.g. Ca^{2+}, Fe^{3+}, Cu^{2+}) are chelated at least partly by low molecular chelating agents such as citrate, polyphenols, etc. The metal ions complexed with various synthetic low-molecular ligands also exhibit some important biological effects; they are at the centre of current attention, e.g. the anti-inflammatory effect of the complexes of Cu^{2+} with indomethacin, D-penicillamine or some salicylates was described.

Na$_2$-EDTA or sodium citrate is used in the collection of non-coagulable blood. The principle of their action is the formation of chelates with calcium ions.

Chelating agents, which form stable complexes with heavy metal ions, are used as **antidotes in case of heavy metal poisoning**; soluble complexes can be eliminated from the body with normal waste products. For example, dimercaptopropanol forms complexes through its two sulfur atoms and facilitates the excretion of As, Hg, Sb, and Au ions from the body, cysteine is used in Pb and Cu poisoning. Complexes can also be used to insert some substances, which do not cross the cell membranes in their free form, into the body (e.g. Fe^{3+} ions in the form of a complex with 8-hydroxyquinoline).

19 Oxidation-Reduction Reactions

In oxidation and reduction (redox) reactions, electrons are transferred between reactants. Whenever a substance (e.g. atom or ion) is oxidised, it loses one or more electrons and increases its oxidation number. On the other hand, reduction means that a substance gains an electron(s) and the oxidation number of the element involved decreases.

The **oxidation number** is a formal electric charge that an atom would have if shared electrons are assigned to the atom of higher electronegativity. It reaches zero values or positive or negative, usually whole numbers. The value of the oxidation number is represented by a Roman numeral, placed as a right superscript to the element symbol, e.g. Fe^{III}, or in parentheses after the name of the element, e.g. iron(III). To determine the value of the oxidation number we should follow these rules:

· For an atom in its elemental form the oxidation number is always zero.

· For a monoatomic ion, the oxidation number equals the charge on the ion.

· The sum of the oxidation numbers of all atoms in the electroneutral molecule is zero, in the polyatomic ion equals the charge of the ion.

The process of oxidation is represented by the general equation:

$$A_{red} - n\,e^- \rightarrow A_{ox}$$
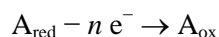
where n is the number of electrons.

This process is real only when the substance B is present at the same time, which is able to accept the released electrons and to be reduced:

$$B_{ox} + n\,e^- \rightarrow B_{red}$$
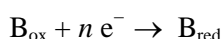
Oxidation and reduction always proceed at the same time. Whenever one substance in the system is oxidised, some other substance in the same system must be reduced. The overall redox reaction is obtained by adding both partial reactions:

$$A_{red} + B_{ox} \rightleftarrows A_{ox} + B_{red}$$
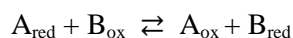
Chemical changes in redox processes are usually more complex than the mere transfer of electrons from the donor to the acceptor (see table below). Oxidation often proceeds in the form of **oxygenation** (gain of oxygen) or **dehydrogenation** (removal of two hydrogen atoms). On the other hand, a common example of reduction is **hydrogenation** (gain of 2H atoms). However, hydration and dehydration (i.e. the addition or elimination of water) are not redox processes as the mean oxidation number of all atoms involved does not change.

The pairs A_{ox}/A_{red} and B_{ox}/B_{red} are called **redox pairs**. Contrary to the conjugate pairs, which differ only in one proton, the components of the redox pair can differ in the number of electrons as well as in the number of hydrogen, oxygen atoms, or by the presence/absence of other elements. The substance which donates electrons is called **the reducing agent**. The reducing agent is oxidised during the redox reaction. The substance, which accepts electrons during the redox reaction (and is reduced itself), is **the oxidising agent**.

To be able to evaluate the course of the redox reactions we have to determine which substance can be oxidised and which reduced. For this reason, we need to have some criterion of the capability of substances to accept or release electrons. A measure of this capability is the oxidation-reduction potential, shortly **redox potential E** which can be defined for each redox pair.

The examples of redox processes

Example of redox process	Type of reaction
$Zn + CuCl_2 \rightarrow ZnCl_2 + Cu$	electron transfer
$CH_3CH=O + \frac{1}{2} O_2 \rightarrow CH_3COOH$	oxygenation
$HOOC−CH=CH−COOH + 2\,H \rightarrow HOOC−CH_2−CH_2−COOH$	hydrogenation*
$CH_3CH_2OH \rightarrow CH_3CH=O + 2\,H$	dehydrogenation*
$C + O_2 \rightarrow CO_2$	oxygenation
$cytochrome\text{-}Fe^{3+} + e^- \rightarrow cytochrome\text{-}Fe^{2+}$	electron transfer

*During enzymatic hydrogenations and dehydrogenations, the H atoms are transferred with the help of cofactors NAD^+ and FAD (see later).

Electrode (Redox) Potential

The electrode (redox) potentials express the ability of the reducing agent to lose electrons or of the oxidising agent to accept electrons. Principally, any redox reaction can be formally divided into two half-reactions, i.e. oxidation which produces electrons and reduction which consumes electrons. Each of these electrochemical half-reaction is characterised by the electrode potential. The redox potential of the redox pair $x\,A_{ox} + n\,e^- \rightarrow y\,A_{red}$ is expressed by the **Nernst-Peters** equation:

$$E = E° + \frac{RT}{nF} \ln \frac{[A_{ox}]^x}{[A_{red}]^y} \qquad\qquad \text{at 25°C:} \quad E = E° + \frac{0.06}{n} \log \frac{[A_{ox}]^x}{[A_{red}]^y}$$

E electrode potential in volts;
$E°$ standard electrode potential in volts (for examples see the table below);
R universal gas constant (8.314 J mol^{-1} K^{-1});
T thermodynamic temperature in Kelvins (273 + temperature in °C);
n number of electrons in the half-reaction $x\,A_{ox} + n\,e^- \rightarrow y\,A_{red}$ (by convention written as reduction);
F Faraday constant (96,485 C mol^{-1});
$[A_{ox}]$ and $[A_{red}]$ molar concentrations of oxidised and reduced form (mol l^{-1}), respectively.

Standard electrode potential $E°$ equals to the value of the electrode potential of the redox pair in the standard state, i.e. at the given temperature and pressure, and 1 M concentration of the reduced and oxidised component of the pair. The absolute value of the electrode potential cannot be measured directly, a reference point must be chosen. The proton-hydrogen ($2H^+/H_2$) redox pair was defined as the reference for the relative scale of electrode potentials. This electrode, referred to as the standard hydrogen electrode (SHE), is assigned a standard potential of 0.000 V at all temperatures.

$$2\,H^+\,(1\,\text{M}) + 2\,e^- \rightarrow H_2\,(g,\,101.4\,\text{kPa}) \qquad E° = 0.000\,\text{V}$$
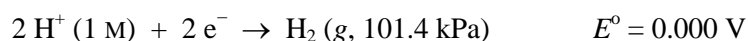
The standard hydrogen electrode (SHE) is the platinum electrode covered by platinum black, partly submerged into an acidic solution with 1 M activity of H^+ ions, partly bathed by gaseous hydrogen with a pressure of 101.3 kPa.

The entire potential of a cell that combines the SHE with a second electrode is assigned to the second electrode. The potential of 0.000 V for the $2H^+/H_2$ pair and all other potentials established relative to it under the standard state conditions are called standard electrode potentials or standard redox potentials.

In practice, the standard hydrogen electrode is usually replaced by other reference electrodes (calomel, silver chloride), if its potential referred to the hydrogen electrode is known.

The table on the left presents the standard redox potentials ($E°$) of some inorganic redox pairs. By convention, all redox pairs are presented as reductions (e.g. $K^+ + e^- \rightarrow K$). The pairs are arranged according to the increasing oxidising ability of the oxidised form.

Values of the standard redox potentials indicate whether the redox pair has the tendency to release rather than to accept electrons.

Values of $E°$ of selected redox pairs (25 °C)

Redox pair	$E°$ (V)
$K^+ + e^- \rightarrow K$	−2.92
$Ca^{2+} + 2\,e^- \rightarrow Ca$	−2.87
$Na^+ + e^- \rightarrow Na$	−2.71
$Mg^{2+} + 2\,e^- \rightarrow Mg$	−2.37
$Al^{3+} + 3\,e^- \rightarrow Al$	−1.66
$Zn^{2+} + 2\,e^- \rightarrow Zn$	−0.76
$Fe^{2+} + 2\,e^- \rightarrow Fe$	−0.44
$\mathbf{2\,H^+ + 2\,e^- \rightarrow H_2}$	**0.00**
$Cu^{2+} + 2\,e^- \rightarrow Cu$	0.34
$I_2 + 2\,e^- \rightarrow 2\,I^-$	0.54
$Fe^{3+} + e^- \rightarrow Fe^{2+}$	0.76
$NO_3^- + 3\,H^+ + 2\,e^- \rightarrow HNO_2 + H_2O$	0.94
$Br_2 + 2\,e^- \rightarrow 2\,Br^-$	1.07
$ClO_4^- + 2\,H^+ + 2\,e^- \rightarrow ClO_3^- + H_2O$	1.19
$O_2 + 4\,H^+ + 4\,e^- \rightarrow 2\,H_2O$	1.23
$Cr_2O_7^- + 14\,H^+ + 6\,e^- \rightarrow 2\,Cr^{3+} + 7\,H_2O$	1.33
$Cl_2 + 2\,e^- \rightarrow 2\,Cl^-$	1.36
$MnO_4^- + 8\,H^+ + 5\,e^- \rightarrow Mn^{2+} + 4\,H_2O$	1.51
$H_2O_2 + 2\,H^+ + 2\,e^- \rightarrow 2\,H_2O$	1.77
$F_2 + 2\,e^- \rightarrow 2\,F^-$	2.56

The more positive value of $E°$, the higher the ability of the oxidised form to accept electrons; the oxidised forms of such pairs are effective oxidation agents (see the positions of the common oxidising agents MnO_4^-, H_2O_2 in the table). The more negative value of $E°$, the higher the ability of the reduced form to release electrons – the reduced forms of those pairs are effective reducing agents (see the positions of the strong reductants Na, Ca, Zn in the table).

A reduced form of the pair can reduce an oxidised form of all pairs with more positive redox potentials. On the other hand, the oxidised form of a given pair can oxidise, i.e. remove electrons from the reduced forms of all pairs with more negative potentials.

Example
Under standard conditions, permanganate oxidises iodide to iodine, even to iodate, and nitrites to nitrates.

It is valid that:

$$E°(MnO_4^-/Mn^{2+}) = 1.51\ V \quad > \quad \begin{aligned} E°(I_2/2I^-) &= 0.54\ V \\ E°(IO_3^-/I^-) &= 1.08\ V \\ E°(NO_3^-/NO_2^-) &= 0.94\ V \end{aligned}$$

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Under standard conditions, the reduction ability of a ferrous ion is sufficient to reduce chlorine into chloride and thallic salts into thallous:

$$E°(Fe^{3+}/Fe^{2+}) = 0.77 \text{ V} \qquad < \qquad E°(Cl_2/2Cl^-) = 1.36 \text{ V}$$
$$E°(Tl^{3+}/Tl^-) = 1.28 \text{ V}$$

However, Fe^{2+} ions cannot reduce sulfate into sulfite:

$$E°(Fe^{3+}/Fe^{2+}) = 0.77 \text{ V} \qquad > \qquad E°(SO_4^{2-}/SO_3^{2-}) = 0.17 \text{ V}$$

If the concentrations of the redox components are different from standard concentration 1 mol l^{-1}, the values of electrode potentials E can be calculated using the Nernst-Peters equation.

It is again valid that the reduced form of the pair with more negative potential E will act as a reducing agent and *vice versa*. It can be proven that if the difference between the standard electrode potentials of the participating redox systems is greater than 0.4 V, the actual concentrations of components will not affect the direction of the reaction predicted with the help of $E°$. The redox reaction can then proceed practically in only one direction (the reaction is practically irreversible). If the difference between the standard potentials is lower than 0.4 V, the direction of the reaction can be affected by the concentrations of components. If the difference between the potentials is lower than 0.1 V, the reactions are fully reversible.

Example

The actual concentrations of substances in the reaction system will be decisive for the direction of reaction:

- whether bromine will oxidise NO to nitrate, or
- whether nitrate will oxidise bromide to bromine

$E°(Br_2/2Br^-) = 1.09 \text{ V}$ $\qquad\qquad$ $E°(NO_3^-/NO) = 0.96 \text{ V}$ $\qquad\qquad$ $\Delta E° = 0.13 \text{ V}$

From the above-mentioned examples we can see that the direction of the course of redox reactions depends on the values of the standard redox potentials, and if their difference is not very large, also on the initial (actual) concentrations of reactants.

Potential Difference (ΔE) and Gibbs Energy (G)

The oxidation-reduction reactions between two redox pairs proceeds only when there is a difference between their redox potentials ($\Delta E = E_2 - E_1$). The reaction proceeds until the equilibrium state is reached and the potentials of both systems are levelled. Electrons are transferred from the reduced form of the redox pair with the more negative potential (reducing agent) to the oxidised form of the pair with the more positive potential.

The change of Gibbs energy ΔG (in joules) of redox reaction can be expressed as useful work connected with the transfer of n moles of electrons with the charge nF across the potential difference ΔE (in volts):

$$-\Delta G = nF(E_2 - E_1) = nF\Delta E$$

After substituting the values of the standard redox potentials we obtain a similar equation for $-\Delta G°$:

$$-\Delta G° = nF(E_2° - E_1°) = nF\Delta E°$$

We know that in the case of spontaneous reactions $\Delta G < 0$. It implies that the expression $nF\Delta E$ in the previous equation will reach a positive value, that is $\Delta E > 0$. By convention, the subscript 2 is always assigned to the potential of the redox pair with the more positive value.

Example

Calculate $\Delta E°$ and $\Delta G°$ for the (not balanced) redox reaction: $MnO_4^- + 5\,Ag + 8\,H^+ \rightarrow Mn^{2+} + 5\,Ag^+ + 4\,H_2O$

Solution:

$E°(MnO_4^-/Mn^{2+}) = +1.51$ V, $E°(Ag^+/Ag) = +0.80$ V, $n = 5$ mol, $F \approx 96,500$ C mol^{-1}

$\Delta E° = +1.51 - (+0.80) = +0.71$ V $\Rightarrow$ $\Delta G° = -(5 \cdot 96,500 \cdot 0.71) = -342,575$ J $\cong -342.6$ kJ

Standard Potentials and Equilibrium Constant (K)

If we know the standard potentials of redox pairs in the redox equation, we can derive the equilibrium constant K.

Consider a general redox reaction: $A_{red} + B_{ox} \rightleftarrows A_{ox} + B_{red}$

The equilibrium constant of this reaction is: $\quad K = \dfrac{[A_{ox}][B_{red}]}{[A_{red}][B_{ox}]}$

The potentials of the redox pairs are:

$$E_1 = E_1^o + \frac{RT}{nF} \ln \frac{[A_{ox}]}{[A_{red}]} \qquad\qquad E_2 = E_2^o + \frac{RT}{nF} \ln \frac{[B_{ox}]}{[B_{red}]}$$

In the equilibrium state, $\Delta E = 0$ V, from that $E_2 = E_1$. If we substitute the above-mentioned equations instead of E_2 and E_1, we find that K is related to the difference between the standard potentials of both redox pairs:

$$RT \ln K = nF\,(E_2° - E_1°)$$

Biochemically Important Redox Systems

Biochemical redox processes are usually catalysed by enzymes oxidoreductases. The principle of degradation of basic nutrients (lipids, saccharides, and proteins) is the gradual oxidation of carbon they contain to carbon dioxide. This degradation process is analogical to the inorganic process of carbon combustion which, as we know, is an important source of energy. However, the difference between the inorganic and biochemical "combustion" is in the fact that useful energy released by biochemical oxidations is released gradually and therefore it is maximally utilisable.

The most frequent form of oxidation in the biological processes is dehydrogenation. Two hydrogen atoms removed from substrates are bound to cofactors of the corresponding oxidoreductases. The typi-

cal cofactors include **NAD⁺** ($\underline{\text{n}}$icotinamide $\underline{\text{a}}$denine $\underline{\text{d}}$inucleotide) and **FAD** ($\underline{\text{fl}}$avin $\underline{\text{a}}$denine $\underline{\text{d}}$inucleotide). Hydrogen atoms bound to cofactors (so called reduction equivalents) are directed to the respiratory chain localised in the inner mitochondrial membrane. Here several enzymatic systems and their cofactors differing in their redox potentials are found (see the table below). Electrons removed from the hydrogen atoms are transported from the carriers with the most negative values of potentials to the positive ones, and finally to the molecules of dioxygen. Energy released by this process is used in the coupled process of oxidative phosphorylation to synthesise ATP.

The redox potentials related to physiological pH value, i.e. pH = 7, are indicated as E', $E°'$. The potential of the standard hydrogen electrode at pH = 7 related to the standard hydrogen electrode at pH = 0 is equal to −0.420 V.

Redox pairs of the respiratory chain (two-electron processes, pH = 7, 30°C)

Redox pairs in the respiratory chain	$E°'$ (V)
$NAD^+ + 2\,H^+ + 2\,e^- \rightarrow NADH + H^+$	−0.320
$FAD + 2\,H^+ + 2\,e^- \rightarrow FADH_2$	[a]
$FMN^b + 2\,H^+ + 2\,e^- \rightarrow FMNH_2$	[a]
2 cytochrome b (Fe^{3+}) + 2 $e^- \rightarrow$ 2 cytochrome b (Fe^{2+})	+0.030
ubiquinone + 2 H^+ + 2 $e^- \rightarrow$ ubiquinol	+0.100
2 cytochrome c (Fe^{3+}) + 2 $e^- \rightarrow$ 2 cytochrome c (Fe^{2+})	+0.235
2 cytochrome a_3 (Fe^{3+}) + 2 $e^- \rightarrow$ 2 cytochrome a_3 (Fe^{2+})	+0.385
½ O_2 + 2 H^+ + 2 $e^- \rightarrow H_2O$	+0.816

[a] Flavoproteins have variable values of $E°'$ depending on the protein part (0.003 − 0.091 V).
[b] FMN flavin adenine mononucleotide.

Examples of biochemically important redox reactions

Enzymatic redox reaction	Commentary
ethanol + $NAD^+ \rightarrow$ acetaldehyde + NADH + H^+	oxidation of ethanol in the liver
pyruvate + NADH + $H^+ \rightleftarrows$ lactate + NAD^+	anaerobic glycolysis
acetoacetate + NADH + $H^+ \rightleftarrows$ β-hydroxybutyrate + NAD^+	conversion of ketone bodies
$R\text{-}CH_2\text{-}CH_2\text{-}CO\text{-}CoA + FAD \rightarrow R\text{-}CH{=}CH\text{-}CO\text{-}CoA + FADH_2$	β-oxidation of fatty acids
phenylalanine + O_2 + $BH_4{}^a \rightarrow$ tyrosine + BH_2 + H_2O	hydroxylation of phenylalanine
succinate + FAD $\rightleftarrows$ fumarate + $FADH_2$	reaction of the citrate cycle
$H_2O_2 + 2\,GSH^b \rightarrow 2\,H_2O + G\text{–}S\text{–}S\text{–}G$	reduction of H_2O_2 in erythrocytes
glyceraldehyde 3-P + P_i + $NAD^+ \rightleftarrows$ 1,3-BPG^c + NADH + H^+	reaction of glycolysis

[a] BH₄ tetrahydrobiopterin, BH₂ dihydrobipterin.
[b] GSH reduced glutathione; G–S–S–G oxidized glutathione.
[c] 1,3-BPG 1,3-bisphosphoglycerate.

20 Latin names

The World Health Organisation (WHO) created Latin names of drugs as international non-proprietary names (INN). They are presented in national pharmacopoeias.

The Latin names of acids consist of the noun *acidum* (neutrum) and an adjective determining the electronegative part. The adjective is different for binary and oxo acids:

· binary acids (without oxygen) have the prefix *hydro–*, the Latin name for element, and the suffix *–icum*.
· inorganic oxo acids have the suffix *–icum* for a higher oxidation number, *–osum* for a lower one.
· all organic acids have the uniform suffix *–icum*.

Latin names of selected inorganic and organic acids

Acid	Acidum	Acid	Acidum
Sulfuric	sulfuricum	Formic	formicum
Sulfurous	sulfurosum	Acetic	aceticum
Nitric	nitricum	Oxalic	oxalicum
Nitrous	nitrosum	Malic	malicum
Hydrochloric	hydrochloricum	Succinic	succinicum
Phosphoric	phosphoricum	Lactic	lacticum
Boric	boricum	Butyric	butyricum
Folic	folicum	Pyruvic	pyruvicum
Benzoic	benzoicum	Ascorbic	ascorbicum

The names of salts consist of two nouns; if needed, numerical prefixes *di-*, *tri-*, *tetra-* etc. are applied to express stoichiometry.

The name of the **electropositive** (first) component (cation) is in genitive. If there is a need to distinguish between the oxidation numbers of a cation, the higher value is indicated by the suffix *–i*, the lower oxidation number gets the suffix *–osi* (ferri *vs.* ferrosi, cupri *vs.* cuprosi).

The name of the **electronegative** (second) component (anion) is in nominative. Binary salts, oxides, peroxides, and hydroxides have the suffix *–idum*. The water of crystallisation ends with *–um* (neutrum).

$NaCl$	natrii chloridum	$CaCl_2.2H_2O$	calcii chloridum dihydricum
$Mg(OH)_2$	magnesii hydroxidum	KI	kalii iodidum
CO_2	carbonei dioxidum	N_2O	dinitrogenii oxidum
$CuCl$	cuprosi chloridum	$CuCl_2$	cupri chloridum

Oxo anions have the suffix *–as* for the higher oxidation number and *–is* for the lower one (both masculinum). If there is a need to distinguish between the oxidation numbers of the central element in an oxo

anion, the highest value is indicated by the prefix *per-*, the lowest oxidation number gets the prefix *hypo-*.

All anions of organic acids have the suffix *–as*. Anions of polyprotic acids containing hydrogen have the prefix *hydrogeno–*.

$MgCO_3$	magnesii carbonas anhydricus	$Na_2B_4O_7$	natrii tetraboras
$NaNO_2$	natrii nitris	$FeSO_4$	ferrosi sulfas
$AgNO_3$	argenti nitras	$Fe_2(SO_4)_3$	ferri sulfas
KH_2PO_4	kalii dihydrogenophosphas	$Al(CH_3COO)_3$	aluminii acetas
$KMnO_4$	kalii permanganas	$NaClO$	natrii hypochloris

The names of non-electrolytes (alcohols, ketones, amides, saccharides, some vitamins, etc.) usually have the suffix *–um* (glucosum) with some exceptions (e.g. urea). The names of esters are constructed like the salts of oxo acids (ethylis acetas).

Latin names of selected non-electrolytes:

glucose	glucosum	caffeine	coffeinum
glycerol	glycerolum	paracetamol	paracetamolum
ethanol	ethanolum	tocopherol	tocopherolum
starch	amylum	retinol	retinolum
menthol	mentholum	retinol acetate	retinoli acetas
acetone	acetonum	glycerol trinitrate	glyceroli trinitras
camphor	camphora	ethyl acetate	ethylis acetas
urea	urea	choline salicylate	cholini salicylas

Elements in their elementary states are also non-electrolytes; their names have the suffix *–um* (ferrum, cuprum), *–ium* (natrium, kalium), or *–eum* (carboneum), exceptions are phosphorus and sulfur.

Latin names of selected elements:

hydrogen	hydrogenium	sodium	natrium
oxygen	oxygenium	potassium	kalium
sulphur	sulfur	calcium	calcium
selenium	selenium	magnesium	magnesium
nitrogen	nitrogenium	aluminium	aluminium
phosphorus	phosphorus	iron	ferrum
carbon	carboneum	manganese	manganum
silicon	silicium	copper	cuprum
fluorine	fluorum	zinc	zincum
chlorine	chlorum	silver	argentum
iodine	iodum	gold	aurum

21 Essential Macroelements

Living organisms extract from the natural environment and selectively cumulate some elements necessary for their existence. They are **essential elements**. Besides, trace elements are also found in living organisms. Other elements regularly found in the human body are taken as constituents without any biological importance, as contaminants absorbed from the environment. Elements regularly occurring in the human body can be in the form of inorganic compounds (water, carbon dioxide, etc.) and organic compounds (glucose, fatty acids, peptides, nucleic acids).

Essential macroelements make up about 99% of human body mass. Daily intake of each macroelement in food is more than 1 g in adults. There are eleven macroelements:

carbon, hydrogen, oxygen, nitrogen, phosphorus, sulfur, calcium, magnesium, sodium, potassium, chlorine

Carbon

Carbon (20% of body mass) is an element bounded in all organic compounds. Saccharides, lipids, and proteins are the main source of carbon for animals (heterotrophic organisms). On the other hand, autotrophic organisms (green plants) use atmospheric CO_2 and reduce it to saccharides in the process of photosynthesis. In the human body, the oxidation of nutrients produces large amounts of carbon dioxide. CO_2 is transformed in erythrocytes by enzyme carbonic anhydrase to H_2CO_3, which is under physiological pH values (~ 7.4) almost completely dissociated. Thus, we obtain distinctly higher concentration of HCO_3^- that is transported to the lungs where it is transformed back to CO_2 and removed by ventilation. HCO_3^- is the most important buffer base of the extracellular fluids and has a major role in maintaining acid-base balance.

Elemental carbon can be prepared from plant or animal material by burning in the absence of air as activated carbon, an effective non-polar adsorbent. The large surface area of carbon particles dispersed in the solution binds non-polar substances (gases and organic compounds) by intermolecular interactions. **Adsorption coal** (carbo adsorbens, charcoal) obtained by burning animal waste, mostly blood or skin, is used as a non-toxic intestinal adsorbent, e.g. during diarrhoea or poisoning, it adsorbs gases and non-polar toxins. In overdosing by some drugs, it limits their absorption, however, it must be administered in sufficiently high doses (about 50 g, dispersed in water).

Selected Inorganic Carbon Compounds

Carbon monoxide (CO) is a toxic gas (see Chapter 33).

Carbon dioxide (CO_2, carbonei dioxidum) is a colourless gas. It can be easily liquefied. It does not burn and does not support burning; therefore, it serves as the filling of fire extinguishers. It is relatively stable; it decomposes to CO and O_2 only at temperatures over 1,000°C.

It is produced during complete combustion of carbon and carbon compounds, produced during the aerobic metabolism of nutrients. It is heavier than air; therefore, it cumulates at the bottom of cellars, in wells, etc. The atmosphere contains approximately 0.03 vol. % of CO_2, air exhaled from the lungs about 3.5%, alveolar sacs 5–6%.

Carbon dioxide is not directly toxic, however, it regulates breathing. In lower concentrations it stimulates the breathing centre in the medulla oblongata (breathing becomes deeper and quicker); in higher concentrations it suppresses the breathing centre. Staying in the environment with 3 vol. % of CO_2 can cause sleepiness, headache, or muscle weakness. Inhalation of air with 7–10 vol. % of CO_2 for several minutes leads to unconsciousness and the stop of breathing, without the symptoms of suffocation. Concentrations higher than 30 vol. % are lethal even if the amount of oxygen is sufficient.

Carbon dioxide is used for medical purpose as an insufflation gas for minimal invasive surgery (e.g. laparoscopy) to enlarge and stabilise body cavities. In its liquid phase it can also be used to provide temperature down to -76°C for cryotherapy or for local analgesia by external application onto the skin surface. Solid carbon dioxide, so called "dry ice", which sublimes (at -79°C) is used as a cooling medium for frozen transport of biological materials.

Carbon dioxide reacts with strong hydroxides to form carbonates. This reaction is used to remove CO_2 from the mixture of gases, e.g. from the exhaled air in the respirators or anaesthesia machines with a closed cycle. Gas passes through the solid granulated mixture (e.g. NaOH and $Ca(OH)_2$) which captures carbon dioxide: $CO_2 + 2\ NaOH \rightarrow Na_2CO_3 + H_2O$.

Carbonic acid (H_2CO_3, acidum carbonicum) is a weak diprotic acid formed from CO_2 and H_2O. Carbon dioxide is not very soluble in water; its solubility depends on temperature and partial pressure pCO_2 above the solution. The majority of CO_2 molecules are not changed in the solution; they are just physically dissolved.
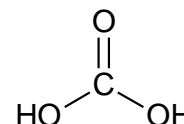
Only a very small portion of CO_2 is converted to weak carbonic acid H_2CO_3:

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

The solution containing carbonic acid, HCO_3^- ion, and physically dissolved CO_2 is a hydrogen carbonate buffer, the major buffer of extracellular fluids (see Chapter 16).

Hydrogen carbonates (bicarbonates) are water-soluble salts with all cations. Due to hydrolysis, their solutions are weakly alkaline. Calcium and magnesium hydrogen carbonates exist only in solutions, and by evaporation or heating they are converted into insoluble carbonates.

Sodium hydrogen carbonate $NaHCO_3$ (natrii hydrogenocarbonas, baking soda) is sometimes used as an antacid to neutralise hyperacidic gastric secretion. It is not very suitable for this purpose. Solutions of $NaHCO_3$ in infusions are used in the treatment of some types of acidoses, e.g. from the excessive losses of HCO_3^- or in some kinds of poisoning (salicylates). $NaHCO_3$ is administered orally in chronic states accompanied by acidosis or for the alkalisation of urine.

Sodium and potassium carbonates are soluble; their solutions have a strong alkaline reaction, while the other carbonates are practically insoluble. Introducing CO_2 into suspensions of carbonates produces their corresponding hydrogen carbonates, the excess of acid causes the complete decomposition of carbonates, for example:

$CaCO_3 + 2\ HCl \rightarrow CaCl_2 + H_2O + CO_2\uparrow$.
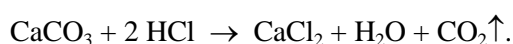
Hydrogen

Hydrogen is the component of water and organic compounds (10% of body mass). In living organisms, it is extremely important in the processes providing energy. Photosynthesising plants and some microorganisms absorb the light energy of the Sun and use it to decompose water (photolysis of water). The hydrogen atoms of water serve as reductants of CO_2 in the synthesis of saccharides and other hydrogen-rich carbon compounds; oxygen molecules are released.

The majority of other organisms are chemoorganotrophs that utilise hydrogen-rich compounds as nutrients. The requirements of these organisms for energy are covered by oxidation of saccharides, triacylglycerols, and proteins (most effectively by aerobic oxidation, oxygen in the air is the oxidant). Nutrients undergo dehydrogenations; H atoms in the form of reduced coenzymes NADH and $FADH_2$ enter the mitochondrial electron-transferring chain. Electrons of the hydrogen atoms reduce oxygen to the oxide anion O^{2-}, from which water is formed by accepting H^+ ions. When hydrogen burns in air, under laboratory conditions (*in vitro*), the synthesis of water ($H_2 + \frac{1}{2} O_2 \rightarrow H_2O$) is a strongly exothermic reaction ($\Delta H° = -286$ kJ mol^{-1}). Approximately the same amount of energy is obtained by the gradual aerobic oxidation of nutrients in many sequential reactions.

The concentration of H^+ ions in most body fluids in humans is normally kept within very close limits. The pH of blood is very strictly regulated. The lungs and the kidneys together maintain overall acid-base balance.

Gaseous dihydrogen (H_2) is one of the products of bacterial fermentation in the large intestine; hydrogen is partly reabsorbed and breathed out.

Oxygen

Oxygen is the most abundant element in inanimate nature and the human body (63% of body mass). The majority of oxygen is bound in compounds, mainly in water. The volume fraction of oxygen in dry air is approximately 21%, other components are nitrogen (78%), carbon dioxide (0.03%), and noble gases (argon, neon).

Dioxygen (O_2) is a common form of oxygen in the air. Oxygen is an almost universal oxidant (acceptor of electrons) in the biosphere. It is essential for organisms that obtain energy by the aerobic oxidation of the hydrogen-rich nutrients. Dioxygen passes from the lungs to the blood (bound to hemoglobin) during breathing and is transported to the tissues. The O_2 then diffuses into the cells and it becomes the acceptor of electrons in the terminal respiratory chain in the mitochondria. The four-electron reduction of dioxygen catalysed by cytochrome-*c*-oxidase produces two oxide anions; water is created by accepting two hydrogen ions:

$$O_2 + 4\,e^- \rightarrow 2\,O^{2-} \qquad\qquad 2\,O^{2-} + 4\,H^+ \rightarrow 2\,H_2O$$

Oxygen also exerts its oxidation properties in a number of other metabolic reactions, catalysed by oxygenases or oxidases (e.g. in hydroxylations, degradations of the aromatic ring of amino acids, synthesis of eicosanoids, oxidative deamination of amines and amino acids).

Tissue hypoxia with all serious consequences occurs within a few minutes when breathing is stopped. In medicine, inhalation of the oxygen-enriched air is used when either the pulmonary ventilation is restricted or tissue hypoxia originates due to insufficient blood circulation, anaemia, etc. It is im-

portant to know that inhalation of pure oxygen or air with more than a 40-volume % of O_2 results in the damage of the respiratory system after more than 20 hours. Oxygen is also used for therapy in hyperbaric chambers (hyperbaric oxygenotherapy), in which pure oxygen at the pressures up to 300 kPa can be inhaled without any harmful effect for almost two hours. Oxygen serves for breathing support whenever its availability is restricted (e.g. aircraft and aeronautics, diving or submarines, climbing at extreme heights, respirators used by rescuers). Compressed cylinders with oxygen for medical purposes are identified by a white strip near the top, (generally, cylinders with gases for medical applications are white and are identified by the colour strip near the top).

The ground stable state of the dioxygen molecule is **triplet** oxygen $^3O_2$. The excited and unstable molecule of singlet oxygen $^1O_2$, formed by delivering energy to the triplet dioxygen, is very reactive.

Ozone (O_3, trioxygen) is a highly reactive gas that has toxic effects to the human organism (Chapter 33). However, ozone also has a positive effect. In the stratosphere, ozone is irreplaceable in the protection of living organisms against the intensive UV radiation from the Sun. The ozonosphere absorbs the majority of short-wave radiation and acts as a UV filter. Chlorofluoroalkanes (freons) and nitrogen oxides, which diffuse from the Earth's surface into the ozonosphere, accelerate the decomposition of ozone and diminish the effectiveness of the filter. An ozone hole arises and UV light reaching the Earth's surface is higher. Increased intensity of UV light negatively influences the Earth's flora and fauna; it is in close connection with the incidence of human skin cancer and eye diseases.

Hydrogen peroxide (H_2O_2, H–O–O–H) is an unstable compound that decomposes easily to give water and oxygen ($2\ H_2O_2^{-I} \rightarrow 2\ H_2O^{-II} + O_2^{0}$). It is a redox reaction, one oxygen atom of the peroxide molecule is reduced to water, the second oxygen atom is oxidised to elemental oxygen (such reactions are called disproportionations or dismutations).
Hydrogen peroxide acts as an oxidising agent. A 30% solution of hydrogen peroxide is harmful to skin and serves only for some oxidation processes. A 3% solution (hydrogenii peroxidum) is used rarely in medicine. It has a weak bactericidal effect, and due to the release of oxygen can cleanse abrasions from unwanted particles.

Reactive Oxygen Species (ROS)

Partially reduced oxygen anions (superoxide anion-radical and peroxide anion) originate in the cells during various reactions. Other reactive oxygen species (hydroxyl radical and singlet oxygen) arise from these anions that can be harmful to cell structures by unfavourable oxidations or by the elicitation of production of other free radicals. They probably play an important role in the process of ageing.

The **negative effect** of reactive oxygen species is based on the fact that they damage all types of biomolecules. The most effective are hydroxyl radical and singlet oxygen. In lipids, they cause peroxidation of PUFA, which can distinctly alter the functions of cell membranes or lipoprotein particles. In proteins, they oxidise sulfanyl (–SH) or other groups of amino acid side chains. The presumable consequences are changes of enzyme activities, modified function of regulatory proteins and receptors, alteration of antigenic determinants, etc. In deoxyribonucleic acids, the reactive oxygen species are able to attack the bases of nucleotides or modify the sugar component with subsequent fragmentation of DNA and thus change the genetic information.

There is also a **positive effect** of the reactive oxygen species – the bactericidal and cytotoxic effect of superoxide in the phagocytes during the respiratory burst in inflammation. However, in chronic inflammation, an insufficient control of the process can result in the long-term stimulation of the phagocytes and thus cause undesirable damage to the other cells of the host.

Superoxide anion-radical ($\cdot O_2^-$) originates from one-electron reduction of dioxygen:

$$O_2 + e^- \rightarrow \cdot O_2^-$$
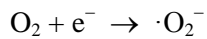
Superoxide is not very reactive by itself, but the extent of its production is rather important. The subsequent reactions of superoxide result in the formation of more ROS, namely hydroxyl radical, hydrogen peroxide, singlet oxygen. Therefore, the production of superoxide is decisive for the formation of other ROS.

In living organisms, superoxide can be formed, for example, in mitochondria (complex III). Superoxide is generally produced during non-enzymatic oxidations of some compounds by dioxygen, e.g. during oxidation of ubiquinone (component of the terminal respiratory chain), other hydroquinones, semiquinones, flavines, or thiols (including glutathione), hemoproteins, and glycated proteins.

Superoxide is also produced during phagocytosis. It is well known that phagocytosis of the foreign cells by neutrophilic granulocytes or macrophages is accompanied by so-called respiratory burst; dioxygen and coenzyme NADPH produce superoxide in the reaction catalysed by NADPH oxidase:

$$2\,O_2 + NADPH \rightarrow 2\,\cdot O_2^- + NADP^+ + H^+.$$
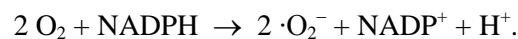
However, formation of superoxide is desirable during phagocytosis, which results in favourable bactericidal or cytotoxic effects.

The most important defence against superoxide is the dismutation of superoxide to dioxygen and hydrogen peroxide catalysed by the metalloenzyme **superoxide dismutase** (SOD):

$$2\,\cdot O_2^- + 2\,H^+ \rightarrow O_2 + H_2O_2$$
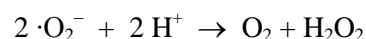
Hydroxyl radical ($\cdot OH$) is mostly produced in cells from hydrogen peroxide in the so-called Fenton reaction, if free Fe^{2+} (or Cu^+) are present:

$$H_2O_2 + Fe^{2+} \rightarrow \cdot OH + OH^- + Fe^{3+}$$
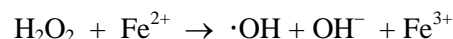
In cells, hydroxyl radical is also formed from superoxide and hydrogen peroxide (in the presence of reduced metal ions, Fe^{2+} or Cu^+):

$$\cdot O_2^- + H_2O_2 \rightarrow \cdot OH + OH^- + O_2$$
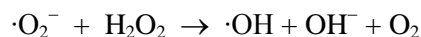
In the phagocytes, the $\cdot OH$ radical is produced during the respiratory burst even in the absence of metal ions, if superoxide reacts with nitrogen oxide or hypochlorite anion.

The hydroxyl radical is extremely reactive and makes most of its harmful effects close to the site of its origin. It removes hydrogen atoms from various molecules or it binds to them by addition. In both cases, reactive **secondary free radicals** (e.g. alkanyl $R\cdot$, peroxyl $ROO\cdot$, alkoxyl $RO\cdot$) originate. Further reactions then either initiate propagation of free radicals production, if the radicals react with other molecules, or terminate the production, if pairs of free radicals (all with unpaired electrons) link to one another by covalent bonds into molecules. Hydroxyl radicals can attack bases in nucleic acids,

unsaturated fatty acids in membrane phospholipids. Due to its high reactivity, hydroxyl is considered to be the most toxic ROS.

Singlet oxygen ($^1O_2$). In organisms, during some reactions, the excitation of a molecule of common triplet dioxygen $^3O_2$ results in the production of a highly reactive species – singlet oxygen $^1O_2$.

Also *in vitro*, singlet oxygen can be produced during photosensitisation reactions. A photosensitiser absorbs the photon to form excitation states with a subsequent transfer of energy to oxygen resulting in the formation of singlet oxygen. This phenomenon can be used for the photodynamic therapy of tumours.

Hydrogen peroxide is also included among the reactive oxygen forms even though it is not a radical. In cells, peroxide is the product of the two-electron reduction of dioxygen during some dehydrogenations catalysed by oxidases; coenzyme FAD transfers the hydrogen atoms of the substrate to dioxygen producing peroxide. The other cellular source of hydrogen peroxide is dismutation of superoxide by superoxide dismutase (SOD). In cells, hydrogen peroxide is decomposed by some enzymes. The enzyme catalase (its activity is high in peroxisomes) decomposes peroxide by dismutation to water and oxygen. Enzymes peroxidases (e.g. glutathione peroxidase) catalyse the reduction of peroxide to water.

Hydrogen peroxide is a less reactive oxygen species. It can diffuse through phospholipid membranes, shows weak oxidation effect (e.g. oxidises –SH groups of enzymes and inhibits their biological activity). Hydrogen peroxide also can be the substrate of the Fenton reaction producing the hydroxyl radical ·OH. In phagocytosing cells, hydrogen peroxide oxidises chloride ions to hypochlorite ions in the reaction catalysed by myeloperoxidase.

$$H_2O_2 + Cl^- \rightarrow ClO^- + H_2O.$$
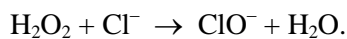
Hypochlorite ions exhibit bactericidal effects.

Oxidative Stress

Reactive oxygen species and the other free radicals are produced in all cells. However, under physiologic conditions, there is a balance between their formation and destruction by protective mechanisms, which keep the concentration of reactive radicals low and the damage caused by these radicals need not be necessarily expressed.

If imbalance occurs between the formation and elimination of ROS, the radicals are generated excessively and cause oxidative stress. This condition is either because of the increase of the formation of reactive radicals caused by external factors or pathological processes (mainly inflammations), or because of the lower effectivity of the antioxidant mechanisms. There are many known external evoking factors such as ionising radiation or excessive exposure of the skin to sunlight, foreign substances (xenobiotics) consumed with food, insufficient amount of antioxidants in the diet, long-time inhalation of air with high partial pressure of oxygen (above 40 kPa).

The formation and elimination of ROS is shown in the simplified scheme:

$$O_2 \xrightarrow{e^-} \cdot O_2^- \xrightarrow{SOD} H_2O_2 \xrightarrow{catalase} H_2O + {}^1/_2 O_2$$
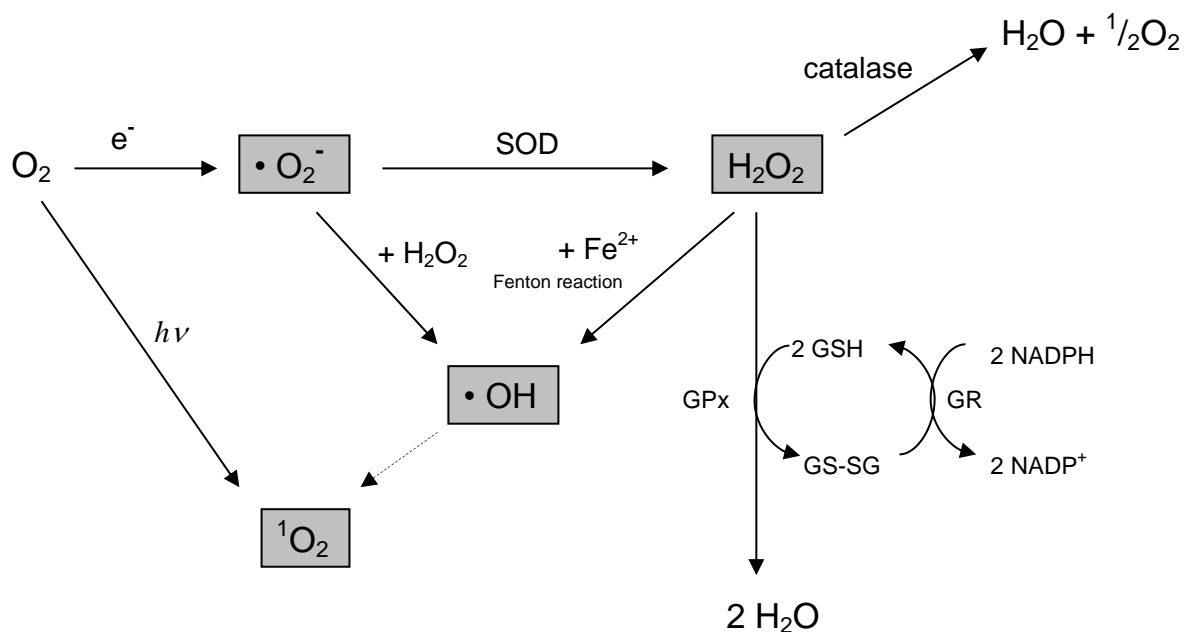
Figure: The formation and elimination of reactive oxygen species
(SOD superoxide dismutase, GPx glutathione peroxidase, GR glutathione reductase, GSH glutathione)

Protective Antioxidant Systems

The evolutional adaptation to life in the earth's atmosphere resulted in certain antioxidant mechanisms, which to a certain extent protect aerobic cells against the toxicity of ROS and thus make their survival easy. Several antioxidant systems acting in various ways are available in the organism. There are three main approaches: enzymes, low-molecular antioxidants, and substances binding metal ions.

Antioxidant enzymes belong to primary systems eliminating free radicals. They have a principal role in the organism. The summary of these enzymes is given in the following table.

Examples of antioxidant enzymes

Enzyme	ROS eliminated
Superoxide dismutase (SOD)	$\cdot O_2^-$
Glutathione peroxidase (GSHPx)	H_2O_2 , hydroperoxides of lipids
Catalase	H_2O_2

Low-molecular antioxidants reduce reactive oxygen species. Some of these antioxidants can be regenerated to the effective reducing form. According to their structure and polarity, antioxidants can be divided into lipophilic and hydrophilic types. The examples of antioxidants are given in the table below.

Some low-molecular antioxidants

Lipophilic antioxidants	Hydrophilic antioxidants
Tocopherols (vitamin E)	L-Ascorbate (vitamin C)
β-Carotene (provitamin A)	Flavonoids
Lycopene and other carotenoids	Uric acid [a]
Xanthophyls	Glutathione [a]
Ubiquinol (coenzyme Q) [a]	Lipoate [a]

[a] Endogenous compounds

Substances decreasing the availability of Fe^{2+} and Cu$^+$ ions. The reduced ions of iron and copper enter the Fenton reaction and support the formation of hydroxyl radicals. The examples of these substances are both low-molecular compounds (e.g. chelating agents) and high-molecular compounds (proteins, e.g. ceruloplasmin).

Water

Water is the most common oxygen compound, essential for living systems. The covalent bonds O–H are polar, due to the different electronegativities of both atoms, and the bond angle formed by this bond is 105°. Therefore, water is a polar molecule with a distinct dipole moment. Liquid water is a solvent of high polarity. The boiling and melting points of water are considerably higher than those of other covalent hydrides, because of associating water molecules through hydrogen bonds. These intermolecular bonds in liquid water form and reorient continuously and very quickly. Hydrogen bonding among water molecules also influences hydrophobic interactions between non-polar parts of biomolecules (proteins, etc.).
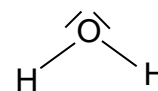
Chemically pure water is a very weak electrolyte. Water can act as both a weak acid and a weak base; it is an ampholyte. It ionises to a small extent to form a hydrogen ion (H$^+$ is hydrated to the hydronium ion H$_3$O$^+$), and a hydroxide anion: $2 H_2O \rightleftarrows H_3O^+ + OH^-$. As equal amounts of conjugate acid and conjugate base are produced, pure water is neutral. The ion product of water ($K_w = 10^{-14}$) expresses the equilibrium of water autoprotolysis and is used instead of the equilibrium constant of water dissociation.

In aqueous solutions of salts, anions of weak acids take H$^+$ from water; cations of weak bases take OH$^-$ ions. These reactions cause the **hydrolysis of ions**, while the aqueous solution of such salts are slightly alkaline or acidic, respectively.

In addition, all ions are hydrated in aqueous solution, enveloped in a **hydration shell** (without any change in pH). The electric charge of ions attracts the opposite ends of the water dipoles that surround them.

Water in the organism. Water is a principal solvent and is important for transport of compounds, helps to regulate body temperature, etc. Water makes up 55–60% of body weight in adults, from which two-thirds is intracellular fluid (ICF), and one-third remains in extracellular fluid (ECF). Extracellular fluid consists of plasma (1/4 of ECF) and interstitial fluid (3/4 of ECF). The recommended daily intake of water is approximately 2,000 ml and there has to be a balance between the amount of

water gained and the amount of water lost by the body. Water balance is regulated by hormones and is connected with electrolyte balance. The intake and excretion of water is affected by antidiuretic hormone (ADH, vasopresine) and also other hormones – the steroid hormone aldosterone and atrial natriuretic peptide (ANP) contribute to the regulation of water balance.

Natural waters always contain dissolved inorganic and organic substances. In chemical laboratories, **deionised water** is mostly used. **Drinking water** is water unobjectionable to health in long-term consumption, it has to meet standards for chemical, physical, and microbiological parameters. To prepare drug solutions, **aqua purificata** is used (the procedure is described in the Pharmacopoeia). Drugs for parenteral applications are dissolved in **aqua pro iniectione**, sterilised and purified from organic substances called pyrogens (apyrogenic water).

Nitrogen

Nitrogen makes up about 3% of body mass (mainly as amino acids, proteins, and nucleic acids with their nitrogen bases). Animals are able to use only dietary proteins as the source of nitrogen to build up their bodies. The recommended daily intake for adults is 0.7–0.8 g of proteins per kilogram of body weight, i.e. approximately 50 g of high-quality proteins daily. The breakdown of body proteins and deamination of amino acids, mainly glutamate, results in the release of ammonia:

$$\text{glutamate} + NAD^+ + H_2O \rightarrow \text{2-oxoglutarate} + NADH + H^+ + NH_3$$
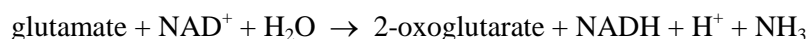
Ammonia is toxic in higher concentrations (mainly for the nervous system). In mammals, the detoxication of ammonia proceeds in the liver – ammonia is transformed to harmless urea $CO(NH_2)_2$ excreted by the kidneys to urine.

Selected Inorganic Compounds of Nitrogen

Ammonia (NH_3) is a toxic gas, a typical weak base ($pK_B = 4.75$), $NH_3 + H_2O \rightleftarrows NH_4^+ + OH^-$. In the human body, it is constantly produced by the catabolism of amino acids.

Dinitrogen monoxide (N_2O, dinitrogenii oxidum, nitrous oxide, laughing gas) is a colourless gas with weak anaesthetic properties. In medicine (surgery) it is used as an inhalation anaesthetic. The mixture of nitrous oxide and oxygen (50% N_2O and 50% O_2) guarantees good oxygenation; the risk of excessive sedation decreases.

Nitrogen monoxide (NO, nitric oxide) is a very reactive substance, a radical, nitrogen has one unpaired electron ($\cdot N=O$). It is formed during the oxidation of all nitrogen compounds with the oxidation number $-III$, as well as the reduction (or disproportionation) of nitrogen compounds with the oxidation numbers III–V. NO is produced physiologically in blood vessel endothelium, phagocytosing cells, and some neurons by the oxidation of arginine catalysed by nitric oxide synthase:

$$\text{arginine} + O_2 + NADPH + H^+ \rightarrow \cdot NO + \text{citrulline} + NADP^+$$
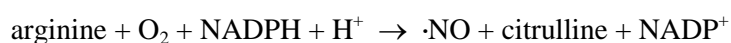
One of its functions is the relaxation of smooth muscle cells of arterial walls. The vasodilatation effect of NO on the coronary vessels is used in the treatment of ischemic heart disease – nitric oxide is released from the esters of nitric acid (e.g. isosorbide dinitrate and glyceryl trinitrate) in the vessel wall.

Nitrogen dioxide (NO_2). NO rapidly combines with oxygen to form red brown nitrogen dioxide. Nitrogen dioxide introduced in water undergoes dismutation:

$$3\ NO_2 + H_2O\ \rightarrow 2\ HNO_3 + NO$$
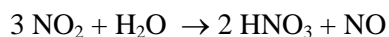
Nitrogen dioxide is the main component of irritating and toxic nitrous fumes, released during the reduction of HNO_3 and nitrites, in industrial exhalations and exhaust fumes. Concentration of nitrogen oxides (NO_x) in the atmosphere is monitored as an indicator of air pollution. Nitrogen oxides contribute to the acidity of rainfall; they are washed by rain into soil.

Nitrous acid (HNO_2, HO–N=O, acidum nitrosum) exists only in diluted solutions as a weak and unstable acid ($pK_A = 3.3$), it is decomposed by disproportionation: $3\ HNO_2 \rightarrow HNO_3 + 2\ NO + H_2O$. It has oxidation effects. The salt **nitrites** are stable in a solid state, mostly soluble in water. They are formed by the moderate reduction of nitrates, they are rather toxic (see Chapter 33).

Nitric acid (HNO_3, HO–NO_2, acidum nitricum) is a strong acid with oxidation properties. The salt **nitrates** are well soluble in water. They are used as oxidation agents, they release oxygen in higher temperatures. Sodium, potassium, and ammonium nitrate, so called saltpetre, are used as industrial fertilisers.

Nitrates are not very toxic; their daily intake up to 300 mg is harmless for adults. We usually consume about 100 mg of NO_3^- daily, mostly from vegetables and tap water. They are also formed from endogenous NO in a small amount in the body. Nitrates are quickly excreted by urine.

A high intake of nitrates poses a certain danger. Part of consumed nitrates (about 4–7%) and nitrates released from blood back into saliva can be **reduced to nitrites** by the enzymes of oral microflora (sooner than enzymes are inactivated by strongly acidic gastric secretion). Therefore, nitrite methemoglobinaemia from the nitrate load occurs in infants in the first four months of life. Their stomach mucosa does not produce a sufficient amount of HCl and foetal hemoglobin F, which is more easily oxidised than adult hemoglobin A, has not yet been removed. Consumed nitrates can be reduced to nitrites also in the intestine during some intestinal infections.

Phosphorus

In the human body phosphorus occurs entirely in the form of phosphoric acid derivatives (1% of body mass). Most phosphates in vertebrates (over 80%) are components of **insoluble** extracellular matrix of bones and teeth in the form of calcium phosphates, mainly hydroxylapatite $Ca_5(PO_4)_3OH$.

Extracellular fluids contain about 1 mmol l^{-1} of hydrogen phosphate and dihydrogen phosphate ions (inorganic phosphates). Their concentration is maintained by exchange with bone tissues and renal excretion, regulated mainly by the parathyroid hormone. The concentration of organic phosphates (phosphate diesters in blood plasma phospholipids) is almost double. Local alkalisation is important for the precipitation of insoluble calcium phosphates, e.g. during the mineralisation of bone tissues or formation of calcium phosphate urinary concretions.

The **intracellular environment** contains a high concentration of inorganic phosphates and many types of phosphate esters – phospholipids, nucleic acids, phosphorylated proteins, phosphoesters of sugars.

Together with proteins, they represent the main intracellular buffer bases. Cells in the human body contain special high-energy compounds where phosphates are linked by anhydride bonds. The most important is ATP, adenosine triphosphate. The hydrolysis of ATP releases energy that is used to power endergonic (energy-requiring) biological processes (cell growth, muscle movements, endergonic syntheses):

$$ATP + H_2O \rightarrow ADP + P_i + energy$$

The recommended amount of phosphorus is 700 mg for adults. The sources of phosphates in food are all foodstuffs of animal origin. Diluted phosphoric acid is consumable; it is used in the food industry instead of citric acid to flavour some soft drinks (*Coca-Cola*).

Selected Phosphorus Compounds

Phosphoric acid (H_3PO_4, acidum phosphoricum) is the common name for trihydrogenphosphoric acid. It is a weak triprotic acid, in the third dissociation a very weak acid.

$$H_3PO_4 \rightleftarrows H^+ + H_2PO_4^- \qquad pK_{A1} = 2.12$$
$$H_2PO_4^- \rightleftarrows H^+ + HPO_4^{2-} \qquad pK_{A2} = 7.21$$
$$HPO_4^{2-} \rightleftarrows H^+ + PO_4^{3-} \qquad pK_{A3} = 12.37$$
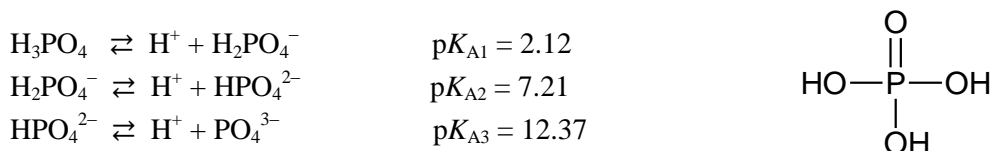
An anhydride formed from phosphoric acid is diphosphoric acid (diphosphate) that is contained in the structures of many nucleotides (e.g. ADP) and enzyme cofactors (e.g. NAD^+, FAD).
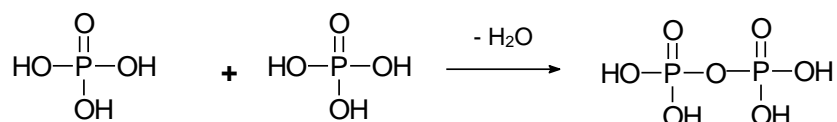
Energy is released after the breaking of an anhydride bond in diphosphate, therefore it belongs to high-energy compounds. In the organism, the most important high-energy compound is ATP containing two phosphoanhydride bonds.

Phosphates. Phosphoric acid forms three groups of salts – dihydrogen phosphates with $H_2PO_4^-$ anion, hydrogen phosphates with HPO_4^{2-} and phosphates with PO_4^{3-} ion. All dihydrogen phosphates are soluble in water and their solutions have a weakly acidic reaction, the $H_2PO_4^-$ anion quite readily releases H^+. Sodium, potassium, and ammonium hydrogen phosphates and phosphates are soluble, their solutions have an alkaline reaction caused by hydrolysis. Hydrogen phosphates and phosphates of other metals are insoluble, but they can be transformed by acids to soluble dihydrogen phosphates and *vice versa*. If acidic solutions containing $H_2PO_4^-$ and Ca^{2+} (Mg^{2+}) ions are alkalised, we obtain insoluble hydrogen phosphates or phosphates by precipitation.

Zinc phosphate cement is used in stomatology for the cementation of inlays, crowns, and other dental restorations. It is made from the solution of phosphoric acid (40%), zinc oxide, and magnesium oxide.

Sulfur

In the human body sulfur occurs mainly in organic compounds (proteins with cysteine and methionine, some vitamins and cofactors, coenzyme A, tripeptide glutathione, etc.). A decisive biological role is played by the organic compounds of sulfur with the oxidation number –II, mainly thiols with –SH groups, they form disulfides by oxidation. Compounds with S^{-II} are catabolised to sulfate SO_4^{2-} ions present in all biological fluids. In cells, the sulfate ion is activated to PAPS (3'-phosphoadenosine-5'-phosphosulfate) and used in sulfation reactions (production of proteoglycans of connective tissue, sulfoglycosphingolipids, and other sulfoesters of many natural and foreign substances). The rest of the sulfates are excreted by the kidneys to urine. The only relevant dietary source of sulfur includes the amino acids **methionine** and **cysteine** consumed in food proteins.

Elemental sulfur (S) is used in dermatology for its fungicidal and anti-seborrheic effects (regulation of sebum production).

Selected Sulfur Compounds

Hydrogen sulfide (H_2S, sulfane) is a toxic gas (see Chapter 33). Hydrogen sulfide dissolved in water is a very weak diprotic hydrosulfuric acid ($pK_{A1} = 7.0$; $pK_{A2} = 12.5$). Salts of H_2S are hydrogen sulfides (anion HS^-) and **sulfides** (anion S^{2-}). Sulfides of heavy metals are insoluble.

Sulfur dioxide SO_2 is a pungent smelling colourless gas. It is a harmful component of fumes from the burning of all fossil fuels (mainly brown coal) and other substances containing sulfur. It is used for whitening wool, fabrics, as a fungicide agent in viniculture. It is **toxic**, irritates conjunctivae and mucosa of the respiratory tract, and in high concentrations it causes serious damage to the lungs. The concentration of SO_2 in the atmosphere is an important indicator of the quality of the environment. SO_2 participates (together with NO_x) in the acidity of rainfall. This results, e.g. in the devastation of forest crops.
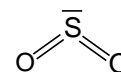
Aqueous solutions of SO_2 contain small amounts of weak and unstable **sulfurous acid** H_2SO_3 ($pK_{A1} = 1.84$). Its salts are hydrogen sulfites (anion HSO_3^-) and sulfites (anion SO_3^{2-}). Solutions of SO_2 and sulfites are common **reducing agents**; they are gradually oxidised to sulfates even by air oxygen.

Sodium sulfite Na_2SO_3 (natrii sulfis) or **sodium disulfite** $Na_2S_2O_5$ (natrii disulfis) act as stabilising antioxidants in the solutions of easily oxidisable substances. Amounts lower than 100 mg kg^{-1} are used for the production of fruit juices, wine, dried fruits or vegetables as preservatives preventing undesirable fermentations and changes of colour.

Sulfur trioxide SO_3 is formed by oxidation of SO_2 and with water it creates **sulfuric acid** H_2SO_4 (acidum sulfuricum). Dissolving SO_3 or concentrated H_2SO_4 in water is an extremely exothermic process. Sulfuric acid is strong only to the first degree of dissociation when the HSO_4^- anion is formed ($pK_{A2} = 1.92$). Complete release of H^+ ions occurs only in solutions with a pH > 4. Concentrated sulfuric acid has strong oxidation properties.
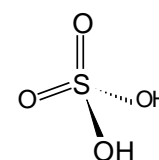
Salts are **hydrogen sulfates** (anion HSO_4^-) and **sulfates** (anion SO_4^{2-}). Most sulfates are easily soluble in water. Calcium sulfate $CaSO_4$ and lead sulfate $PbSO_4$ are poorly soluble. Barium sulfate $BaSO_4$ (barii sulfas) is practically insoluble and is used as a contrast substance for x-ray examination of the

gastrointestinal tract. Soluble sulfates have limited absorption after their consumption and they bound water in the intestine acting as osmotical laxatives: **sodium sulfate** Na_2SO_4 (natrii sulfas) and **magnesium sulfate** $MgSO_4$ (magnesii sulfas) are contained in some mineral waters.

Thiosulfuric acid $H_2S_2O_3$ is another example of an oxo acid of sulfur, the salts are **thiosulfates** (anion $S_2O_3^{2-}$). **Sodium thiosulfate** $Na_2S_2O_3$ (natrii thiosulfas), given intravenously, is applied for the treatment of cyanide or thallium poisoning and for the moderation of the toxic manifestations of cis-platin.

Calcium

Most of the body calcium (99%) is in the insoluble mineral components of the extracellular bone matrix, tooth cement, dentin, and tooth enamel. An adult human body contains approximately 1 kg of calcium (about 1.5% of body mass). In hard tissues, the various calcium phosphates are found and the predominant compound is hydroxylapatite $Ca_5(PO_4)_3OH$. The simple formula cannot express its composition exactly, because it also contains small amounts of other contaminating ions (CO_3^{2-}, F^-, HPO_4^{2-}, Mg^{2+}, Na^+, etc.) because of ion exchange. The rest of the calcium (1%) is in the other tissues and body fluids where ions Ca^{2+} are unequally distributed.

In **extracellular fluids**, the concentration of Ca^{2+} oscillate in the close range around 2.5 mmol l^{-1} and ions Ca^{2+} exist in the three forms: ionised Ca^{2+} (50%), bound to plasma proteins (albumin) (45%), chelated (10%) to citrate, malate, etc. A biologically active form of calcium is only ionised Ca^{2+}. The main role of plasma calcium consists in blood clotting; ions Ca^{2+} are one of the coagulation factors.

The **intracellular** concentration of Ca^{2+} is very low (about 0.1 µmol l^{-1}). In contrast to the cytosol, some organelles (endoplasmic reticulum, mitochondria) contain a high concentration of Ca^{2+} ions that serve as an intracellular calcium store. This unequal distribution of calcium has an important signal role. The regulated opening of the specific ion channels in the cell membranes increases intracellular Ca^{2+} concentration. This signal may initiate several cell processes as, for example, the contraction of muscle cells, secretory activity, the release of neurotransmitters on nerve terminals.

Three hormones are involved in the regulation of calcium metabolism. The hormones parathyrine (parathyroid gland), calcitonin (thyroid gland), and calcitriol (formed by the transformation of calciols, vitamins D) effectively control plasma Ca^{2+} concentrations. They act through actions on bone resorption and formation, reabsorption of Ca^{2+} in the renal tubules (regulation of urinary calcium excretion), and the efficiency of calcium absorption in the small intestine. Hypocalcaemia (low calcium in plasma) results in demineralisation of bone (osteomalacia or osteoporosis).

Food sources. The recommended daily intake of calcium in healthy adults is 1.0–1.2 g depending on age. Pregnancy and lactation have an increased need for calcium. The main sources of calcium in food are milk and dairy products. Foodstuffs of plant origin are relatively poor in calcium; if taken predominantly, phytates or some carboxylate anions (oxalates, citrates) included in foodstuffs can limit the intestinal calcium absorption by binding Ca^{2+}. Lack of calcium usually results from its insufficient intake because of the inappropriate composition of the diet, also from malabsorption of fat (lack of calciol, insufficient calcium absorption) and from the extreme urinary losses of calcium in chronic renal failure.

Ca^{2+} and Mg^{2+} ions in water cause the formation of insoluble hard scale in water mains and kettles. Total concentration of Ca^{2+} and Mg^{2+} (mmol l^{-1}) is also called hard water or water hardness. The recommended concentration of Ca^{2+} + Mg^{2+} in drinking water is 0.9–5 mmol l^{-1}, 1.3–2.5 mmol l^{-1} is required for a community water supply. For laundering and industrial operations, these undesirable cations can be removed or bound chemically in complexes by various methods of water softening.

Water can be softened partly by boiling, if it contains HCO$_3^-$ ions; the equivalent amount of insoluble CaCO$_3$ precipitates in the reaction Ca^{2+} + 2 HCO$_3^-$ → CaCO$_3$ + CO$_2$ + H$_2$O. A common method of softening water is its **demineralisation** by ion exchange. Water passes through columns of solid cation exchangers in the Na$^+$-cycle. Calcium and other polyvalent ions present in the water are exchanged for sodium ions and are removed from the liquid phase.

Calcium ions make some poorly soluble salts; their solubility often depends on pH (see the table below, Chapter 17, and Practicals).

Examples of poorly soluble calcium salts

Poorly soluble calcium salt	Comments
CaCO$_3$	Soluble in acidic environment to Ca^{2+} and HCO$_3^-$
CaHPO$_4$, Ca$_3$(PO$_4$)$_2$	Soluble in acidic environment to Ca^{2+} and H$_2$PO$_4^-$
CaSO$_4$	calcium sulfate hemihydrate (CaSO$_4 \cdot ^1/_2$H$_2$O) is plaster
Calcium oxalate	It can easily make kidney stones
Calcium soaps	The reason why washing in hard water is less effective

Organic di- and tricarboxylic acid react with ions Ca^{2+} to form chelates. For example, Ca^{2+}-citrate is soluble salt, however, it does not dissociate in water. The addition of citrate when collecting blood results in non-clotting blood because ions Ca^{2+} are necessary for coagulation. After centrifugation of non-clotting blood, the plasma is obtained. Substances that form chelate complexes with calcium ions such as sodium citrate and Na$_2$-EDTA are the common laboratory anticoagulants used to obtain non-coagulating blood and blood plasma.

Calcium oxalate, poorly soluble in water, is another example of a chelate. To compare Mg-oxalate and Ca-oxalate, magnesium oxalate is more soluble in water, therefore magnesium acts as the inhibitor of lithogenesis, reducing the saturation of urine by calcium oxalate.

Selected Calcium Compounds

Calcium sulfate dihydrate CaSO$_4 \cdot$2H$_2$O (calcii sulfas dihydricus, gypsum) is only slightly soluble in water (about 2.3 g l^{-1}). Water of crystallisation may be partly removed from dihydrate by long-term heating to 110–120°C so that **plaster**, **calcium sulfate hemihydrate** CaSO$_4 \cdot ^1/_2$H$_2$O (calcii sulfas hemihydricus) is obtained. Plaster mixed with a proper quantity of water sets to a hard mass due to retransformation of the hemihydrate into dihydrate. This setting of plaster is accompanied with the release of heat as well as volume expansion by approximately 1%. It is necessary to count upon this expansion when preparing plaster bandages in traumatology because of an increase of pressure that might result in the damage of tissues on the exposed areas of the immobilised body parts.

In medicine, solutions of **calcium chloride** $CaCl_2$ (calcii chloridum) are used for intravenous application.

Calcium carbonate $CaCO_3$ (calcii carbonas) is administered orally to make up an insufficient dietary calcium intake or as an antacid; it is also a common component of powders.

The granulated mixture of solid CaO and NaOH is called **soda lime**. It serves as an absorbent of exhaled carbon dioxide in respirators and anaesthetic appliances with a closed cycle.

Magnesium

A body of an adult man contains about 25 g (1 mol) of magnesium. The majority of this amount (approximately 60%) occurs in the bones and teeth.

In plants, it is especially important as a component of the leaf pigment chlorophyll.

Mg^{2+} ions are present in all biological fluids, though distributed very unevenly. Magnesium concentration is relatively low in the **extracellular** fluid, about 1 mmol l^{-1}. In the **intracellular** fluid, Mg^{2+} are next to K^+ the second main cation, partly bound by negative charges of proteins and nucleotides in the cells. They are activators of many essential enzymes, mainly of the enzymes participating in glycolysis, metabolism of nucleic acids, and proteosynthesis. Magnesium ions decrease the excitability of neurons, slow off the neuromuscular transmission; they can be taken as natural antagonists of Ca^{2+} ions. Symptoms of the magnesium deficiency may include, e.g. heart arrhythmia, vasoconstriction, muscular weakness, and tetany.

Food Sources. Adults should consume at least 300–400 mg of magnesium (30 mmol) per day. The main sources are leafy vegetables, meat, other animal proteins, and walnuts. Magnesium deficiency is the disturbance of electrolyte balance, which is often overlooked. It occurs when the dietary intake of Mg^{2+} is insufficient or as a result of increased urinary loss (mainly during treatment by diuretics or in osmotic diuresis). To make up for a magnesium deficit, oral administration of $MgCO_3$, magnesium citrate or lactate (magnesii carbonas, citras, and lactas) is initiated. In an acute shortage of magnesium, intravenous application of magnesium aspartate (magnesii aspartas) is useful.

Magnesium sulfate heptahydrate $MgSO_4 \cdot 7H_2O$ (magnesii sulfas heptahydricus, also bitter salt) occurs in some mineral waters. The absorption of magnesium sulfate from the intestine is limited, it binds water and, therefore it acts as a strong osmotic **laxative**.

Sodium (Natrium)

Sodium (Na^+) ions are, together with potassium (K^+) ions, the major cations of the human body. However, their distribution in the body is very uneven. Both ions are hydrated, the degree of their hydration varies, and influences their size and permeability across membranes. Generally, the permeability of substances through cell membranes is given by their size and polarity (charge). A hydrated potassium ion is smaller than a hydrated Na^+ ion and is therefore more permeable across cell membranes (see the table below). A different situation is in the case of ionic channels where ions diffuse without hydrations shells.

Hydration of sodium and potassium ions

Ion	Diameter of the ion (nm)	Diameter of the hydrated ion (nm)
Na^+	0.19	0.52
K^+	0.27	0.46

Sodium is the main cation of **extracellular fluid**, essential for the maintenance of the osmotic pressure and the management of body water. Its concentration in human blood plasma is kept in a narrow range of about 140 mmol l^{-1}. The osmolality of blood plasma (approximately 285 mmol per kilogram of water), the total volume of body water, and shifts of water between extra- and intracellular fluids depend mainly on the concentration of sodium ions. The renal excretion of Na^+ ions into urine (related closely to the excretion of water) is regulated by complicated mechanisms in which the steroid hormone of the adrenal cortex aldosterone, natriuretic peptides, and antidiuretic hormone (vasopressin) play the most important roles.

Food Sources. The most important source of sodium ions in the human diet is sodium chloride, common table salt. The recommended intake of sodium is less than 2 g for adults, which is less than 5 g NaCl. In industrially developed countries, the intake of NaCl is higher and the participation of high salt intake on the origin of some diseases, e.g. arterial hypertension with its circulatory consequences, is undeniable. The inevitable daily intake is 1.2 g of NaCl. The common mixed diet contains this amount even without adding salt to the food.

Sodium chloride NaCl (natrii chloridum) is common table salt. The **isotonic solution** of NaCl (solutio natrii chloridi isotonica), improperly called physiological saline solution, contains 154 mmol of NaCl (i.e. 9 g of NaCl) per litre of water. It is one of the basic solutions used (besides the solutions of sodium lactate and sodium hydrogen carbonate) in infusions to maintain ion, water, and acid-base balance.

Potassium (Kalium)

Potassium is the major cation of the **intracellular fluid.** Concentration of K^+ in blood plasma varies in close range around 4 mmol l^{-1}. This uneven distribution of both ions, maintained by the energy-demanding transport across cytoplasmic membranes of cells (Na^+,K^+-ATPase), has among others the decisive role in the excitability of smooth muscles and the spreading of action potentials along neurons. The higher permeability of K^+ ion through cell membranes (when compared with that of Na^+) is given its less degree of hydration (see Sodium).

In extracellular fluid, the concentration of K^+ ions is relatively low, but it is decisive, e.g. for the contractility of myocardium and skeletal muscles. Great deviations in the blood plasma concentration of K^+ may have unpropitious effects. Some states of body, mainly the increased urinary excretion of K^+ (e.g. during treatment by some diuretics or steroids), require the administration of potassium salts orally or in infusions to substitute the losses of K^+. KCl (kalii chloridum) and potassium salts of malic or aspartic acids (kalii malas, kalii aspartas) serve mostly to this purpose.

Food Sources. The intake of K^+ is about 1–3 g/day, which common mixed food ensures. The inevitable daily intake of K^+ is supposed to be 0.5 g/day. Potassium-rich foodstuffs are those of plant origin (potatoes, beans, fruits); there is a very low content of K^+ in white pastry, fats and oils.

Chlorine

Chlorides (Cl^-) are the most abundant anions **in blood plasma**, their concentration is about 100 mmol l^{-1}. Their concentration is lower in the intracellular fluid, only 15–30 mmol l^{-1}. Together with Na^+ ions Cl^- ions are decisive for the maintenance of osmolality of extracellular fluids. The concentration of Cl^- in plasma is in close relation to acid-base balance; its change causes the change of concentration of HCO_3^- ions in the opposite direction. The increase of concentration of Cl^-, e.g. during higher NaCl intake (in infusions or orally), causes the decrease of concentration of HCO_3^- in plasma and the acidification of the internal environment (metabolic acidosis). Losses of large amounts of Cl^-, caused by, e.g. some diuretics or long-term vomiting, lead to metabolic alkalosis.

Food sources. Chlorides are mostly consumed in the form of table salt NaCl. The intake of chlorides corresponds to the intake of salt (NaCl contains 60% of Cl^-). Chlorides are excreted mainly by urine and sweat, together with the equivalent amount of Na^+ or K^+.

Isolated loss of chlorides, such as repeated vomiting, impairs acid-base balance with a shift to the higher values of pH. Administration of large amount of chlorides (NaCl, orally also $CaCl_2$ or NH_4Cl) relatively lowers the concentration of buffer bases and acidifies extracellular fluids.

Dichlorine (Cl_2) is a yellow green choking gas (used by the German army as the first chemical warfare in 1915). It has distinct oxidation effects, e.g. it oxidises bromides and iodides to elementary halogens ($Cl_2 + 2\ I^- \rightarrow 2\ Cl^- + I_2$). It is used for the disinfection of water.

Selected Chlorine Compounds

Hydrochloric acid HCl (acidum hydrochloricum) is a common laboratory agent. Gastric juice contains HCl in the concentration of approximately 0.1 mol l^{-1} (pH 1–2).

Sodium chloride NaCl (natrii chloridum) is a frequent component of various types of infusions. NaCl solution with the concentration of 154 mmol l^{-1} is isotonic with blood plasma (see Sodium).

Potassium chloride KCl (kalii chloridum) is administered orally to substitute the lack of potassium (chronic diarrhoeas, etc.), which is often connected with the loss of chlorides and metabolic alkalosis, and in severe cases of K^+ deficiency potassium is added to infusion solutions.

Sodium hypochlorite NaClO (natrii hypochloris) is a common disinfectant, also used in households. Hypochlorite ions ClO^- are produced by phagocytosing cells in a myeloperoxidase reaction:

$$H_2O_2 + Cl^- \rightarrow ClO^- + H_2O$$
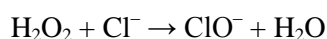
Calcium chloride-hypochlorite CaCl(ClO) is so called bleaching lime, prepared by the introduction of chlorine into calcium hydroxide and is used as a crude disinfectant and decontaminant.

Potassium perchlorate $KClO_4$ (kalii perchloras) limits the intake of iodides by the thyroid gland cells and therefore it is used for the protection of the thyroid gland during the functional examination by substances containing radioiodine.

22 Essential Microelements

Essential microelements make up about 1% mass of the human body. Dietary intake of essential microelements reaches maximally tens of milligrams in adults per day. The recommended daily intake of microelements is stated as the necessary daily intake of all essential microelements, which is as yet to be definitely determined; the search for values of the safe daily intake continues. Problems also include the fact that the usual intake of an element can be insufficient in periods of increased demands such as stress, or intercurrent disease, and on the other hand, the long-term overrunning of the optimal intake can result in harmful effects on health. It must be stressed that the parenteral nutrition (intravenous feeding) of patients unable to take normal food should supply all the essential elements in the appropriate amounts. Most usual European mixed diets provide sufficient amounts of the essential microelements.

There are ten microelements known:

> iron, copper, zinc, cobalt, chromium, molybdenum, manganese, selenium, iodine, fluorine

Iron

Adult bodies contain about 4–5 g of iron, mainly in hemoproteins and other iron-proteins. Their examples are given in the tables below. Iron is mostly contained in hemoproteins capable of binding dioxygen, hemoglobin, and muscle myoglobin. Remaining iron occurs in cell reserves, bound in ferritin (and hemosiderin). Small amounts, however important from the biological point of view, are the components of cytochromes and other enzymes catalysing redox reactions.

Hemoproteins

Protein	Redox state	Function
Hemoglobin	Fe^{2+}	transport of O_2 from the lungs to tissues, tetramer, 4 Fe^{2+}
Myoglobin	Fe^{2+}	binding O_2 in muscles, monomer, 1 Fe^{2+}
Cytochromes	$Fe^{2+} \rightleftarrows Fe^{3+}$	transfer of electrons in the terminal respiratory chain
Catalase	Fe^{3+}	decomposition of H_2O_2
Peroxidase	Fe^{3+}	decomposition of peroxides

Other proteins containing iron

Protein	Redox state	Function
Transferrin	Fe^{3+}	transport of Fe in blood plasma
Ferritin	Fe^{3+}	storage of Fe in tissues, mainly in liver
Hemosiderin	Fe^{3+}	storage of Fe (?), degradation product of ferritin
Lactoferrin	Fe^{3+}	antimicrobial effect
FeS-proteins	$Fe^{2+} \rightleftarrows Fe^{3+}$	transfer of electrons in the respiratory chain

Hemoproteins contain heme as a prosthetic group. Heme is a chelate where Fe^{2+} or Fe^{3+} is bound to a porphyrin ring. **Hemoglobin** contains heme-Fe^{2+}. If it does not bind dioxygen, it is called deoxygenated hemoglobin; iron has a coordination number of 5 (four covalent bonds with nitrogen atoms of porphyrin, the fifth one with protein component globin). Binding O_2 by the coordination bond to heme iron produces **oxyhemoglobin**; iron keeps the oxidation number II (thus it is not oxidation); however, the coordination number is six. Oxidation of Fe^{II} of hemoglobin to Fe^{III} (e.g. by nitrites) produces hemiglobin or **methemoglobin**, which is not able to transport dioxygen – a molecule of water is usually bound to Fe^{III} as the sixth ligand.

Hemes of **cytochromes** are in contrast to hemoglobin and myoglobin electron transporters. Their oxidised forms with Fe^{III} (ferri-forms) are changed to reduced ferro-forms with Fe^{II} by accepting electrons.

Iron in the oxidation state II and III very easily forms **complex ions**, the coordination number being mostly 6, e.g. potassium hexacyanoferrate(II) $K_4[Fe(CN)_6]$ (potassium ferrocyanide), which is a sensitive agent for the detection of Fe^{3+} ions. An undesirable effect of Fe^{2+} ions is their participation in the Fenton reaction resulting in the formation of hydroxyl radicals, which damage cell membranes and DNA (see Chapter 21).

Food sources. The common diet of adults contains about 10–20 mg of iron daily. From these about 1–2 mg are absorbed in the gastrointestinal tract, and they are sufficient to make up physiological losses and to keep the body stores. If the body store of Fe decreases, the intestinal absorption of Fe increases. The best available, though not the richest source, is the heme iron of meat, 20–30% of Fe is absorbed. Non-heme Fe from the liver (ferritin), cereals, and vegetables is absorbed only in 1–6%. Absorption of Fe ions, also Ca, Mg, from plant sources is inhibited by binding phytate and carboxylate anions, mainly oxalate.

An insufficient intake of iron, impaired absorption, or higher losses gradually exploit the cell reserves of iron. Synthesis of hemoglobin and formation of erythrocytes is insufficient and it leads to the development of sideropenic anaemia. Patients with proven iron insufficiency (sideropenia), blood donors preventively, and pregnant and lactating women are given iron supplements such as iron(II) sulfate (ferrosi sulfas), iron(II) chloride (ferrosi chloridum), or iron(II) fumarate (ferrosi fumaras) orally. Only exceptionally, the body reserves of iron are completed by supplying a calculated essential dose of iron parenterally (Fe^{III} compounds). As a natural way of elimination of the iron surplus does not exist, it can easily result in the accumulation of ferritin and hemosiderin in tissues and thus their damage.

Sodium nitroprusside (natrii nitroprussias) is sodium pentacyanonitrosylferrate(III) $Na_2[Fe(CN)_5NO]$. It is used in infusions as a vasodilator. It is also a reagent for the detection of ketone bodies (acetone and acetoacetic acid) in urine used in clinical laboratories.

Copper

Adult bodies contain about 80–100 mg of copper. It is the component of many oxidoreductases, e.g.:

· cytochrome-*c*-oxidase ($O_2 \rightarrow 2\ O^{2-}$, four-electron reduction of dioxygen),
· monoamine oxidases (primary amine $\rightarrow$ aldehyde),
· dopamine hydroxylase (dopamine $\rightarrow$ noradrenaline),
· tyrosinase (DOPA $\rightarrow$ $\rightarrow$ melanins),

· lysyl oxidase (lysine → allysine, maturation of collagen),

· ceruloplasmin (ferroxidase, $Fe^{2+} \rightarrow Fe^{3+}$, acts in blood plasma).

If the entry of Cu into the liver cells and its binding to ceruloplasmin is disrupted, the condition leads to the accumulation of copper in the body (Wilson disease).

Food sources. Copper occurs in meat, liver, sea fish, cereals, and beans. The daily intake in common food is sufficient, about 20 mg, from which 1–2 mg are absorbed.

Copper deficiency results from malnutrition. Patients suffering from diarrhoea and sucklings either feed insufficiently or are artificially in danger. Symptoms of the lack of copper can include anaemia, a decrease of the number of white blood cells, bad healing of wounds, etc.

Copper(II) sulfate pentahydrate, $CuSO_4.5H_2O$ (cupri sulfas pentahydricus) is used as a fungicide (growing of grapevine) and for the extermination of some algae. A very diluted solution of $CuSO_4$ can be used as an antidote during rare white phosphorus poisoning or burns (it transforms white phosphorus into insoluble copper(II) phosphide Cu_3P_2). Copper(II) sulfate is the substance for the preparation of the **Benedict reagent** which contains Cu^{2+}-chelate. The Benedict reagent is used for the proof of reducing substances; they reduce the Cu^{2+} chelate to insoluble copper(I) oxide/hydroxide $Cu_2O/CuOH$.

Zinc

Adult bodies contain 1.5–2.5 g of Zn, mostly in muscles. It is the component of numerous enzymes:

· alcohol dehydrogenase (ethanol → acetaldehyde),

· carbonic anhydrase ($H_2CO_3 \rightleftarrows CO_2 + H_2O$),

· carboxypeptidase (hydrolytic cleavage of an amino acid from the *C*-terminal of polypeptides),

· Cu,Zn-superoxide dismutase (superoxide → $O_2 + H_2O_2$).

Zinc ions stabilise the secondary and tertiary structures of many proteins. The recommended daily intake in food is 10 mg for adults.

Food sources. The most abundant source is beef, other red meat, and sea products (oysters, lobsters). Zinc is not stored in the liver, therefore its limited intake or increased loss (to interstitial fluid during extensive burns or by urine of top athletes) can rapidly result in zinc deficiency. It may be manifested by muscle weakness, increased tendency to infection, bad wound healing, sometimes skin lesions, and disturbed growth in children. In cases of proven deficiency, or when high losses are detected during any catabolic state, zinc sulfate is supplemented orally.

Zinc oxide ZnO (zinci oxidum) is because of its moderate anti-inflammatory effects used in dermatology as a component of powders, liquid powders, and pastes. In stomatology, the mixture of phosphoric acid with zinc oxide is used as **zinc phosphate cement**.

Zinc sulfate heptahydrate $ZnSO_4.7H_2O$ (zinci sulfas heptahydricus) is in the 0.25% solution used as an auxiliary remedy in ophthalmology, has mild antiseptic and astringent effects.

Zinc chloride $ZnCl_2$ (zinci chloridum) is added into infusions in parenteral nutrition.

Cobalt

Adult bodies contain about 1 mg of cobalt, mostly in muscles and bones. The cobalt cation is the component of **cobalamine** (**vitamin B_{12}**), where it is bound by coordination bond to the tetrapyrrole ring corrin (see Chapter 32). Vitamin B_{12} is essential for the methylation of the amino acid homocysteine to methionine. The daily requirement is approximately 3 µg of cobalamine (contains 0.13 µg of cobalt). The organism has usually high deposits (2–4 mg) of cobalamine.

Food sources. The main dietary sources are liver, meat, milk, and eggs, generally foods of animal origin. Cobalamine deficiency is presented as megaloblastic anaemia (see Chapter 46).

Chromium

The adult body contains about 10 mg of chromium. It potentiates the effects of insulin on glucose metabolism; this is called the "glucose tolerance factor". Chromium deficit may develop in total parenteral nutrition; it is manifested by impaired glucose tolerance (prediabetes) and peripheral neuropathy. The recommended daily intake of chromium in food is 40 µg. It is contained in wholemeal cereals, yeast, and egg yolk.

Molybdenum

The human body contains about 5 mg of molybdenum. It is essential for the function of xanthine oxidase (xanthine $\rightarrow$ uric acid) and sulfite oxidase (sulfites $\rightarrow$ sulfates). Molybdenum deficiency manifests by the impaired metabolism of purine bases and sulfur amino acids. Molybdenum compounds (the most frequent are molybdates with the MoO_4^{2-} anion) are well absorbed in the gastrointestinal tract, they do not cumulate in the tissues and are excreted by urine and bile. The recommended daily intake (RDI) of molybdenum is 50 µg; the multiple exceeding of this dose increases the loss of copper by urination. Molybdenum is found in beans, milk, offal, greens, and wholemeal cereals.

Manganese

The adult body contains about 12–20 mg of manganese. It is a component of some enzymes, e.g. mitochondrial superoxide dismutase (superoxide $\rightarrow O_2 + H_2O_2$), enzymes of proteoglycan synthesis (in bones and cartilages), and hepatic arginase (arginine $\rightarrow$ urea and ornithine). Mn^{2+} ions act as activators of other enzymes; they increase their catalytic properties. The sufficient and safe daily intake of manganese in adult nutrition is 2 mg. It is contained mainly in cereals. The symptoms of manganese deficiency include bone deformations from impaired bone remodelling, malfunctions of the nervous system, and blood coagulation.

Potassium permanganate $KMnO_4$ (kalii permanganas) is an effective oxidation agent. $KMnO_4$ has mild antiseptic and deodorant properties; we use very diluted (light pink) solutions. The concentrated solutions of $KMnO_4$ have caustic effects especially on the conjunctiva and cornea; they penetrate mucous membranes or the skin and cause deep necroses.

Selenium

Adult bodies contain about 5–15 mg of selenium, bound in selenoproteins, containing amino acid seleno-cysteine (exceptionally selenomethionine). They include glutathione peroxidase in erythrocytes (reduction of H_2O_2, hydroperoxides), deiodases of iodothyronines (thyroxine → triiodothyronines), or selenoprotein P. Selenium belongs to substances with an antioxidant effect; it influences the regulation of the metabolism of the thyroid gland and it is supposed to have an anti-carcinogenic effect.

The recommended daily intake is 55 μg. It is stated that the intake of food selenium in Europe is low, about 30–40 μg daily; this depends on the content of selenium in the soil. Good sources of Se are nuts, Brazil nuts, seafood, and wholemeal cereals. Selenium compounds consumed orally are well absorbed, excreted mostly in urine, and a smaller part in the stool. Symptoms of mild deficiency primarily include muscle slackness and weakness.

Severe and long-term selenium deficiency causes serious damage of the myocardium or joints. Insufficient selenium intake is substituted by the administration of **sodium selenite** Na_2SeO_3 (natrii selenis) or organic compounds of selenium, the most convenient is **selenomethionine**. The range between the required intake and the toxic dose of selenium is relatively narrow; the daily intake of more than 800–2,000 μg of selenium is evidently **toxic**.

Acute selenium poisoning is similar to that of arsenic. One of the striking symptoms of poisoning is a garlic smell, caused by the release of volatile dimethyl selenide (CH_3–Se–CH_3).

Iodine

The total amount of iodine in the adult body is 15–20 mg, from this 70–80% is in the thyroid gland. Its follicular cells actively capture iodide ions from circulation and use them to iodinate thyreoglobulin stored in the colloid of follicles. Amino acids **thyroxine** (tetraiodothyronine, T_4) and **triiodothyronine** (T_3) are gradually split off from thyreoglobuline and subsequently released into the blood. However, a larger part of T_3 is formed by the deiodination of thyroxine in peripheral tissues. About 67% of iodine is excreted in urine, some non-polar metabolites in bile.

The recommended daily intake of iodine is 150 μg for adults. A high amount of iodides is contained in ocean products (fish, algae, etc.). The intake of iodine in inland regions depends mainly on the amount of iodine in drinking (tap) water, which is often insufficient. The Czech mineral water *Vincentka* is quite exceptional, containing about 600 μg of I^- in 100 ml.

Mild iodide deficiency from dietary deficit is presented by goitre (enlargement of the thyroid gland), rather common in in-land regions (endemic goitre). It is also responsible for the more frequent occurrence of cognitive disorders in schoolchildren. More serious iodine deficiency causes the insufficient function of the thyroid gland – hypothyroidism. Its extreme form in adults is myxoedema.

Severe congenital hypothyroidism, cretinism, occurs only in some remote mountain parts of the world. Iodine deficiency is prevented by **iodination of salt** in the form of potassium iodate KIO_3 (35 mg of iodine = 60 mg of KIO_3/kg). Iodine deficiency can be substituted by long-term administration of NaI or KI (150 μg daily).

In medicine, elementary iodine is also used as a common disinfectant agent. Ethanol iodine solutions are now replaced by aqueous solutions of iodised organic polymers – iodophors (Jodisol, Betadine) which gradually release elementary iodine. For the disinfection of a mouth cavity or nasopharynx we can use a iodine solution in an aqueous solution of KI (Lugol solution).

Solutions of elementary iodine used in medicine

English name	Latin name	Composition
Tincture of iodine	tinctura iodi	solution of I_2 in ethanol
Lugol solution	solutio iodi aquosa	solution of I_2 and KI in water
Povidone-iodine	solutio povidoni iodinati	iodine-polyvinylpyrrolidone complex in water

Fluorine

Adult bodies contain about 2–3 g of fluorides. The fluoride ion (F^-) is the component of fluorapatite in bones and teeth (100–200 mg kg^{-1}). It has a favourable strengthening effect on dentine and tooth enamel, mainly during the development of teeth. A decrease in solubility of bone minerals limits their demineralisation and supports mineralisation of bone tissue (important for the treatment of osteoporosis).

The only known symptom of fluoride deficiency is the **increased incidence of tooth decay**. It is distinct in regions where the content of fluorides in drinking water is lower than 0.7 mg l^{-1}. The prophylaxis of tooth decay in children can be done by individual administration of tablets with NaF. Another possibility is the topical application of fluoride solutions on the dentition several times a year. Fluorides inhibit the growth of bacteria and plaques on the surface of teeth; therefore, they are often added to toothpastes, dental flosses, chewing gums or oral rinses.

The daily intake of fluorides in the common diet varies between 0.5–1.5 mg. Its sources primarily include tap water, ocean products, quality black tea (*Camellia sinensis*) – a cup prepared from 1.5 g of tea contains 0.05–0.25 mg of F^-. Fluorides are contained in a number of mineral waters. Soluble fluorides are easily absorbed and excreted in urine. In the presence of calcium and magnesium ions, poorly soluble fluorides precipitate and they are not absorbed (see Chapter 17). Estimation of the safe and adequate daily intake for adults is 1.5–4.0 mg.

Long-term intake of higher doses is harmful; it can result in chronic **fluorosis**. It is firstly recognised on teeth (chalk stains, teeth gradually darken, are more easily abrased and become fragile). If high doses are taken for several years, we notice bone changes – osteosclerosis (decalcification of bones and their rebuilding with local petrification), and spontaneous fractures are frequent.

Sodium fluoride NaF (natrii fluoridum) in tablets is administered in small doses to treat increased caries, mainly in children; multiple doses are used in the prophylaxis or treatment of osteoporosis.

23 Introduction to Organic Compounds

Organic chemistry includes the vast majority of carbon compounds except the simplest ones like CO_2, H_2CO_3, etc. In the basic state, the electron configuration of the carbon atom is $1s^2 2s^2 2p^2$; therefore it has four valence electrons. It can form four single covalent bonds or one double and two single, or one triple and one single bond. The formation of these bonds, their parameters, and bond angles are explained by quantum chemistry and the theory of hybridisation. The hybrid orbitals of the carbon atom are derived on the base of the energetic unification of the 2s orbital with one, two or three 2p orbitals to form the same number of equivalent hybrid orbitals. Those 2p orbitals, which form multiple bonds, do not take part in hybridisation.

1s		2s	$2p_x$	$2p_y$	$2p_z$
↑↓		↑↓	↑	↑	

Basic state of carbon atom

1s		2s	$2p_x$	$2p_y$	$2p_z$
↑↓		↑	↑	↑	↑

Excited state of carbon atom

According to bond character, three different combinations (hybridisations) of 2s and 2p orbitals are formed. Hybridisation of all three 2p orbitals with 2s orbital creates four hybrid sp^3 orbitals, which point to the corners of the regular tetrahedron (angle 109.5°). This type of hybridisation exists in compounds, where carbon forms four single bonds. The example of such compound is chloroform $CHCl_3$.

1s	hybridisation sp^3			
↑↓	↑	↑	↑	↑

Valence state of carbon atom
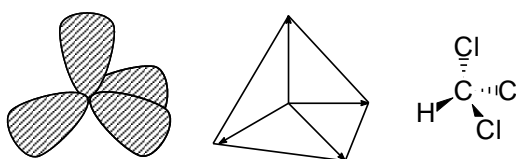
Hybridisation of the 2s orbital with two 2p orbitals ($2p_x$, $2p_y$) results in three hybrid sp^2 orbitals, which lie in the plane and make a 120° angle (trigonal hybridisation). The fourth orbital $2p_z$, which does not hybridise, forms the double bond and directs perpendicularly above and below the plane of the hybrid orbitals. This hybridisation of carbon is found in compounds with the double bond (C=C, C=O, C=N) and in compounds with the aromatic ring (benzene). The example is ethene $CH_2=CH_2$.

1s	hybridisation sp^2			$2p_z$
↑↓	↑	↑	↑	↑

Valence state of carbon atom
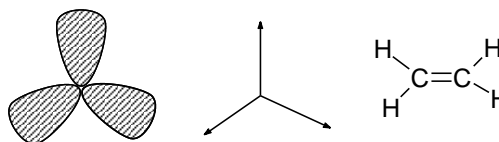
Hybridisation of the 2s and $2p_x$ orbitals results in two sp orbitals, which lie in one line (linear hybridisation, 180° angle). The remaining 2p orbitals participate in the formation of two π-bonds in the triple bond. They are oriented perpendicularly to the hybrid orbital sp and to each other. The example is ethyne (acetylene) H-C≡C-H.

1s	hybridisation sp		$2p_y$	$2p_z$
↑↓	↑	↑	↑	↑

Valence state of carbon atom
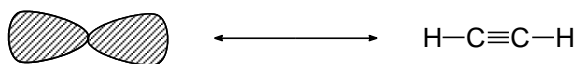
Structure and Isomerism of Organic Compounds

Constitution describes the sequence of atoms and bonds in the molecule. Examples of constitutional isomers are ethanol × dimethyl ether. Constitutional isomers can differ in the branching of the carbon chain (butane × 2-methylpropane), in the position of the multiple bond (but-1-ene × but-2-ene) or in the position of substituent (1,2-dichlorobenzene × 1,4-dichlorobenzene).

$$CH_3-CH_2-OH \qquad CH_3-O-CH_3 \qquad CH_3-CH_2-CH_2-CH_3$$
$$\text{ethanol} \qquad\qquad \text{dimethyl ether} \qquad\qquad\qquad \text{butane}$$

2-methylpropane

but-1-ene   but-2-ene   1,2-dichlorobenzene   1,4-dichlorobenzene
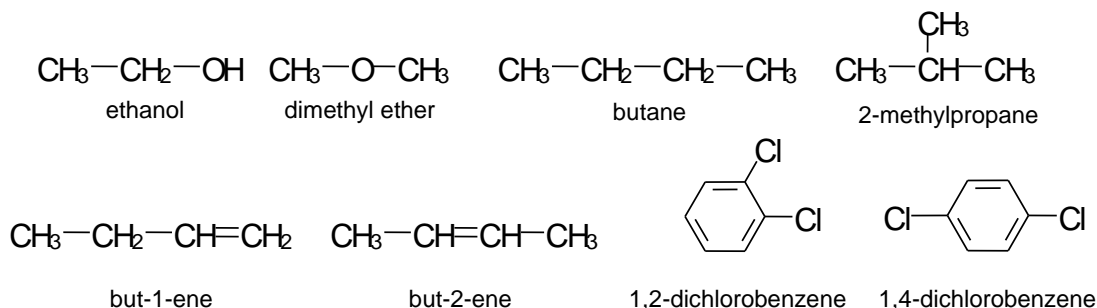
A special example of dynamic constitutional isomerism is **tautomerism**. Some substances exhibit equilibrium between two forms (tautomers), usually distinctly shifted in favour of one of these substances depending on conditions (solvent, pH). Tautomers are formed by concurrent H-atom migration and a double bond. They cannot be separated as chemical individuals. The example is **keto-enol tautomerism** of carbonyl compounds (pyruvate vs. enolpyruvate). Another type, **lactam-lactim tautomerism**, exists in purine and pyrimidine bases (see Heterocycles).

pyruvate   enolpyruvate
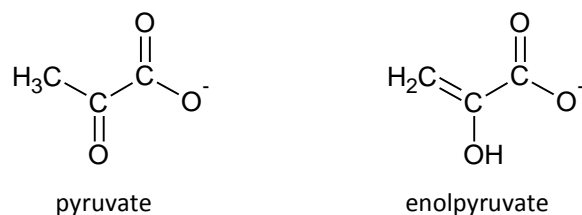
Configuration describes the spatial arrangement of atoms. Thus configurational isomers are compounds with the same constitution that differ in their configuration. Configuration isomerism occurs on the double bond (*cis/trans*), and in chiral compounds (D/L, *R/S*).

 Cis-trans **isomerism.** Two different substituents are in the relative *cis* or *trans* position if they are on the same or opposite sides, respectively, of the plane interlaid through the two compared isomers. In compounds with a double bond, this plane goes through the double bound atoms and is perpendicular to the plane in which all four atoms bound to the double bond lie. In cyclic compounds it is the plane of a cycle.

cis-but-2-enedioic acid   *trans*-but-2-enedioic acid
(maleinic)   (fumaric)

94
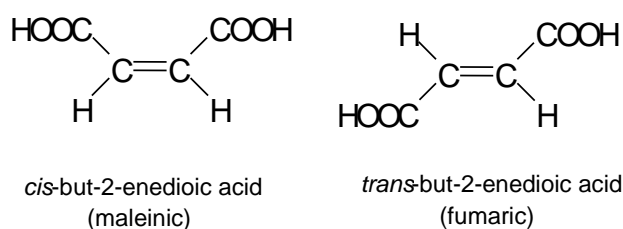
Stereoisomerism. Molecules, which cannot be superimposed with their mirror image, like human hands, are called **chiral** and are optically active, i.e. they turn the plane of linearly polarised light. The most frequent type of chiral compounds are substances with one or more asymmetric carbon atoms (C*). This is the carbon atom in sp^3 hybridisation, which has four different substituents. Two stereoisomers with different configuration on the asymmetric carbon atom are called **enantiomers**. They differ only in optical activity, i.e. one is dextrorotatory and the other levorotatory; other physicochemical properties are identical.

One example of a common chiral compound is lactic acid. Configuration on C* is marked according to the **Fischer projection**. The carbon chain is written in the vertical direction, the most oxidised group is at the top; the top C atom is given number 1. The horizontal bonds from the C* point above the plane of the paper, the vertical bonds are directed below the plane of the paper. If in this orientation the -OH group is on the right, it is a D-enantiomer, while -OH on the left-hand side means an L-enantiomer.

D-lactic acid   L-lactic acid
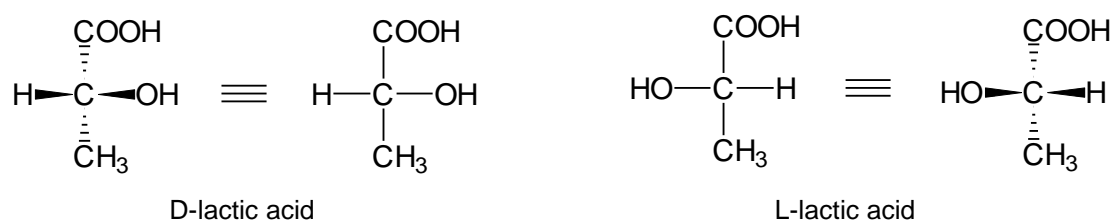
There is also an *R/S* system based on proton numbers of substituents on C* (not discussed here). The *R/S* and D/L symbols have no relation to the direction of the optical rotation. D-lactic acid is laevorotatory, which is expressed as (-)-D-lactic acid, the dextrorotatory enantiomer is then (+)-L-lactic acid. The mixture of both enantiomers in a 1:1 ratio is an optically inactive **racemate**, which can be expressed as (±)-lactic or D,L-lactic acid. If a molecule contains more chiral carbons, the number of stereoisomers is given by the expression 2^n, where *n* is the number of C*.

Enantiomers and biological activity. Enantiomers generally differ in the velocity and character of interaction with other chiral molecules or biomolecules like enzymes or membrane receptors in the human organism. Enzymes and receptors often prefer only one of the two enantiomers. The activity of enantiomers differs quantitatively (intensity of effect) or qualitatively (different effects). Differences in the interaction with the biological systems could be shown in the sensational properties (smell, taste), or in case of drugs in the varying pharmacological activity.

An example is levodopa (L-enantiomer), a drug to treat Parkinson disease, while D-enantiomer is ineffective.

L-DOPA (levodopa)   D-DOPA
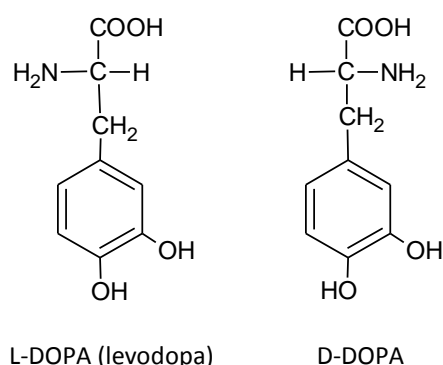
24 Alcohols and Phenols

Alcohols are derived from hydrocarbons by substituting one or more H atoms (on different carbons) with a hydroxyl group -OH. We distinguish **alcohols** (the hydroxyl group is bound to an alkyl, carbon in sp^3 hybridisation) and **phenols** (the hydroxyl group bound to an aromatic ring, carbon in sp^2 hybridisation). **Enols** have the hydroxyl group on the double bond (sp^2 hybridisation) which does not belong to the aromatic ring. Enols are unstable tautomeric forms of carbonyl compounds. Compounds with two hydroxyls on one carbon atom are not alcohols, but unstable hydrates of carbonyl compounds.

Nomenclature. Names of alcohols contain the suffix *–ol* (*–diol*, *–triol*, etc.) added to the name of the parent hydrocarbon, together with the locant describing the position of the -OH group (butanol, propan-2-ol). The RO- group is called *alkoxy*, e.g., CH_3O- methoxy, CH_3CH_2O- ethoxy. The name for the -$(CH_2)_n$-OH group is hydroxyalkyl, e.g., -CH_2OH hydroxymethyl, -CH_2CH_2OH 2-hydroxyethyl. Some simple phenols have common names (phenol, pyrocatechol).

Alcohols

According to the character of the functional group we divide alcohols into primary, secondary and tertiary. According to the number of the hydroxyl groups we distinguish monofunctional alcohols (alkanols), bifunctional alcohols (alkandiols), etc. Polyalcohols with four and more -OH groups (tetrols, pentols, hexols, etc.) are called alditols (sugar alcohols).

$$R—CH_2—OH \qquad R—\overset{\displaystyle R}{\underset{}{CH}}—OH \qquad R—\overset{\displaystyle R}{\underset{\displaystyle R}{C}}—OH$$

primary alcohol   secondary alcohol   tertiary alcohol
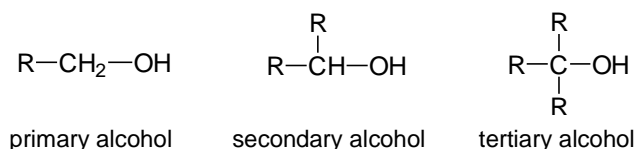
Properties. The lowest alcohols (C_1–C_3) are flammable, very polar liquids, completely miscible with water. Alcohols form hydrogen bonds. In the aqueous solution, the hydrogen bonds are not only formed among the molecules of alcohols, but also between alcohol and water. With the growing portion of the hydrocarbon chain, the solubility of alcohol in water decreases. A higher number of the -OH groups in the molecule increases its solubility in water. Higher alcohols (from C_{12}) are solid substances, odourless, insoluble in water. Alcohols do not dissociate in water, therefore they behave as **non-electrolytes**.

hydrogen bonds   aqueous solution of alcohol
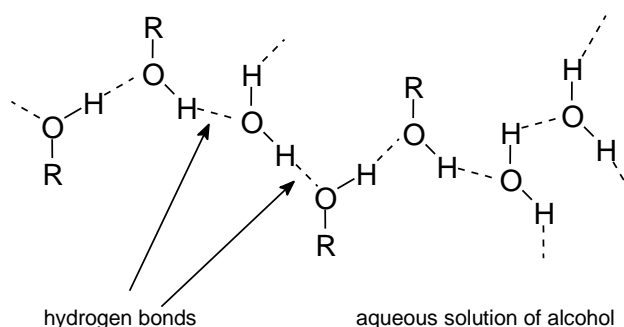
Oxidation (dehydrogenation). The -OH group of primary and secondary alcohols can be oxidised to form carbonyl compounds. Primary alcohols form aldehydes, secondary alcohols provide ketones. The oxidation proceeds by the dehydrogenation mechanism and is reversible; reduction (hydrogenation) transforms the resulting carbonyl compounds back to alcohols. The arising aldehyde can be further oxidised to a carboxylic acid, whereas ketones are resistant to mild oxidation agents (see Chapter 27).

$$R-CH_2-OH \underset{+2H}{\overset{-2H}{\rightleftarrows}} R-C\overset{O}{\underset{H}{\diagup}} \overset{^1/_2\,O_2}{\longrightarrow} R-C\overset{O}{\underset{OH}{\diagup}}$$

primary alcohol   aldehyde   carboxylic acid

$$R-\overset{R'}{\underset{}{\overset{|}{CH}}}-OH \underset{+2H}{\overset{-2H}{\rightleftarrows}} R'-C\overset{O}{\underset{R}{\diagup}}$$

secondary alcohol   ketone
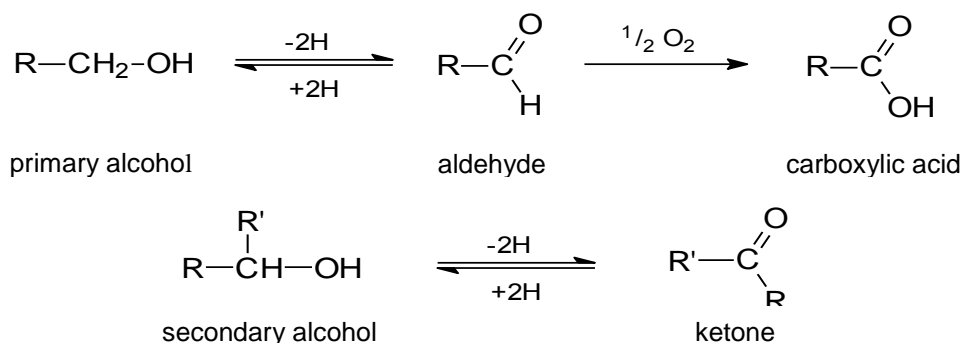
Tertiary alcohols are stable in reactions with mild oxidation agents (they cannot be dehydrogenated), strong agents cleave the C–C bond and form ketone and carboxylic acid.

In laboratory conditions, we use common oxidation agents to oxidise alcohols (e.g. the oxidation of ethanol by potassium dichromate – see Practicals). In biochemical processes, the dehydrogenation of alcohols is catalysed by enzymes dehydrogenases. The acceptor of hydrogen atoms in these reactions is the cofactor nicotinamide adenine dinucleotide (NAD^+).

Other reactions. Alcohols add to the carbonyl bond of aldehydes to form unstable **hemiacetals**, which can react with another alcohol molecule to give more stable **acetals**. Alcohols react with acids to make esters (see the next chapters).

Important Alcohols

Methanol CH_3OH is a colourless liquid with a typical alcohol-like smell and taste. Subjectively, it is not distinguishable from ethanol, so it can be the cause of dangerous replacements. After consumption methanol acts similarly to ethanol; however the lethal dose is 30-70 ml of pure methanol (see Chapter 33).

Ethanol CH_3CH_2OH is a colourless liquid with a typical alcoholic smell. It is completely miscible with water and lighter than water (density 0.8 g/ml). It belongs to common solvents. Ethanol causes reversible coagulation of proteins in aqueous solutions, destabilises their hydration shell. It is not used for disinfection because of its insufficient efficiency. Ethanol is produced by alcoholic fermentation of sugars ($C_6H_{12}O_6 \rightarrow 2\ CH_3CH_2OH + 2\ CO_2$). About 90% of the consumed alcohol is degraded in the liver. Alcohol is oxidised primarily by alcohol dehydrogenase (in the presence of coenzyme NAD^+) to acetaldehyde, which is oxidised in the subsequent reaction to acetic acid. The rest of ethanol is excreted by the lungs and in urine (see Practicals).

$$CH_3CH_2-OH + NAD^+ \overset{\text{alcohol dehydrogenase}}{\rightleftarrows} CH_3-C\overset{O}{\underset{H}{\diagup}} + NADH + H^+$$
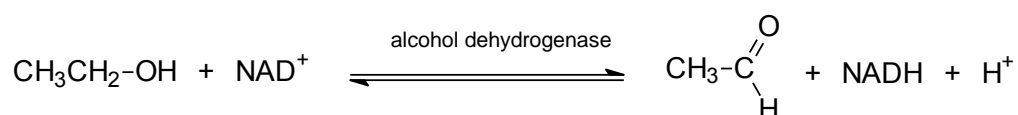
Polyalcohols

Alkanediols (glycols) and alkanetriols are viscous liquids, well soluble in water, with a relatively high boiling point and sweet taste (γλυκος, from the Greek sweet).

Ethylene glycol (ethane-1,2-diol) $HO-CH_2CH_2-OH$ is a common component of non freezing mixtures. Consumed instead of ethanol it causes dangerous poisoning, it is enzymatically dehydrogenated to oxalic acid (see Chapter 33).
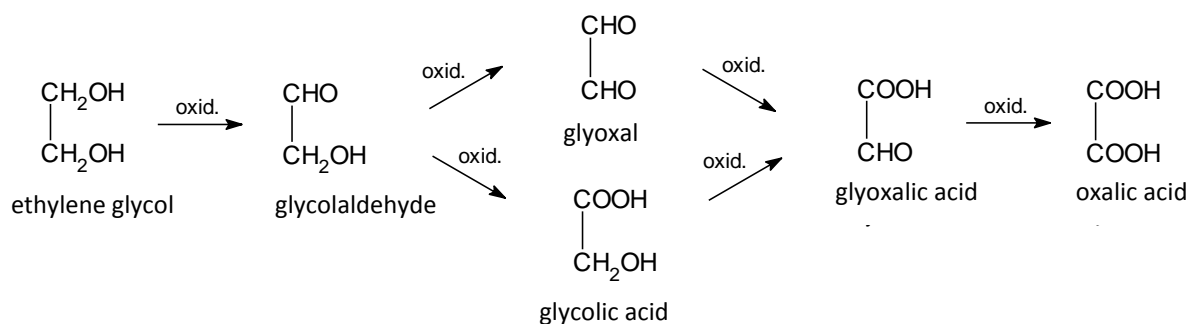
Glycerol (propane-1,2,3-triol) is a viscous liquid with a sweet taste. It is the structural component of lipids (triacylglycerols) and glycerophospholipids, in which it forms ester bonds with higher fatty acids. It is obtained by alkaline hydrolysis of fats. During digestion it is released from fats by the action of hydrolytic enzymes (lipases). Glycerol has hygroscopic properties; it is used as an additive to soaps and cosmetic preparations. Glycerol suppositories are used as osmotically active laxatives.

Dehydrogenation of the primary alcohol group in glycerol leads to glyceraldehyde, and with further oxidation to glyceric acid (glycerate). Glycerol oxidised on the secondary alcohol group gives dihydroxyacetone.
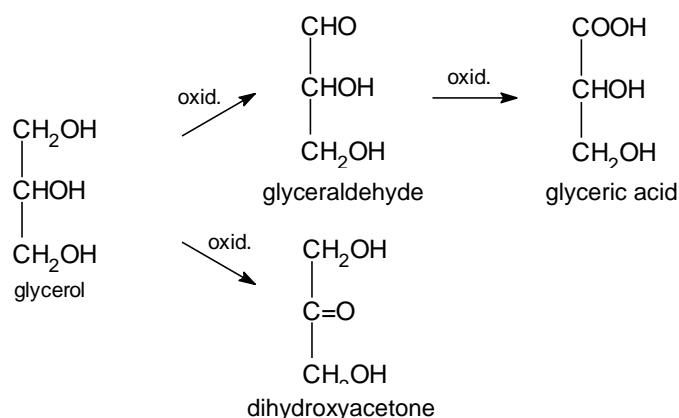
Inositol (cyclohexane-1,2,3,4,5,6-hexol) forms theoretically eight isomeric forms. Only one steroisomer, *myo*-inositol, is important in the human body, because it is the component of phosphatidylinositols. It is found in plants free or in the form of hexaester with phosphoric acid (**phytic acid, phytate**).

myo-inositol
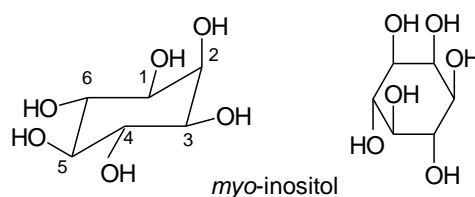
Enols

Enols have the -OH group bound to a C=C double bond. They are relatively unstable and easily tautomerise to carbonyl compounds (keto-enol tautomerism). Enols release protons (H^+) more easily than water; enol hydroxyl has approximately the same acidity as phenolic hydroxyl. The example is **L-ascorbic acid (vitamin C)**, which contains two enol hydroxyl groups ($pK_{A1} = 4.2$, $pK_{A2} = 11.6$).

enol   keto   ascorbic acid
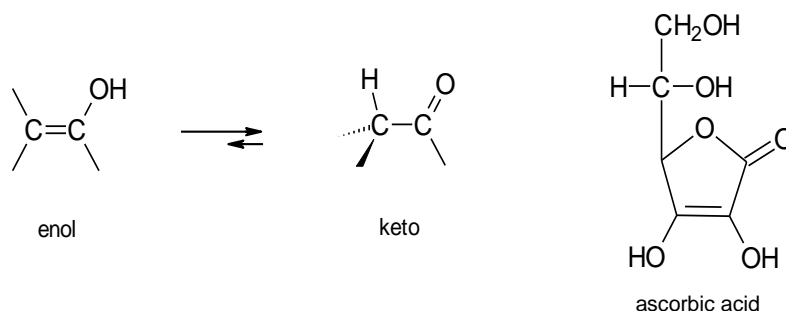
Phenols

Phenols are compounds with the hydroxyl group bound to a carbon of the aromatic ring. Phenols are mostly crystalline substances, with limited solubility in water, they often have a characteristic smell. In contrast to alcohols, phenols behave as very weak acids in the aqueous solution ($pK_A \sim 10$). Insoluble phenols can be dissolved in the aqueous solution of a strong hydroxide, because they form phenolate ions. The acidity of phenols substantially increases, if their aromatic ring contains *o*- or *p*- substituents, which attract electrons, e.g., 2,4,6-trinitrophenol (picric acid, $pK_A = 0.4$).

Oxidation. Phenols are easily oxidised, but they do not form aldehydes and ketones like alcohols. Oxidation of monoprotic phenols produces phenoxyl radicals (stabilised by the delocalisation of the free electron in the aromatic system), which are transformed to products with the quinoid arrangement of bonds.

Diphenols with the -OH groups in the *ortho* and *para* position are easily reversibly dehydrogenated to coloured **quinones**, unsaturated cyclic diketones. During dehydrogenation, the aromatic ring disappears and the quinoid system (two double bonds in the cycle conjugated with two oxo groups) is formed.

hydroquinone   *p*-benzoquinone   pyrocatechol   *o*-benzoquinone
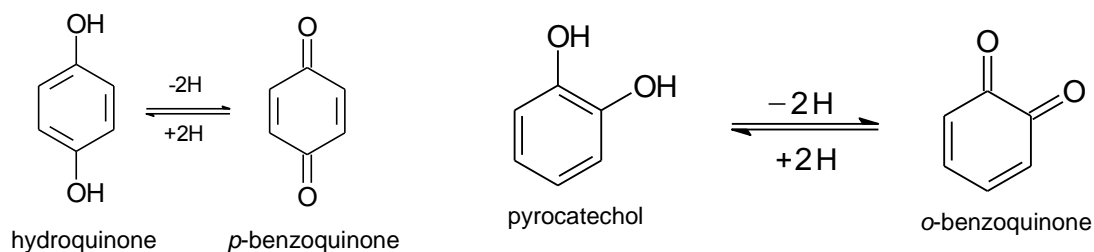
Diphenols

Three isomeric diphenols are derived from benzene: **pyrocatechol** (benzene-1,2-diol), **resorcinol** (benzene-1,3-diol), and **hydroquinone** (benzene-1,4-diol).

phenol   pyrocatechol   resorcinol   hydroquinone
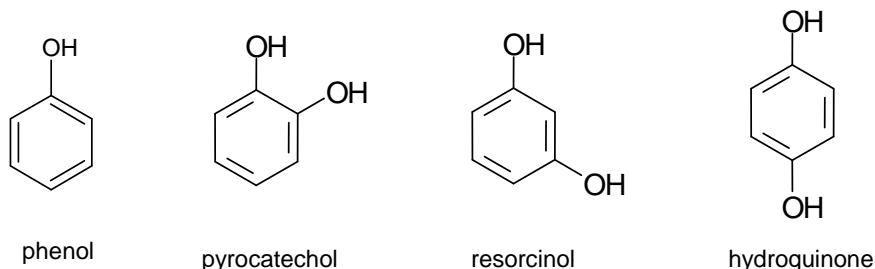
Biochemically Important Quinones

Ubiquinone (coenzyme Q) is the substituted derivative of 1,4-benzoquinone. Besides three simple groups it has one very long hydrophobic, polyisoprenoid chain. It is widespread in living organisms (prefix *ubi-*, from Latin *ubique* anywhere) and plays an important role in the electron transfer in the terminal respiratory chain in mitochondria. It is reversibly reduced by two hydrogen atoms to diphenol **ubiquinol (QH$_2$)**.

ubiquinone
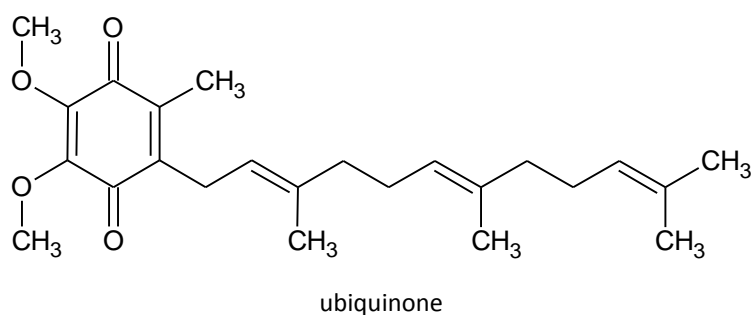
Phylloquinone (vitamin K) is a derivate of 2-methyl-1,4-naphtoquinone. A long polyisoprenoid chain makes the compound lipophilic. Phylloquinone is essential for the biosynthesis of the some protein factors needed for blood coagulation, therefore, vitamin K is used in the prevention and therapy of increased bleeding (see Vitamins).

phylloquinone

100
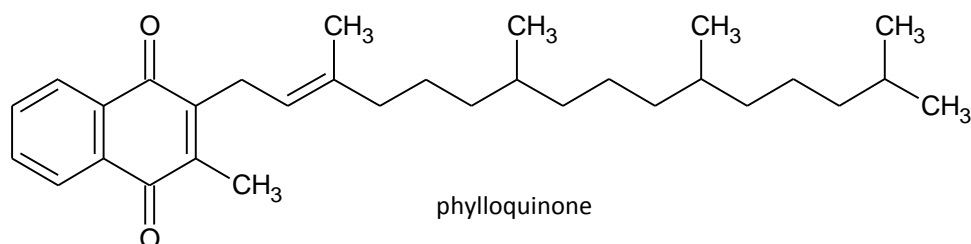
25 Esters of Inorganic Acids

Esters of Sulfuric Acid

Sulfuric acid H_2SO_4 (HO-SO$_2$-OH) is a strong diprotic acid. It gives two types of esters: monoesters and diesters. Monoesters are **alkyl sulfates** (alkylsulfuric acids, strong electrolytes), diesters are dialkyl sulfates (non-electrolytes).

sulfuric acid   alkyl sulfate   dialkyl sulfate
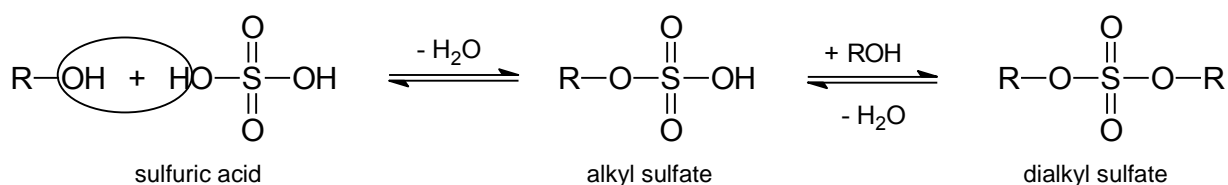
Sodium salts of higher alkyl sulfates (C_{12}-C_{18}) are **anionic tensides**, effective even in the case of high contents of Ca^{2+} and Mg^{2+} ions in water, e.g. sodium dodecyl sulfate (SDS).

SDS
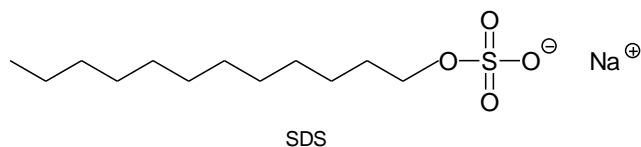
Ester-bound sulfate groups are common in natural substances. They are contained in saccharide components of **glycosaminoglycans**, where some sugar units are esterified by sulfuric acid (e.g. dermatan sulfate, keratan sulfate, chondroitin sulfate, Chapter 36). Sulfate esters occur also in **glycolipids** (Chapter 39). Many phenolic compounds of endogenous origin (estrogens, catecholamines) or exogenous drugs (e.g. Panadol) are transformed to more polar products that can easily be excreted from the body. One of these reactions is **sulfation** – the formation of organic sulfates.

Esters of Phosphoric Acid

Esters of trihydrogenphosphoric acid H_3PO_4 are very important as intermediate products of many metabolic pathways (e.g. glucose 6-phosphate, glyceraldehyde 3-phosphate, phosphoenolpyruvate).

phosphoric acid   alkyl phosphate   dialkyl phosphate   glucose 6-phosphate
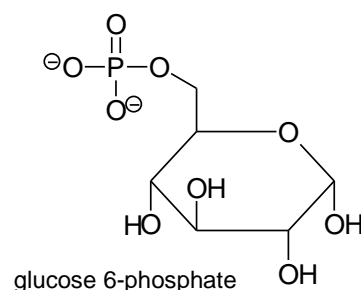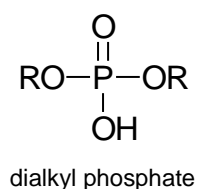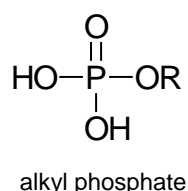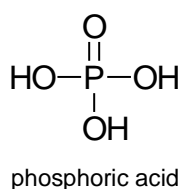
Nucleotides are phosphoesters of nucleosides. Their phosphate groups can make phosphodiester bonds form polynucleotides and nucleic acids. The phosphate group in **phospholipids** forms the bond between two –OH groups. Moreover, it is also the carrier of a negative charge. In proteins, formation of phosphoesters in the side chains of serine, threonine, or tyrosine (phosphorylation of proteins) changes the conformation of a protein molecule. It is one of the mechanisms for the regulation of the catalytic activity of enzymes (protein-OH + ATP $\rightarrow$ protein-O-P + ADP).

Organophosphates are synthetic esters of substituted phosphoric acid, thiophosphoric $(HO)_3P=S$, fluorophosphonic acid $(HO)_2FP=O$, and other acids of phosphorus. From the medical point of view, they have a very important common feature: they are all very strong nerve paralysing poisons, which can be absorbed through the skin. This effect was discovered in Germany just before World War II, when looking for new insecticides among the esters of phosphoric acid. After finding the extreme toxicity of some organophosphates, the research was concentrated on those which could be used as chemical warfare. The example of toxic insecticide, commonly used in agriculture (for the treatment of indoor plants) is **parathion** or the most toxic **mevinfos**. Chemical warfare agents are **sarin**, **soman**, **tabun, VX** (nerve gases).

The effect of organophosphates is based on the fact that they irreversibly inhibit the enzyme acetylcholinesterase, which catalyses the hydrolytic breakdown of acetylcholine in the synaptic gap. If acetylcholine is not sufficiently removed, it cumulates and causes long-term stimulation of the receptors in the postsynaptic membrane. Therefore organophosphate poisoning is viewed as a long-term stimulation (overstimulation) of the motor neurons (muscle weakness, cramps, paralysis) and the stimulation of the parasympathetic nervous system (sweating, salivation, diarrhoea, miosis). If organophosphates penetrate to the brain, there are also neurological symptoms (dizziness, convulsions, coma).

Treatment of organophosphate poisoning involves alkaloid atropine, the principal antidote (antagonist of acetylcholine receptors), and the reactivators of acetylcholinesterase (pralidoxime, obidoxime), which can release organophosphate from the enzyme complex.
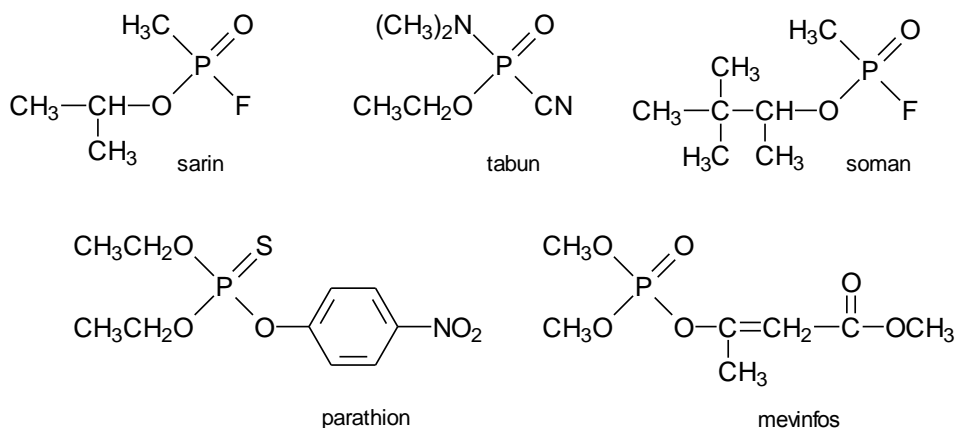
Esters of Nitric Acid

Nitric acid HNO_3 ($HO-NO_2$) is a strong monoprotic acid; its esters are **alkyl nitrates** $R-O-NO_2$. The structural formula of nitric acid is not easily expressed because of existing resonance forms:
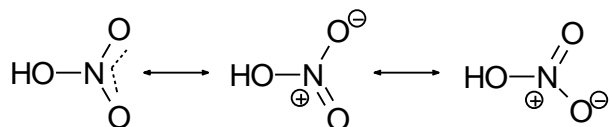
The most famous ester is **glyceryl trinitrate** (glyceroli trinitras). It is a yellowish oily liquid, extremely explosive; explosion is provoked by even mild impact. Its adsorption to an inert material produces relatively safe explosives (including Nobel´s dynamite).
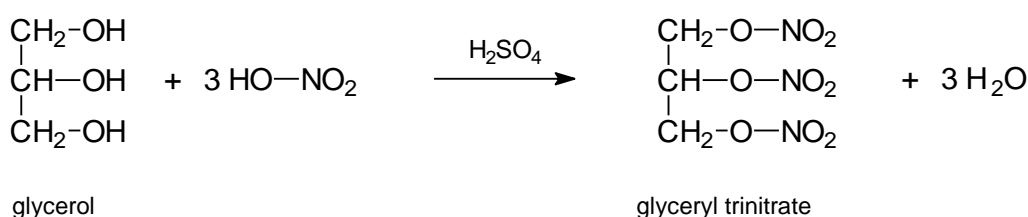
It is used as a rapidly acting vasodilator in the therapy and moderation of difficulties accompanying myocardial ischemia. At present, we prefer other nitrates having the same effect but acting longer, e.g., **isosorbide dinitrate** (isosorbidi dinitras). The effect of all these nitrates is based on the release of nitrogen monoxide from their molecules into the smooth muscle cells of arterioles.

isosorbide dinitrate
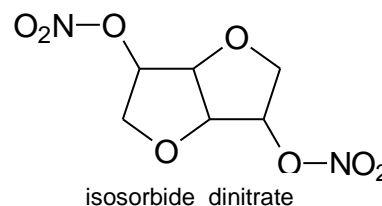
Nitrogen monoxide NO (N=O, nitrogenii oxidum, nitroxide) is a signal molecule, which is naturally formed in some cells by oxygenation of the amino acid arginine. Alkyl nitrates substitute this process. They release the nitrate anion, which is reduced by cellular thiols to nitrite, and this is then decomposed to nitroxide. Nitroxide increases the activity of the enzyme, which catalyses the synthesis of the proper vasodilatation substance – cyclic guanosine monophosphate (cGMP). The effect of sildenafil (Viagra) is based on keeping the higher concentration of NO in the smooth muscle cells of *corpora cavernosa penis*, though its mechanism is different (inhibits the hydrolysis of cGMP).

Esters of Nitrous Acid

Nitrous acid HNO_2 (HO-N=O) is a weak monoprotic acid; its esters are **alkyl nitrites** R-O-N=O.
Amyl nitrite (amylis nitris) is made from nitric acid and 3-methylbutanol. It is a very volatile liquid, used as the inhalation antidote in case of cyanide poisoning. It oxidises haemoglobin to methemoglobin, which produces the stable complex methemoglobincyanide with the CN⁻ ions.

amyl nitrite
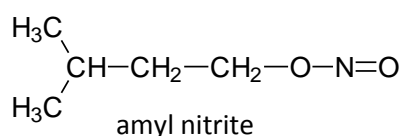
26 Organic Sulfur Compounds

Organic sulfur compounds comprise molecules with the covalent bond C–S. Esters of sulfuric acid are not included in this group. In organic compounds, sulfur has the oxidation number –II (thiols R-SH, disulfides R-S-S-R, sulfides R-S-R) or higher oxidation numbers (sulfonic acids R-SO$_3$H).

Thiols

Nomenclature. Thiols are sulfur analogues of alcohols; they contain monovalent groups -SH. The names of thiols are formed similarly to alcohols: the suffix –*thiol* is added to the name of the parent hydrocarbon (ethanethiol, propanethiol, butanethiol). If -SH is not the main group, we add the prefix *sulfanyl*- (2-amino-3-sulfanylpropanoic acid = cysteine).

Properties and reactions. Low-molecular thiols are more volatile and less soluble in water than corresponding alcohols. They have a characteristic unpleasant and pungent smell. E.g., methanethiol CH$_3$SH is one of the components of intestinal gases, ethanethiol CH$_3$CH$_2$SH is added to natural gas to indicate gas leakage. Propanethiol CH$_3$CH$_2$CH$_2$SH is contained in onion. The main components of the protective secretions of polecats and skunks are 3-methylbutanethiol (CH$_3$)$_2$CHCH$_2$CH$_2$SH and but-2-ene-1-thiol CH$_3$CH=CHCH$_2$SH. Natural thiols are made by decomposition of sulfur amino acids cysteine and methionine. Thiols participate in many reactions similar to alcohols: alkylation produces dialkyl sulfides, they form thioesters and thioacetals in the reactions with acids and aldehydes, respectively. In comparison to alcohols, thiols are more acidic, like hydrosulfuric acid H$_2$S (pK_{A1} = 7.0) is substantially more acidic than water (pK_A = 15.7).

Oxidation. Alcohols and thiols also differ in oxidation reactions; they do not produce analogues of carbonyl compounds. **Mild oxidation** of thiols (air oxygen is enough) causes dehydrogenation only on the sulfur atoms, thus two molecules of thiols form **dialkyl disulfides** R-S-S-R with the covalent disulfide bond -S-S-. The reaction is reversible; disulfides can easily be reduced back to thiols.

$$2\ \text{R}-\text{SH} \quad \underset{+\ 2\text{H}}{\overset{-\ 2\text{H}}{\rightleftharpoons}} \quad \text{R}-\text{S}-\text{S}-\text{R}$$

thiol   disulfide
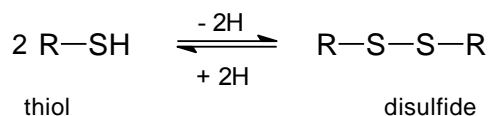
Natural substances containing -SH groups play the role of **reducing agents** in biochemical processes; in some cells (e.g. glutathione in the erythrocytes) they are important for the elimination of reactive oxygen radicals. Besides that, the formation of disulfide bonds between the side chains of cysteine in a polypeptide chain stabilises the spatial arrangement of proteins (their tertiary structure).

$$2\ \text{HS}-\text{CH}_2-\underset{\underset{\text{NH}_2}{|}}{\text{CH}}-\text{COOH} \quad \underset{+\ 2\text{H}}{\overset{-\ 2\text{H}}{\rightleftharpoons}} \quad \text{HOOC}-\underset{\underset{\text{NH}_2}{|}}{\text{CH}}-\text{CH}_2-\text{S}-\text{S}-\text{CH}_2-\underset{\underset{\text{NH}_2}{|}}{\text{CH}}-\text{COOH}$$

cysteine   cystine
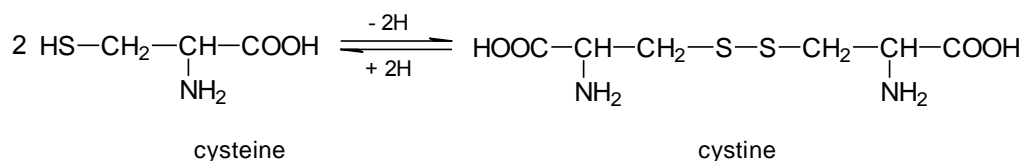
This effect is used by hair dressers. Hair contains a protein keratin whose polypeptide chains are stabilised by disulfide bonds between the cysteine side chains. During the formation of permanent waves, at first, hair undergoes treatment by a reducing agent (thioglycolic acid HS-CH$_2$-COOH). When disulfide bonds -S-S- are reduced to -SH groups, the hair is then given the required shape and after the treatment with an oxidation agent new disulfide bonds are formed in different positions compared to the old ones, which then keep the hair in its new shape.

Strong oxidation agents increase the oxidation number of sulfur. Thiols (S^{-II}) give **sulfonic acids** R–S^{VI}O$_3$H via intermediate products (sulfenic R-S^{II}-OH and sulfinic R-S^{IV}O-OH acids). In the human body, the end product of the metabolism of substances with -SH groups is sulfate (SO$_4^{2-}$) ion.

Biochemically Important Thiols and Disulfides

Cysteine (2-amino-3-sulfanylpropanoic acid) belongs to twenty coded amino acids. Its oxidation produces the disulfide cysteine.

Glutathione (γ-glutamyl-cysteinyl-glycine, GSH) is a tripeptide, participates in biochemical redox reactions, e.g. in the reduction of hydrogen peroxide to water, catalysed by glutathione peroxidase:

H-O-O-H + 2 GSH $\rightarrow$ 2 H$_2$O + G-S-S-G.
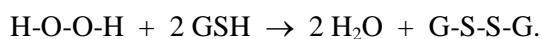
Coenzyme A (HS–CoA) is essential for the metabolism of all nutrients. Its reactive group is the –SH group of cysteamine, which is bound to pantothenic acid and adenine nucleotide. The endergonic reaction with carboxylic acid (in the presence of ATP) produces a **thioester** bond between acyl and coenzyme, i.e. acyl coenzyme A (acyl-S-CoA).

R-COOH + HS-CoA + ATP $\rightarrow$ R-CO-S-CoA + AMP + PP$_i$
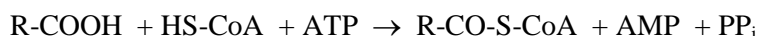
Acyl coenzymes A are macroergic compounds (activated acids), which enter other metabolic reactions more easily.

Lipoic acid (thiooctic acid, 1,2-dithiolane-3-pentanoic acid) is a cyclic disulfide, the oxidised form of dihydrolipoic (6,8-disulfanyloctanoic) acid:

lipoic acid   dihydrolipoic acid
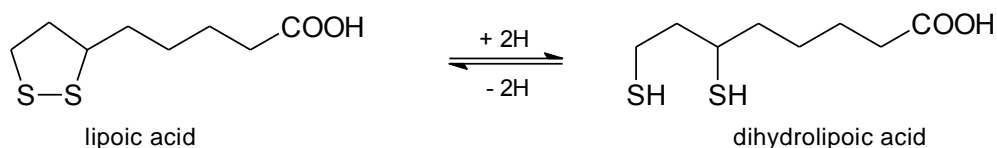
Lipoic acid bound as amide (lipoamide) is the cofactor of enzymatic complexes, which catalyse the oxidation decarboxylation of 2-oxoacids. It has two functions – redox and transfer. Its oxidised form binds to the intermediate product in the course of the reaction, causes its oxidation and transfers it in the form of acyl to coenzyme A. For example, acetyl-CoA is produced from pyruvate.

Dimercaprol (2,3-disulfanylpropan-1-ol) HS-CH$_2$-CH(SH)-CH$_2$OH is used as an antidote in cases of heavy metal poisoning (e.g. Sb, As, Bi, Hg).
The metal ions are bound to the –SH groups as thiolate which is soluble and the kidneys can excrete it.
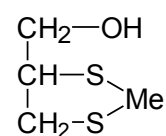
Sulfides

have general formula R^1-S-R^2, e.g. CH_3-S-CH_3 dimethyl sulfide, CH_3-S-CH_2CH_3 ethyl methyl sulfide. The most important sulfide in biochemistry is **methionine**, amino acid with the -S-CH_3 group, the donor of methyl groups for many methylation reactions (see Amino acids).

Sulfonic Acids

contain the sulfonic group -SO_3H, where the sulfur atom is bound directly to carbon. They are produced by the oxidation of thiols or sulfonation of hydrocarbons. They are strong acids. Sulfonic acids and their salts – sulfonates are mostly well soluble in water. If the sulfonic group is bound to larger hydrocarbon residue, they have properties of **anionic surfactants (tensides)**. They are essential components of detergents. An example of such a compound is sodium 4-dodecylbenzenesulfonate:
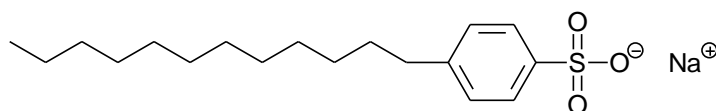
Taurine (2-aminoethanesulfonic acid) NH_2-CH_2-CH_2-SO_3H is a naturally occurring sulfonic acid. It is formed by oxygenation and decarboxylation of cysteine. It forms (like the amino acid glycine) an amide bond with the carboxyl of bile acids in the liver and these conjugated bile acids are excreted into bile. Bile acids are the main anionic surfactants for the digestion of fats in the small intestine.

Sulfonamides are generally amides of sulfonic acids, they have the -SO_2NHR group on the aromatic ring. A special group of sulfonamides are **sulfonamide chemotherapeutics** which are derived from sulfanilic (4-aminobenzenesulfonic) acid and have bacteriostatic effects. This effect is based on the structural similarity with *p*-**aminobenzoic acid** (PABA). PABA is the growth factor of bacteria, an essential component for the bacterial synthesis of folate (folic acid). In all living organisms, tetrahydrofolate is the cofactor of transferases that transport the one-carbon units (e.g. formyl) required in inevitable biosynthetic processes, including the biosynthesis of nucleic acids. Sulfonamides act in bacteria as competitive inhibitors of the incorporation of PABA into folate molecule. The resulting restriction of the synthetic processes dependent on tetrahydrofolate inhibits growth of bacteria. Examples are sulfafurazole or sulfamethoxazole, administered orally in the course of some urological infectious diseases. At present, the use of sulfonamides in medicine is not very common because a wide spectrum of antibiotics is available.

benzenesulfonic acid   sulfanilamide   sulfonamide general formula   PABA
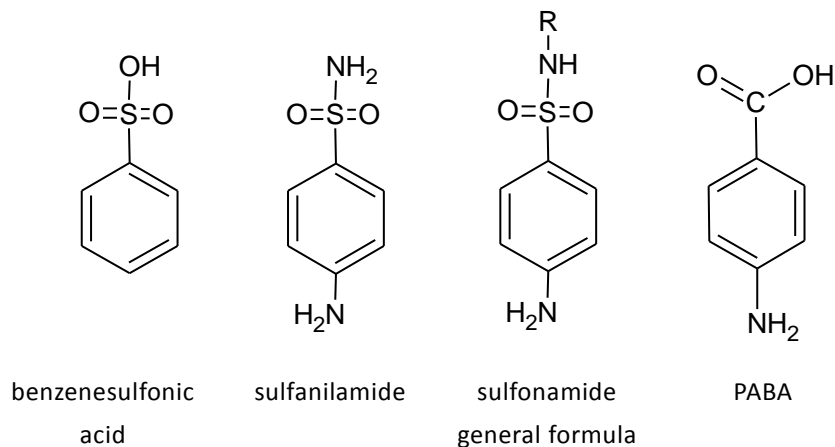
27 Aldehydes and Ketones

Aldehydes and ketones contain the polar and reactive **carbonyl group** >C=O. The carbonyl group in aldehydes is always bound to one H atom and one hydrocarbon residue (alkyl or aryl), ketones contain two hydrocarbon residues.
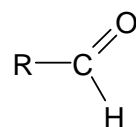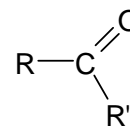
aldehyde   ketone

Nomenclature. The systematic names of aldehydes are formed by adding the suffix –*al* to the name of hydrocarbon (butanal, propanedial etc.). In cyclic aldehydes, the suffix –*carbaldehyde* is added to the name of the cyclic system (cyclohexanecarbaldehyde, naphthalenecarbaldehyde). Common names are used for some aldehydes (formaldehyde, acetaldehyde, benzaldehyde, glyceraldehyde, malondialdehyde, glyoxal). The names of ketones are formed by adding the suffix –*one* to the name of hydrocarbon (propanone, cyclohexanone, pentane-2,4-dione). For unsaturated cyclic diketones the suffix *quinone* is used (*p*-benzoquinone). Some ketones have common names, e.g. acetone CH_3-CO-CH_3.

Properties. Except for formaldehyde (gas), aldehydes and ketones are liquid or solid substances with a characteristic smell. Their molecules are not able to form hydrogen bridges; therefore the boiling point is lower in comparison to the corresponding alcohols.

Keto-enol tautomerism. Carbonyl compounds may exist as an equilibrium mixture of two forms, called the keto and enol. Usually the keto form is the prevailing and more stable species. The two forms differ in the location of the H atom and the double bond.

keto form of acetone   enol form of acetone
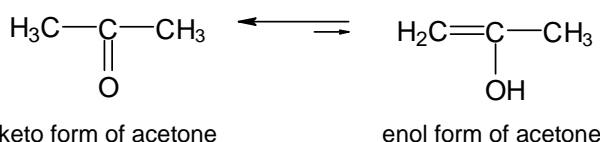
Reactions of Aldehydes and Ketones

Addition. The oxygen atom of the carbonyl group attracts electrons more strongly than the carbon atom and therefore this bond is strongly polarised. The carbon atom gets a partial positive charge and the oxygen atom a partial negative charge. This distribution of electrons determines the main reaction of aldehydes and ketones – **the addition of nucleophilic agents** to the carbonyl carbon.
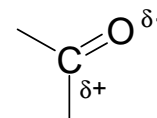
The addition of water to the carbonyl group produces **aldehyde hydrates** (ketone hydrates), unusual compounds with two hydroxyls on one carbon, stable only in aqueous solutions.

aldehyde hydrate
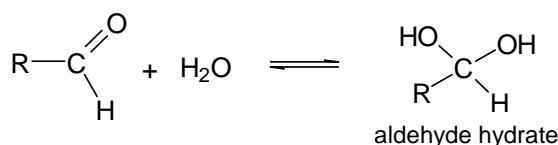
The addition of alcohol to carbonyl produces an unstable **hemiacetal** (1-alkoxyalkan-1-ol) or hemiketal, which can react with another molecule of alcohol to produce a stable **acetal** (1,1-dialkoxyalkane) or ketal and water.
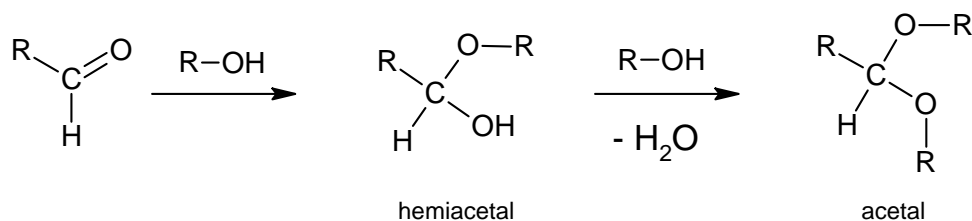
hemiacetal   acetal

The hydroxyl group can add to the carbonyl group within the same molecule. This addition produces **stable cyclic hemiacetals**. These reactions are very important in the chemistry of saccharides. Monosaccharides exist in cyclic hemiacetal forms as pyranoses or furanoses (see Chapter 34).

The addition of primary amines produces very unstable aminohemiacetals. They are stabilised by the elimination of water to form **imino compounds** (aldimines or ketimines, Schiff bases).

aminohemiacetal   aldimine
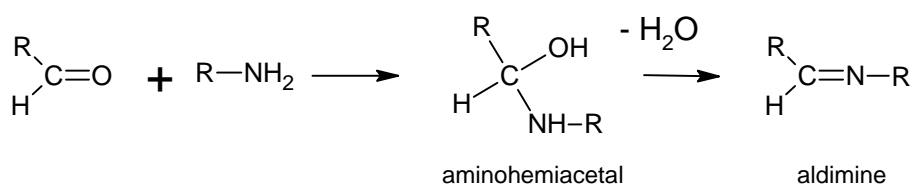
These reactions occur in the metabolism of amino acids (**transamination**, reaction of amino acid with pyridoxal phosphate), the **maturation of collagen** and elastine (see later), and the glycation of proteins. Increased concentration of glucose in the body fluids, in case of inadequately compensated diabetes, is the cause of the pronounced **non-enzymatic glycation of proteins**. Glucose forms through its aldehyde group unstable Shiff bases with the free -NH$_2$ groups of proteins, which are then isomerised to stable amino derivatives. In the course of time, reactions with the other groups in the side chains of proteins produce so called advanced glycation endproducts (AGE), which change the function of proteins. Determination of the glycated hemoglobin in blood is used in diabetology to evaluate the efficiency of the treatment or the adherence to diet.

glucose   labile Schiff base   glycated protein
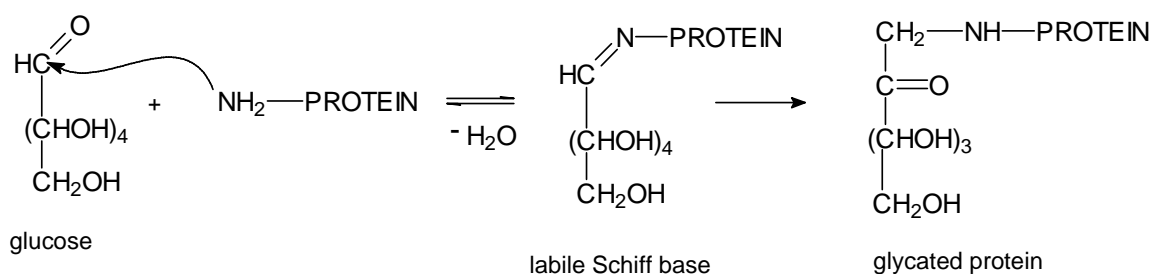
Aldol condensation. In an alkaline environment, carbonyl compounds are able to release a proton from the α-carbon to form a carbanion. The addition of the carbanion to the carbonyl group of another aldehyde produces a new C-C bond and creates a compound with longer chain, **β-hydroxyaldehyde (β-aldol)**. An example of biochemical aldol condensations is the reaction of dihydroxyacetone phosphate with glyceraldehyde phosphate in the process of gluconeogenesis. Aldols stabilise by dehydration to α,β-unsaturated aldehydes.

3-hydroxyaldehyde (β-aldol)
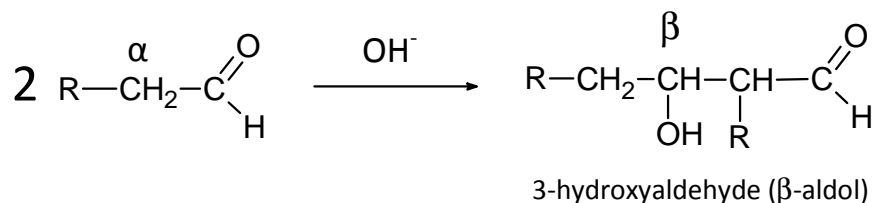
Redox reactions. Aldehydes are **easily oxidised** to carboxylic acids; this oxidation proceeds typically as the dehydrogenation of aldehyde hydrates (see the scheme).

| aldehyde | aldehyde hydrate | carboxylic acid |
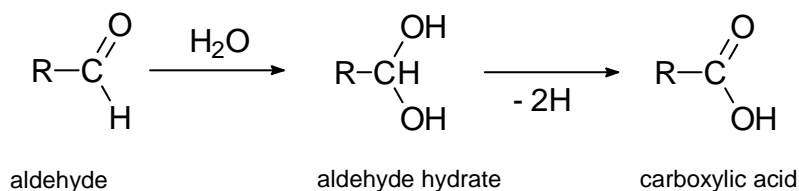
Therefore aldehydes have substantial reducing properties. These reducing properties are used in analytical reactions with the Benedict's reagent (reduction of Cu^{II} to Cu^{I}). In contrast to aldehydes, ketones do not have reducing properties. Strong oxidation agents cleave the C-C bond between the carbonyl group and the α-carbon; products are two molecules of carboxylic acids.

Reduction of aldehydes and ketones (hydrogenation, addition of hydrogen) is achieved only by strong reducing agents. Aldehydes are transformed to primary alcohols, ketones to secondary alcohols.

Important Aldehydes and Ketones

Formaldehyde HCH=O is a colourless irritant gas with disinfection properties (precipitates proteins). It is classified as a known human carcinogen. In small amounts, it is released from plywood and particle board (chipboard) furniture, and its elevated indoor level can negatively influence health condition. Its 40% aqueous solution is used for the conservation of anatomic samples (formalin).

Acetaldehyde $CH_3CH=O$ is a pungent, volatile, and flammable liquid. Acetaldehyde is formed by the oxidation of ethanol. Its further oxidation produces acetic acid. Acetaldehyde is the intermediate product of alcohol fermentation; it is formed by the decarboxylation of pyruvic acid.

pyruvic acid   acetaldehyde
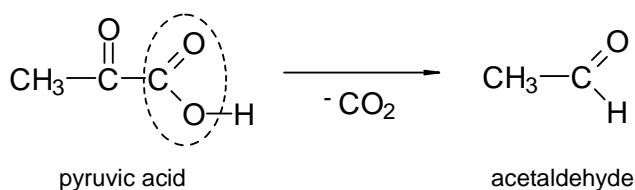
Glyceraldehyde $CH_2(OH)-CH(OH)-CH=O$ (2,3-dihydroxypropanal) is the simplest sugar belonging to the group of aldoses (trioses). Glyceraldehyde 3-phosphate is an intermediate of glucose metabolism.

Allysine (2-amino-6-oxohexanoic acid) is an amino acid found in the proteins of connective tissues, collagen and elastin. It is produced during the posttranslational modification by the oxidation deamination of the ε-amino group of lysine. The aldehyde groups make inter-chain covalent bonds (crosslinks) during the maturation of collagen and elastin. Aldol condensation between two allysine residues or the formation of the Shiff base between allysine and lysine residues creates crosslinks between peptide chains. Crosslinks are responsible for the properties of collagen (strength) and elastin (elasticity).
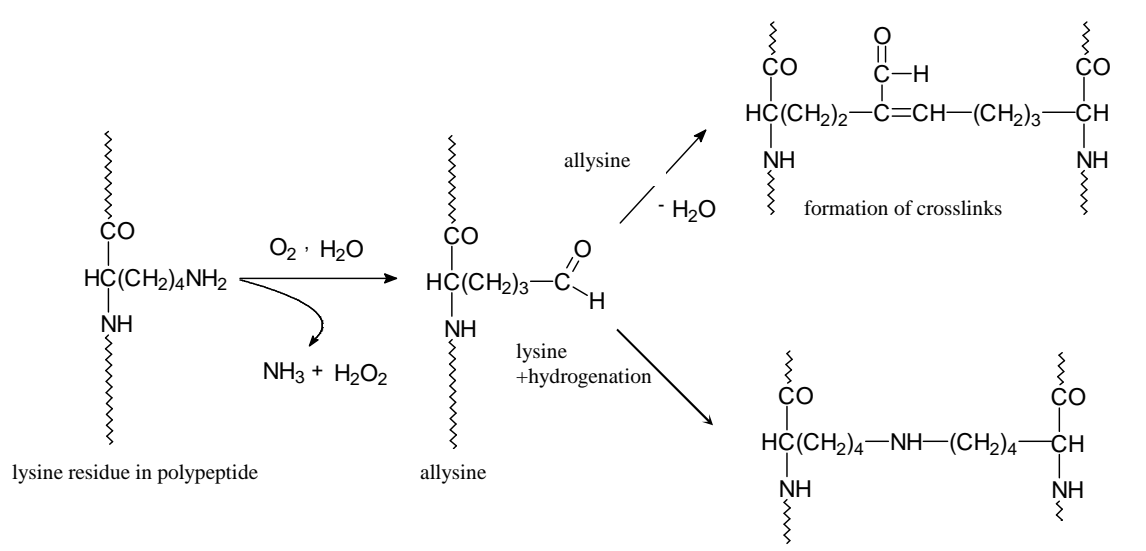
Malondialdehyde $O=HC-CH_2-CH=O$ (MDA, propanedial) is one of the products of lipoperoxidation, a non-specific non-enzymatic oxygenation of unsaturated fatty acids (containing at least three double bonds) bound in lipids and phospholipids. Lipoperoxidation accompanies not only the rancidification of fats (*in vitro*), but it occurs also in the organism (*in vivo*) by the action of hydroxyl radical $\cdot OH$ and other oxygen radicals. Malondialdehyde is a highly reactive compound. It causes the crosslinking of membrane phospholipids and the formation of unfavourable covalent bonds between protein molecules. Malondialdehyde is used as a diagnostic marker of lipoperoxidation (oxidation stress).

Acetone $CH_3-CO-CH_3$ (propanone) is a colourless, volatile, and flammable liquid with a characteristic smell. It is an excellent solvent with low polarity, although miscible with water. It irritates the skin and also the upper respiratory tract when inhaled. In the human body, acetone is formed during spontaneous, non-enzymatic decarboxylation of acetoacetic (3-oxobutanoic) acid.
Together with 3-hydroxybutyrate these three substances are called ketone bodies.

Dihydroxyacetone $HO-CH_2-CO-CH_2-OH$ (1,3-dihydroxypropanone) is formed by the dehydrogenation of the secondary hydroxyl group in glycerol. Dihydroxyacetone phosphate (DHAP) is an intermediate of glycolysis.

28 Carboxylic Acids and Their Derivatives

The carboxyl –COOH is the characteristic functional group of carboxylic acids, in which carbon reaches the highest oxidation state in organic molecules (III), except formic acid.

| carboxylic acid | acyl | carboxylate anion | sodium salt of carboxylic acid | calcium salt of carboxylic acid |
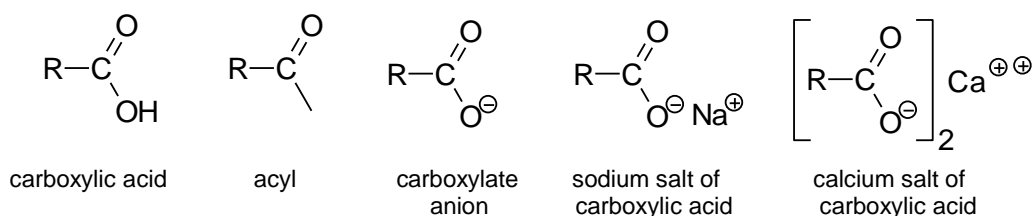
Nomenclature. To create the systematic name of a carboxylic acid, the final *–e* in the name of parent hydrocarbon is replaced with the suffix *–oic* (or *–dioic*) *acid* (e.g. butane → butanoic acid). For cyclic acids, the suffix *–carboxylic acid* is used, e.g. benzenecarboxylic acid (benzoic acid). The names of tri- and polycarboxylic acids are formed in the similar way (propane-1,2,3-tricarboxylic acid). A number of carboxylic acids have common names, namely those of biochemical importance.

The names of anions and salts of acids are derived from the systematic or common names by replacing the final *–ic* in the name of acid with the suffix *–ate*, e.g., acetate, butanoate (butyrate), hexanoate, butandioate (succinate), stearate. The names of salts consist of the name of a cation and an anion: sodium benzoate, calcium oxalate, magnesium stearate.

Acyl R-CO- is the residue of acid after removal of hydroxyl from R-COOH. To derive the name of an acyl, the final *–ic* in the name of acid is replaced with the suffix *–oyl* (*–yl*): hexanoic acid – hexanoyl, formic acid – formyl, acetic acid – acetyl, succinic – succinyl, palmitoic acid – palmitoyl, oleic acid – oleoyl. The systematic and common names of some acids, the names of their anions, and acyls are presented in the tables below.

Saturated monocarboxylic acids

Number of C	Formula	Acid	Anion	Acyl
1	HCOOH	formic	formate	formyl
2	CH$_3$COOH	acetic	acetate	acetyl
3	CH$_3$CH$_2$COOH	propanoic (propionoic)	propionate	propionyl
4	CH$_3$CH$_2$CH$_2$COOH	butanoic (butyric)	butyrate	butyryl
5	CH$_3$(CH$_2$)$_3$COOH	pentanoic (valeric)	pentanoate	pentanoyl
6	CH$_3$(CH$_2$)$_4$COOH	hexanoic (caproic)	hexanoate	hexanoyl
12	CH$_3$(CH$_2$)$_{10}$COOH	dodecanoic (lauric)	dodecanoate	dodecanoyl
14	CH$_3$(CH$_2$)$_{12}$COOH	tetradecanoic (myristic)	tetradecanoate	tetradecanoyl
16	CH$_3$(CH$_2$)$_{14}$COOH	hexadecanoic (palmitic)	palmitate	palmitoyl
18	CH$_3$(CH$_2$)$_{16}$COOH	octadecanoic (stearic)	stearate	stearoyl
20	CH$_3$(CH$_2$)$_{18}$COOH	eicosanoic (arachidonic)	eicosanoate	eicosanoyl

Unsaturated monocarboxylic acids

No. of C	Formula	Acid	Anion	Acyl
3	$CH_2=CH–COOH$	propenoic (acrylic)	acrylate	propenoyl
18	$CH_3(CH_2)_7CH=CH(CH_2)_7COOH$	*cis*-octadec-9-enoic (oleic)	oleate	oleoyl

Dicarboxylic acids

No. of C	Formula	Acid	Anion	Acyl
2	HOOC-COOH	ethanedioic (oxalic)	oxalate	oxalyl
3	$HOOC-CH_2-COOH$	propanedioic (malonic)	malonate	malonyl
4	$HOOC-(CH_2)_2-COOH$	butanedioic (succinic)	succinate	succinyl
5	$HOOC-(CH_2)_3-COOH$	pentanedioic (glutaric)	glutarate	glutaryl
6	$HOOC-(CH_2)_4-COOH$	hexanedioic (adipic)	adipate	adipoyl
4	HOOC-CH=CH-COOH	*trans*-butenedioic (fumaric)	fumarate	fumaroyl
4	HOOC-CH=CH-COOH	*cis*-butenedioic (maleic)	maleate	maleoyl

There are two types of aromatic carboxylic acids: arenecarboxylic acids, e.g. benzoic (benzenecarbox-ylic), phthalic (benzene-1,2-dicarboxylic), 1-naphthoic (naphthalene-1-carboxylic), and arylalkanoic acids, e.g. phenylacetic acid.

benzoic acid   phtalic acid   1-naftoic acid   phenylacetic acid
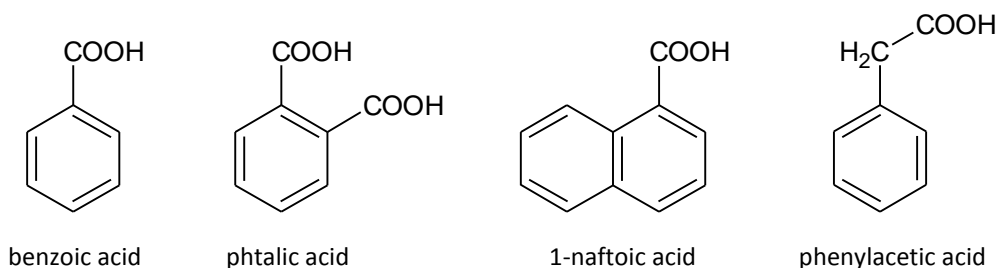
Properties. Carboxyl groups are highly polar. The resulting polarity of molecules depends on the number of carboxyls, as well as on the length of the aliphatic chain and/or the presence of an aromatic ring. The three shortest aliphatic monocarboxylic acids are liquids well soluble in water (formation of hydrogen bonds between carboxyls and water). Long aliphatic chains or aromatic rings decrease the polarity of the acids distinctly. Higher aliphatic acids are according to the degree of saturation oily liquids or solid substances (fatty acids). Di- and tricarboxylic acids and aromatic acids are crystalline substances. The acids insoluble in water are soluble in solutions of strong hydroxides: a strong base supports dissociation of carboxyls and solutions of salts of carboxylic acids are obtained.

Reactions of Carboxylic Acids

Acidity of carboxylic acids. Carboxylic acids dissociate only partially in aqueous solutions:

carboxylate ion
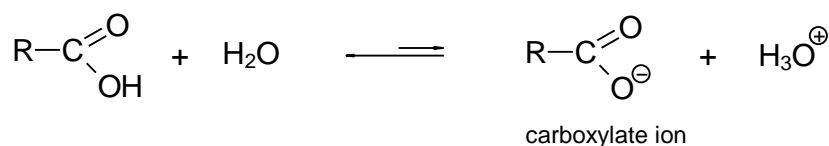
In body fluids (pH ~ 7.4), carboxylic acids are fully dissociated and exist only in the form of anions. Therefore, to describe the reactants in metabolic pathways, it is more correct to use the names of ani-ons instead of the names of acids, for example, the dehydrogenation of succinate gives fumarate.

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Neutralisation. Carboxylic acids, if treated with a strong base, form salts. The salts of alkali metals are usually well soluble in water; therefore, the acids that do not dissolve in water are soluble in the solutions of NaOH. The aqueous solutions of these salts are alkaline (due to hydrolysis of the carboxylate ion), treating with strong acids again releases the free non-ionised acid. The salts of long-chain fatty acids are **soaps**; soluble soaps are anionic surfactants.

Decarboxylation. Under certain conditions, also enzymatically, carboxylic acids undergo decarboxylation, CO_2 is eliminated from the carboxyl group. The carbon chains of products are then shorter by one-carbon: alkanoic acids give saturated hydrocarbons, 3-oxoacids give **ketones**, α-amino acids provide biogenic **amines**.

Formation of derivatives. The hydroxyl group of the carboxyl can be substituted with some other groups in a number of reactions. Such derivatives of acids (sometimes called functional derivatives) are no longer acidic (acid anhydrides, esters, amides).

Biochemical conversions. Metabolic pathways include reversible transformations of saturated carboxylic acids to unsaturated, and further into various types of substituted acids. The following scheme shows, as an example, the sequence of three reactions of the citric acid cycle, from succinate to oxaloacetate, and the following transamination of oxaloacetate to aspartate:

succinate (saturated)   fumarate (unsaturated)   malate (hydroxy acid)   oxaloacetate (oxo acid)   aspartate (amino acid)
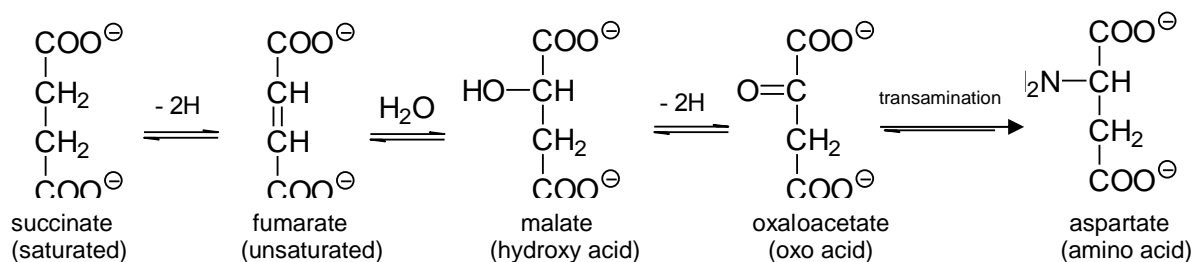
Some Important Carboxylic Acids

Formic acid H-COOH (acidum formicum, salts formates) is a pungent smelling colourless liquid with caustic effects. It is present in the poisonous gland secretion of ants (Lat. *formica*) or in the trichomes of stinging nettle. It is formed by the oxidation of methanol, also in the body, after it was consumed. Acyl of formic acid H-CO– **formyl** is an important building unit in some biosynthetic processes; it is transported attached to tetrahydrofolate. The product of oxidation of formic acid is carbonic acid, which decomposes immediately to CO_2 and water.

Acetic acid CH_3-COOH (acidum aceticum, salt acetates) is formed by oxidation of ethanol, also in the body after alcohol consumption. Vinegar contains acetic acid in 5–8% concentration. The acyl of acetic acid bound to the –SH group of coenzyme A is **acetyl coenzyme A**, which is the central intermediate of the metabolism of glucose, fatty acids, and some amino acids.

Propionic acid CH_3CH_2-COOH (propanoic acid) is an unpleasant smelling liquid. A number of biochemically important acids (lactate, pyruvate, and alanine) are formally derivatives of propionic acid. Molecules of heme and bile pigments contain the residues of propionic acid attached through C3 to pyrrole rings of porphin.

Butyric acid $CH_3CH_2CH_2$-COOH (acidum butyricum, salts butyrates) is an unpleasantly smelling oily liquid. Triacylglycerols of milk fat contain also esterified butyric acid (Lat. *butyrum* butter). Free butyric acid is released by hydrolysis of milk fat, e.g. in old cheese and rancid butter.

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Oxalic acid (acidum oxalicum, salts oxalates) is a crystalline substance, quite toxic. It is the simplest dicarboxylic acid, found in some plants (spinach, sorrel, rhubarb). Oxalates are also formed in the human body during catabolism of glycine, after the consumption of large doses of L-ascorbate, or in ethylene glycol poisoning by its oxidation.

In body fluids, oxalate anions combine with calcium ions and form poorly soluble **calcium oxalate** $(COO)_2Ca$, which does not dissociate in water. Calcium oxalate is often one of the components of urinary stones (renal calculi). Sodium oxalate is soluble and used in laboratories as an anticoagulant, which prevents blood plasma from clotting.
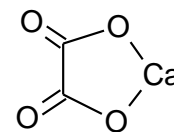
Succinic acid (butanedioic, ac. succinicum, salts succinates) is an intermediate of the citric acid cycle.

Fumaric acid (*trans*-butenedioic acid) is a product of dehydrogenation of succinate in the citric acid cycle.

Benzoic acid (benzenecarboxylic acid, acidum benzoicum) is a white crystalline substance, which has certain antimycotic properties. The benzoates salts are found in many plants. Sodium benzoate is used as a food additive that extends the shelf life of some food. Ingested benzoates are excreted into urine after biotransformation into **hippuric acid** (*N*-benzoylglycine, amide bond with glycine).

Ibuprofen, 2-(4-isobutylphenyl)propanoic acid, is the synthetic derivative of 2-phenylpropanoic acid. It is an analgesic (pain killer) and antipyretic with anti-inflammatory effects.

ibuprofen
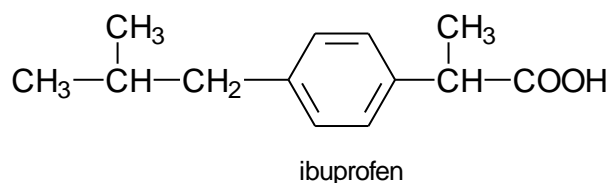
Carboxylic Acid Derivatives

Carboxylic acid derivatives are compounds in which other groups replace the hydroxyl of the carboxyl group. These derivatives (e.g. anhydrides, esters, thioesters, amides) are no longer acidic and all can be hydrolysed to the corresponding acid.

| anhydride | ester | thioester | *N*-alkylamide | amide |
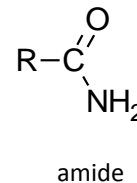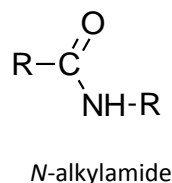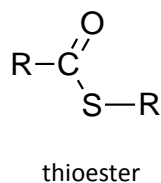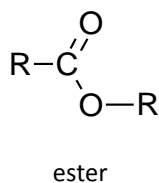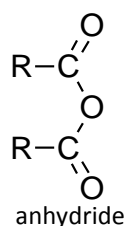
Acid anhydrides are derived from two acid molecules by eliminating water (also from two different acids). The anhydride bond is characterised by the typical arrangement of atoms R-CO-O-CO-R. Hydrolysis of acid anhydrides gives the original acids.

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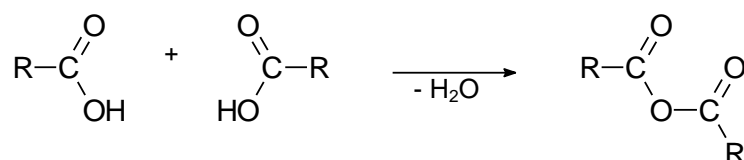
Some dicarboxylic acids easily form **intramolecular** cyclic anhydrides, e.g. succinic anhydride and phtalic anhydride. Acid anhydrides are very reactive compounds; they are mainly used as acylating agents, e.g. acetylation of salicylic acid by acetic anhydride gives acetylsalicylic acid.

acetic anhydride   succinic anhydride   phthalic anhydride
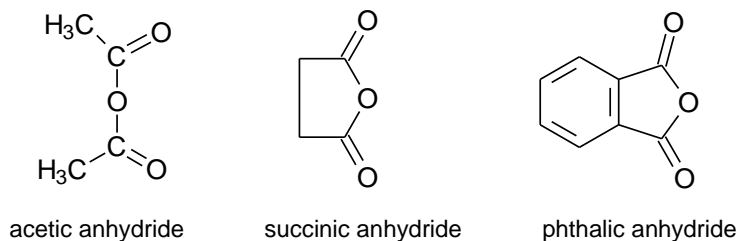
Mixed anhydrides (**acyl phosphates**, $R-CO-O-PO(OH)_2$) are formed from some carboxylic acids and phosphoric acid. They are high-energy phosphate intermediates, their hydrolysis is strongly exergonic. **1,3-Bisphosphoglycerate** (3-phosphoglyceroyl phosphate) is formed during glycolysis by dehydrogenation from glyceraldehyde 3-phosphate and inorganic phosphate (P_i). One phosphate is attached to C3 through an ester bond; the carboxyl group forms a mixed anhydride with phosphoric acid. **Carbamoyl phosphate** – the mixed anhydride of carbamic acid and phosphoric acid – is the intermediate in the synthesis of urea and pyrimidine bases.

acyl phosphate   1,3-bisphosphoglycerate   carbamoyl phosphate
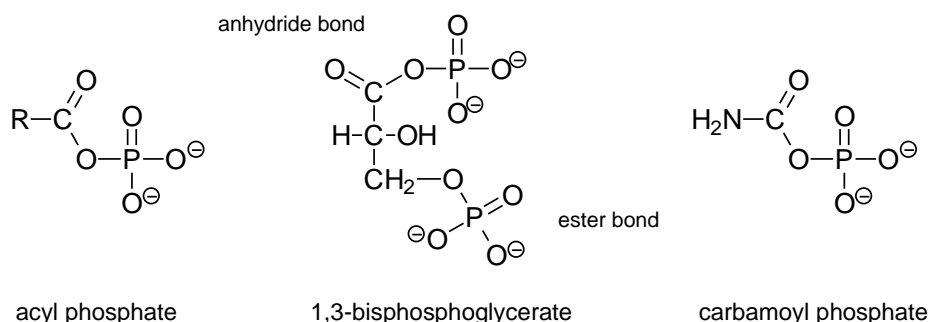
Esters of carboxylic acids $R-CO-O-R$ are derived from acids by replacing the -OH group with an alkoxy group -OR of alcohols. The names of esters are formed similarly to the names of the salts: the name of alkyl is followed by the name of the carboxylate anion, e.g., methyl salicylate, phenyl acetate. Esters are products of the reversible reaction of an alcohol with a carboxylic acid in the presence of catalytical amounts of H^+ ion. The example is the esterification of acetic acid by a general alcohol:
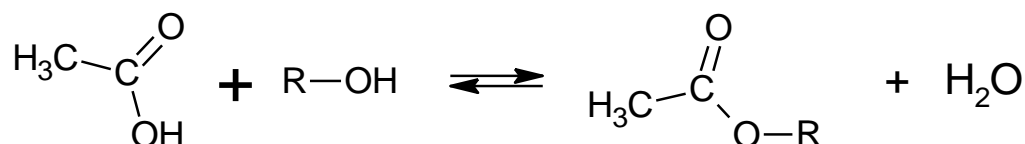
Cyclic esters, called **lactones**, arise from intramolecular esterification of some hydroxy acids, if hydroxyl is sufficiently distant from the carboxyl. For example, 5-hydroxypentanoic acid gives pentano-

5-lactone. Another example of lactone is L-ascorbic acid (vitamin C, see Chapter 46), the dehydrogenation of glucopyranose gives gluconolactone (see Chapter 34).

5-hydroxypentanoic acid   pentano-5-lactone
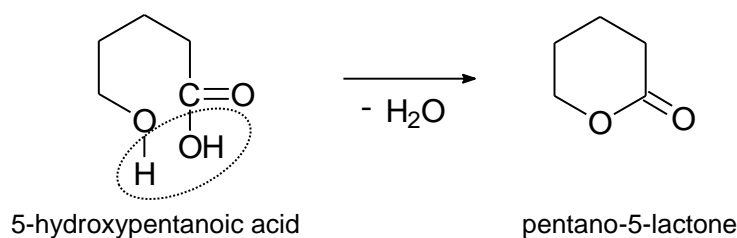
Esters are **non-electrolytes.** Esters are mostly volatile liquids, non-polar, insoluble in water. The hydrolytic cleavage of esters in the acidic aqueous environment gives alcohols and acids. Hydrolysis of esters in the presence of a strong base (saponification) produces alcohols and salts of acids. Biochemically the most important group of esters are **lipids (triacylglycerols)**, triesters of glycerol and fatty acids.

Thioesters R-CO-S-R, sulfur analogues of esters, are more reactive than esters. Acyl is bound to the alkylsulfanyl group -S-R. Thioesters belong to high-energy (macroergic) compounds and serve as the active acyl-transfer agents in the cell. The formation of a thioester bond activates the carboxylic acid. Examples of thioesters are **acetyl coenzyme A** and **acetyl lipoate** that transports acetyl during the aerobic decarboxylation of pyruvate.

acetyl coenzyme A   acetyl lipoate
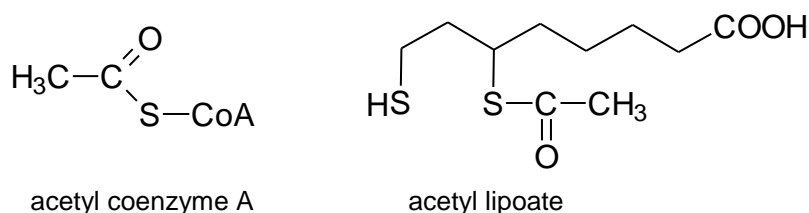
Amides are derivatives of carboxylic acids that can be formally derived by substitution of the -OH group in carboxyl by the $-NH_2$ group which can be substituted ($R-CO-NH_2$, R-CO-NHR, $R-CO-NR_2$). Some amides can be prepared by condensation of carboxylic acids with ammonia or an amine.

N-alkylamide
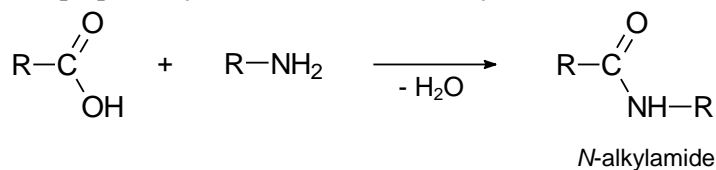
The acid-base reaction of acids with amines gives ammonium salts of acids:

$$R\text{–}COOH + R\text{–}NH_2 \rightarrow R\text{–}COO^- \ H_3N^+\text{–}R.$$
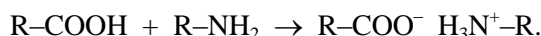
Amides are named by replacing the *–ic/–oic acid* ending of the carboxylic acid with the *–amide* suffix, e.g., CH_3-CO-NH_2 acetamide, CH_3-CH_2-CO-NH_2 propanamide. If the nitrogen atom binds alkyl groups, they are introduced by a locant *N*, for example:
CH_3-CO-NH-CH_3 *N*-methylacetamide, CH_3-CO-N(CH_3)$_2$ *N,N*-dimethylacetamide.

Cyclic intramolecular amides are called **lactams**. 4-Amino acids and 5-amino acids can release water and form intramolecular cyclic amides; e.g. 5-aminopentanoic acid gives pentano-5-lactam. In these compounds, lactam-lactim tautomerism occurs. Lactim forms with the enolic hydroxyls are less stable than lactams.

| 5-aminopentanoic acid | pentano-5-lactam | pentano-5-lactim |
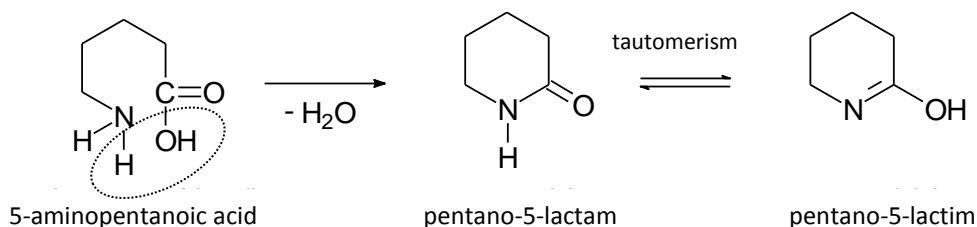
The amide group is **highly polar** and **non-basic**; amides, in contrast to amines, are **non-electrolytes**, because the unshared electron pair on a nitrogen atom is in conjugation with π-electrons of the carbonyl group. Some amides are soluble in water and only slightly reactive. Hydrolysis of amides provides carboxylic acids and ammonia (or amine).
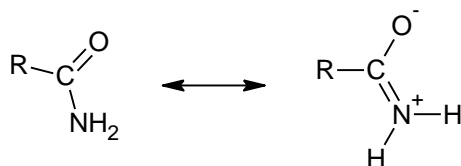
Biochemically Important Amides

Peptides and **proteins** are very common, the peptide bond is a special type of amide bond (Chapter 43).

Ceramides (*N*-acylsphingosines) make the basic structure of sphingolipids, fatty acid is attached to amino alcohol sphingosine by amide bond (Chapter 39).

N-acetylated amino sugars (e.g. *N*-acetylglucosamine) are neutral compounds (Chapter 34).

Asparagine (amide of aspartic acid) and **glutamine** (amide of glutamic acid) belong to twenty coded amino acids; their side chain is polar, but unionised (Chapter 42).

Urea, the catabolite of amino acids, is diamide of carbonic acid (Chapter 29).

Nicotinamide is the hydrophilic vitamin, the component of NAD^+ (Chapter 37, 46).

Folic acid and **pantothenic acid** are hydrophilic vitamins with amide group (Chapter 46).

Melatonin (*N*-acetyl-5-methoxytryptamine) is a signal molecule regulating daily biorhythm (Chapter 31).

Paracetamol (acetaminophen), *N*-(4-hydroxyphenyl)acetamide, is a synthetic analgesic and antipyretic (e.g. Panadol). Its effects are similar to those of acetylsalicylic acid; on the contrary, it does not damage the gastric mucosa. However, higher doses of paracetamol could be hepatotoxic.

paracetamol
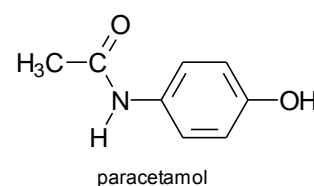
29 Derivatives of Carbonic Acid

Carbonic acid and its salts carbonates and hydrogen carbonates are traditionally classified as inorganic compounds. On the other hand, functional derivatives of this acid belong to organic compounds.

Carbonic acid H_2CO_3 (acidum carbonicum, the hydrate of carbon dioxide) is very unstable, so it cannot be isolated. The H_2CO_3 molecules exist only in the aqueous solution of CO_2 where physically dissolved CO_2 prevails ($H_2CO_3 \rightleftarrows H_2O + CO_2$). Both hydroxyls of carbonic acid can be modified to form organic derivatives. Generally, diderivatives of carbonic acid (e.g. urea) are substantially more stable than extremely unstable monoderivatives (e.g. carbamic acid).
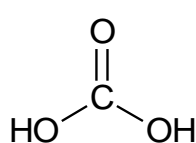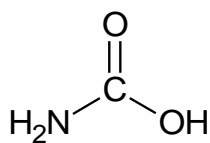
| carbonic acid | carbamic acid | urea | guanidine (iminourea) |
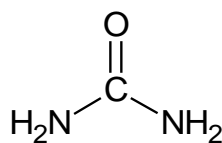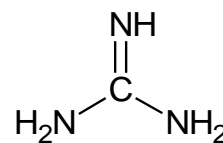
Carbamic acid NH_2-COOH (monoamide of carbonic acid) exists only in the form of salts and esters. Mixed anhydride of carbamic and phosphoric acid, **carbamoyl phosphate**, is a high-energy intermediate of the biosynthesis of urea and pyrimidine bases in the human body.
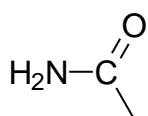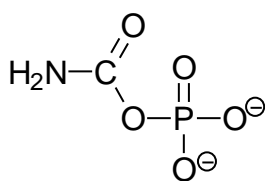
| carbamoyl (acyl) | carbamoyl phosphate (mixed anhydride) | alkyl carbamate (ester) |
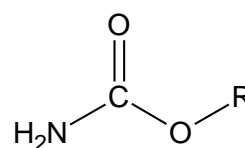
N-Substituted esters of carbamic acid, both natural and synthetic, are called **carbamates**. Many of them inhibit acetylcholinesterase – the enzyme that catalyses the quick decomposition of acetylcholine on the nerve synapses. Many other carbamates are used as insecticides and herbicides.

Neostigmine is the ester of *N,N*-dimethylcarbamic acid and 3-hydroxyphenyltrimethylammonium. It acts as a reversible inhibitor of acetylcholinesterase, and also in the treatment of post-surgical atonia of intestines and some neuromuscular diseases.
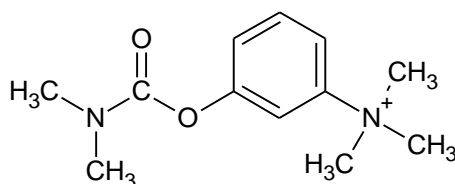
neostigmine

Urea NH_2CONH_2, a diamide of carbonic acid, is the final nitrogenous product of the catabolism of amino acids in humans. Urea is excreted into urine (20-35 g/d) as a waste product in dependence on the consumption of dietary proteins. Urea is a highly **polar** compound, perfectly soluble in water and easily diffused through biological membranes. It is a **non-electrolyte**, therefore its aqueous solutions are neutral. Infusions of hypertonic solutions of urea cause the osmotic movement of water from the intracellular into the extracellular compartment, which is used, e.g. in the treatment of brain oedema. Hydrolysis of urea produces ammonia and carbon dioxide:

$$NH_2CONH_2 + H_2O \rightarrow 2\ NH_3 + CO_2$$

Guanidine $(NH_2)_2C=NH$ is iminourea. In contrast to urea, guanidine is a strong base ($pK_B = 0.5$), because accepting H^+ to the imino nitrogen stabilises the guanidinium cation by resonance energy. The guanidino group is included in a strongly basic amino acid arginine, which is also the intermediate of the biosynthesis of urea.

Creatine (*N*-methylguanidine-*N*-acetic acid) is another guanidine derivative of biochemical importance. Three coded amino acids are required for the biosynthesis of creatine: glycine (donor of its entire skeleton) and arginine (donor of $H_2N-C(=NH)-$ group) react to give guanidinoacetate as an intermediate. Methionine (donor of methyl) accomplishes the synthesis of creatine.

In the muscles, ATP phosphorylates creatine to **creatine phosphate**, an amide of phosphoric acid. The reversible reaction is catalysed by creatine kinase:

$$\text{creatine } + \text{ATP} \rightleftarrows \text{ creatine-P } + \text{ADP}.$$
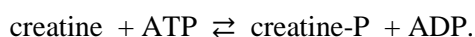
The standard Gibbs energy change $\Delta G°$ of hydrolysis of the unusual nitrogen-phosphorus bond is very high. Creatine phosphate serves as a reservoir of high-potential phosphoryl groups that can be readily transferred to phosphorylate ADP (see also Chapter 3).

Creatine is transformed very slowly into a cyclic anhydride **creatinine**, which is excreted into urine. Creatine concentration in blood serum (and urine) is the marker of the filtration functions of kidneys.

glycine   guanidinoacetic acid   creatine

creatine phosphate   creatinine
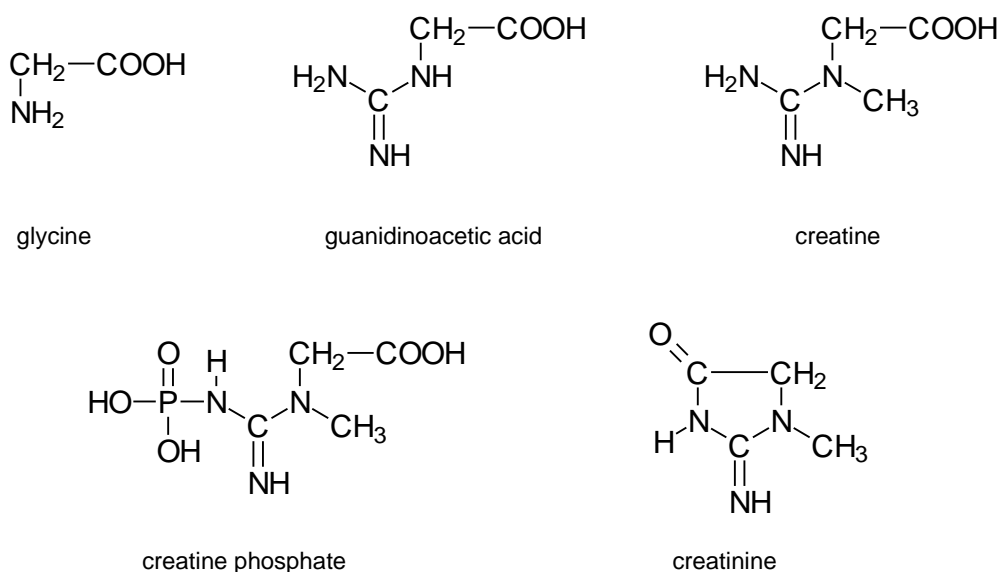
30 Hydroxy Acids and Oxo Acids

Substituted carboxylic acids possess another functional group such as hydroxyl, oxo or another group. Both the carboxyl and another functional group keep all their properties in substituted acids, though they can influence or react with each other to make a heterocyclic product (lactone, lactam).

Hydroxy acids are mostly crystalline substances of pleasantly acidic taste. Their polarity is higher than that of the corresponding alkanoic acids; therefore, they dissolve well in water. Many of them are chiral compounds, with asymmetric carbon atoms.

Dehydrogenations of hydroxy acids play very important roles in many biochemical pathways. Hydroxy acids containing a secondary alcohol group undergo reversible dehydrogenation to give oxo acids by removing two H atoms. Similarly, dehydrogenation of the primary alcohol group -CH_2-OH provides an aldehyde acid. The reactions are catalysed by dehydrogenases, which require the coenzyme NAD^+ as an acceptor of hydrogen atoms. The reversible dehydrogenation can be expressed by the following general scheme:

| 2-hydroxy acid | | 2-oxo acid |
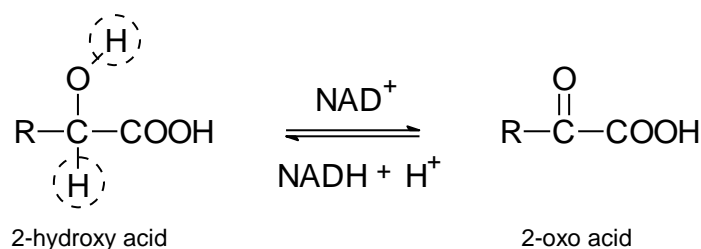
Dehydration. Aliphatic hydroxy acids easily release water when heated. The product of dehydration depends on the distance between the hydroxyl and the carboxyl group. 3-Hydroxyalkanoic acids (β-hydroxy acids) produce **2,3-unsaturated** acids by dehydration. Similarly, β-hydroxyacyl coenzyme A reacts in the same way during biosynthesis of fatty acids.

3-hydroxy acid   2,3-unsaturated acid
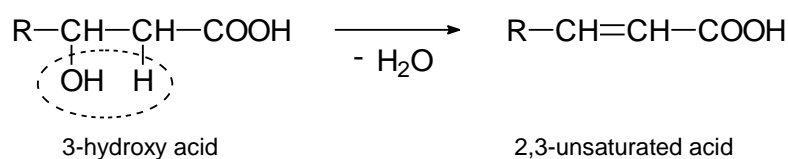
4-Hydroxyalkanoic acids and 5-hydroxyalkanoic acids dehydrate in a different manner so that they form cyclic intramolecular esters called lactones (see Carboxylic acids).

Oxo acids have at least one oxo group in addition to the carboxyl. If the oxo group is located at the terminal C atom, the compound is an aldehyde acid, for example, O=HC-COOH is glyoxylic acid, O=HC-CH_2-CH_2-COOH is succinaldehydic acid (semialdehyde of succinic acid). The reactivity of oxo acids depends on the position of the oxo group. The difference can be seen, for example, in how easily they undergo decarboxylation (elimination of CO_2 from the carboxyl group). 3-Oxo acids (β-ketoacids) are spontaneously decarboxylated to the corresponding ketones. These decarboxylations proceed also in the organism (e.g. acetoacetate decarboxylates to acetone).

Biochemically Important Redox Pairs "Hydroxy Acid / Oxo Acid"

Glycolic acid / Glyoxylic acid (Glycolate / Glyoxylate, 2C)
Glycolic acid $HO-CH_2-COOH$ (hydroxyacetic acid) is the simplest hydroxy acid. In intoxication with ethylene glycol, it is the cause of severe acadosis, which is produced by its oxidation.
Glyoxylic acid $O=HC-COOH$ (oxoacetic acid) is the simplest aldehyde acid, the intermediate of the oxidation of ethylene glycol or glycolate to oxalate; it is also produced by transamination of glycine.

Lactic acid / Pyruvic acid (Lactate / Pyruvate, 3C)
Lactic acid $CH_3-CH(OH)-COOH$ (2-hydroxypropanoic acid, acidum lacticum, $pK_A = 3.86$, anion lactate) is a syrupy liquid. It is chiral, exists in two enantiomers. L-Lactate is the end product of glycolysis under anaerobic conditions, e.g. in muscles working with high intensity. The racemic D,L-lactate is the product of lactic fermentation of sugars (kefir milk, yoghurt, sauerkraut). If the gastric secretion is not sufficiently acidic (anacidity), lactic acid may be produced in the stomach. Dehydrogenation of lactate gives pyruvate; the reaction is reversible, catalysed by lactate dehydrogenase.
Pyruvic acid $CH_3-CO-COOH$ (2-oxopropanoic acid, acidum pyruvicum, $pK_A = 2.39$, anion pyruvate) is biochemically very important oxo acid. It is formed in cells by glycolysis of sugars, and also by transformation of some amino acids. Its further fate depends on the availability of oxygen. In case of anaerobic conditions in the animal cells (or in lactic acid fermentation), it is hydrogenated to lactate. During alcohol fermentation it gives acetaldehyde by decarboxylation and then by the following hydrogenation ethanol. Under aerobic conditions, pyruvate usually undergoes oxidative decarboxylation to acetyl-coenzyme A. Carboxylation of pyruvate gives oxaloacetate.

lactate   pyruvate
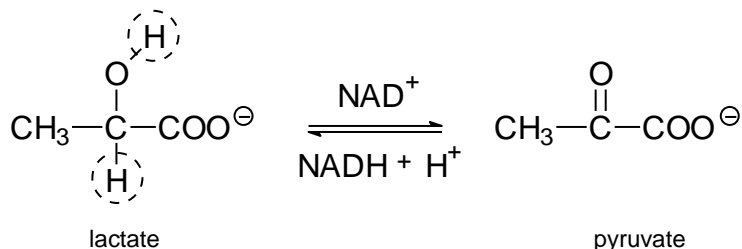
β-Hydroxybutyric acid / Acetoacetic acid (β-Hydroxybutyrate / Acetoacetate, 4C)
β-Hydroxybutyric acid $CH_3-CH(OH)-CH_2-COOH$ (3-hydroxybutanoic acid, $pK_A = 4.70$, anion β-hydroxybutyrate). It is formed by hydrogenation of acetoacetate as one of the three ketone bodies (see below). Under pathological conditions it is excreted in increased amounts into urine.
Acetoacetic acid $CH_3-CO-CH_2-COOH$ (3-oxobutanoic acid, $pK_A = 3.52$, ion acetoacetate) is formed in the body during the catabolism of fatty acids and by transformation of some amino acids. Like other 3-oxo acids, it is not stable; its spontaneous decarboxylation produces acetone. Acetoacetate is enzymatically hydrogenated to β-hydroxybutyrate, a small portion is decarboxylated to acetone.

β-hydroxybutyric acid   acetoacetic acid   acetone
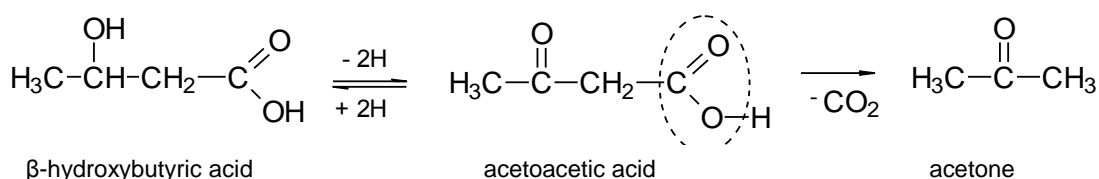
In biochemistry, these three substances with common metabolic origin – acetoacetic acid, β-hydroxybutyric acid, and acetone – are called **ketone bodies.** In the blood and urine of healthy individuals, only very small amounts of ketone bodies are present. The production of ketone bodies increases, if the cells have to utilise fatty acids as the main energy source, mainly during non-compensated diabetes or during long-term starvation. In some cases, large amounts of ketone bodies are excreted in urine, sweat, and the breath gives out a smell of acetone. Acetoacetate and acetone are detected in urine by a colour test with nitroprusside.

Malic acid / Oxaloacetic acid (Malate / Oxaloacetate, 4C)

Malic acid $HOOC-CH_2-CH(OH)-COOH$ (hydroxybutanedioic acid, acidum malicum, anion malate) was originally isolated from apples (Lat. *malum*). It is an intermediate of the citric acid cycle, in which it is produced by the hydratation of fumarate. Dehydrogenation of malate gives oxaloacetate.

Oxaloacetic acid $HOOC-CO-CH_2-COOH$ (oxobutanedioic acid, anion oxaloacetate) is the starting substrate of the citric acid cycle. Condensation with acetyl-coenzyme A gives citric acid. Oxaloacetate is regenerated in the last reaction of this cycle by dehydrogenation of malate; it is also formed by carboxylation of pyruvate or transamination of the amino acid aspartate.

malate NAD^+ / $NADH + H^+$ oxaloacetate
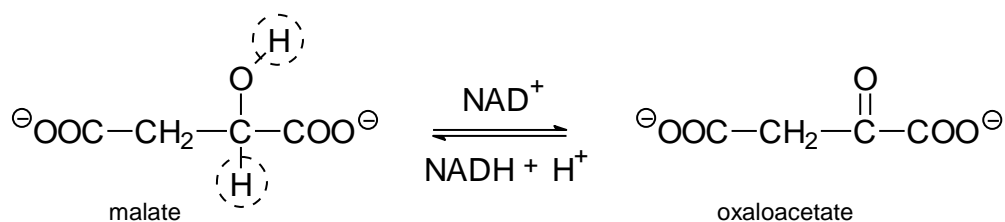
Other Hydroxy Acids and Oxo Acids

Glyceric acid (2,3-dihydroxypropanoic acid, anion glycerate) is formed by oxidation of glyceraldehyde. Its esters 3-phosphoglycerate and 2-phosphoglycerate and macroergic mixed anhydride 1,3-bisphosphoglycerate are the intermediates of glycolysis.

glycerate   2-phosphoglycerate   3-phosphoglycerate   1,3-bisphosphoglycerate
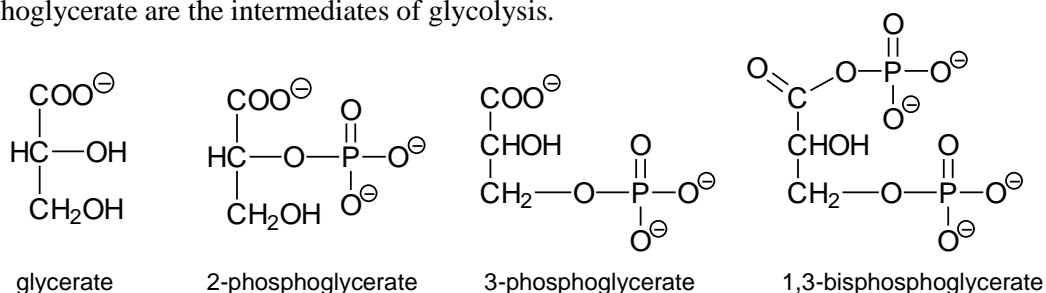
Citric acid (2-hydroxypropane-1,2,3-tricarboxylic acid, acidum citricum, anion citrate) is a triprotic hydroxy acid ($pK_{A1} = 3.13$, $pK_{A2} = 4.76$, $pK_{A3} = 6.40$). It is abundant in fruits, mainly in the citrus species.

In mitochondria, it is formed by the condensation of acetyl-coenzyme A with oxaloacetate – in the first reaction of the citric acid cycle. Citric acid possesses the tertiary alcohol group that cannot undergo the above mentioned dehydrogenation. Therefore in this cycle, the citric acid is isomerised to **iso-**

citric acid (1-hydroxypropane-1,2,3-tricarboxylic acid) that has the secondary alcohol group and after its dehydrogenation and decarboxylation produces 2-oxoglutarate (see later).

Citric acid is produced by industrial biotechnology in the huge quantities required for the food industry; e.g., citric acid is an acidulant in numerous soft drinks. The solution of trisodium citrate (natrii citras) serves as an anticoagulant, because citrate binds Ca^{2+} ions and prevents blood clotting (taking of non-coagulable blood, e.g. from blood donors). Sodium citrate administered orally can be used for alkalisation of urine (preventively against formation of uric acid calculi).

$$
\begin{array}{cc}
CH_2COOH & HO-CH-COOH \\
HO-C-COOH & CH-COOH \\
CH_2COOH & CH_2COOH \\
\text{citric acid} & \text{isocitric acid}
\end{array}
$$
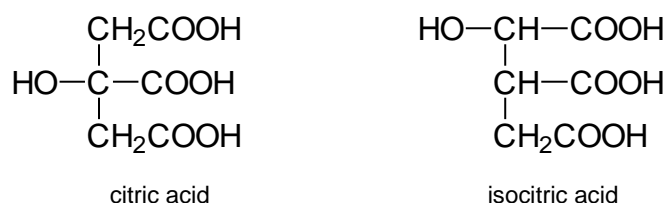
2-Oxoglutaric acid $HOOC\text{-}CO\text{-}CH_2\text{-}CH_2\text{-}COOH$ (2-oxopentanedioic, α-ketoglutaric acid) is the intermediate of the citric acid cycle, formed by dehydrogenation and subsequent decarboxylation of isocitrate.

$$
\begin{array}{ccc}
HO-CH-COOH & & O=C-COOH \\
CH-COOH & \xrightarrow[-CO_2]{-2H} & CH_2 \\
CH_2COOH & & CH_2COOH \\
\text{isocitric acid} & & \text{2-oxoglutaric acid}
\end{array}
$$
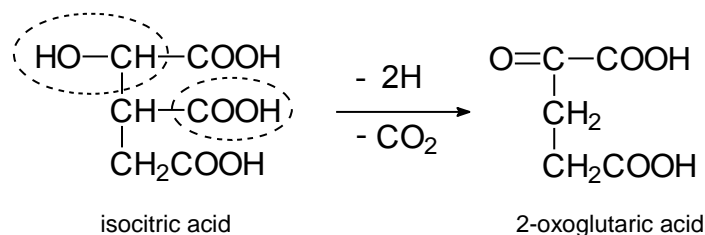
2-Oxoglutarate is produced from glutamate during transamination of amino acids, also in glutamate dehydrogenase reaction (see Chapter 42).

Salicylic acid (2-hydroxybenzoic acid, acidum salicylicum) was first isolated from the willow tree (Lat. *salix*). It is a colourless crystalline substance, almost insoluble in water. It has antiseptic, antipruritic, and anti-inflammatory effects. It is used mainly in dermatology. At concentrations lower than 5%, it exhibits keratoplastic properties (stimulates the growth of skin epithelium), in higher concentrations it acts keratolytically or keratocaustically.

Acetylsalicylic acid (2-acetoxybenzoic acid, acidum acetylsalicylicum) is an important and famous derivative of salicylic acid. In doses about 0.5–3 g/day it is a commonly used analgesic and antipyretic drug (e.g. Aspirin). In doses of approximately 100 mg/day it prevents aggregation of blood platelets.

salicylic acid   acetylsalicylic acid
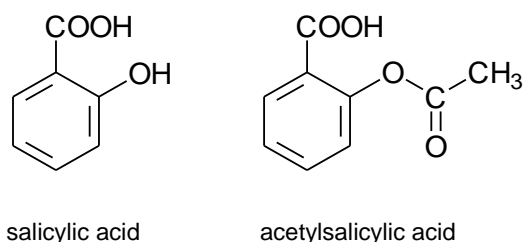
31 Amines

Amines can be derived from ammonia by replacing one or more H atoms with alkyl groups (aliphatic amines) or aryl groups (aromatic amines). Amines are divided into primary R-NH$_2$, secondary R^1-NH-R^2, and tertiary R^{1}R^{2}R^{3}N amines. Some secondary or tertiary amines may have nitrogen enclosed in a cycle. Alkylation of tertiary amine produces **quaternary ammonium salts** R$_4$N$^+$ X$^-$. The tetravalent nitrogen atom does not have the lone electron pair, therefore it is not basic. Tetraalkylammonium salts with one long alkyl belong to the group of cationic surfactants.

R—CH$_2$—NH$_2$ primary amine   secondary amine   tertiary amine   quaternary ammonium salt   cyclic amine
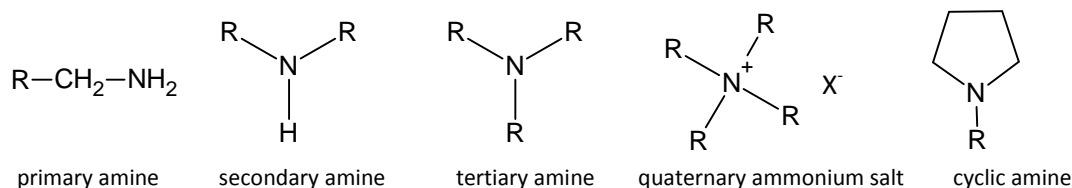
Nomenclature. The names of primary amines consist of the names of alkyl (or alkane) and the suffix –*amine* or –*diamine*, e.g. CH$_3$-CH$_2$-NH$_2$ ethylamine (ethanamine), NH$_2$-CH$_2$CH$_2$CH$_2$CH$_2$-NH$_2$ tetramethylenediamine (butane-1,4-diamine). The names of secondary (tertiary) amines are formed according to schemes: *di(tri)alkylamine*, *alkyl^{1}alkyl^{2}amine*, and *alkyl^{1}alkyl^{2}alkyl^{3}amine*, or as *N*-substituted primary amines. For example, CH$_3$-NH-CH$_3$ dimethylamine, (CH$_3$)$_2$N-CH$_2$CH$_2$CH$_3$ dimethylpropylamine (*N,N*-dimethylpropanamine).

Properties. The lowest amines are gases with a pungent smell, well soluble in water. With growing molecular mass the water-solubility of amines decreases.

Basicity of amines. The lone electron pair of nitrogen gives a weakly basic character and nucleophilic properties to amines. In the aqueous solutions, amines behave as bases and their solutions are alkaline (R-NH$_2$ + H$_2$O $\rightleftarrows$ R-NH$_3^+$ + OH$^-$). Aliphatic amines are stronger bases compared to ammonia. On the other hand, aromatic amines, due to the conjugation of the nitrogen electron pair with the aromatic system, are weaker bases compared to ammonia. In the series of aliphatic amines we can observe the steric effect of alkyls influencing the basicity of trialkylamines. Thus, the basicity of amines decreases in the following order:

$$\text{dialkylamines} > \text{alkylamines} \gtrsim \text{trialkylamines} > \text{NH}_3 > \text{arylamines}$$
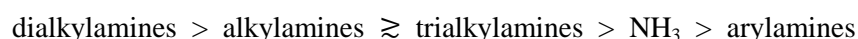
The reaction of amines with acids produces alkylammonium salts: R-NH$_2$ + HCl $\rightarrow$ R-NH$_3^+$ Cl$^-$.
Bases liberate free amines from these salts: R-NH$_3^+$ Cl$^-$ + NaOH $\rightarrow$ R-NH$_2$ + H$_2$O + NaCl.
Ammonium salts hydrolyse producing a weakly acidic solution: R-NH$_3^+$ + H$_2$O $\rightleftarrows$ R-NH$_2$ + H$_3$O$^+$.

Reaction with aldehydes. The reaction of primary amines H$_2$N–R^1 with carbonyl compounds produces unstable aminohemiacetals, which become stabilised by the removal of water to form *N*-substituted imino compounds called Shiff bases R-CH=N-R^1. Amines react with the anomeric (hemiacetal) hydroxyl of saccharides to produce *N*-glycosides.

Dehydrogenation. Some amines are sensitive to oxidation agents. They can produce various compounds. **Oxidative deamination** of primary amines, catalysed by monoamine oxidases, is the way of inactivation of some biogenic amines. The course of reaction is similar to the deamination of amino acids. Dehydrogenation of an amine in the presence of a flavine cofactor gives an imino compound, which is hydrolysed to ammonia and aldehyde. The side-product is hydrogen peroxide.
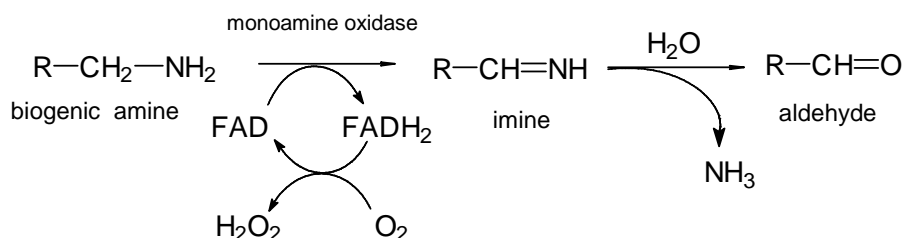
Biogenic Amines

are formed by decarboxylation of coded (and non-coded) amino acids. This reaction needs an enzyme (decarboxylase) and a cofactor (pyridoxal phosphate). Biogenic amines usually exhibit various physiological effects (see also Amino acids).
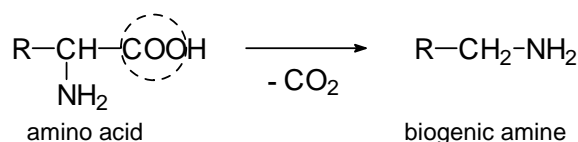
Ethanolamine $H_2N\text{-}CH_2CH_2\text{-}OH$ (2-aminoethanol) is produced by the decarboxylation of serine, which belongs to the structural components of some phospholipids (phosphatidylethanolamine). If methylated three times in nitrogen, it produces choline.

Cysteamine $H_2N\text{-}CH_2CH_2\text{-}SH$, a product of cysteine decarboxylation, makes the terminal part of coenzyme A (CoA-SH); the –SH group binds acyl by thioester linkage (CoA-S-CO-R).

Tyramine is formed by tyrosine decarboxylation. It occurs widely in some foods, especially if fermented (cheeses, sauerkraut, wine, beer, chocolate, fish, processed meat). In predisposed people, it may cause unpleasant effects (headache, hypertension).

β-Alanine $H_2N\text{-}CH_2CH_2\text{-}COOH$ is a non-coded amino acid (isomer of alanine), produced by α-decarboxylation of aspartate. It is a structural part of pantothenic acid (vitamin B), coenzyme A, and muscular dipeptide carnosine.

GABA $H_2N\text{-}CH_2CH_2CH_2\text{-}COOH$ (gama-aminobutyric acid) arises by elimination of α-carboxyl from glutamate. GABA works as an inhibitory neurotransmitter at synapses.

Cadaverine $NH_2\text{-}(CH_2)_5\text{-}NH_2$ (pentane-1,5-diamine) is a decarboxylation product of lysine, originally described as the toxin of dead bodies (Lat. *cavader*). Its toxicity is relatively low.

Putrescine $NH_2\text{-}(CH_2)_4\text{-}NH_2$ (butane-1,4-diamine) is made by the decarboxylation of ornithine. It is a typical product of putrefaction (animal protein decay). Putrescine is a precursor of two polyamines **spermidine** $NH_2\text{-}(CH_2)_3\text{-}NH\text{-}(CH_2)_4\text{-}NH_2$ and **spermine** $NH_2(CH_2)_3NH(CH_2)_4NH(CH_2)_3NH_2$ which exhibit certain physiological effects for cell proliferation and growth.

Histamine, 2-(imidazole-4-yl)ethylamine, is the decarboxylation product of histidine. It plays an important role in inflammatory and allergic reactions and acts as a neurotransmitter in the CNS.

Agmatine, 1-(4-aminobutyl)guanidine, derived from arginine, functions as a signal molecule.

Tryptamine, from tryptophan, is structurally related to **serotonin** (5-hydroxytryptamine), a neuro-transmittor in CNS. Serotonin is a precursor of **melatonin** (*N*-acetyl-5-methoxytryptamine), which plays several biological roles, e.g. regulates seasonal and daily rhythms.

agmatine   tyramine   histamine

tryptamine   serotonin   melatonin
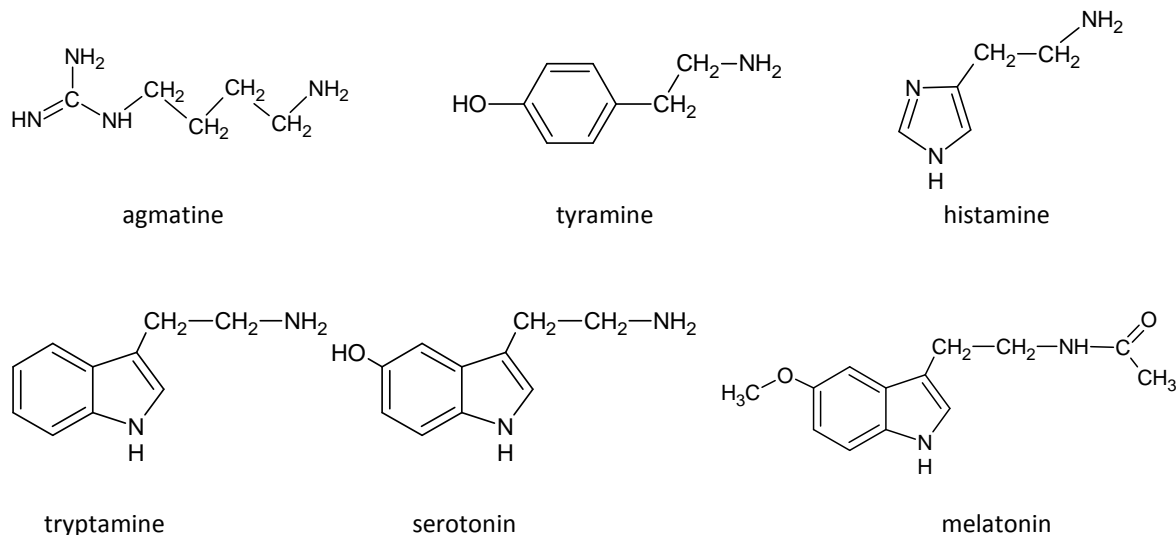
Catecholamines

The decarboxylation of 3,4-dihydroxyphenylalanine (DOPA) produces **dopamine** (3-hydroxytyramine). Further transformations produce hormones of the adrenal cortex **noradrenaline** and **adrenaline**. Moreover, noradrenaline is the neuromediator at adrenergic synapses. The above-mentioned three substances with important physiological and pharmacological effects are called cate-cholamines, and have the structure of *o*-diphenols similar to pyrocatechol (benzene-1,2-diol).

dopamine   noradrenaline   adrenaline
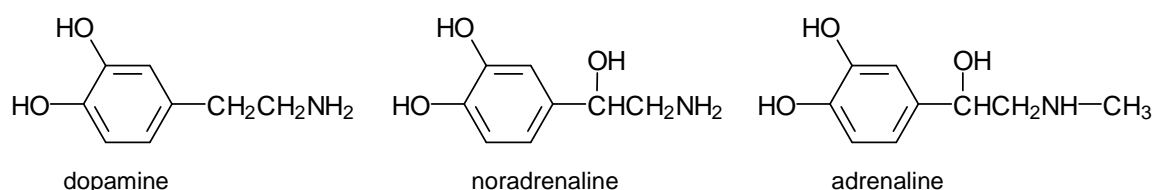
Phenethylamines

are plant or synthetic derivatives of phenethylamine (2-phenylethylamine), which is produced by de-carboxylation of phenylalanine. Their common feature is the substantial central stimulative effect. They have a considerable addictive potential. **Ephedrine** (1-phenyl-2-methylaminopropan-1-ol) be-longs to plant alkaloids. It is contained in the shrub *Ephedra vulgaris*, and its use was already known in China 2,000 years ago. The salt of ephedrine with hydrochloric acid (ephedrini hydrochloridum) is used as a bronchodilatatory drug, locally as a decongestant of the nasal mucose. However, it has con-siderable psychostimulatory effects with the risk of drug addiction. The molecule of ephedrine has two asymmetric carbon atoms and, therefore, it occurs in four stereoisomers. **Amphetamine** (1-phenylpropan-2-amine) is a synthetic substance with strong stimulation effect, it is not used in medi-cine.

Pervitin (methamphetamine, *N*-methyl-1-phenylpropan-2-amine) is illegally produced from ephedrine. Pervitin is a quite "hard" stimulatory drug; its long-term use can result in serious damage of psyche (the paranoid syndrome).
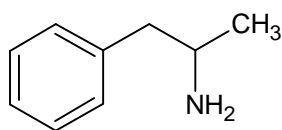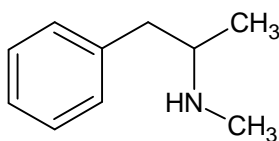
amfetamine   methamphetamine (pervitin)   ephedrine
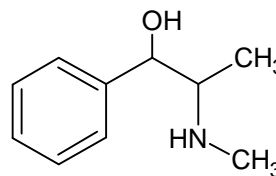
Quaternary Ammonium Salts

Choline (2-hydroxyethyl)trimethylammonium, is the component of phosphatidylcholines and sphingomyelins. The positive charge on the nitrogen atom is compensated by any physiological anion, most often by a chloride.
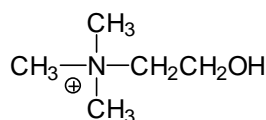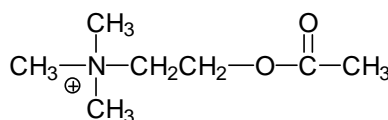
choline   acetylcholine

Acetylcholine (ester of choline and acetic acid) belongs among important neurotransmitters; it is the transmitter substance on cholinergic synapses. It participates in the transport of nerve impulses through its binding to receptors (nicotinic and muscarinic).

Bisquaternary ammonium salts include a group of important drugs – **peripheral muscle relaxants**, called curarimimetics according to **curare**, the arrow poison of the South-American Indians. It acts on the postsynaptic membrane of the neuromuscular junctions of the striated muscles (including the muscles of the respiratory system) and causes their relaxation. The example is **succinylcholine** (diester of choline and succinic acid).
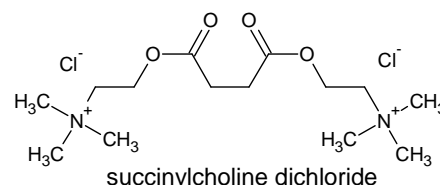
succinylcholine dichloride

Carnitine, (2-hydroxy-3-carboxypropyl)trimethylammonium, is important for the transport of acyls of fatty acids across the inner mitochondrial membrane into the matrix, where β-oxidation occurs. The fatty acid is bound as ester to the hydroxyl group of carnitine.
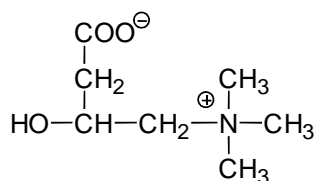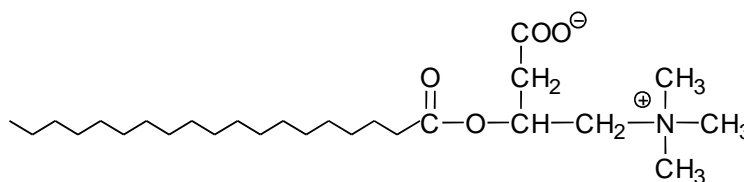
carnitine   acylcarnitine

32 Biochemically Important Heterocycles

Pyrrole Derivatives

Pyrrole is a five-membered heterocycle with one nitrogen atom. The free electron pair of nitrogen participates in the conjugation with two double bonds. The consequence is the planarity and aromatic character of the ring and the fact that basicity of nitrogen is practically gone. Biochemically important derivatives are **cyclic tetrapyrroles** (red heme, green chlorophyll, pink cyanocobalamine). Degradation of heme results in the formation of **linear tetrapyrroles** (green biliverdin, orange bilirubin, colourless urobilinogen, brown urobilin).
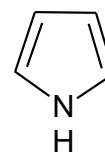
The basic structure of heme is cyclic tetrapyrrole **porphin** – four pyrrole rings are joined by four methine bridges =CH− in the positions 2 and 5 (positions alpha). The derivatives of porphin with substituents in the positions 3 and 4 (beta) are called **porhyrins**. Porphin and porphyrins are planar, completely conjugated systems of double bonds and therefore intensively coloured.
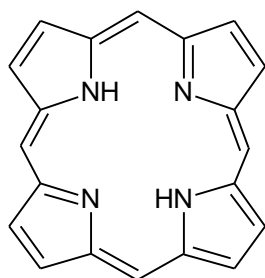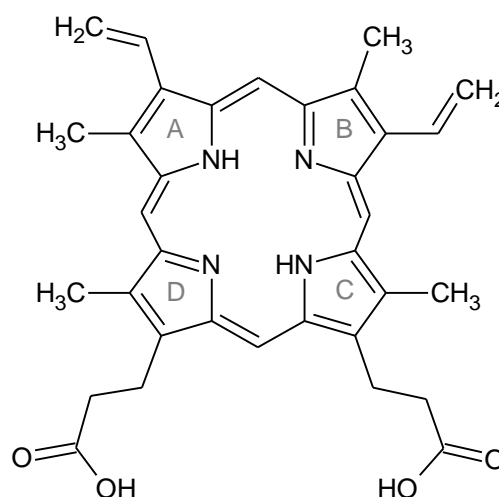
porphin   protoporphyrin IX

The porphin skeleton has eight positions for substitution. The most important is **protoporphyrin IX** which has four methyl groups, two vinyl groups, and two propionic acids. **Heme**, the coloured prosthetic group of hemoglobin, is a chelate of protoporphyrin IX and Fe^{2+} cation. The same heme is contained in **myoglobin** of muscle cells. Other hemoproteins with similar hemes are **cytochromes** (redox components of the respiratory chain), heme enzymes **peroxidase**, **catalase** (they decompose hydrogen peroxide), and **cytochrome P450** (hydroxylating system). The metabolic breakdown of heme produces linear tetrapyrrole pigments **biliverdin**, with three methine bridges between four pyrrole rings, and **bilirubin**, with the central =CH− group hydrogenated to methylene (−CH$_2$−). The structure of bilirubin is not linear as in the presented formula. The central methylene bridge exhibits free rotation and partial tilt of the molecule. The resulting conformation is stabilised by six intramolecular hydrogen bonds. All polar groups of bilirubin (2 × COOH, 2 × C=O, 4 × NH) are involved in these H-bonds. This is why carboxyl groups are not dissociated; bilirubin is non-polar, water insoluble, in blood transported with albumin.
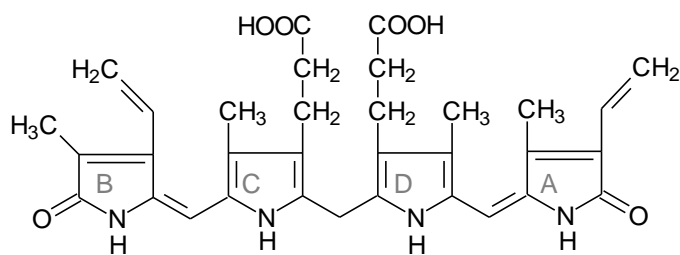
bilirubin
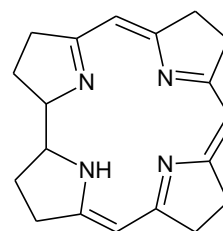
corrin

Cyclic tetrapyrroles also occur in **corrinoids**. The skeleton is called **corrin**. It has four partially reduced pyrrole rings and only three methine bridges. Two rings are linked directly. The most important corrinoid is **cyanocobalamin** (vitamin B_{12}) with the central ion of cobalt.

Pyrrolidine Derivatives

Pyrrolidine is formed by complete hydrogenation of pyrrole and has the properties of secondary amines. It is markedly basic ($pK_B = 2.7$); protonation produces the pyrrolidinium cation. Its derivatives include the amino acids **proline** (pyrrolidine-2-carboxylic acid) and **4-hydroxyproline**, both contained in the protein collagen.
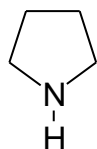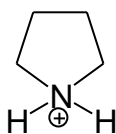
pyrrolidine   pyrrolidinium   proline   4-hydroxyproline
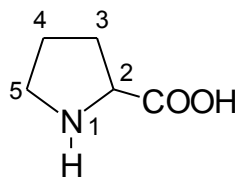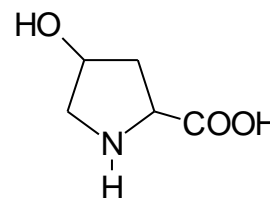
Indole Derivatives

Indole is benzo[*b*]pyrrole; the benzene ring is connected to the C2-C3 (*b*) bond of pyrrole. Similarly to pyrrole, it is not basic. The essential amino acid **tryptophan** is 2-amino-3-(3-indolyl)propanoic acid. Its derivative is the biogenic amine **tryptamine** and **serotonin** (5-hydroxytryptamine), the mediator of the nervous excitement in the brain-stem and the nervous plexus of the digestive tract. The hormone **melatonin** (*N*-acetyl-5-methoxytryptamine) is also synthesised from tryptophan. It is produced in response to the light-dark cycle. It probably has a role in the regulation of the seasonal and daily biorhythm (see Chapters 31 and 42).
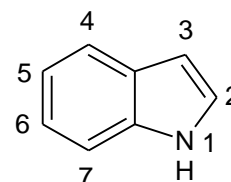
indol

Imidazole Derivatives

Imidazole is weakly basic. The nitrogen atom N3 can bind proton to form imidazolium cation. The N1 nitrogen atom is not basic because its electron pair participates in conjugation with the double bonds.
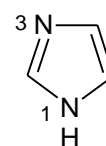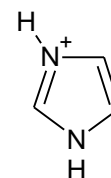
The imidazole derivative is the amino acid **histidine**. The protonated imidazole (imidazolium) in histidine has $pK_A = 6.0$, and its acid-base

imidazol   imidazolium

conversions are the principle of the buffer function of proteins. The decarboxylation of histidine leads to **histamine**, a mediator of inflammatory and allergic reactions (see Chapters 31 and 42).

Pyridine Derivatives

Pyridine is a six-membered heterocycle with one nitrogen. The free electron pair of N is not included in the conjugation of the aromatic ring and it is available for binding H^+. Pyridine is weakly basic (pK_B = 8.7), therefore it can be protonated to the pyridinium cation or alkylated to *N*-alkylpyridinium salts.

Nicotinic acid (pyridine-3-carboxylic) and its amide, **nicotinamide**, are included in the group of B vitamins. Sometimes they are given a common name niacine. Nicotinamide is the component of NAD^+ (nicotinamide adenine dinucleotide), cofactor of many dehydrogenases (see Chapter 37).

nicotinic acid   nicotinamide   nicotine
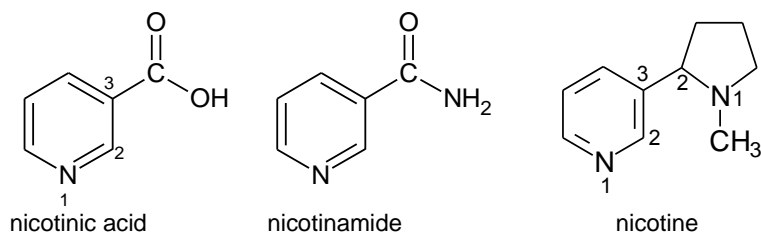
Nicotinamide (vitamin) must be distinguished from nicotine (alkaloid). Alkaloids are secondary nitrogen metabolites of plants. **Nicotine** is 3-(1-methylpyrrolidin-2-yl)pyridine. It is contained in the leaves of tobacco (*Nicotiana tabacum*). The average content of nicotine in one cigarette is about 1 mg. Nicotine is strongly addictive, in its pure state very toxic. In small quantities, it increases cognitive abilities and relaxation, however, it also increases blood pressure, vasoconstriction, the secretion of saliva and gastric juice, and stimulates peristalsis. Its effects are not used in medicine. Only in the case of smokers, after they stopped smoking, are nicotine patches applied for the prevention of withdrawal syndrome.

Vitamine B₆ is the name given to three related derivatives of 3-hydroxy-5-hydroxymethyl-2-methylpyridine. All of them have a primary alcohol group and phenolic hydroxyl, and the same biological activity but differ in the group bound at C4. **Pyridoxine** has a primary alcohol group, **pyridoxal** an aldehyde group, and **pyridoxamine** an aminomethyl group. Pyridoxal-5-phosphate is the coenzyme of aminotransferases and decarboxylases in the transformation of amino acids.

pyridoxine   pyridoxal   pyridoxamine
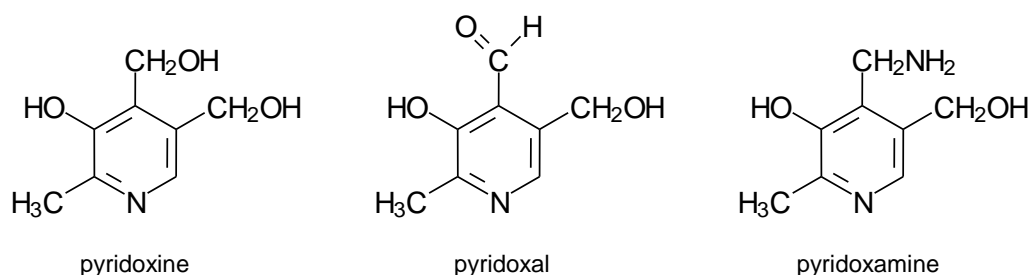
Pyrimidine Derivatives

Three pyrimidine derivatives, **cytosine** (2-hydroxy-4-aminopyrimidine), **uracil** (2,4-dihydroxy pyrimidine), and **thymine** (2,4-dihydroxy-5-methylpyrimidine) are components of nucleic acids and some important nucleosides and nucleotides. These compounds exist in two tautomeric forms. The lactam form is the more stable and prevailing to the lactim form with enolic hydroxyl. In nucleotides, lactam form bases are linked to pentose through the *N*-glycosidic bond from N1.

cytosine   thymine   uracil (lactim)   uracil (lactam)
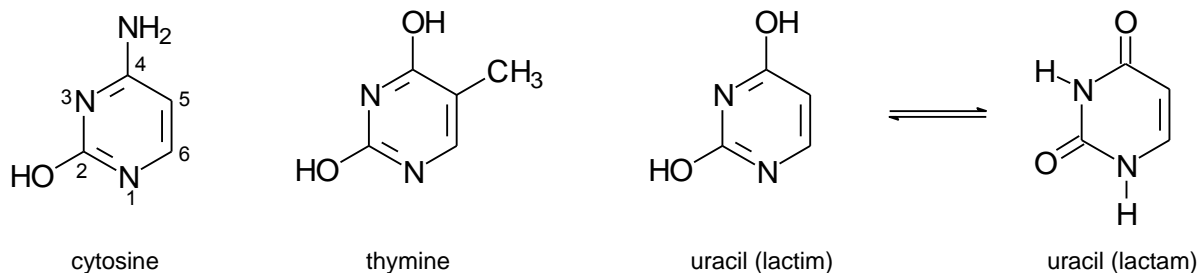
Barbituric acid (perhydropyrimidine-2,4,6-trione) is a synthetic derivative of pyrimidine. It has the character of a weak monoprotic acid; the -NH-CO- groups easily undergo tautomerisation and in lactim form can release H^+ and form salts. By replacing both H atoms of the CH_2 group with various alkyls, different barbiturates were prepared, used as hypnotics and sedatives. Due to numerous side effects, the current use of barbiturates in medicine is very limited.

barbituric acid   barbiturate (general formula)
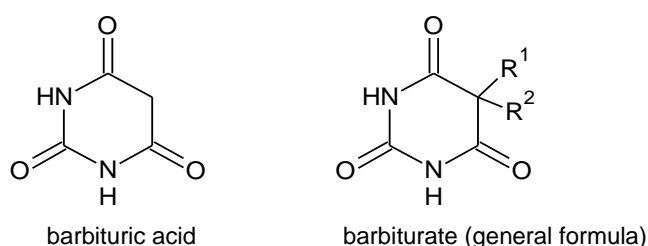
Purine Derivatives

Purine is a condensed system of pyrimidine and imidazole. It has special numbering. Its derivatives are **adenine** (6-aminopurine) and **guanine** (2-amino-6-hydroxypurine), the most widespread purine bases in the nucleic acids and nucleotides. Both bases are linked by *N*-glycosidic bond through nitrogen N9 to pentose.

purine   adenine   guanine
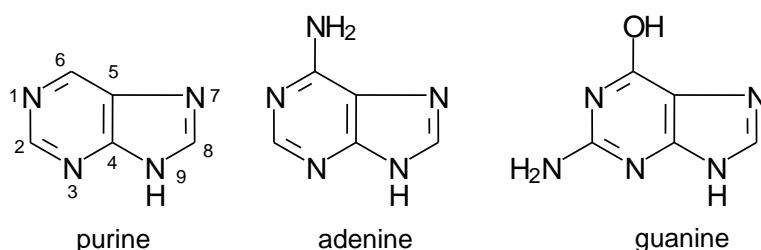
Hydroxyderivatives of purine are formed from the purine bases during the catabolism of nucleotides in cells, adenine produces **hypoxanthine** (6-hydroxypurine) and guanine **xanthine** (2,6-dihydroxypurine). Both these substances are hydroxylated by xanthine oxidase to uric acid.

hypoxanthine   xanthine   uric acid   ammonium hydrogen urate
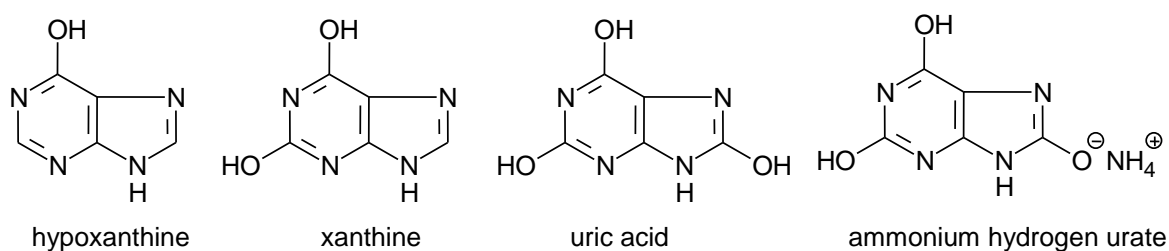
Uric acid (2,6,8-trihydroxypurine, acidum uricum, salts urates) is a weak diprotic acid (pK_{A1} = 5.4; pK_{A2} = 10.3) with substantial reducing properties. It belongs to the natural antioxidants which reduce reactive oxygen species in the body. It is only slightly soluble in water, mainly at pH < 5.5. The solubility of uric acid rapidly increases with alkalisation, when its salts hydrogen urates are formed, in the strongly alkaline environment also urates. Uric acid and hydrogen urates can precipitate in the efferent urinary tract and form renal stones (urate urolithiasis). An adult man excretes up to 1 g of uric acid daily. Under pathological conditions it is deposited into the joints and tissues (gout, arthritis urica).

There are many synthetic derivatives of pyrimidine and purine bases used as drugs in medicine. For example, **allopurinol** is the synthetic isomer of hypoxanthine with a different position of one nitrogen atom. It is used to treat gout. It inhibits xanthine oxidase and thus blocks the transformation of hypoxantine and xanthine to uric acid. Hypoxanthine and xanthine are more soluble than uric acid and are more rapidly excreted in urine. Mercaptopurine and 8-azaguanine are used as cytostatics.

allopurinol   mercaptopurine   8-azaguanine
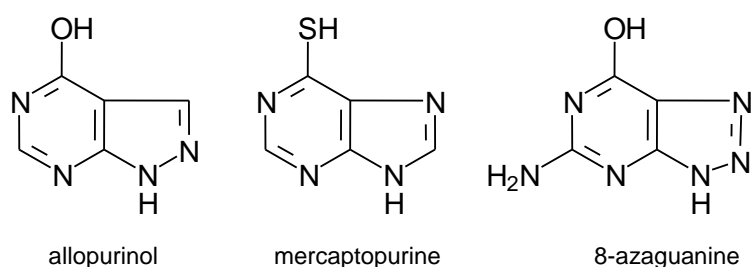
Methylxanthines. Caffeine (1,3,7-trimethylxanthine) is contained in coffee (*Coffea arabica*), tea (*Camelia sinensis*), cola (*Cola acuminata*), and guarana (*Paullinia cupana*). Caffeine is only slightly basic (pK_B = 13.40). It has a well-known stimulatory effect on the CNS (removes the feeling of fatigue), it also acts as a weak diuretic drug. It also increases the secretion of HCl by stomach mucose.

Theophylline (1,3-dimethylxanthine) is found in tea. For its substantial relaxation effect on the smooth muscles it is used to treat bronchial asthma, it also improves the perfusion of a brain.

Theobromine (3,7-dimethylxanthine) is obtained from cocoa powder (*Theobroma cacao*).

caffeine   theophylline   theobromine
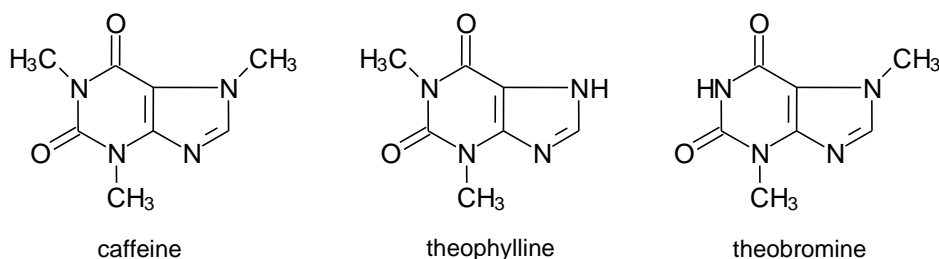
Pteridine Derivatives

Pteridine is a condensed system of pyrimidine and pyrazine. The pteridine derivatives include cofactors of many enzymes: pterin cofactors, folate, and flavine coenzymes. **Tetrahydrobiopterin** is the donor of two H atoms in hydroxylation of phenylalanine to tyrosine, hydroxylation of tyrosine to DOPA). It is oxidised to dihydrobipterin during this reaction.
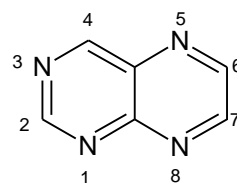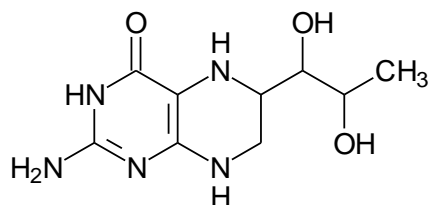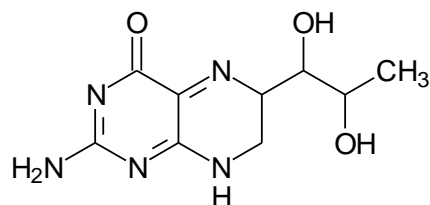
tetrahydrobiopterin   dihydrobiopterin

Folic acid (acidum folicum, anion folate) contains trisubstituted pteridine, *p*-aminobenzoic acid (PABA) linked to glutamic acid through an amide bond. Animals are not able to synthesise this acid, it is an important essential factor (vitamin). Its lack in food causes anaemia.
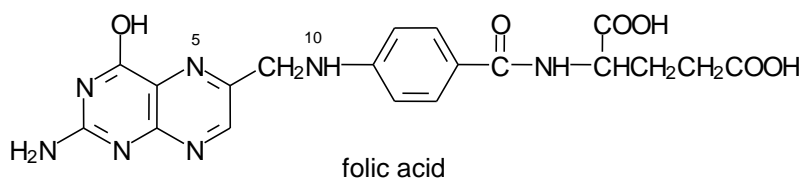
folic acid

Folic acid absorbed from food produces **5,6,7,8-tetrahydrofolate** through hydrogenation in the body. It is the carrier of one carbon fragment (formyl, methyl), which binds to atoms N5 or N10 (biosynthesis of purine bases, methylation of uracil to thymine).

Benzopteridine (benzo[*g*]pteridine) has one benzene ring attached to the *g* (C6-C7) pteridine bond. Its 2,4-dioxoderivative is **isoalloxazine**, from which we derive **riboflavin** (6,7-dimethyl-9-ribitylisoalloxazine, vitamin B_2).
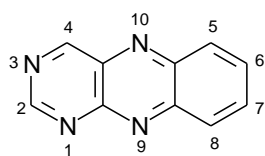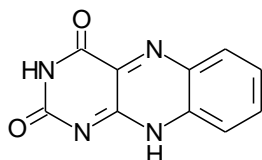
benzo[*g*]pteridine   isoalloxazine   riboflavin
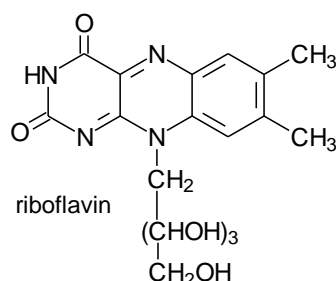
Riboflavin is a yellow vitamin (from the Latin *flavus*), the component of cofactors FAD (flavin adenine dinucleotide) and FMN (flavin mononucleotide) of some dehydrogenases (see Chapter 37).

Oxygen and Sulfur Heterocycles

Tetrahydrofuran, totally hydrogenated furan, makes the skeleton of cyclic monosaccharides, especially pentoses, called **furanoses** (e.g. ribofuranose, fructofuranose).

Tetrahydropyran occurs in cyclic sugars called **pyranoses** (glucopyranose, see Chapter 34).

Chroman (benzodihydropyran) is comprised in the structure of tocopherol (vitamin E).

Thiolane (tetrahydrothiophene) makes part of the structure of biotin (vitamin H).

Thiazole, five-membered heterocycle with sulfur and nitrogen, is the part of **thiamine** (vitamin B_1) with tetravalent (positively charged) nitrogen – see Chapter 46.

33 Toxicologically Important Substances

Toxic substances can cause health damage or death. Toxic effects depend on the dose, i.e. the amount of substance entering the body, and the length of exposition. Legislature imposes duties to all individuals and corporations which process or use chemical substances. Current legislative norms distinguish the following categories of dangerous substances: highly toxic, toxic, harmful for human health, corrosive, irritating, sensitising, carcinogenic, mutagenic, and toxic for reproduction. Instructions for the work with these substances are comprised in the special course Handling chemical substances (ZC011), for more information see http://www.rect.muni.cz/nso/.

Selected Inorganic Substances

Ozone (trioxygen O_3). Near the Earth's surface, ozone defined as **down-to-earth ozone**, is taken as the undesirable component of the atmosphere. In agglomerations with heavy traffic and industrial exhalations, nitrogen oxides and hydrocarbons pollute the air. Bad conditions for air dispersion and intensive sunlight can result in the formation of **photochemical smog** with high concentrations of ozone. Atomic oxygen is released by the photochemical decomposition of nitrogen dioxide ($NO_2 \rightarrow \cdot NO + \cdot O$) and it then combines with dioxygen ($\cdot O + O_2 \rightarrow O_3$). If the concentration of ozone reaches 0.18 ppm, the inhabitants of the affected area must be warned against long-lasting outdoor activities (sports and child games, etc.). Ozone already irritates the eyes (conjunctives) and the respiratory system from 0.06 ppm; it also acts on the CNS, reduces performance and concentration ability. If over 1 ppm, it may seriously damage the lungs.

Silicon dioxide (SiO_2, silica) is a very stable substance. Long-term inhalation of silicious dust with particles < 5 μm (they penetrate to the alveolus and interstitium of the lungs) results in the serious type of lung dusting – **silicosis**. It mainly endangers miners, polishers of casts in steel-foundries, stonecutters, workers in the ceramic industry, and similar professions. Finely fibrous types of some natural silicates are called **asbestos**. For its heat and chemical resistance, asbestos was industrially used for the production of heat-resistant and building materials. However, it is a dangerous substance, included among **carcinogens**. Dust containing asbestos fibres, once inhaled, is not effectively removed from the lungs. Long-term inhalation of asbestos dust, common in some professions, may lead to the serious occupational disease **asbestosis** (fibrotic changes of the lungs with the risk of tumours).

Hydrogen cyanide (HCN) is an extremely toxic volatile liquid. In water, HCN behaves as a weak hydrocyanic acid. Its salts **cyanides**, easily binding by coordination, bond as ligands to the cations of transition metals to form complex compounds. The consequence is the high **toxicity** of HCN and cyanides (a lethal dose for adults is approximately 70 mg of HCN or 250 mg of KCN). CN^- anion binds to Fe^{3+} of mitochondrial cytochrome-c-oxidase and inhibits the respiratory chain. Patients can be rescued by quick intravenous application of substances binding CN^- ions in biological fluids (e.g. hydroxocobalamin is converted to stable and non-toxic cyanocobalamin) or by conversion of haemoglobin to methaemoglobin (by amyl nitrite or sodium nitrite) capable of binding CN^- ions. Intravenously applied sodium thiosulfate converts cyanide to harmless thiocyanate ($CN^- + S_2O_3^{2-} \rightarrow SCN^- + SO_3^{2-}$).

Carbon monoxide (CO) is very toxic. Even 0.1 vol. % of CO in the inhaled air can be life threatening after several tens of minutes. CO binds to Fe^{2+} of heme in haemoglobin and muscle myoglobin, as well as to cytochromes in the mitochondrial respiratory chain. Binding CO to haemoglobin produces **carbonylhemoglobin** – it restricts the transport of oxygen from the lungs to tissues. Binding CO to cytochromes blocks the function of the mitochondrial respiratory chain. CO is not easily released from the bond to heme; in haemoglobin, it has 250 times greater affinity to heme than oxygen, but it can be released gradually by the inhalation of air enriched by oxygen. Therefore, the first aid during CO poisoning is to remove the patient from the place containing CO, relieving the respiratory tract and frequent inhalation of oxygen; heavy poisoning is treated by oxygenation in hyperbaric chambers.

Nitrites (NO_2^-). Nitrites are absorbed in the gastrointestinal tract after consumption. They oxidise Fe^{II} of haemoglobin in erythrocytes to Fe^{III} (**methemoglobin**), which is not able to transport dioxygen. **Sodium nitrite** (natrii nitris) given intravenously oxidises a part of blood haemoglobin to methaemoglobin. This is one of the therapeutic interventions in cyanide poisoning. Methaemoglobin binds cyanide ions with high affinity. Thus, the inhibition of cell breathing is prevented, because CN^- ions are gradually released from cyanmethaemoglobin and the slow transformation to harmless thiocyanate SCN^- can proceed in the liver.

Another unwanted effect of nitrites is the formation of nitrosamines, some of which are carcinogens. In the strongly acidic stomach environment, nitrosamines $R^1R^2N-N=O$ are produced in the reaction of secondary amines R^1R^2NH with nitrous acid HNO_2. Nitrosamine formation is inhibited by vitamin C.

Nitrites are contained in small amounts in some meat products, smoked meat, salamis, pates, etc. Nitrite is reduced to NO during meat processing. NO binds to myoglobin and prevents its oxidation to greybrown metmyoglobin, therefore products obtain an attractive red pink colour. Aside from this, the presence of nitrites has an inhibitory effect on some unwanted microorganisms (*Clostridium botulini*). Daily intake of nitrites up to 15 mg is considered harmless for adults. We accept the concentration of nitrites up to 0.5 mg l^{-1} in drinking water.

Hydrogen sulfide (H_2S, sulfane) is a colourless gas with the unpleasant smell of rotten eggs. It is released from sulfides by the action of acids or during putrefaction of proteins (from amino acids cysteine and methionine). It is **very toxic**, combines with haemoglobin to sulfhemoglobin, and with cytochrome-*c*-oxidase, which results in the inhibition of the respiratory chain in mitochondria. If we do not follow the safety precautions when entering unventilated sewers or wastewater collectors we are in high danger of lethal acute poisoning.

Barium. All soluble barium compounds are toxic. Ba^{2+} ions seriously damage the myocardium and nervous system (Ba^{2+} ions block potassium channels). The only exception is barium sulfate $BaSO_4$ (barii sulfas), it is not dissolved in water nor in acids. Suspension of barium sulfate is used as a contrast substance for the x-ray examination of the gastrointestinal tract.

Cadmium and all its compounds are toxic even in doses of about 10 mg; Cadmium is gradually accumulated in the body, esp. in the kidneys (biological half-life is about 20 years). The most serious effects include damage of the respiratory system (acute inflammation or even oedema of the lungs, chronic emphysema) and the kidneys. In chronic exposition, cadmium has carcinogenic effects (activates the formation of reactive oxygen species, with subsequent DNA damage).

Mercury. Long-term **inhalation of mercury vapours** leads to chronic poisoning. Mercury is not very volatile; however, the concentration of vapours could reach toxic values, mainly in areas where the cracks in the floor are contaminated by finely dispersed mercury. Elemental mercury easily crosses the blood brain barrier and cumulates in the brain and becomes oxidised to Hg^{2+} ions, which bind with – SH groups of proteins. The symptoms of poisoning could be generated slowly (months or years) during work with metallic mercury. They include inflammations of the gums and neural disorders (irritability, indistinct pains, and gentle tremor). When working with elementary mercury (dental ordination, dropping electrodes in laboratories) it is necessary to precisely collect all small drops of mercury which escape during careless work outside the collecting vessels. All mercury compounds are toxic, especially those which are soluble. Acute poisoning by inorganic compounds causes serious damage to the mucous membrane of the gastrointestinal tract and the renal tubuli cells. The example is **mercury(II) chloride** $HgCl_2$, a white substance, soluble in water. Dental amalgams containing metallic Hg are considered to be non-toxic. On the other hand, methylmercury (CH_3-Hg^+ X^-) and dimethylmercury (CH_3-Hg-CH_3) are extremely toxic to the environment.

Lead and all its compounds are toxic. Chronic lead poisoning (**saturnism**) seriously damages the synthesis of haemoglobin (anaemia, increased excretion of porphyrins), the nervous system (dyskinesia, neurasthenic syndrome, judgement disorders), and the gastrointestinal tract. We usually notice a grey margin on the gums from lead(II) sulfide PbS. Lead compounds are also deposited in bone tissues.

Selected Organic Substances

Benzene (C_6H_6) is a volatile, flammable liquid with an unpleasant smell and narcotic effects. Benzene is highly toxic, its chronic exposition destroys hemopoiesis in the bone marrow (agranulocytosis or conversion to leukaemia) therefore it belongs among **carcinogens**. The proof of chronic benzene exposition is the increased excretion of phenol in urine.

Toluene (C_6H_5-CH_3) is a non-polar solvent and raw material for chemical syntheses. Inhalation of toluene vapours has narcotic effects. It is less toxic than benzene because it is oxidised to benzoic acid in the organism. Intoxication is similar to that of alcohol, causing frequent headaches, dizziness or unconsciousness, and it could cause the inhibition of the respiratory centre and death. Repeated inhalation leads to drug addiction (sniffing). Urine of toluene addicts contains a higher concentration of benzoic and hippuric acid (*N*-benzoylglycine).

Polycyclic aromatic hydrocarbons (PAH) of various types are components of coal and other fossil fuels. They are formed by incomplete combustion of organic substances. They are found in coal-tar, soot, cigarette smoke, exhaust fumes, smoked, longer baked, and burnt food-stuffs, burnt vegetable oils, etc. Some of these PAH are indirect **carcinogens**. PAH themselves are inert, however, in the body (especially in the liver) they are

benzo[*a*]pyrene

transformed by detoxication enzymes to reactive compounds (epoxides), which react with DNA and could destroy the genetic material of cells. An example of PAH is benzo[*a*]pyrene.
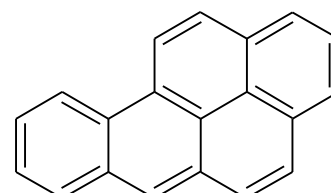
Methanol (CH$_3$OH), if consumed, acts similarly to ethanol. Methanol is contained in small amounts (0.1–0.5%) in alcoholic drinks obtained by fermentation of sugar solutions. A lethal dose is 30–70 ml of pure methanol. It is degraded like ethanol by oxidation catalysed by alcohol dehydrogenase. The enzyme has more isoforms (liver, stomach, lungs, retina). The product is formaldehyde and subsequently formic acid which causes metabolic acidosis and serious damage to brain nerves. After 12 hours lesion of the visual nerve (impairment of sight) occurs with the possibility of irreversible blindness in the course of 14 days. Methanol poisoning is treated by **ethanol**, administered orally or by infusion. Because of the higher affinity of ethanol to alcohol dehydrogenase, ethanol is metabolised preferentially and the methanol oxidation slows down, so that methanol can be excreted by the lungs and into urine. Another antidote is the specific inhibitor of alcohol dehydrogenase **fomepizole** (4-methylpyrazole), which has a number of advantages, does not suppress CNS, does not cause inebriation (drunkenness) or hypoglycaemia, can reduce the need of haemodialysis.

Ethylene glycol (ethane-1,2-diol, HO-CH$_2$CH$_2$-OH) is a common component of non-freezing mixtures. In spite of its viscosity and sweet taste it resembles alcoholic drinks. If consumed instead of ethanol, it causes dangerous poisoning. A lethal dose is less than 100 ml. After intestinal absorption, it is enzymatically dehydrogenated (alcohol dehydrogenase) to acidic metabolites, first to glycolic acid, which causes severe metabolic acidosis, and then gradually to oxalic acid. Calcium oxalate is excreted in urine, where it can precipitate in the kidneys and be the cause of renal failure. Ethanol or fomepizole are antidotes, similarly to methanol poisoning.

Carcinogenic substances

can provoke irreversible changes of cellular DNA and malignant proliferation either directly or after biotransformation (indirect carcinogens – procarcinogens, e.g. polycyclic aromatic hydrocarbons, PAH). Their structures are diverse and include a number of synthetic compounds, e.g., aromatic amines, epoxides, dialkyl sulfates, halogenalkyl ethers. Some effective carcinogens are produced by fungi (e.g. aflatoxins) and some plants, there are also carcinogens produced by pyrolysis of fats or during grilling food. Some inorganic compounds are also carcinogenic, e.g. compounds of Cr^{VI}, Ni^{II}, As^{III}, As^{V}, Cd^{II}, **asbestos** – fibrous species of magnesium silicates, e.g. chrysotile Mg$_6$Si$_4$O$_{10}$(OH)$_8$. Results of carcinogenic effects tested on the experimental animals can be applied to humans very carefully. The reliable proof for the carcinogenic effect on humans is only substantially higher occurrence of a certain type of malignant proliferation in a large group of people, which were in contact with the observed substance. Proven organic carcinogens include, for example, **formaldehyde**, **benzene**, **benzidine**, **bis(chloromethyl) ether** (Cl-CH$_2$-O-CH$_2$-Cl), **2-naphtylamine**, and **coal tar** – a complex mixture of polycyclic aromatic hydrocarbons (PAH), phenolic compounds, and aromatic nitrogen heterocycles.
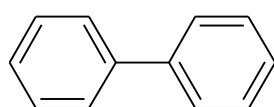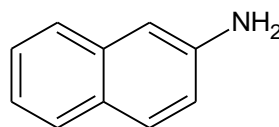
benzidine   2-naphtylamine

Teratogens

are harmful substances from the environment, or also some drugs, which can endanger normal embryonic development during the first trimester of gravidity and thus cause congenital development defects. Examples are PCBs (polychlorinated biphenyls), heavy metals (methylmercury), ethanol, cytostatic drugs, some antibiotics, warfarin, marijuana, pervitin, many components of cigarette smoke.

Addictive Substances

are a special group of substances harmful to human health. They include narcotic (psychotropic) substances which very often cause addiction. They influence the function of the central nervous system in such way that they suppress or stimulate its function. At the same time they change in various ways, mostly pleasantly, the mental state of mind, or they cause transient hallucinations. Their repeated use causes addiction and psychical changes which not only endanger the consuming individuals but also their surroundings and society.

Addiction is characterised by the presence of several of the following features:
· Pronounced desire or urge to use the psychotropic substance
· Tolerance to increasing doses of a substance
· Withdrawal symptoms physical and mental
· Limited control of personal behaviour
· Limitation or neglecting of other interests and hobbies
· Continuation in taking the substance even when its harmful effects are known

Addiction is a serious problem. It can easily lead to the destruction of the personality of a drug addict, their social and physical degradation with other undesired social consequences. The treatment of drug addicts is difficult and expensive, the result is uncertain. The handling of addictive narcotic and psychotropic substances is restricted by law. Examples of the main addictive substances are presented in the following table.

Examples of addictive substances

Category	Examples of substances
Tobacco	nicotine (see Heterocycles)
Opiates	opium, morphine, heroin (diacetylmorphine), codeine, fentanyl, pethidine
Stimulants	amphetamine, pervitin, ecstasy, ephedrine, phenmetrazine, cocaine, khat (see Amines)
Sedatives	benzodiazepins (diazepam, flunitrazepam), GHB (γ-hydroxybutyrate), barbiturates, propofol
Hallucinogens	LSD (trip), magic mushrooms, mescaline, datura (angel's trumpet), phencyclidine (angel dust)
Cannabis	THC (tetrahydrocannabinol), marijuana (leaves), hashish (resin)
Inhalants	solvents (toluene), glues, cleaning products, paints, sprays (propellant gases), etc.
Alcohol	ethanol, alcoholic beverages

Morphine is the main alkaloid of opium. Morphine itself, i.e. the free base, is not soluble in water and therefore it cannot be used in therapy. Only its soluble salts are applied, e.g. morphine hydrochloride (morphinium chloride) or morphine sulfate. Morphine and morphinium differ in only one proton bound to a nitrogen atom. Morphine strongly suppresses the feeling of pain. It is used parenterally in cases of strong acute pain, and also orally in cases of long-term treatment of pains, especially those caused by tumours. Besides the strong analgesic effect it markedly suppresses the respiratory centre, decreases peristalsis and vigilance. If it is repeatedly abused as a narcotic drug, it causes pathological addiction, both mental and physical. Morphine belongs to addictive substances; its use in medicine follows special rules.

morphine (free base)   morphinium chloride
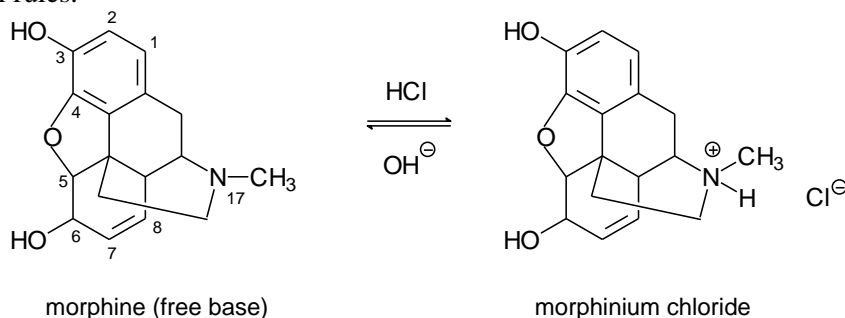
Codeine is 3-*O*-methylmorphine, monomethyl ether of morphine. Codeine strongly suppresses cough (antitussive); its analgesic effect is weak, but it increases the effect of other analgesics. In case of long-term use there is a risk of addiction. It is often sought by drug addicts as a compensatory drug, because it is partially demethylated in the body to morphine.

Heroin is 3,6-diacetylmorphine. It is not a natural alkaloid, but a semisynthetic derivative. It is the most dangerous addictive drug. Heroin is prepared by acetylation of morphine, which is illegal and prosecuted as a criminal offence throughout the world. The illegally prepared salt of heroin is usually applied in the form of a solution, injected subcutaneously or intravenously. It is not used in medicine, though it is six time more effective than morphine.

Cocaine is contained in the South American coca shrub (*Erythroxylon coca*). Its application to mucosa causes their anaesthesia, however it had been completely driven out from medicine by synthetic local anaesthetics (trimecaine, lidocaine). It has transitory psychostimulative effects after absorption, which cause dangerous addiction. It is abused as a stimulant drug. Cocaine in the form of a salt is usually snorted or injected subcutaneously in an aqueous solution. So called *crack* is a free base of cocaine, precipitated from the aqueous solution of the salt by the action of sodium carbonate and heating (crystals "crack" during heating). Crack cocaine has a low melting point, volatile above 90°C, and is abused by smoking.

codeine   heroin   cocaine
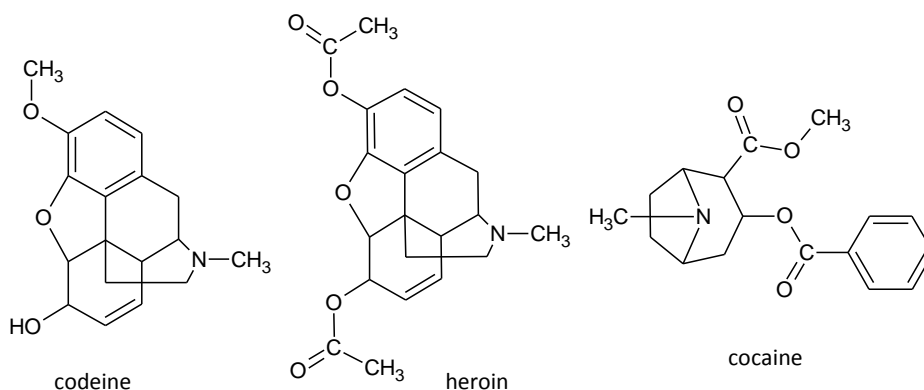
34 Monosaccharides

Carbohydrates are the most abundant organic compounds in the biosphere. They are produced during photosynthesis in green plants from CO_2 and H_2O; the required energy is taken from the Sun. Saccharides are the **source of energy** for humans. Carbohydrates are important **structural components** of living organisms. Proteoglycans are components of the connective tissue of higher animals. Ribose and deoxyribose are components of nucleotides and nucleic acids. The carbohydrate components of glycoproteins have distinctive functions (recognition of cell types, immunogens, cell adhesion).

Carbohydrates are defined as **polyhydroxyaldehydes** and **polyhydroxyketones**; they are divided into three groups. Monosaccharides (simple sugars) cannot be hydrolysed. Oligosaccharides contain 2-10 monosaccharide units joined by *O*-glycosidic bonds (e.g. disaccharides). Polysaccharides contain hundreds to thousands of monosaccharide units.

Monosaccharides are classified according to the nature of the carbonyl group and number of C atoms. If it is an aldehyde, the sugar is called aldose. In case of the oxo group, the sugar is a ketose. The general names are formed from the Greek numerals referring to the number of C atoms. An aldoses has the ending *-ose* (triose, tetrose, pentose, hexose), a ketose has the ending *-ulose* (tetrulose, pentulose). **Glyceraldehyde** and **dihydroxyacetone** are the two simplest sugars. Glyceraldehyde has one chiral carbon (C2), therefore it forms two enantiomers.

D-glyceraldehyde   dihydroxyacetone
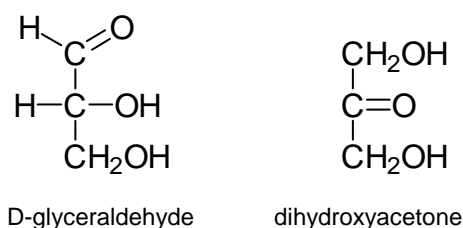
Configuration of Monosaccharides

Monosaccharides with the higher number of carbon atoms have a more chiral centre in the molecule, and the number of their possible stereoisomers is also higher. Generally, the number of stereoisomers is 2^n, where *n* is the number of chiral centres.

Monosaccharide is assigned to D or L series according to the configuration at the highest-numbered centre of chirality. This asymmetrically substituted C atom is called the **configurational carbon**, e.g., in pentoses it is C4, in hexoses C5. If the hydroxy group projects to the right in the Fischer projection (on the same side as in D-glyceraldehyde), the sugar belongs to the D series and receives the prefix D-. If the -OH on the configurational C is to the left, it is L-sugar. The classification D-/L- has no relation to the direction of optical rotation, some D-sugars are dextrorotatory (+), other levorotatory (-).

Diastereoisomers differ in configuration at least at one chiral carbon and are equal in configuration at least at one chiral carbon. If two diastereoisomers differ in configuration at just one chiral C, they are called **epimers**, e.g., D-mannose is the 2-epimer of D-glucose.

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Enantiomers are pairs of stereoisomers which differ in configuration at all chiral centres. They rotate the plane of polarised light by the same value, but in the opposite direction. As seen from the formulas bellow, D- and L-glucose differ in configuration at all four chiral carbon atoms. **Racemate** is the optically inactive mixture of two enantiomers in the ratio 1:1.

D-glucose   L-glucose
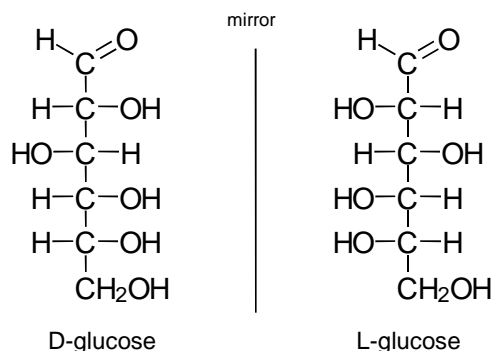
Cyclic Forms of Monosaccharides

Molecules of monosaccharides spontaneously form cyclic forms in solutions. The intramolecular addition of the hydroxyl group to carbonyl occurs and **cyclic hemiacetals** are produced. In case of aldohexoses it is usually the hydroxyl group at C5, in aldopentoses at C4. The resulting oxygen heterocycle is the derivative of hydrogenated pyran or furan and is generally called **pyranose** (six-membered) or **furanose** (five-membered), respectively. Cyclic forms are prevailing species in solutions.

D-glucose
(Fischer projection)

β-D-glucopyranose
(Fischer projection)

β-D-glucopyranose
(Haworth projection)
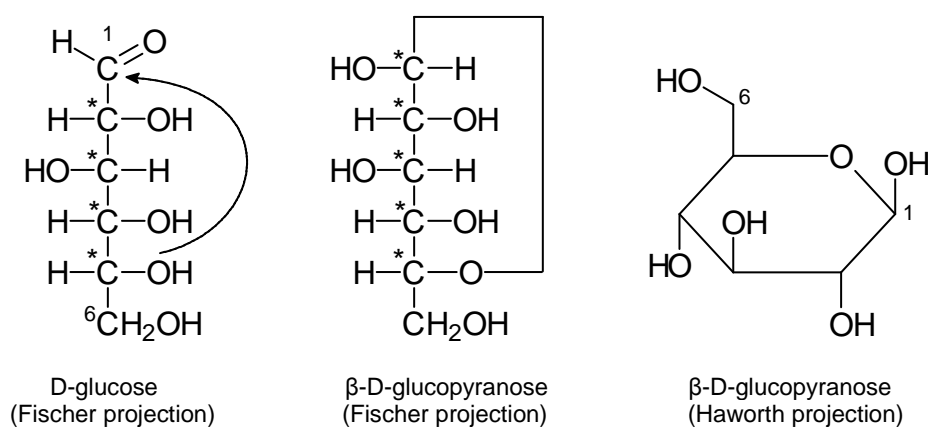
As a monosaccharide makes a cycle, the carbonyl carbon becomes a new chiral centre (C1 in aldoses, C2 in ketoses) called **anomeric** carbon. Four different substituents are now instead of the >C=O group. Therefore, two stereoisomers which differ only by the configuration of anomeric carbon are called α and β anomers. The configuration of cyclic forms can be expressed by Fischer projection (α-anomer has the same configuration on anomeric and configurational atom, β-anomer has reverse configuration), but Haworth projections are used more often.

Primary rules for Haworth formulas of D-monosaccharides:

- anomeric carbon is located on the right side, oxygen heteroatom up

- bonds going to left in the Fischer formula go up in the Haworth formula (and *vice versa*)

- the primary alcohol group -CH$_2$OH in on the left side and up (above the imaginary plane)

- α-anomer has anomeric -OH down (below the plane), β-anomer has it up.

Conformation of Monosaccharides

In the Haworth projection, the pyranose heterocycle is presented as a planar hexagon. It does not correspond with reality; pyranoses are always in the chair conformation. The predominating conformation has bulky substituents at equatorial positions rather than at the more crowded axial positions. β-D-Glucopyranose has all five non-H substituents in equatorial positions. α-Anomer has the hemiacetal hydroxyl in the axial position.
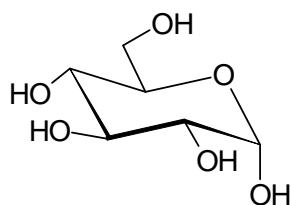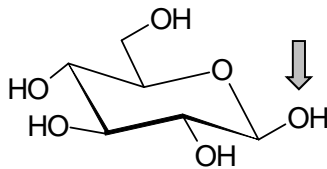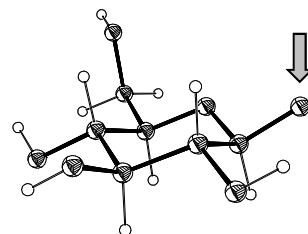
α-D-glucopyranose   β-D-glucopyranose   real structure of β-D-glucopyranose

Properties and Biochemical Conversions of Monosaccharides

All monosaccharides, like other compounds with many hydroxyl groups, are highly polar, well soluble in water, on the other hand, insoluble in non-polar organic solvents. They do not dissociate in aqueous solutions, and are typical **non-electrolytes**. They are mostly sweet.

Esterification

Monosaccharides are metabolised primarily in phosphorylated forms. The synthesis of phosphoric esters is catalysed by enzyme kinases; the donor of the phosphate group is ATP. Under physiological values of pH, the phosphate group $-PO(OH)_2$ dissociates to $-PO_3^{2-}$ and carries two negative charges. The phosphorylation changes the nature of the molecule from a neutral species to a dianion which cannot leave the cell (no membrane transporter), and is ready to interact with corresponding enzymes and undergo metabolic conversions. Phosphorylation typically occurs on the primary alcohol group, e.g., in glucose 6-phosphate, fructose 1,6-bisphosphate or ribose 5-phosphate, which is also the basic component of nucleotides. Sometimes the phosphate group can be attached to anomeric hydroxyl (glucose 1-phosphate, glycoside of the ester type).
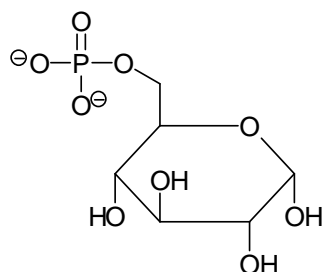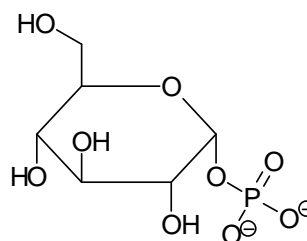
glucose 6-phosphate   glucose 1-phosphate

Esters of hexoses with **sulfuric acid** are frequent building components of many proteoglycans of the extracellular matrix in connective tissues and in some glycolipids. A sulfate group bound to sugars carries one negative charge.

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Isomerisation

Enzymatic reactions include transformations of aldoses to ketoses and *vice versa*, as well as various epimerisations. Reactants are not free monosaccharides but their phosphoric esters or the activated forms, in which the sugar is bound to nucleoside-phosphate, e.g., uridine diphosphate glucose (UDP-glucose), is transformed to UDP-galactose and *vice versa*. This is the method of formation of galactose for the synthesis of lactose in a lactating mammary gland and for the production of glycoproteins.

Oxidation

Monosaccharides (aldoses) are relatively easily oxidised, mainly in the alkaline environment, therefore they have substantial reducing properties. Oxidation of ketoses is connected with the cleavage of the carbon chain.

D-gluconic acid   D-glucuronic acid
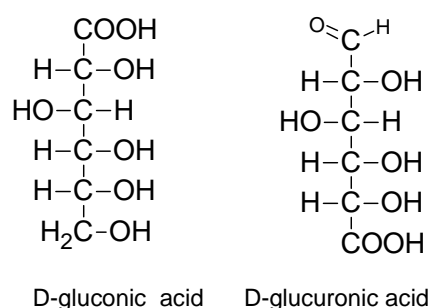
If an aldehyde group is oxidised, **aldonic acid** is the product, for example D-glucose converts to D-gluconic acid. Aldonic acids can easily produce cyclic esters, lactones, by elimination of water, e.g., D-gluconic acid forms D-glucono-5-lactone, which can hydrolyse to gluconic acid (gluconate). Glu-cono-5-lactone is also produced by direct dehydrogenation of the C^1H-OH group of glucopyranose.

D-gluconic acid   D-glucono-5-lactone   β-D-glucopyranose
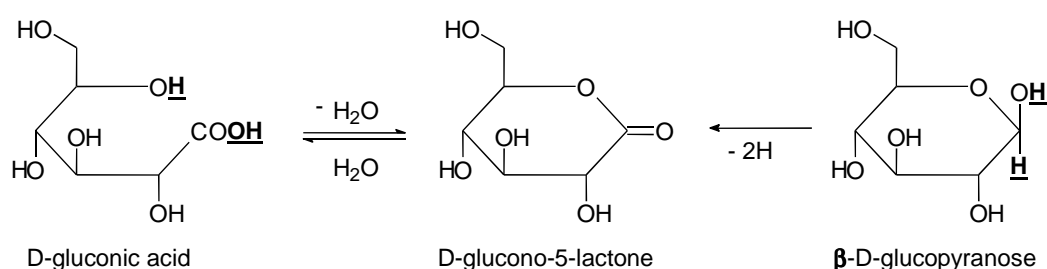
If the aldehyde group is protected against oxidation, only the primary alcohol group is oxidised to provide **uronic acids** (aldehydic acids).

D-Glucuronic acid is the basic component of heteropolysaccharides glycosaminoglycans. They form the carbohydrate component of proteoglycans of the connective tissue. Glucuronic acid also forms glycosides with a number of substances increasing their solubility and their excretion to urine or bile

(conjugation reactions). In the form of **glucuronide conjugates** some natural substances (bilirubin, oestrogens, catecholamines) as well as many foreign substances (drugs) are eliminated from the body. **D-Galacturonic acid** is present in plants and fruits as the component of pectins.
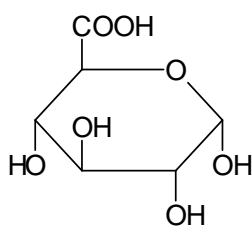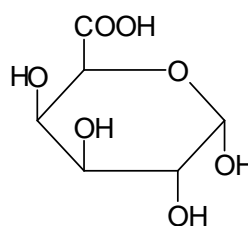
α-D-glucuronic acid   α-D-galacturonic acid

Reduction

Hydrogenation of the carbonyl group of aldoses and ketoses produces **sugar alcohols** (alditols). They have no reducing properties. The trivial names of alditols express their configuration and they are made from the name of the corresponding aldose by substituting the suffix –*ose* with –*itol*.

Aldoses give only one sugar alcohol by reduction, e.g., D-glucitol is formed from D-glucose. Reduction of ketoses gives a pair of epimeric alditols, because carbon of the carbonyl group (hybridisation sp^2) becomes chiral by hydrogenation (hybridisation sp^3). For example, D-fructose gives a mixture of D-glucitol and D-mannitol, which differ in configuration at C2.

D-glucitol   D-mannitol   ribitol
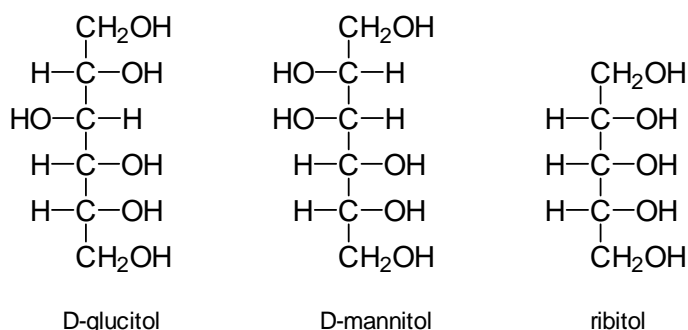
D-Glucitol (sorbitol) is used as an artificial sweetener in foodstuffs for diabetics.
D-Mannitol is widespread in plants. It is easily absorbed, but it does not penetrate into the cells and is excreted into urine unchanged. Therefore the intravenous infusions of hypertonic, up to 20% solutions of mannitol are used to transport water from the tissues into the bloodstream, e.g., in the case of brain oedema or to trigger osmotic diuresis.
Ribitol is produced by the reduction of ribose. It is bound in the molecule of riboflavin and cofactor FAD.

Deoxysugars are products of the more complicated way of reduction – deoxygenation. Most important is **2-deoxy-D-ribose**, a component of DNA. In cells, 2'-deoxyribonucleoside diphosphates are produced by complex deoxygenation of ribonucleoside diphosphates.

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6-Deoxyhexoses have the methyl group at C-6 instead of the primary alcohol group. They are relatively widespread in nature as components of plant glycosides. **L-fucose** (6-deoxy-L-galactose) is a typical component of membrane glycoproteins and glycolipids in erythrocytes (determining blood group substances A-B-0).

β-D-2-deoxyribose   α-L-fucose
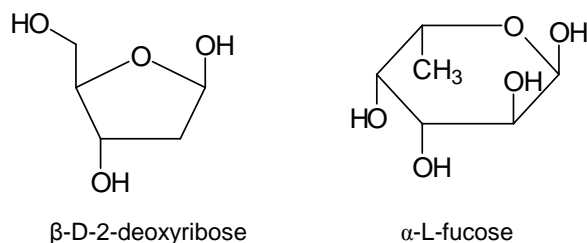
Formation of amino sugars

Amino sugars have an amino group -NH$_2$ instead of one hydroxyl group. This is most frequently at C-2 of aldose. Glucosamine, the major amino sugar, is formed as glucosamine 6-phosphate from fructose 6-phosphate, using glutamine as the donor of the amino group. Amino sugars have, like all the other primary amines, basic properties. In living organisms, they are found in the form of non-basic *N*-acetylderivatives (non-electrolytes), mostly bound in hetero-polysaccharides. Primarily, the amides of glucosamine, galactosamine, and neuraminic acids are biochemically important. ***N*-acetylglucosamine** and ***N*-acetylgalactosamine** are common components of glycosaminoglycans.

β-D-glucosamine   *N*-acetyl- β-D-glucosamine   *N*-acetyl-α-D-neuraminic acid
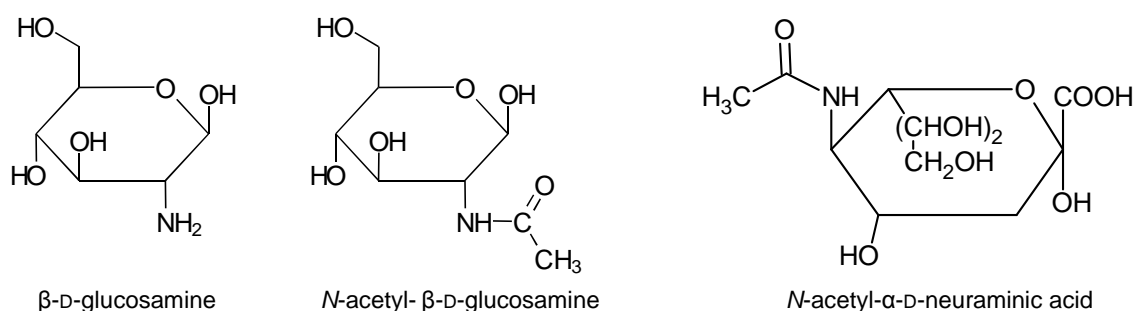
Neuraminic acid is a nine carbon amino acid. The structure can be derived from D-mannosamine (2-epimer of glucosamine), to which the third carbon atom of pyruvate is attached by condensation. The oxo group of pyruvate forms a pyranose hemiacetal cycle.

D-mannosamine + pyruvate   D-neuraminic acid (acyclic form)   α-D-neuraminic acid (pyranose form)
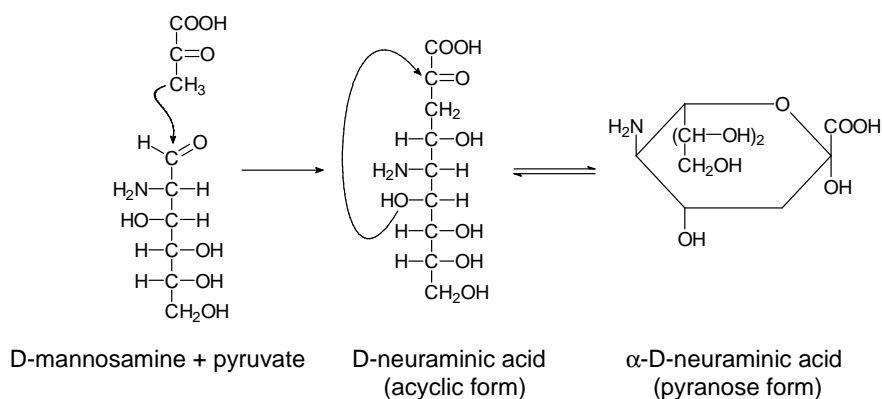
Neuraminic acid is rather acidic ($pK_A = 2.2$). It is not free in nature, but in the form of *N*-acetyl-, *N*-glycolyl-, and also *N,O*-diacetylneuraminic acid. These derivatives of neuraminic acid have the common name **sialic acids**, although the term sialic acid is usually only used for ***N*-acetylneuraminic acid**. Sialic acids are components of oligosaccharides found in the membrane and plasmatic glycoproteins or membrane glycolipids. They are bound at the terminals of oligosaccharide chains (Chapter 45).

Non-enzymatic glycation of proteins

In an environment rich in free glucose and proteins, like in the blood of diabetics, the aldehyde group of glucose reacts with free -NH$_2$ groups of plasmatic and tissue proteins. The product is 1-amino-1-deoxyfructose (fructosamine) linked through the amino group to the carbon of the polypeptide chain. This can result in serious changes in the biological function of proteins (see Chapter 27).

Formation of glycosides

Glycosides are derivatives of the cyclic forms of monosaccharides, which are formed by reaction of anomeric hydroxyl with an oxygen or nitrogen containing compound with the simultaneous release of water. Glycosides formed by reaction of anomeric hydroxyl with alcohol, phenol or carboxyl hydroxyl (glycosides of ester type) are generally ***O*-glycosides**; the bond is *O*-glycosidic. If the glycosidic bond is formed between monosaccharides, it leads to oligosaccharides or polysaccharides. The simplest *O*-glycoside is methyl-β-D-glucoside.

Glycosides formed by reaction of anomeric hydroxyl with the nitrogen of amines, amides, and nitrogen heterocycles are ***N*-glycosides**; the bond is *N*-glycosidic. Acids cleave the glycoside bond hydrolytically with a simultaneous release of components from which the glycoside was formed. The glycosidic bond is relatively stable in an alkaline environment. Enzymes catalysing the hydrolysis of glycosidic bonds (glycosidases) are stereospecific, they catalyse cleavage of only α- or β-glycoside bonds.

The residue formed from cyclic forms of monosaccharides after the removal of anomeric hydroxyl is generally called *glycosyl* (e.g glucopyranosyl). The names of glycosides are formed in various ways, if the non-sugar component (aglycon) is simple, the name is *alkyl-glycoside* or *glycosylamine*.

methyl-β-D-glucoside   α-D-glucosylamine   glucosiduronate of paracetamol
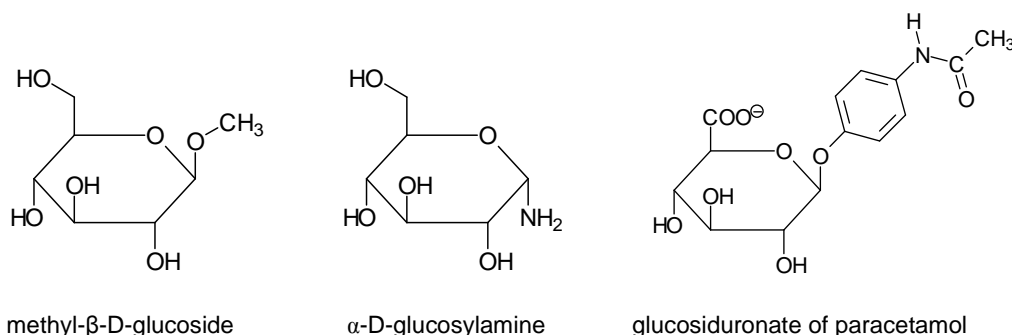
In higher animals, the simple glycosides are most often **glucosiduronates**. Glucuronidation is a major inactivating pathway for a variety of exogenous and endogenous molecules, including drugs, bilirubin, steroid hormones, etc. The formation of the β-glycosidic bond with glucuronic acid increases the po-

larity and solubility of hydrophobic compounds. The process is generally named **conjugation**. This substantially facilitates the excretion of the above mentioned substances into bile or urine.

Important Monosaccharides

D-Ribose and **2-deoxy-D-ribose** are structural components of nucleotides and nucleic acids. They both occur as furanoses. Deoxyribose lacks the hydroxyl group in position 2 (see later).

Ribulose (ketose corresponding to ribose) and **xylulose** (3-epimer of ribulose) are the intermediates of the pentose cycle.

α-D-ribofuranose

D-Glucose (Glc, glucosum, grape sugar) occurs in fruits (1-5%) and honey (~ 30%). Humans have free glucose in blood (3.3-5.5 mmol/l), lymph, and cerebrospinal fluid, and its content is regulated by the hormones insulin and glucagon. Under some pathological conditions, e.g., in case of diabetes mellitus, it is also found in urine. Glucose is rapidly absorbed after consumption, and therefore it is used as a quick energy source. It is usually manufactured by acidic hydrolysis of starch. Phosphoric esters of glucose, glucose-1-phosphate and glucose-6-phosphate, are important intermediates. Glucose solutions are often used as intravenous infusions. The 5% glucose solution, isotonic with

α-D-glucopyranose

blood plasma, is often the carrier of drugs or used to cover the loss of water. Hypertonic solutions (10-60%) are used for parenteral nutrition or in therapy for lung or brain oedema.
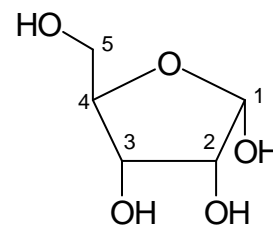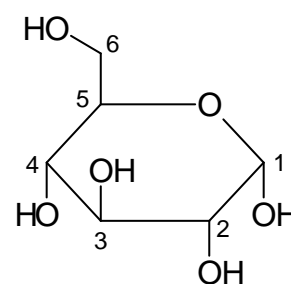
D-Mannose (Man, 2-epimer of D-glucose) is present in some plants, e.g., in polysaccharides of carob (*Ceratonia siliqua*). In the animal organism, it is a component of blood plasma glycoproteins.

D-Galactose (Gal, 4-epimer of glucose) occurs in lactose (milk sugar), cerebrosides, and glycoproteins. In plants it is found in gums (fibre).

D-Fructose is the most widespread ketohexose. Configuration at C3-C5 is identical with D-glucose. It is found mainly in fruits (3-6%) and honey (~ 40%). Free fructose exists mostly in the pyranose form; fructofuranose occurs only in sucrose, polysaccharides (inulin), and phosphoric esters. It forms colourless crystals with a strongly sweet taste. Two esters are of biochemical importance; fructose-6-phosphate and fructose-1,6-bisphosphate, the intermediates of glycolysis and gluconeogenesis.
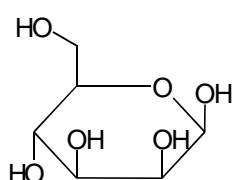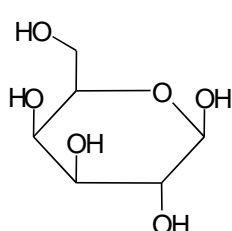
β-D-mannopyranose   β-D-galactopyranose   D-fructose   β-D-fructofuranose
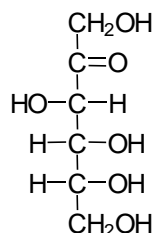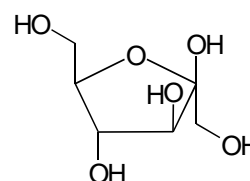
35 Disaccharides

Disaccharides are formed from two identical or different monosaccharides joined by an *O*-glycosidic bond. Anomeric hydroxyl of one molecule can react with an alcoholic or anomeric hydroxyl of a second molecule. In the first case, the result is **reducing disaccharide**, which exists in two anomeric forms and is able to form glycosides with other compounds. If the glycosidic bond is formed between two anomeric hydroxyls, it results in the formation of **non-reducing disaccharide**, which does not reduce the Benedict reagent.

Nomenclature of Disaccharides

Many disaccharides have common names; if needed, they are described as α/β-anomers.
The general name of the reducing disaccharide is *glycosyl-glycose*. The locants of the glycosidic linkage and the anomeric descriptor(s) must be given in the full name. The carbon number, whose alcoholic hydroxyl participates in the glycosidic bond, is placed in front of the glycosyl prefix.
The general name of the non-reducing disaccharide is *glycosyl-glycoside*; the monosaccharide components have an alphabetic order.

Non-Reducing Disaccharides

Sucrose (β-D-fructofuranosyl-α-D-glucopyranoside, saccharosum, table sugar, beet/cane sugar) is the only nutrient we take in crystalline pure form. Sucrose occurs in a range of plant species, sugar beet (*Beta vulgaris*) and sugar cane (*Saccharum officinarum*) and is used in the food industry. Sucrose has a perfectly sweet taste; its sensorial qualities can hardly be overcome by any sweetener.

sucrose
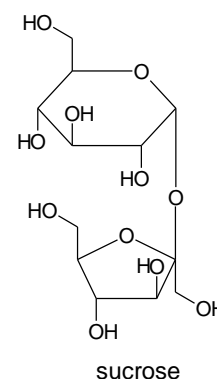
Reducing Disaccharides

Maltose (4-*O*-(α-D-glucopyranosyl)-D-glucopyranose, maltosum, malt sugar) has two glucosyl residues linked by the α(1→4) glycosidic bond. Maltose is formed during acidic hydrolysis or digestion of starch and is present in malt.
Cellobiose (4-*O*-(β-D-glucopyranosyl)-D-glucopyranose) has two glucosyl residues linked by the β(1→4) bond. It is produced during partial hydrolysis of cellulose.

α-maltose

β-cellobiose
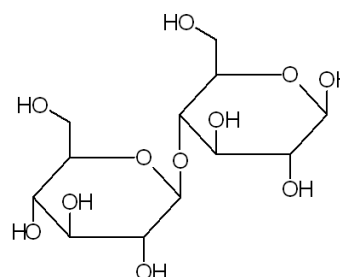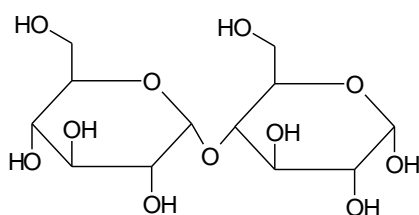
Lactose (4-*O*-(β-D-galactopyranosyl)-D-glucopyranose, lactosum, milk sugar) consists from galacto-syl and glucosyl residues connected by β(1→4) bond. It is the most important sugar contained in the milk of all mammals; in cow milk 4-6%, in human milk 6%. Lactose is substantially less sweet than sucrose.

β-lactose   β-lactulose
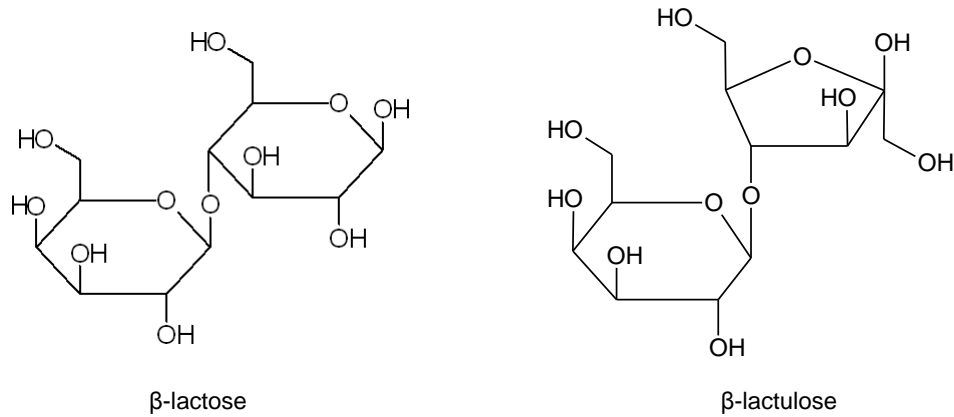
Lactulose (4-*O*-(β-D-galactopyranosyl)-D-fructofuranose, lactulosum) is the synthetic derivative of lactose and is used as an osmotic laxative and probiotics. Digestive enzymes of the small intestine cannot cleave lactulose; it is practically not absorbed, and acts as dietary fibre. Saccharolytic bacteria of the large intestine break down lactulose to short chain organic acids. Intestinal content becomes a more acidic and hyperosmotic environment – water travels from enterocytes to lumen (osmosis), in-creases stool volume, and causes the laxative effect. Lactulose administration supports the predomi-nance of saccharolytic bacteria over proteolytic (putrefactive) bacterial strains. The production of ammonia in the large intestine and its concentration in portal blood decreases, and the liver is protect-ed against toxic ammonia and its detoxification to urea. Lactulose is used in the therapy of some liver diseases.

36 Polysaccharides

Polysaccharides consist of several hundreds or thousands of monosaccharide units. The molecules of monosaccharides are linked by α- or β-*O*-glycosidic bonds, most often 1→4 or 1→6. Polysaccharide chains can be linear or branched. In contrast to simple sugars, polysaccharides are not soluble in water; some of them can form colloid solutions. They do not display reducing properties and they are not sweet. Acidic hydrolysis creates monosaccharide units. Homopolysaccharides consist of molecules of only one monosaccharide. Heteropolysaccharides contain two or more different monosaccharides or their derivatives (e.g. uronic acids, amino sugars).

Homopolysaccharides

Starch (amylum) is the storage substance of plants contained mainly in cereals, potatoes, and rice. Starch dissolves in hot water to make a colloidal solution. It is the mixture of amylose and amylopectin. **Amylose** forms 20-30% of common starches and consists of 250 to 1,000 glucose units joined by α-1,4-glycosidic bonds. Due to α-glycosidic linkage the polysaccharide chain is not linear, but turned to a helix. Enzyme pancreatic amylase mainly hydrolyses amylose to maltose. **Amylopectin** is the main component of starch (70-80%). Its molar mass is high, according to the origin up to 1,000,000. Amylopectin is branched, side chains bind by α-1,6 bond to the main α-1,4 chain. Side chains can contain other side chains. Branching occurs approximately in every 20-25 glucose residue. Amylase hydrolyses amylopectin to maltose and a mixture of tri- to pentasaccharides called **limit dextrins**, some of which are branched (amylase does not cleave 1→6 bonds).

a part of amylopectin
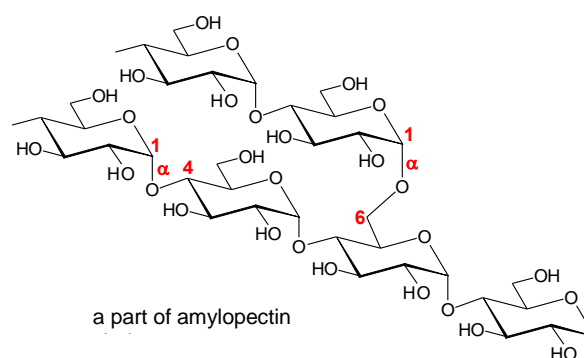
Glycogen, also called "animal starch", is the storage substance in the liver (5-10%) and in the muscles (1%) of animals. It is formed by synthesis from sugars taken in food. It is the ready source of glucose during fasting. Structurally it is very similar to amylopectin, but it has shorter side chains and more branches. Each 5-9 glucose unit of the main chain contains a side chain joined by α-1,6 bond which usually consists of 10-14 glucose units. The molar mass is very high; muscle glycogen has about 1,000,000, liver glycogen 16,000,000 (it corresponds to about 100,000 glucose units). In spite of that glycogen is soluble in water to a colloid solution. Enzyme phosphorylase cleaves glycogen by phosphorolysis (i.e. by the action of inorganic phosphate) to glucose 1-phosphate:

$$(Glc)_n + P_i \rightarrow (Glc)_{n-1} + Glc\text{-}1\text{-}P.$$
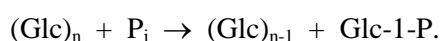
Cellulose is the most abundant organic substance on the Earth. Almost pure cellulose is cotton (*Gossypium hirsutum*); technical cellulose is obtained from wood. Cellulose makes unbranched chains where the glucose units are joined by a β-1,4-glycosidic bond. The fibrous macromolecules of cellulose are

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several times folded and linked by hydrogen bridges; therefore cellulose is not soluble in water. It is indigestible for humans and most animals as they do not have enzymes to cleave β-glycosidic bonds. Cellulose passes through the digestive tract as fibre (see later). Enzymes cleaving cellulose occur in bacteria. If some animals use cellulose as a nutrient, its cleavage is performed by bacterial microflora; e.g., in the paunch of ruminants. Pure cellulose is used in medicine as dressing cotton-wool. Powder microcrystalline cellulose is used externally to sponge surface exudates.
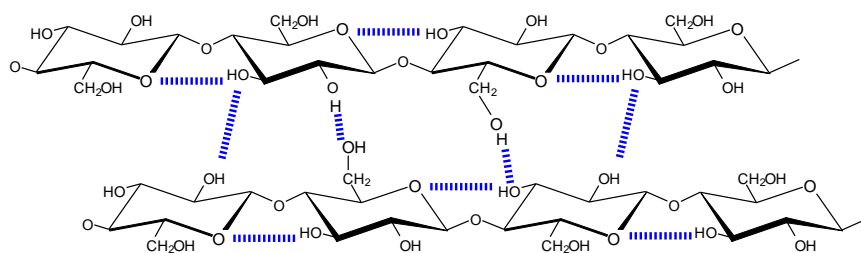
Two cellulose chains with H-bonds

Derivatives of cellulose. Alkylation of the alcohol groups of glucose units produces polyethers methylcellulose, hydroxyethylcellulose and salts of carboxymethylcellulose, used as components of so called bulk-forming laxatives. Moreover **carboxymethylcellulose** is used to prepare oral forms of some drugs or in the ion exchange chromatography. Oxidation and degradation of cellulose produces **carboxycellulose** which is used in powder form for the quick suppression of capillary bleeding.

Dextran is a polysaccharide consisting of D-glucose units linked by α-1,6 bonds with branching in positions 3 and 4. Dextran is formed from sucrose by the action of a bacteria *Leuconostoc mesenteriodes*. Dextran is also produced in the oral cavity by the action of *Streptococcus mutans* and builds up dental plaque on unclean teeth, which are resistant to salivary amylase. The molecular mass of native dextran varies from 10,000 to 1,000,000. The colloid solutions of dextrans are used in medicine as short-term substitutes of blood plasma in cases of acute bleeding or in the therapy of burns. For this purpose the strongly viscose native dextran undergoes hydrolysis to produce fractions with M_r 40,000-70,000. Gels prepared from highly branched dextrans by artificial cross-linking are used as "molecular sieves" in laboratories for gel filtrations or chromatography (Sephadex).

Inulin is a fructan contained as a store substance in the tubers of the Jerusalem artichoke, sunflower, chicory, etc. It consists of a relatively small amount of D-fructose (less than 100), and therefore it is soluble in water. The molecules of fructose in their furanose forms are linked by β-2,1 bonds. Inulin acts as a prebiotic (dietary fibre) supporting the growth of intestinal microflora. It is used experimentally to determine extracellular fluid volume (given intravenously it easily penetrates to intersticium, but not into the cells) and to examine the filtration function of the kidneys (inulin clearance).

Heteropolysaccharides

Glycosaminoglycans (older name mucopolysaccharides) form the saccharide component of large **proteoglycan** macromolecules. They consist of a core protein with one or more covalently attached glycosaminoglycan chain. The saccharide part is coupled to the protein core through an *O*-glycosidic bond to a serine or threonine residue or by *N*-glycosidic linkage to asparagine. The percentage of carbohydrate portion highly overcomes the protein portion (to 95%). Proteoglycans have a supportive function in the extracellular matrix of various types of connective tissues,

Proteoglycan complex

they fill in the space between the collagenous and other fibrilar structures. They are also found in the secretions of glands and mucosal cells.
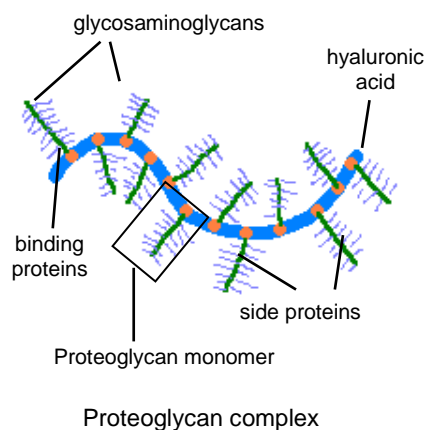
A glycosaminoglycan contain a repeating disaccharide unit: uronic acid and *N*-acetylated aminosugar. One or both sugars contain ester-bonded **sulfate groups** (the only exception is hyaluronic acid), which together with the carboxyl groups of uronic acids give them **strongly acidic properties**. Thus, at usual pH values they have a large number of negative charges. The main types of glycosaminoglycans and their structural components are shown in the table below.

Glycosaminoglycan	Aminosugar	Uronic acid	Aminosugar bond	Acid bond
Hyaluronic acid	GlcNAc	GlcUA	β-1,4	β-1,3
Chondroitin sulfate	GalNAc	GlcUA	β-1,4	β-1,3
Keratan sulfate	GalNAc	-	β-1,3	-
Heparin	GlcNAc	GlcUA / L-IdUA	α-1,4	β/α-1,4
Dermatan sulfate	GalNAc	GlcUA / L-IdUA	β-1,4	β/α-1,3

GlcNAc (*N*-acetylglucosamine), GalNAc (*N*-acetylgalactosamine), GlcUA (D-glucuronic acid), L-IdUA (L-iduronic acid)

Hyaluronic acid, the simplest glycosaminoglycan, consists of D-glucuronic acid and *N*-acetylglucos-amine. The molecule is linear, without sulfate groups. Hyaluronic acid can be found in different types of connective tissues, in the synovial fluid and vitreous humour. It is also present in its free form, i.e. without the covalent linkage to proteins. However, a large number of polypeptide proteoglycan chains can be attached to the hyaluronate chain to make huge proteoglycan complexes.
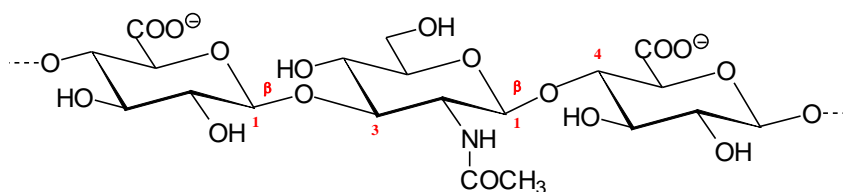
Hyaluronic acid forms very viscous solutions; its presence prevents the penetration of microorganisms and other foreign particles through the extracellular space. β-1,4 Glycosidic bonds in hyaluronate and

chondroitin sulfate can be hydrolytically cleaved by the enzyme **hyaluronidase**, which acts as the spreading factor in the skin and connective tissues. If this enzyme is produced by pathogenic bacteria, they can easily penetrate through the extracellular matrix into tissues and spread the infection.

Chondroitin sulfate participates in the building of connective tissue together with hyaluronic acid; it is contained mainly in the cartilage. Its ionic interactions with the microfibrils of collagen help to strengthen the structure of collagenous fibrils.

Dermatan sulfate contains L-iduronic acid instead of some D-glucuronic acid molecules.

Keratan sulfates are also components of proteoglycans, mainly in the cartilage and cornea. They substantially differ from the above stated structures because they do not contain uronic acids, but galactose units instead.

Heparin is a heteropolysaccharide composed of glucosamine-*N*-sulfate, glucuronic acid, and L-iduronic acid (M_r 17,000-20,000). The content of sulfuric acid is high; the position of sulfate groups is various. Native heparin is found in many tissues and basophilic granules of mast cells as a heterogeneous mixture whose components differ in their molar mass. Heparin has an anticoagulation effect (it is used to obtain non-coagulated blood or plasma). It prevents the blood clotting, inhibits the conversion of prothrombin to thrombin, and the action of thrombin to fibrinogen. Today, heparin is replaced by low molecular mass heparins (fractions with M_r 4,000-6,000) which have retained their antithrombotic properties.
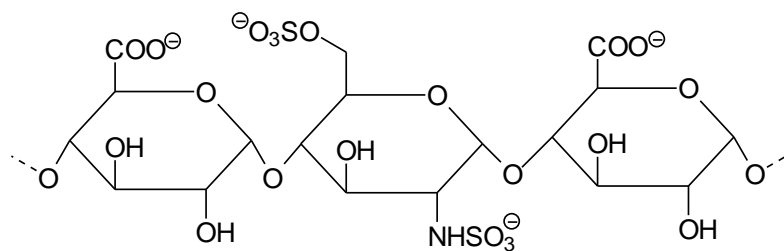
A part of heparin chain

Agar

is a heteropolysaccharide contained in some sea algae. It consists of the linear agarose and branched agaropectin. **Agarose** is formed by regularly repeating and partly methylated residues of D- and L-galactose primarily linked by an α-1,3 bond. **Agaropectin** consists of D-galactose and D-galacturonic acid, which is partly sulfated. Agar forms solid gels and is often used to prepare bacterial cultivation media in microbiology; in laboratories agarose gels are common carriers for the electrophoresis of macromolecular substances.

Dietary Fibre

is a heterogeneous mixture of plant polysaccharides and accompanying substances, which are not cleaved in the human digestive tract. According to water-solubility, we distinguish insoluble fibre (cellulose, hemicelluloses, and non-saccharide polymer lignin) and soluble fibre (inulin, pectins, mucilages).

Hemicelluloses accompany cellulose in plants. These heteropolysaccharides contain, in addition to glucose and mannose, mainly pentoses (D-xylose, L-arabinose), glucuronic and galacturonic acid. Hemicelluloses contained in plant food are very important for the diet. They are especially in wholemeal cereals; its concentrated source is bran.

Pectins are the main components of soluble fibre. They occur in intercellular layers of higher plants, fruits, and vegetables. The highest content of pectin is in apples, currants, gooseberry, tomatoes, carrot. The main type of pectin is **pectic acid**, poly(α-1,4-D-galacturonic acid); unbranched chains contain a certain number of carboxyls of galacturonic acids esterified by methanol. They give colloid, viscous solutions with water, in higher concentration and in cool places they form gels (fruit jelly, jams). Digestive enzymes do not cleave their glycosidic bonds; they are partly decomposed by bacterial microflora of the large intestine.

Plant mucilages contain hexoses, pentoses, uronic acids. They are used as additives in the food industry, some of them as laxatives. The most common examples are mucilages of **oat flakes**, clearly visible when eating porridge. **Psyllium** is the soluble fibre from the seed coat of plantain (*Plantago psyllium*); it is used as a gentle laxative. **Flax seeds** (semen lini) exhibit similar effects.

The Functions of Dietary Fibre

· Hemicelluloses are highly hydrated in the digestive tract. The enlargement of the intestinal content supports intestinal peristalsis, especially that of the large intestine (prevention and treatment of constipation).
· Fibre supports the growth of saccharolytic bacteria in the large intestine, which are responsible for fermentative processes at the expense of proteolytic bacteria, causing putrefactive processes (with production of ammonia and other nitrogen compounds).
· The components of fibre show the adsorbent effect – they adsorb some low molecular nitrogen substances and bile acids (indirect removal of cholesterol from the body).
· The mass of fibre prevents the access of pancreatic enzymes to their substrates and reduces the intestinal absorption of nutrients. It slows down the absorption of glucose and thus decreases the demands for the secretion of insulin, which would otherwise be needed to regulate the level of glucose in the blood.
· Pectins are beneficial mainly because of the fact that they quite strongly bind toxic metal ions from the alkaline intestinal environment, and this is proportional to the number of non-esterified carboxyl groups.

For several years the indirect relation between the amount of fibre in food and the presence of colorectal carcinoma has been observed. The possible anti-tumour effect of fibre is probably caused by the decreased activation of some natural carcinogens by the bacterial microflora of the digestive tract.

37 Nucleosides and Nucleotides

Nucleosides

are compounds in which a heterocyclic nitrogen base and a pentose are linked by a β-*N*-glycosidic bond, formally created by removing water. The pentose is ribose or deoxyribose. The nitrogen hereto-cycles include the purine bases **adenine** and **guanine**, or pyrimidine bases **uracil**, **cytosine**, and **thymine**. These bases exhibit tautomerism, but in cells with a pH of approximately 7.0 the hydroxyl group exists mainly in its lactam (i.e. oxo) form, while amino groups of guanine and cytosine remain
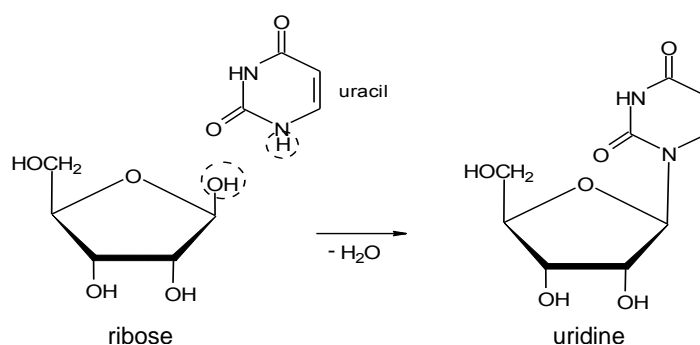
in the –NH$_2$ form.

Formal origin of uridine

Modified purine or pyrimidine bases occur in some nucleic acids and are referred to as **minor bases**. They include, for example, N^6-methyladenine, hypoxanthine, 5-methylcytosine, and 5,6-dihydrouracil. Pseudouridine is not a genuine glycoside; its base is linked to the pentose atypically (C5-C1').

Nucleosides have trivial names derived from the names of bases (see the table below). The names of purine nucleosides end with -*osine* (adenine → adenosine), pyrimidine nucleosides end with **-***idine* (uracil → uridine). Carbon atoms of pentose are numbered 1'- 5', so that they differ from the numbering of the base. Purine bases are usually glycosylated on the nitrogen N9, pyrimidine base on N1. The *N*-glycosidic bond has a β configuration; the glycosyl is β-D-ribofuranosyl.

Base	Nucleoside	Nucleotide
Adenine	adenosine (A)	adenosine 5'-monophosphate (AMP)[a]
Guanine	guanosine (G)	guanosine 5'-monophosphate (GMP)
Hypoxanthine	inosine (I)	inosine 5'-monophosphate (IMP)
Cytosine	cytidine (C)	cytidine 5'-monophosphate (CMP)
Uracil	uridine (U)	uridine 5'-monophosphate (UMP)
Thymine	deoxythymidine (dT)	deoxythymidine 5'-monophosphate (dTMP)

[a]AMP is sometimes called adenylic acid (adenylate).

Nucleosides are highly polar compounds, soluble in water. The *N*-glycosidic bond can be, like in other glycosides, hydrolysed by diluted acids; it is stable in an alkaline environment.

adenosine (A)   guanosine (G)   inosine (I)

cytidine (C)   deoxythymidine (dT)   pseudouridine (Ψ)
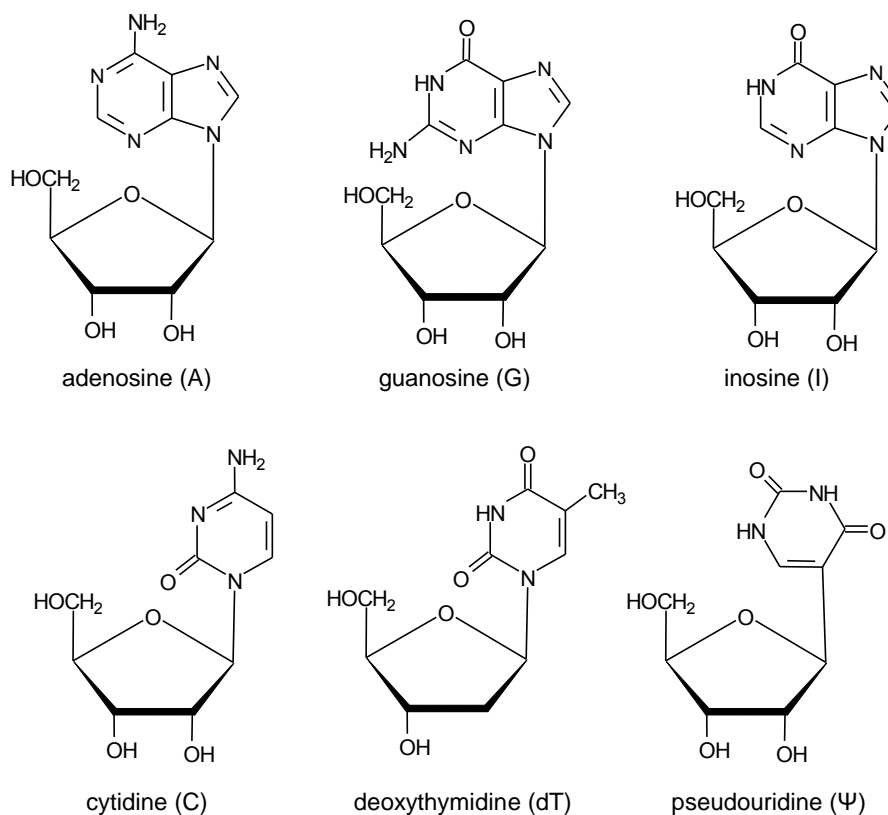
Synthetic nucleosides. Nucleosides with a modified saccharide component are used in therapy. They function as inhibitors of nucleic acid synthesis, for example, **cytarabine** (β-D-arabinosyl is attached to cytosine) or **azidothymidine** (AZT, zidovudinum, with an attached azide group -N_3), which inhibits the reverse transcriptase of HIV.

Nucleotides

are derivatives of nucleosides in which phosphate is bound to ribose by an ester bond, most often in the positions 5' or 3'. The general name of a nucleotide is nucleoside phosphate. In individual names, the position of the phosphate group on ribose is given, e.g., adenosine 5'-phosphate. If the locant is missing (e.g. AMP), we suppose the bond between phosphate and pentose hydroxyl in position 5'. Nucleotides are polar compounds; the phosphate group provides an acidic character. In neutral solutions, nucleoside monophosphates occur mainly as dianions (with two negative charges). Nucleoside diphosphates and nucleoside triphosphates have the hydroxyl group of pentose (usually in position 5') esterified by diphosphate or triphosphate, respectively. Phosphate groups are attached by a **phospho-anhydride bond** to the phosphate of nucleoside monophosphates. A lot of Gibbs energy is needed to form the anhydride bond; it is obtained by the oxidation of nutrients, mostly in the respiratory chain. This process of ATP synthesis from adenosine diphosphate (ADP) and inorganic phosphate (P_i) is called aerobic (oxidative) phosphorylation.

Energy accumulated by the formation of two phosphoanhydride bonds turns nucleoside triphosphates into **high-energy (macroergic)** compounds, the most universal is **adenosine triphosphate (ATP)**. Hydrolysis of ATP to ADP and phosphate is a very exergonic reaction. The Gibbs energy released in this reaction is used to drive many biochemical processes, enzymatically catalysed endergonic syntheses, to create concentration gradients on membranes, for muscle contraction, etc.

Thus the ATP in cells presents a prompt source of chemical energy. Its concentrations are usually about a few mmol/l. It is not much; in a number of tissues this could cover the energetic need for only a few seconds. However, ATP is constantly and quickly supplied by the phosphorylation of ADP, dependent on the oxidative degradation of nutrients. The human body can produce (and cleave) about 70 kg of ATP in 24 hours, that is approximately the same amount, that it weighs.
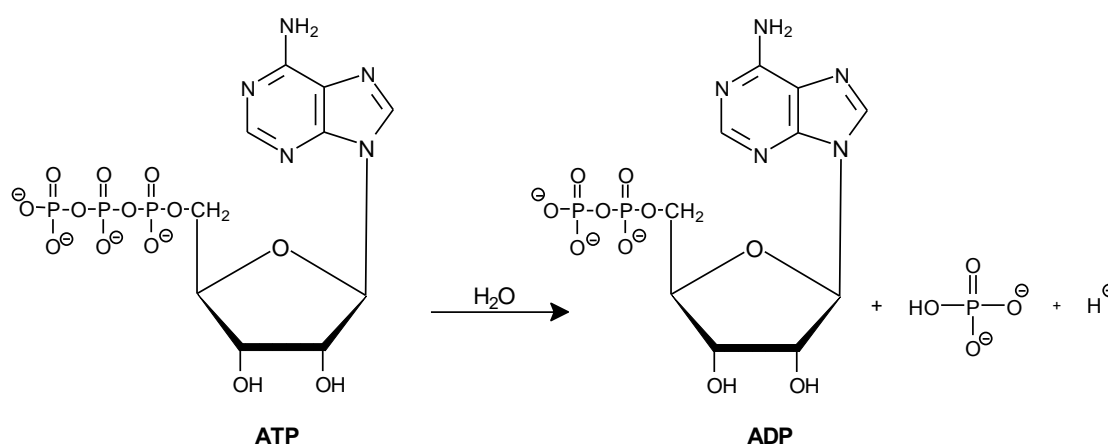
The hydrolysis of ATP

The transfer of a $-PO_3^{2-}$ (phosphoryl) group from ATP to other compounds is of great importance. These **phosphorylation** reactions are catalysed by various specific enzymes, generally called **kinases**. The product can be another high-energy compound if the **anhydride** bond is formed again (synthesis of nucleoside triphosphates or acylphosphates), or if a **phosphoamide** bond is produced (synthesis of creatine phosphate by *N*-phosphorylation of the guanidine group in creatine). Transfer of $-PO_3^{2-}$ from ATP to the hydroxyl group produces **phosphoesters**. They do not belong to high-energy compounds, but they are often reactive intermediates of various metabolic pathways. The reversible phosphorylation of the hydroxyl groups of serine, threonine or tyrosine, catalysed by **protein kinases**, is important for regulation of the function of many proteins (enzyme).

substrate (neutral)   ATP (4-)   phosphorylated substrate (dianion)   ADP (3-)
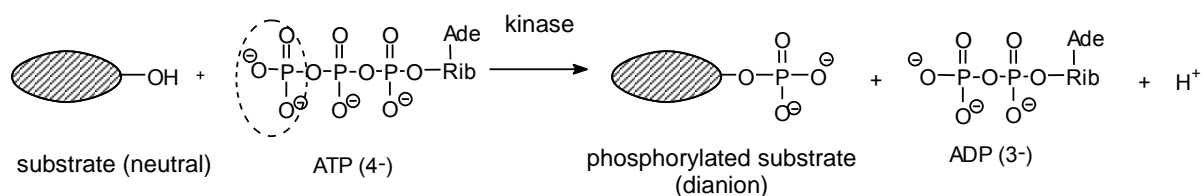
Cyclic nucleotides are a special group of nucleoside monophosphates. In these compounds, phosphate is attached by diester bond between 3'-OH and 5'-OH groups of ribose. Cyclic adenosine 3',5'-monophosphate (**cAMP**) is produced intracellularly from ATP by the catalytic action of the enzyme adenylate cyclase. cAMP belongs to so called second messengers; it mediates the action of various hormones and neurotransmitters in cells in which these signal molecules bound to specific receptors on the outer part of the cytoplasmic membrane. The cAMP then affects the activity of certain types of protein kinases and cell response is induced by phosphorylation of some proteins.

cAMP
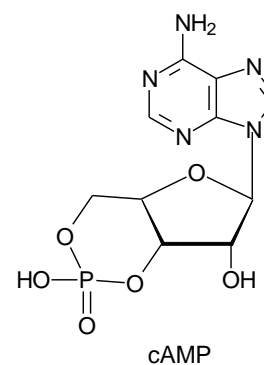
Nucleotide Coenzymes

Nucleotides structurally also include coenzymes of redox reactions NAD^+, $NADP^+$, FMN, and FAD. **Pyridine nucleotides** are the coenzymes **NAD^+** (nicotinamide adenine dinucleotide) and **$NADP^+$** (nicotinamide adenine dinucleotide phosphate). Both these coenzymes of dehydrogenases are dinucleotides. In these compounds, adenosine monophosphate (AMP) is bound by anhydride bond to the phosphate of the second nucleotide whose base is nicotinamide. $NADP^+$ contains one more phosphate group at the C2' hydroxyl of ribose.

Structure of NAD$^+$
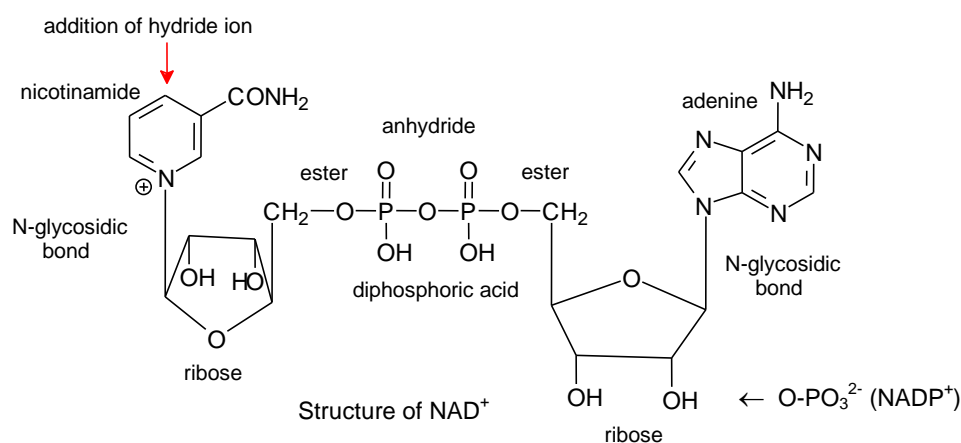
NAD^+ contains a pyridinium ring linked to C1 of ribose; the nitrogen atom is tetravalent with a positive charge (oxidised form of coenzyme). In dehydrogenation reactions, two hydrogen atoms (2H) are removed from the substrate. One is added as a hydride anion (H^-) to the C4 position of the pyridinium ring to produce the electroneutral reduced form NADH. The second hydrogen is released to surroundings as a proton H^+ ($NAD^+ + 2H \rightarrow NADH + H^+$). The pyridine ring loses its aromatic character and the trivalent nitrogen no longer has a positive charge.

part of NAD$^+$
(oxidized form)

part of NADH
(reduced form)

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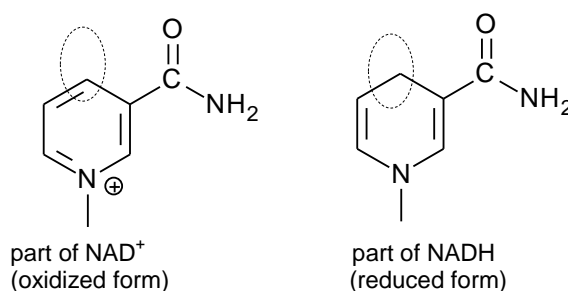
Flavin nucleotides are prosthetic groups of flavin enzymes. They include substituted isoalloxazine, a derivative of benzopteridine, which binds in position N10 a residue of the five carbon sugar alcohol ribitol. The name of this compound is **riboflavin** (vitamin B$_2$, see Chapter 46).

Its phosphorylation produces the cofactor riboflavin 5'-phosphate, called **FMN** (flavin mononucleotide). This name is not precise, because it is not a genuine nucleotide (i.e. *N*-glycoside of ribose); ribitol linkage is not the glycosidic bond. However, this name and abbreviation are very common, because its resemblance with nucleotides is obvious. Most flavoproteins do not contain a "mononucleotide", but the flavin adenine dinucleotide, **FAD**. In FAD, riboflavin phosphate and adenosine monophosphate (AMP) are joined by a phosphoanhydride bond (FAD = FMN + AMP).

Structure of FAD
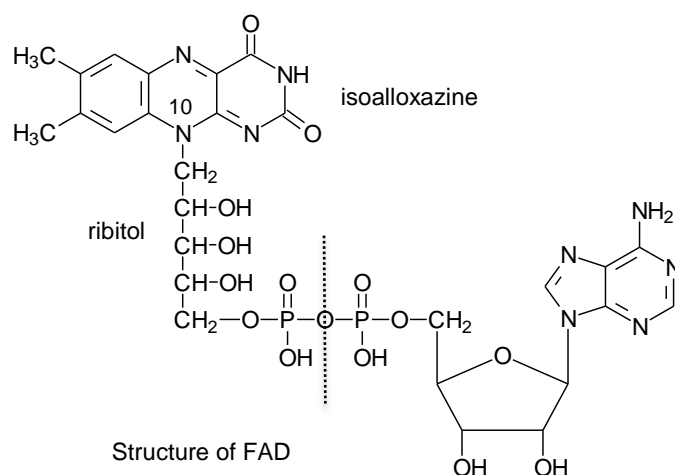
The function of flavin cofactors in the redox reactions (catalysed by flavin dehydrogenases) consists in the transfer of two hydrogen atoms. In both reduced forms, FMNH$_2$, FADH$_2$, the hydrogen atoms are bound in isoalloxazine to the nitrogen atoms N1 and N5. Two conjugated double bonds between N1 and N5 are replaced by one double bond.

oxidized form of FAD   reduced form FADH$_2$
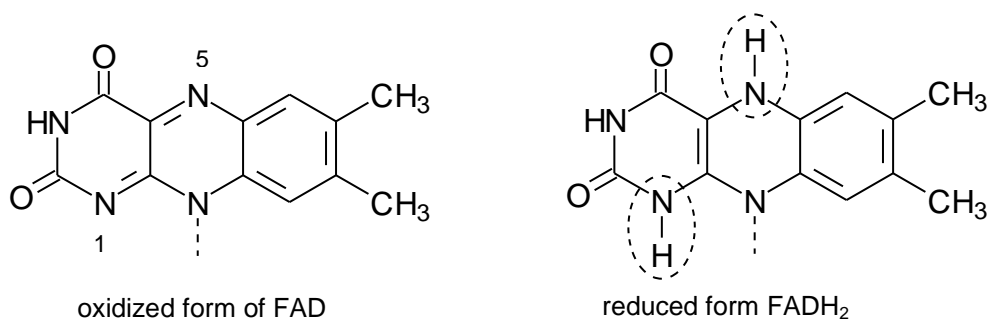
Coenzyme A (CoA-SH) is another important compound which contains the adenine nucleotide. It is the essential cofactor of many enzymatic acetylations; but it is an almost universal transporter of other acyls, e.g., those of fatty and bile acids.

The structure of coenzyme A is rather complicated. It can be divided into adenosine 3',5'-diphosphate and pantetheine phosphate, which are joined by a phosphoanhydride bond. Pantetheine phosphate contains a free -SH group, which can bind an acyl as thioester. CoA-SH is also the prosthetic group of enzymatic complex for the biosynthesis of fatty acids. **Pantetheine** is the growth factor of many microorganisms; it consists of pantoic acid, β-alanine, and cysteamine. The compound, which consists of pantoic acid (2,4-dihydroxy-3,3-dimethylbutyric) and β-alanine, is known as **pantothenic acid**, included in the group of B vitamins (see Chapter 46).
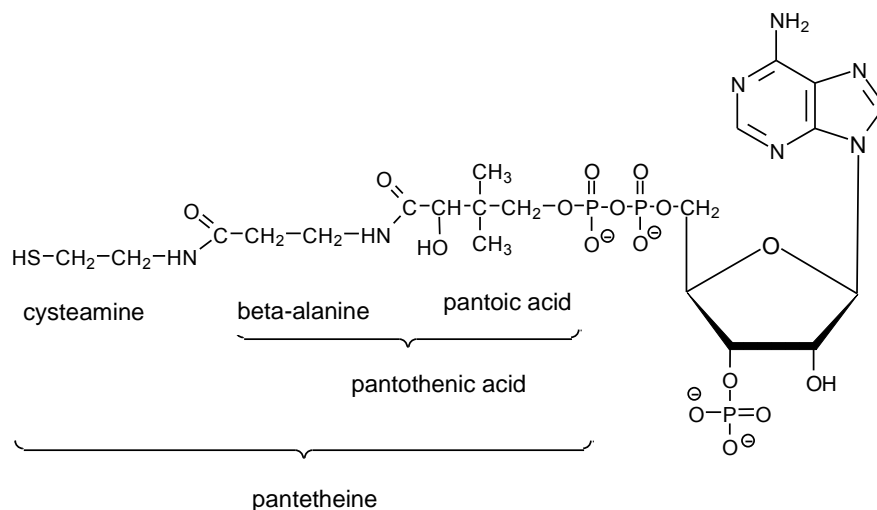
Structure of coenzyme A

Binding acyl to -SH group of coenzyme A produces high-energy thioester **acyl-coenzyme A**, the activated form of acid. Acyls bound to coenzyme A are more easily transformed or transported to other substrates.

Acetyl-CoA (activated acetic acid) is the product of aerobic catabolism of glucose (it is formed by the oxidative decarboxylation of pyruvate), β-oxidation of fatty acids, and breakdown of some amino acids. It is the starting substance for the biosynthesis of fatty acids and cholesterol. Quantitatively the most important reaction, in which acetyl-coenzyme A participates, is the synthesis of citric acid in the citrate cycle.

38 Nucleic Acids

Nucleic acids are polynucleotides made of tens up to millions of nucleotides. Nucleotides are linked by **3',5'-phosphodiester bonds**. A 5'-phosphate group is attached to a 3'-OH group of the following nucleotide. Chains of all nucleic acids are made of regularly sequenced phosphate-pentose groups with purine or pyrimidine bases attached to their anomeric carbons (C1') by *N*-β-glycosidic bonds. Genetic information stored in cells and transcribed to daughter cells is encoded in this specific base sequence of the poly-nucleotide chain; conserving the genetic code is the main function of nucleic acids.

Polynucleotide chains have polarity (direction). Each chain has its 5'-end and 3'-end; there is a free phosphate group at the 5'-end, while on the other side of the chain there is a free hydroxyl group at C3 of a pentose. The sequence of nucleotides in the polynu-cleotide chain (the primary structure) is read from the 5'-end to 3'-end. In cells with a pH of around 7, the phosphoric acid is ionised in diester bonds; nucleic acids are **polyanions**.

5'-end

Part of DNA chain

sequence
–dT-dC-dC-dG-dA-

3'-end
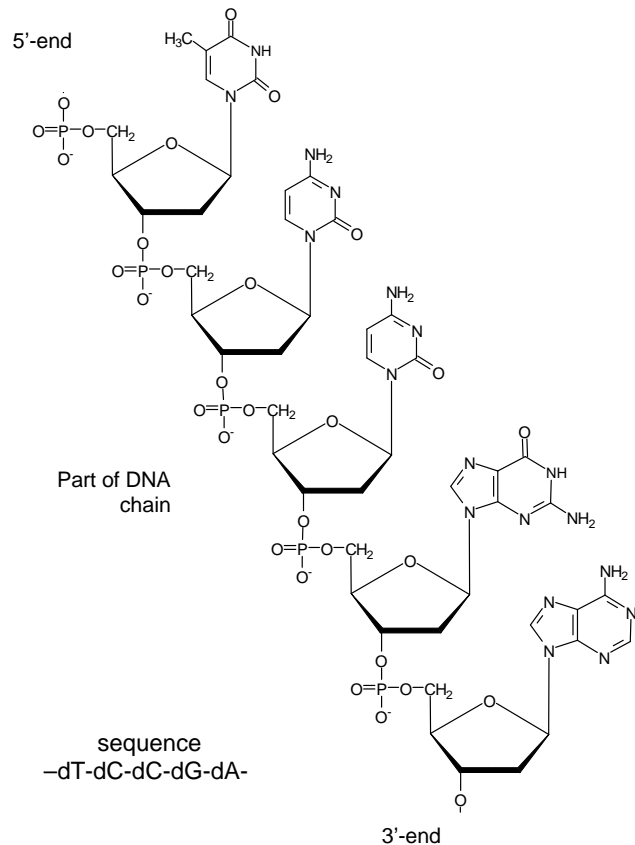
Deoxyribonucleic Acids (DNA)

DNA molecules are carriers of genetic information. Replicated molecules of DNA allow transfor-mation of genetic information into daughter cells (organisms). Parts of DNA carrying genetic infor-mation about protein synthesis are called **structural genes**. In eukaryots, most DNA is located in the nucleus (nuclear DNA, nDNA), while a small portion occurs in mitochondria. In prokaryots, DNA is in cytoplasm.

DNA molecules are extremely long polynucleotides which, besides deoxyribose and phosphate, are made of the purine bases adenine and guanine, and pyrimidine bases cytosine and thymine. Thymine is typical for deoxyribonucleic acids, while ribonucleic acids contain uracil in its place.

DNA molecules are double-stranded (dsDNA); the strands are antiparallel, which means that the direc-tion of bonds is 5'→3' in one strand and 3'→5' in the other strand. Both polynucleotide strands are **complementary** to each other; opposite nucleotides are held by hydrogen bonds between bases.

Hydrogen bonds are formed between adenine – thymine and guanine – cytosine pairs.

thymine-adenine (two H bonds)   cytosine-guanine (three H bonds)
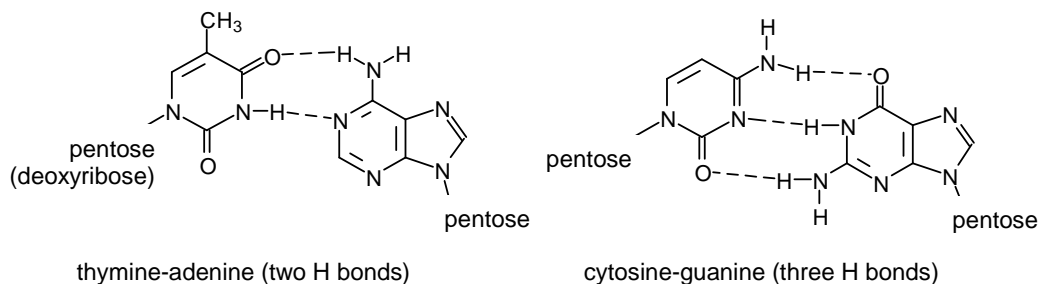
There is always the same amount of purine and pyrimidine bases. The ratio of adenine-thymine pairs to guanine-cytosine pairs varies; usually there are more A-T pairs. DNA molecules in human cell nuclei are extremely long. Each of the 23 chromosome pairs in a diploid cell is made of one double stranded DNA. This DNA molecule is composed of approximately 50-250 million nucleotide pairs.

The two polynucleotide chains are twisted around the longitudinal axis and form a double-helix (Watson and Crick 1953, Nobel Prize 1962). The skeleton of the DNA molecule is formed by diester bonds between deoxyribose and phosphate. Phosphate groups with negative charges protrude from the surface into the space. Purine and pyrimidine bases are oriented inside the double helix. The base planes are oriented perpendicularly to the vertical axis of the DNA molecule.

The deoxyribose plane is oriented perpendicularly to its bases (almost parallel with the axis of the DNA molecule). Base pairs are parallel to allow interactions of their π-electrons. The layout of bases above each other (base-stacking) helps the conformation stability of the double helix. In cells or in solutions under physiological conditions (high degree of hydration and low concentration of salts), the double helix forms the right-handed **B-form** with 10 nucleotide pairs per one turn. On the surface of the B-form of the helix, we can observe a major groove and a

DNA double helix

minor groove. The major one allows interactions of proteins with specific sequences of bases.
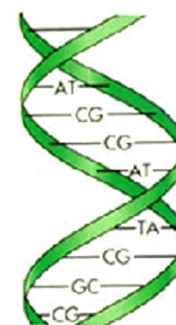
Similarly as with proteins at high temperatures, the double helix is not stable. If we heat DNA in an NaCl solution up to 80-90°C, the hydrogen bonds between the complementary bases are released and the DNA splits into two separate strands (denaturation of DNA). Besides lowering the solution viscosity, denaturation rises the light absorption of the molecule. DNA denaturation is often compared to melting processes when crystal structures are broken down and therefore we talk about DNA melting points which are higher in DNA with more guanine-cytosine pairs. Denaturation of DNA is reversible; renaturation (double helix rearrangement) occurs after slow temperature lowering. A fast temperature decrease does not allow renaturation. **Hybridisation**, connection of complementary or partly complementary strands from different DNA molecules, or DNA and RNA strands, may occur under certain conditions. Newly formed hybrids are either perfect double stranded DNAs (homoduplexes with full complementarity) or hybrids with only partly formed double helix (heteroduplexes). We can also find

heteroduplexes of complementary strands of DNA and RNA. Hybrid molecules are formed *in vivo* (transcription, reverse transcription), or they can be performed *in vitro*.

Mitochondrial DNA (mtDNA) determines the structure of 13 mitochondrial proteins and represents only a small portion of the total DNA in the cell (about 1%). Human mtDNA molecules are also double helices, but they are circulatory and smaller than nuclear DNA. They are probably fragments of genetic material of aerobic organisms, which became cellular organs after long endosymbiosis with the cell and adaptation. This theory is also supported by the fact that mitochondria are capable of self-replication.

Ribonucleic Acids (RNA)

The RNA chain is similar to DNA; 3',5'-phosphodiester bonds connect ribonucleosides. Uracil is found there instead of thymine, and in some RNAs we can find other modified bases. RNA molecules are shorter in comparison to DNA and they are, in animal cells, only single stranded. However, there are some parts which are double stranded – **arms** (if there are antiparallel complementary sequences in the RNA chain.

The synthesis of ribonucleic acids is performed by **transcription**, genetic information is transferred from DNA into RNA. The nucleotide sequence of RNA is formed by transcription of the template strand of DNA with reverse polarisation. The RNA sequence is therefore (with a change of uracil for thymine) the same as the non-transcribed DNA strand. The first products of transcription are called **primary RNA transcripts**. Before they leave the nucleus, primary transcripts undergo many post-transcription modifications. The most important modification is enzymatic cutting, which takes away and digests long parts of the chains (introns). According to their function, there are three types of RNA: messenger RNA (mRNA), ribosomal RNA (rRNA), and transfer RNA (tRNA). RNAs differ by molecule size and the structure of bases. They all have specific functions in protein synthesis.

Messenger RNAs (mRNA) take only a few percent of the total cellular RNA, but they present a very diverse group. The mRNA carries transcribed genetic information from structural genes and serve as a template for protein synthesis on ribosomes. Each protein synthesised in the cell has to have its specific mRNA, which represents a transcript of one gene. The primary structure of the polypeptide chain is defined by the nucleotide sequence of the gene made up by triplets of bases. Each of the 20 coded amino acids is represented in the mRNA by a triplet of bases called **codon**. Codons signalise the starting and ending points of ribosome synthesis and the order of amino acids in the polypeptide. The molecular mass of cytoplasmic mRNA is between 10^5-10^6. There are long modified sequences on the 5′ and mainly on the 3' end that helps to stabilise the molecule and to regulate its function. The biological half time of mRNA in mammal cells varies between tens of minutes up to hours.

Ribosomal RNAs (rRNA) are the most abundant in cells, they form up to 80% of all cellular RNA. They are found in different types which can be distinguished by size, base composition, and specific sedimentation coefficient (unit Svedberg, S). rRNAs are structural compounds of ribosome subunits, on which translation takes place. There are four different types of rRNA in eukaryotic organisms; the small subunit contains one molecule 18S-RNA, the large subunit contains one each of 5S-, 5.8S- and 28S-rRNA. The molecular mass of mRNA corresponds to about 100-5,000 nucleotides and there are many arms and loops made. The biological half time of rRNA is very long compared to mRNA.
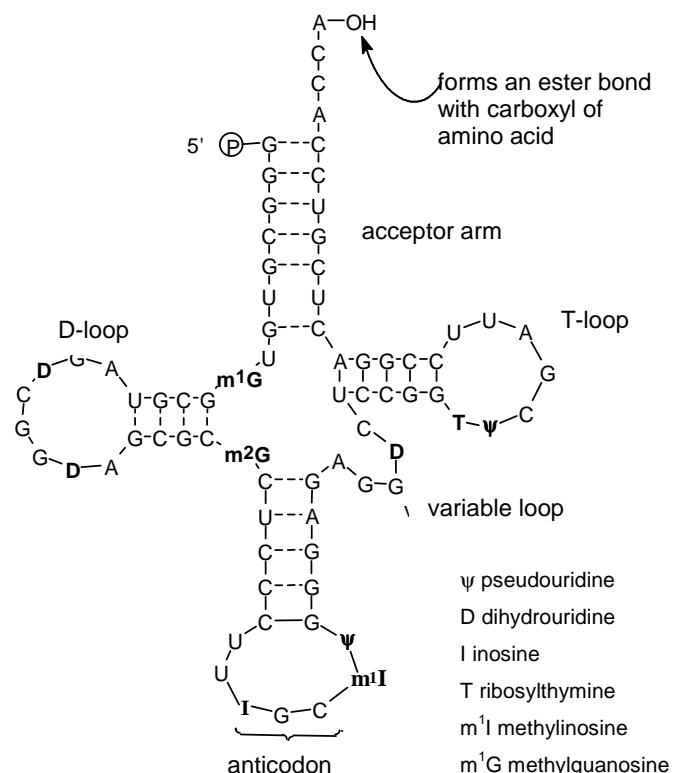
Transfer RNAs (tRNA) are the smallest type of RNA. As like other types of RNA, they are formed by splicing the tRNA precursor. They consist of 60-95 nucleotides. The function of tRNA is to bind a specific amino acid and transfer it to ribosome in the correct order of the polypeptide chain. There are at least 20 different tRNA in each cell, which means there is at least one tRNA for each of the 20 coded amino acids. More than 100 different tRNA are known now. The amount corresponds to the total number of codons for amino acids (61 + 3 termination codons). In all tRNA there is a significant amount of unusual or **minor bases**. Those are chemically modified bases, e.g., methylated (methylguanosine) or hydrogenated (dihydrouridine). Minor bases are necessary for formation of secondary and tertiary structures of tRNA. We suppose that they stabilise (make more resistant to nucleases) the tertiary structure of tRNA and that they are responsible for codon determination and stabilisation of the codon-anticodon interaction. In all types of tRNA there are anti-parallel complementary sequences which allow formation of four short double-helix sequences (arms). On their ends, with one exception, there is a connecting loop made of non-matching nucleotides.

If we draw the secondary structure of tRNA in two dimensions it resembles a cloverleaf. The three-dimensional conformation of the molecule, tertiary structure of tRNA, is more compact; it is formed into a flexible structure, shaped as the letter L. The 5'-end sequence of tRNA forms with its complementary sequence near the 3'-end an **acceptor arm**; the free 3'-end of CCA sequence with 3'-hydroxyl of adenosine forms an **ester bond with the acyl of amino acid**. The correct binding of an amino acid is catalysed by specific enzymes, aminoacyl-tRNA synthetases. If we follow the chain from the acceptor sequence in the 5'→3' direction, the next very short complementary sequence with 8-12 unpaired bases forms a **dihydrouridine** arm. A typical feature of the dihydrouridine loop (D-loop) is two or more dihydrouridine nucleotides in various positions.

The next is the **anticodon** arm with five pairs of bases and a loop with three nucleotides called the **anticodon**. After the anticodon a purine base follows, usually alkylated (methylinosine in the Figure). During **translation** tRNA carrying a corresponding amino acid binds by its anticodon to the complementary codon of mRNA. Hydrogen bonds are formed between chains of opposite polarity.

Following the anticodon region, there is a small variable loop which may be missing sometimes.

The loop can be either unpaired, made of 3-5 nucleotides, or paired with a loop formed by up to 21 nucleotides. The fourth main region is the pseudouridine arm with a loop of seven unpaired bases.

Secondary structure of tRNA

ψ pseudouridine
D dihydrouridine
I inosine
T ribosylthymine
m^{1}I methylinosine
m^{1}G methylguanosine

39 Lipids

Lipids are structurally and functionally a very diverse group of natural compounds of either animal or plant origin. They are esters (or amides) of fatty acids and glycerol (or some higher alcohols, sphingosine). Generally, simple lipids have a lipophilic character, are insoluble in water, soluble in non-polar organic solvents (e.g. toluene, chloroform). Some lipids, called complex lipids, also contain polar groups and thus acquire the polar-nonpolar (amphipathic) character; therefore they are important natural surfactants. Lipids can be classified into two subgroups based on chemical composition: **simple lipids** (acylglycerols, waxes, ceramides) and **complex lipids** (glycerophospholipids, sphingophospholipids, glycosphingolipids).

Fatty acids

Fatty acids (FA) are aliphatic monocarboxylic acids. Almost all fatty acids have an unbranched chain with the even number of carbons, because they are synthesised from two-carbon units, acetyl-coenzyme A (CH_3CO-S-CoA). According to the length of carbon chain, fatty acids are classified as short chain fatty acids with up to 6 carbon atoms, medium chain FA (8-10 C, MCFA), long chain FA (12-20 C, LCFA), and very long chain FA with more than 20 C atoms. According to the number of double bonds, they are distinguished into **saturated fatty acids** (SAFA), **monounsaturated fatty acids** (MUFA), and **polyunsaturated fatty acids** (PUFA). PUFAs are classified according to the position of the terminal double bond relative to the terminal methyl group into two series, i.e. n-3 (ω-3) and n-6 (ω-6) PUFAs, where n (ω) is the number of the carbon atom of the methyl group at the end of the hydrocarbon chain. Double bonds are isolated (in contrast to isoprenoids), separated by a single methylene (-CH_2-) group. Configuration on the double bonds is typically *cis*. Chemical formulas of fatty acids are commonly presented in a simplified way. The abbreviated designation includes the number of carbon atoms, number of double bonds, and locations of the double bonds in parenthesis. For example, α-linolenic acids, is recorded as 18:3(9,12,15), or as 18:3 (*n*-3).
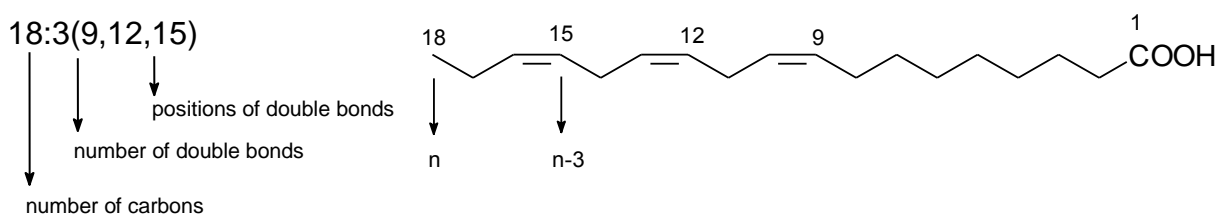
The following table shows the most important fatty acids, their systematic and common names, and also their occurrence.

Selected saturated fatty acids (SAFA)

Abbreviated record	Common name	Systematic name	Occurrence
4:0	butyric	butanoic	milk fat
6:0	caproic	hexanoic	milk fat
12:0	lauric	dodecanoic	coconut fat
14:0	myristic	tetradecanoic	coconut fat
16:0	palmitic	hexadecanoic	nearly all fats
18:0	stearic	octadecanoic	nearly all fats
20:0	arachidic	eicosanoic	nearly all fats
24:0	lignoceric	tetracosanoic	sphingolipids

Selected unsaturated fatty acids (MUFA, PUFA)

Abbreviated record	Series	Common name	Systematic name[a]	Occurrence
16:1(9)	n-7	palmitoleic	hexadec-9-enoic	vegetable oils
18:1(9)	n-9	oleic	octadec-9-enoic	vegetable oils
18:2(9,12)	n-6	linoleic	octadeka-9,12-dienoic	vegetable oils
18:3(9,12,15)	n-3	α-linolenic	octadeka-9,12,15-trienoic	vegetable oils
18:3(6,9,12)	n-6	γ-linolenic	octadeka-6,9,12-trienoic	vegetable oils
20:4(5,8,11,14)	n-6	arachidonic	eicosa-5,8,11,14-tetraenoic	phospholipids
20:5(5,8,11,14,17)	n-3	EPA[b]	eicosa-5,8,11,14,17-pentaenoic	fish oil
22:6(4,7,10,13,16,19)	n-3	DHA[c]	eicosa-4,7,10,13,16,19-hexaenoic	fish oil
24:1(15)	n-9	nervonic	tetracos-15-enoic	sphingolipids

[a] Double bonds have *all-cis*-configurations. [b]Eicosapentaenoic acid. [c]Docosahexaenoic acid.

Unsaturated fatty acids are important for the chemical and physical properties of lipids. It is well known that the more double bonds (*cis*-configuration) in a lipid, the more liquid character the lipid has: the melting point of triacylglycerols becomes lower. A *cis*-double bond in the unsaturated FA introduces a kink in the shape which makes it more difficult to pack the molecules together in crystalline lattice. Dispersion interactions are limited; fats with many MUFA, PUFA are liquid. The chains of saturated fatty acids are fully extended and can pack closely together by the action of dispersion forces, making these fats solid at room temperature (see the scheme below).
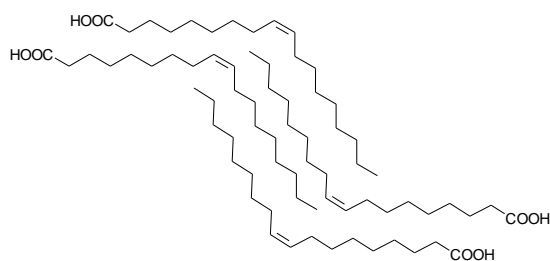
Model arrangement of oleic acid molecules   Model arrangement of stearic acid molecules

Most unsaturated fatty acids in lipids are *cis*-acids, *trans*-isomers are exceptional, e.g., milk fat of ruminants contains 3-10% of them depending on the way of feeding. *Trans* fatty acids (tFA) are formed to a greater extent during hardening vegetable oils by partial catalytic hydrogenation of unsaturated

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fatty acids. Today, new manufacturing processes are introduced that do not elevate the portion of tFA. *Trans* fatty acids are considered to be an undesirable component of the diet, because their higher intake increases blood cholesterol and the risk of coronary heart disease (CHD). The World Health Organization recommends a maximum intake of *trans* fatty acids up to 1% of total energy intake. Polyunsaturated fatty acids easily undergo auto-oxidation. The oxidation on double bonds is the primary cause of rancidification of fats in foods.

Rancidification

Rancidification of fats is a complex of reactions which begins with oxidation by air oxygen, initiated either by light or by trace amount of metal ions. PUFA are much more reactive than MUFA. Therefore, oils with high content of polyunsaturated fatty acids should not be used for frying or deep frying because of the little resistance against auto-oxidation. If food contains water and fat, microorganisms also take part in the process and hydrolyse ester bonds. Rancid fats have a typical unpleasant smell and taste. Foods containing rancid fat are not edible and need to be thrown away after the very first indications of fats becoming rancid. Fats can be protected from rancidification by storing them in a place with little exposure to oxygen or free radicals, at a low temperature, and away from light. The removal of water and air by heating (rendering) or drying, and thermal inactivation of cell enzymes and microorganisms can also increase the resistance of lipids to rancidification. Antioxidants are often added to fat-containing foods in order to retard the development of rancidity due to oxidation. Natural lipophilic antioxidants include tocopherols, β-carotene or some synthetic antioxidants.

Lipoperoxidation

Attack by oxygen is also frequent *in vivo* – lipoperoxidation. Since polyunsaturated fatty acids are often components of membrane phospholipids or circulating lipoproteins, oxidation can lead to changes of their properties. PUFA are the most susceptible to oxidative damage. The process is usually initiated by hydroxyl radical ·OH or other reactive oxygen species, the primary product is often a lipoperoxide. In the continuation of this process, secondary products are formed, e.g., cyclic peroxides, various products of peroxides decomposition like short carboxylic acids, pentane, ethane, and various aldehydes, e.g., malondialdehyde (MDA). Its determination in blood plasma can be used to evaluate the degree of lipoperoxidation and thus the oxidative damage of the body.

Essential polyunsaturated fatty acids

Unsaturated fatty acids are formed from saturated fatty acids by **desaturation**, which is catalysed by oxygenases (not by dehydrogenases). Desaturation of fatty acids in humans can occur only in positions between C-9 and C-1 in the carbon chain. No double bonds beyond C-10 can be created. Formation of double bonds in the positions *n*-3 and *n*-6 is only possible in plants. However, the *n*-3 and *n*-6 series of fatty acids are required for the synthesis of eicosanoids in man. These *n*-3 and *n*-6 fatty acids must be present in the diet or synthesised from other fatty acids in the diet. Essential fatty acids are **linoleic** and **α-linolenic acid**. Linoleic acid (*n*-6) is converted by a series of elongation and desaturation reactions to arachidonic acid and analogically α-linolenic acid (*n*-3) into eicosapentaenoic and eicosahexaenoic acid.

The main source of essential fatty acids are plant oils, in our diet mainly soya, sun-flower, and rape seed. The content of essential fatty acids in olive oil is significantly lower. Other important sources of polyunsaturated fatty acid, especially eicosapentaenoic acid, are fish oils. Long deficiency of essential PUFA (e.g. during parenteral nutrition) is typically manifested by a worse resistance to infections, slow wound healing, ichthyotic skin, and other problems.

Eicosanoids

Eicosanoids originate from polyunsaturated 20-carbon fatty acids (from Greek είκοσι twenty), series *n*-6 or *n*-3. These acids are ester-bound in membrane phospholipids. Eicosanoids are divided into several groups, e.g., prostanoids, leukotrienes, lipoxins, hepoxilins, etc.

Prostanoids include prostaglandins, prostacyclins, and thromboxanes. Prostanoids can be considered as the derivatives of hypothetical **prostanoic acid**. The middle part of its C_{20} skeleton consists of a cyclopentane ring to which two aliphatic chains are connected: the C_7 chain with the carboxyl group and the C_8 alkyl. The hydroxyl in the position C-15 is typical for all prostanoids.

arachidonic acid   prostanoic acid
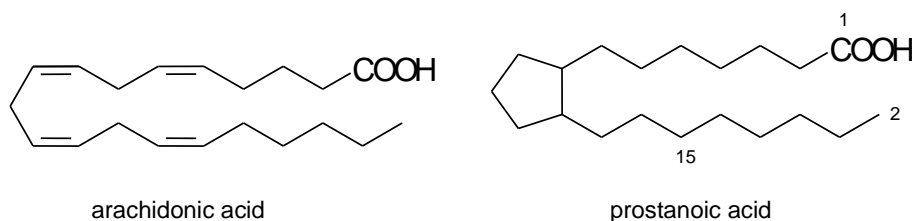
Depending on the structure (the presence of substituents and arrangement of the five-membered ring), the individual prostanoids are indicated by a capital letter and a numerical index that indicates the number of double bonds in the chain attached to the ring (e.g PGE_2, PGI_3). The number of double bonds in the structure of eicosanoids depends on the number of double bonds in the PUFA precursor, e.g., arachidonic acid provides prostanoids with two double bonds and leukotrienes with four double bonds. Thromboxanes have an oxygen atom incorporated in the cyclopentane ring forming the oxane ring; prostacyclins have an additional tetrahydrofuran ring. The following partial formulas show the differences in the five-membered ring of individual prostaglandins (PGE, PGF, PGD).

prostanoid precursor   PGE   PGF   PGD   thromboxane
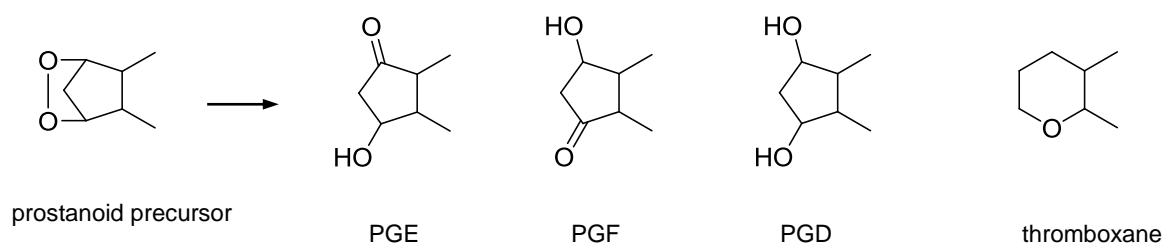
In leukocytes or mast cells, leukotrienes (LT) are formed from precursor PUFAs. Contrary to prostanoids they are not cyclic, contain two hydroxyl groups at positions 5 and 12, and three conjugated double bonds. Like prostanoids, depending on its structure, they are labelled by capital letters and a numerical index that reflects the total number of double bonds. Leukotrienes produced from arachidonic acid contain four double bonds, such as LTB_4 (see formula below). Some leukotrienes contain a peptide (glutathione, cysteinylglycine) or amino acid cysteine. Lipoxins possess four conjugated double bonds and three hydroxyl groups.

leukotriene LTB_4
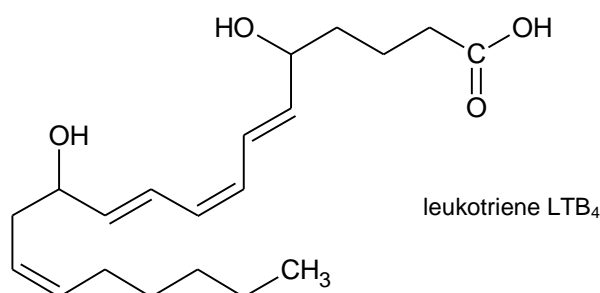
Biological functions of eicosanoids

Eicosanoids are very effective regulators of cellular functions. They function as local hormones with limited actions. After a release from cells, they act in their immediate environment (paracrine effect) or on the cell itself (autocrine effect). They are effective in minute concentrations and have a very short half-life (minutes or tens of seconds). Eicosanoids have various, sometimes even antagonistic effects, they also modulate (increase or decrease) the effects of some hormones. They play a role in the **inflammatory reaction** caused by infectious agents, allergens or other tissue damage. They reduce blood loss at the site of damage and some accompanying signs of inflammation (pain, swelling as a result of changes in vascular permeability, fever). Among other effects, they act on smooth muscles, especially the uterus, intestines, and blood vessels, and also the kidneys functions (stimulate water and sodium excretion). Leukotrienes, especially LTC_4 and LTD_4, play an important role in the origin of bronchial asthma. The table below shows some data on eicosanoids.

Eicosanoid	Structure type	Place of origin	Main effects
PGE_2	prostaglandin	almost all cells	inflammatory reaction, inhibitor of HCl output
$PGF_{2\alpha}$	prostaglandin	almost all cells	contractions of the uterine muscle, vasoconstriction
PGI_2	prostacyclin	endothelial cells	vasodilatation, prevention of thrombo-aggregation
TXA_2	thromboxane	thrombocytes	aggregation of platelets, vasoconstriction
LTC_4	leukotriene	leukocytes	bronchoconstriction
LTB_4	leukotriene	leukocytes	leukocyte chemotaxis, activation of macrophages

Alcohols as lipid components

Glycerol (glycerolum, propane-1,2,3-triol) is a three carbon alcohol; it is a hygroscopic liquid, miscible with water. Its polar character becomes fully suppressed by the formation of three ester bonds.

If two or three different acyl groups are attached to the primary alcohol hydroxyls, the molecule becomes chiral. In lipid chemistry, stereochemical numbering (*sn*) was therefore introduced. If we draw the Fischer projection of glycerol in the L-configuration (carbon chain vertical, C-2 hydroxyl on the left), the upper carbon is then C1.

$$1\;CH_2OH$$
$$HO-C-H$$
$$3\;CH_2OH$$
sn-glycerol
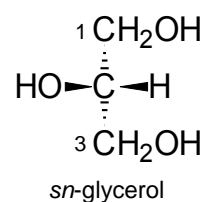
Sphingosine (2-aminooctadec-4-ene-1,3-diol) is unsaturated C_{18} aminoalcohol. Long chain fatty acids form an amide bond between their carboxyl and amino group at C2. The nonpolar 16-carbon chain of sphingosine comes from palmitate, atoms C1 and C2 originate from decarboxylated amino acid serine.
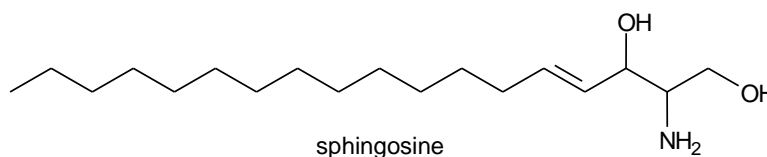
sphingosine

Ceramides (*N*-acylsphingosines) are amides of fatty acids and amino alcohol sphingosine; they are the base for all sphingolipids. Long chain fatty acids attached by an amide bond to sphingosine can be either saturated or unsaturated, typically lignoceric (24:0), 2-hydroxylignoceric (cerebronic), and nervonic 24:1(15) acids. Free ceramides are only found in small amounts in animal cells as intermediates of biosynthesis or degradation of sphingophospholipids and glycosphingolipids.

ceramide
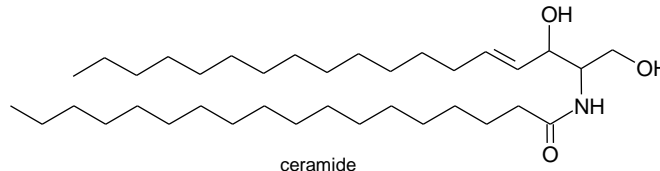
Fatty alcohols are primary alcohols with a long aliphatic, usually saturated chain and an even number of carbon atoms (at least 10). They can be considered as products of fatty acid reduction, e.g., dodecanol (reduction of lauric acid), octadecanol (stearic acid), hexacosanol (cerotic acid). Fatty alcohols are used as non-ionic emulsifiers for the preparation of creams and ointments in dermatology and cosmetics. Octadecanol is a good example (alcohol stearylicus).

Simple lipids

are composed only of fatty acids and alcohol. **Triacylglycerols** are esters of glycerol and fatty acids; they are the main components of naturally occurring fats and oils. They exist always as a mixture of various molecular species of triacylglycerols. The acyl residues in the molecule of triacylglycerols are typically not identical; usually there are two or three different residues in one molecule that vary by chain length and degree of saturation. Their positions are not random; unsaturated fatty acids are primarily bonded to the C-2 atom. They are considered as *O*-acylderivates of glycerol, location of acyl is marked according to stereochemical numbering (*sn*).

1,3-distearoyl-2-oleoyl-*sn*-glycerol
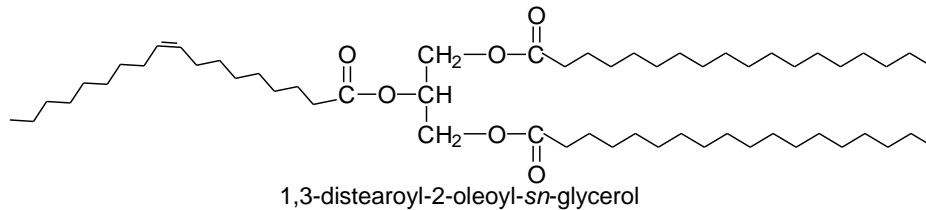
Properties. Triacylglycerols are markedly **nonpolar** compounds, insoluble in water. By shaking oil with water the emulsions are formed. For very fine emulsification of lipids, e.g., in the small intestine, an emulsifier is needed. Natural surfactants in the intestine include bile acids, phospholipids contained in bile, and anions of fatty acids released from dietary lipids by the action of lipase. Milk is another example of a stable emulsion; milk fat is dispersed in small droplets, stabilised by proteins and phospholipids. Chemical properties of triacylglycerols are determined partly by the properties of fatty acids, especially unsaturated (peroxidation of lipids, hydrogenation of double bonds), and partly by common properties of esters.

Hydrolysis of **an** ester bond of triacylglycerols leads to the release of their components. It is performed by heating with mineral acids; glycerol and a mixture of fatty acids are the products. Another method is the hydrolysis of triacylglycerols by boiling with alkaline hydroxides. As a result glycerol and **soap** (a mixture of salts of fatty acids) are obtained. In aqueous solutions, sodium and potassium soaps dissociate to anions of very weak acids (pK_A 9-10), therefore soap solutions react as weakly alkaline due to hydrolysis. Calcium ions precipitate calcium soaps from their solutions (soap precipitation in hard water). Soaps are **anionic tensides**, they form colloidal micellar solutions. The hydrolysis of lipids in the body is catalysed by enzyme lipases (pancreatic lipase, lipoprotein lipase).

Relationship of the triacylglycerol structure to fat properties. Properties of natural fats are derived from their fatty acid composition. The average molecular mass of a mixture of triacylglycerols implies that palmitic, oleic, and stearic acids are the main components. The physical properties of fats depend on the proportion of unsaturated fatty acids. The higher the PUFA/SAFA ratio, the lower the melting point of the triacylglycerols mixture. We distinguish solid fats (e.g. beef or mutton tallow), semisolid fats (pork lard, chicken lard, butter, cocoa butter, and palm oil), and liquid fats with a high proportion of unsaturated FA (plant oils, fish oils). Human fat with a melting point of 17-18°C ranks among oils.

Nutritional value of fats. Compared to other nutrients (saccharides, proteins), there is a relatively high content of hydrogen in fatty acid molecules. Therefore lipids release more chemical energy during catabolism. Fatty acids are ingested in foods as a part of fats and oils. Triacylglycerols containing unsaturated fatty acids should dominate in the diet. Fatty acids with short or medium chains found in milk fat are digested more easily than long chain fatty acids. Fatty acids are synthesised in the organism from acetyl-coenzyme A. Exceptions are the essential fatty acids linoleic and α-linolenic that must be taken in food. Therefore it is recommended to increase the consumption of plant oils and fish oil, and to avoid hardened fats. The amount of triacylglycerols in food should cover 30% of the total energy requirements.

Selected kitchen fats and the average composition of their fatty acids

Fat	SAFA (%)	MUFA (%)	ω−3 PUFA (%)	ω−6 PUFA (%)
Rape seed oil	10	60	10	20
Sunflower oil	10	25	1	64
Soybean oil	16	24	7	53
Olive oil	15	75	1	9
Coconut oil	90	7	0	3
Palm kernel oil	82	14	0	4
Palm oil	50	40	1	9
Lard	43	48	1	8
Butter*	67	28	0	2
Poultry fat	42	37	1	20
Linseed oil	10	12	60	18
Fish oil	28	52	15	5
Cocoa butter	60	38	0	2

*Contains ~ 80% of fat. The remainder of the 100% of FA is made by ~ 3% of *trans*-FA.

Plant oils are an important source of essential fatty acid when used without heat processing. The choice of fat for high temperature processing (frying, fritting) requires special care. High temperature leads to pero-xidation of PUFA and formation of reactive oxygen species (ROS) and other harmful products. Only oleic acid predominating in olive and rape seed oil (see table) is relatively resistant to high temperature. For high temperature processing the best choice are hardened kitchen fats. Oils with a high content of PUFA (sunflower oil, soya oil) are absolutely unsuitable for fritting and frying. During high temperature exposure, carcinogenic compounds are formed. It is therefore necessary to use special oils for fritting with high antioxidative stability, containing mainly oleic acid.

Complex lipids
Their molecules contain two nonpolar tails (formed by two fatty acids and/or sphingosine), to which one polar head is attached. All phospholipids therefore belong to natural tensides. Their overall polarity has a wide range, from the least polar or neutral glycolipids up to ionised dipolar and acidic phospholipids and acidic glycolipids. They have various functions in living organisms. The most significant role is the formation of the phospholipid bilayer of all membranes.

Phospholipids contain phosphoric acid that forms two ester bonds; one with the alcohol group of substituted glycerol or sphingosine, the second binds another component, most commonly choline. The phosphate group has a negative charge at a physiologic pH. If the other component is a nitrogen base, the phospholipid is **dipolar (amphoteric)** – it has a positive charge on the nitrogen. If the component is neutral or acidic, it is an acidic phospholipid. The polar-nonpolar character of phospholipids is important for stabilisation of the phase interface and properties of membranes. At a phase interface,

membranes are oriented with a hydrophilic end into the water phase and with the hydrophobic end to the nonpolar phase (air, lipids or other nonpolar molecules).

two nonpolar tails
(interaction with hydrophobic phase)

one polar head
(interaction with water phase)

phosphatidylcholine
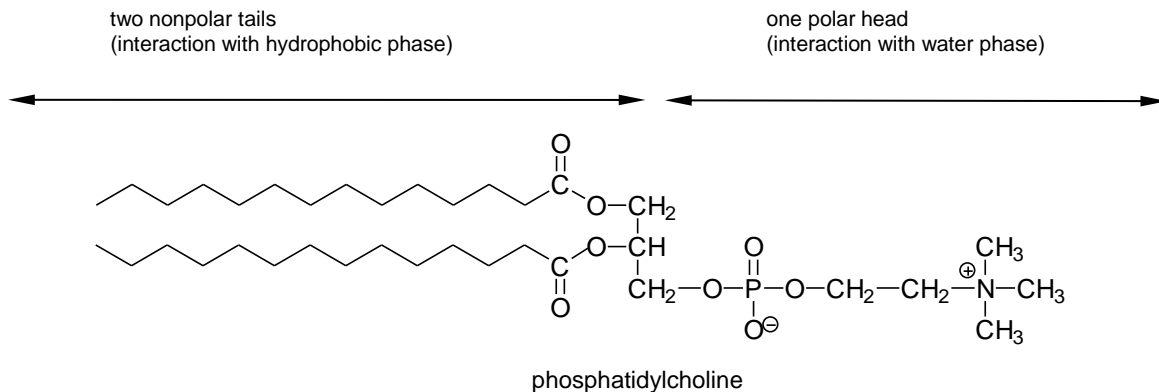
This phenomenon is the reason for the formation of a **monomolecular layer** of phospholipids on the surface of water, alveolar epithelium (pulmonary surfactant), lipoprotein particles, etc. Hydrated phospholipids have a tendency to form spherical micelles or flat lamellas. Under certain conditions flat **bilayers**, biomembranes, are formed.

Glycerophospholipids. The principal structure of glycerophospholipids is **phosphatidic acid** (1,2-diacyl-*sn*-glycerol-3-phosphoric acid), that is phosphoric ester of 1,2-diacylglycerol. In position 2, unsaturated fatty acids are typically located. The ester bonds in glycerophospholipids are cleaved hydrolytically by enzymes phospholipases.

phosphatidic acid (with a marked acyl – phosphatidyl)
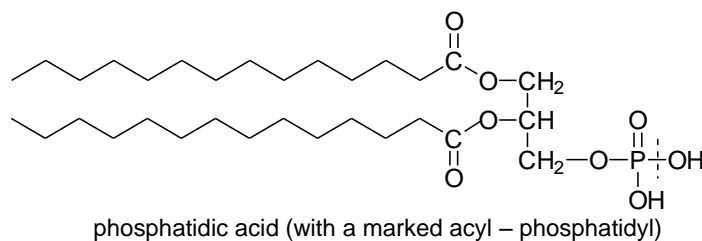
Phosphatidylcholines and **phosphatidylethanolamines** are dipolar phospholipids, the main components of cell membranes and membranes of cellular organelles. Dipalmitoylphosphatidylcholine is the main component of pulmonary surfactant, secreted by the epithelial cells of pulmonary alveoli. It protects alveolus against collapsing during exhalation and thereby makes the inhalation of air into alveoli easier.

Phosphatidylserines are acidic phospholipids; they have two negative and one positive charge.

Phosphatidylinositols (PI) contain *myo*-inositol, a cyclic alcohol with six hydroxyl groups. Small amounts of phosphatidylinositols can be found in all plasmatic membranes. In a great part, mainly on the inside of cell membranes, inositol is further phosphorylated to **phosphatidylinositol 4-phosphate (PIP)** and **phosphatidylinositol 4,5-bisphosphate (PIP$_2$)**.

The hydrolysis of PIP$_2$ (catalysed by phospholipase C) provides two signal molecules (second messengers): **inositol 1,4,5-trisphosphate (IP$_3$)** and **diacylglycerol (DAG),** which change the intracellular concentration of Ca^{2+} ions.

phosphatidylethanolamine

phosphatidylserine

phosphatidylinositol (PI)
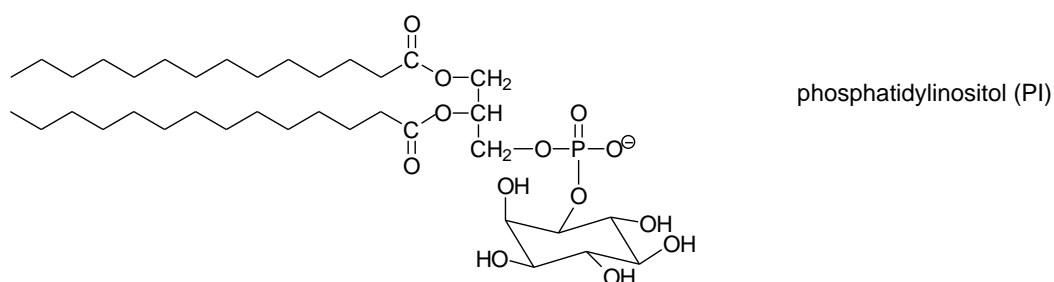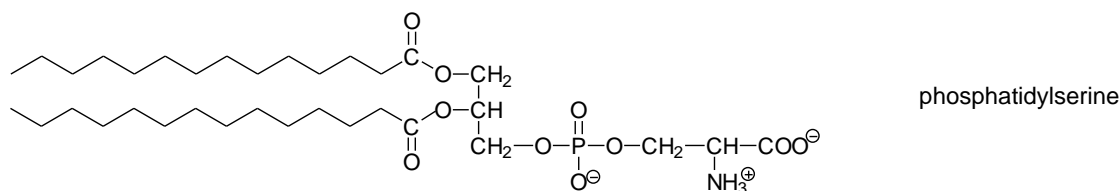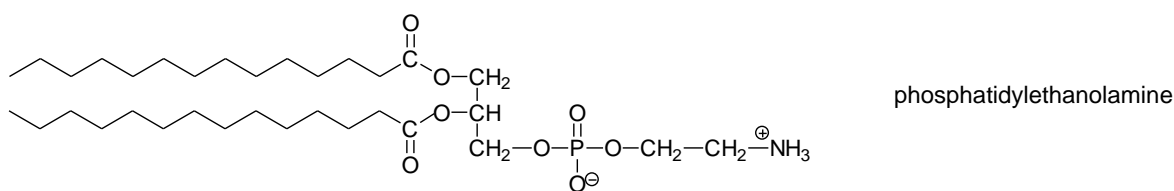
Phosphatidylglycerols of different types belong among the acidic phospholipids and are mainly relevant to plants. One of these types, characteristic for the plasma membrane of bacteria and inner mitochondrial membrane of all eukaryotic cells, is **cardiolipin** (1,3-bisphosphatidylglycerol). It is formed by an ester linkage of phosphatidic acid to the second -CH$_2$OH group of phosphatidylglycerol; it is therefore diphosphatidylglycerol.

cardiolipin
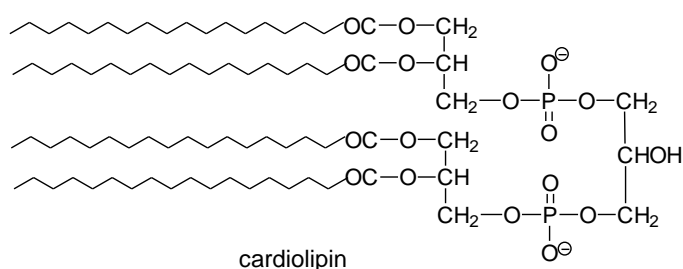
A very important group of glycerophospholipids are **plasmalogens**. Instead of one acyl, they have an ether bond to 1-alkenyl. By hydrolysis of this bond, enol is released and immediately tautomerised to aldehyde. They are one of the main groups of mitochondrial lipids (up to 50% of all phospholipids in mitochondrial cells of cardiac muscle), highly resistant against peroxidation.

ether bond

alkenyl

acyl

plasmalogen

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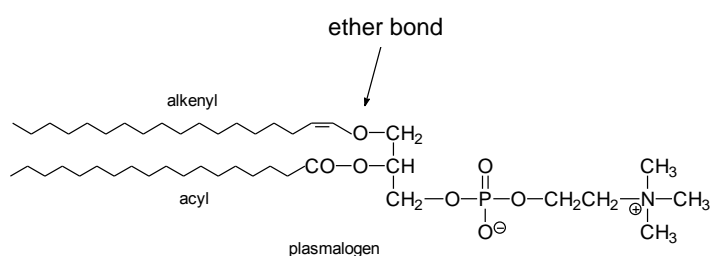
Sphingolipids

The base of their structure is ceramide, *N*-acylsphingosine. There are two types of sphingolipids: sphingophospholipids and glycosphingolipids.

Sphingophospholipids

have phosphoric acid (phosphate) attached to the primary alcohol group of sphingosine.
In **sphingomyelin**, choline is bound as an ester to phosphate.
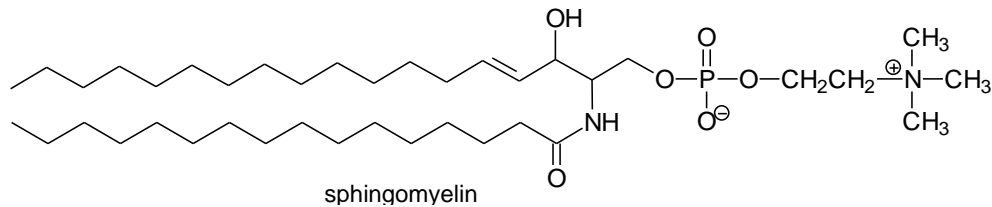
sphingomyelin

Sphingomyelins belong among dipolar (amphotheric) phospholipids. Even though their structures differ from glycerophospholipids, they have a similar shape and physico-chemical properties. Sphingomyelins are the main type of phospholipids in the myelin sheet of axons of neural cells, which gave them their group name. They are regular components of plasma membranes of almost all cell types.

Glycosphingolipids

are polar-nonpolar complex lipids. The saccharide component, monosaccharide or oligosaccharide (polar part of the molecule) is glycosidically linked to the primary alcohol group of ceramide. The saccharide part may contain sialic acids (which are *N*- or *O*-acyl derivatives of neuraminic acid) or may be esterified by sulfate. Glycolipid then becomes acidic and carries negative charges at the physiological pH.

Glycolipids are common constituents of the outer layer of the cytoplasmic membrane of eukaryotic cells and represent there a kind of biological information. Their saccharide units together with glycoproteins take part in the formation of surface **glycocalyx**, which plays many roles in biological processes. For example, glycocalyx is necessary for intercellular contacts or binding extracellular structures. Galactose residues in membranes of epithelial cells lining the colon can capture bacteria from normal intestinal microflora or also pathogenic species.

Glycosphingolipids are subdivided into two groups: neutral and acidic glycolipids.

Neutral glycolipids are divided into cerebrosides and globosides.

Cerebrosides are monoglycosylceramides. The monosaccharide part is either galactose (galacto-cerebrosides, abundant in white matter, around 15% of all lipids of myelin) or glucose (glucocerebro-sides of other tissues). Fatty acids in cerebrosides are of various kinds, usually 24-carbon lignoceric, nervonic, and 2-hydroxynervonic acids.

galactosylceramide
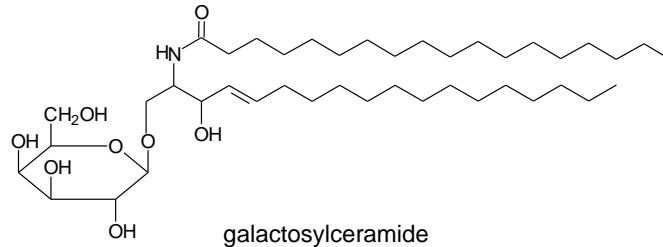
Globosides is an old name for neutral **oligoglycosylceramides**. The saccharide part can vary from disaccharides up to branched oligosaccharides composed of more than ten glycosyl units. Among those *N*-acetylgalactosamine is regularly found.

Acidic glycosphingolipids are sulfatides and gangliosides.

Sulfatides (sulfoglycosphingolipids) are cerebrosides. A monosaccharide residue is esterified by sulfate at one of the hydroxyls (often at C-3). The myelin of oligodendrocytes and the glomerular basement membrane of kidney are mainly sulfogalactosphingolipids.

Gangliosides (sialoglycosphingolipids) are oligoglycosylceramides. Their oligosaccharide part (usually glucose, galactose, *N*-acetylgalactosamine) always contains 1–4 residues of sialic acids. They can be found in the outer part of cytoplasmic membrane of various types of cells, mainly in basal ganglia of neurons.
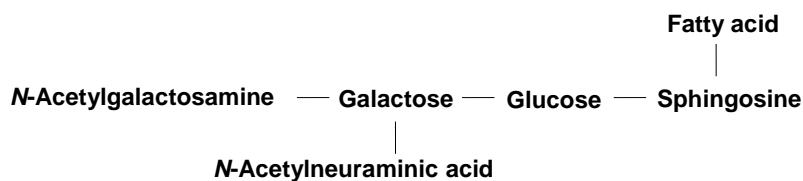
Scheme of ganglioside components

40 Terpenes

Terpenes (isoprenoids) are compounds of various structural types, mainly of plant origin. Their common characteristic is the carbon skeleton of the molecule that consists of five-carbon (C_5) branched building units called **isoprene units** (saturated correspond to isopentane).
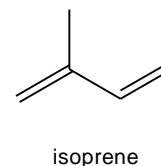
isoprene

After joining isoprene units into bigger molecules, some double bonds always disappear or are transferred. Some biochemically important compounds contain an isoprene chain of variable length, e.g., ubiquinone (coenzyme Q), phylloquinone (vitamin K), tocopherol (vitamin E), and some membrane proteins. The isoprene chain gives them a lipophilic character; therefore they are present in the phospholipid bi-layer of biomembranes. According to the character of the carbon chain it is possible to distinguish aliphatic, cyclic, saturated or unsaturated terpenes. Many of them contain oxygen groups. Terpenes are divided in six groups according to the number of isoprene (C_5) units: mono- (C_{10}), sesqui- (C_{15}), di- (C_{20}), tri- (C_{30}), tetra- (C_{40}) and polyterpenes ($C_{>5000}$).

An example of **monoterpene** is alcohol **menthol**, the main component of ethereal oils of peppermint (*Mentha piperita*). Menthol has anti-itching properties and evokes a sensation of coolness on the mucous membranes; it is added to liquid powders, ointments, creams, toothpastes, mouthwashes, etc. **Camphora**, a bicyclic ketone, is used as an auxiliary remedy. It irritates the skin slightly and evokes hyperaemia, and therefore is often used as a part of massaging and other remedies, e.g., for treating pressure and bed sores.

menthol   camphora
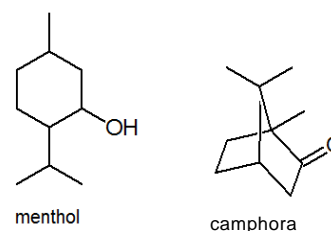
A representative of aliphatic **diterpenes** is alcohol **phytol**, which is bound in chlorophylls. The phytol chain is also a part of **phylloquinone** (vitamin K_1). Diterpenes also include compounds with biological activity of vitamin A called retinoids (see below).

phytol
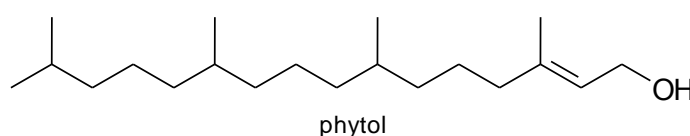
A **triterpene** example is a hydrocarbon **squalene**, an intermediate of cholesterol biosynthesis. It is present in almost all animal fats and oils.

squalene
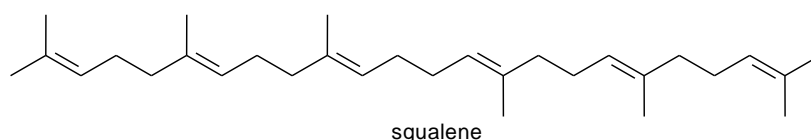
Important **tetraterpenes** are natural pigment **carotenoids**, consisting of eight isoprene units. Unlike other terpenes their molecules have an extensive system of **conjugated** double bonds, which determines their colour. Carotenoids are present in the leaves of all green plants, in chloroplast membranes, alongside with chlorophylls. They are pigments of carrot roots, tomatoes, peppers, oranges, rowanber-

ry, and maize. All carotenoids are very non-polar, lipophilic compounds. Dissolved in lipids they cause their yellow colour, e.g., egg yolk, butter, tissue fat, corpus luteum. Carotenoids are either orange to red coloured hydrocarbons **carotenes** or yellow oxo derivative **xanthophylls**.

The most important is **β-carotene**, which has a polyene chain (with all-*trans*-configuration on double bonds), ended on both ends with cyclohexene, so called β-ionone ring. In animal organisms, β-carotene has two functions. It is a **lipophilic antioxidant** (together with tocopherols). It prevents spontaneous oxidation of lipophilic compounds, and terminates chain reactions of free radicals during lipoperoxidation. The oxidative splitting of the double bond in the symmetry axis of β-carotene affords retinol. β-Carotene is considered as **provitamin A**. Other carotenes, if they contain only one β-ionone ring, have lower provitamin activity.
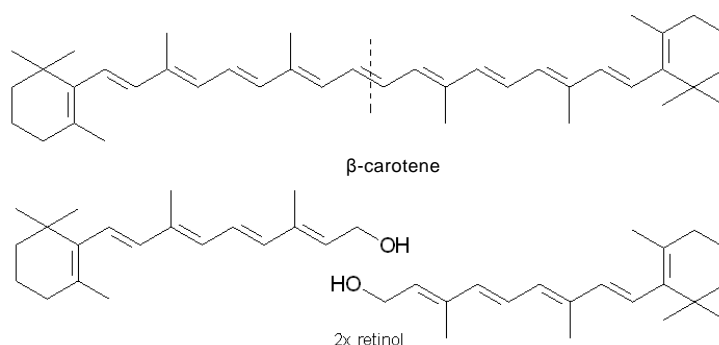
Diterpenes (C_{20}) with a β-ionone ring, which originate from carotenes, are **retinoids**. If they exhibit biological activities, they are called vitamin A (**retinol**). Two products of its oxidation are **retinal** and **retinoic acid**. Liver contains a reserve of retinol in the form of its esters. After ester hydrolysis retinol is transported by blood bound to a specific glycoprotein (RBP, retinol binding protein), retinoic acid to albumin, and in tissue they penetrate into cells. Conversions of retinol to retinal, catalysed by dehydrogenases, are reversible, retinoate is not reduced. Retinal, after isomerisation to 11-*cis*-retinal, becomes a photosensitive component of the eye pigment rhodopsin. Retinoate participates in the gene expression control, supports growth of young individuals, normal reproductive functions, differentiation of tissues, immune system, and other processes.
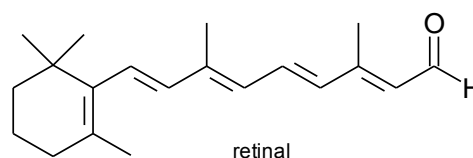
In animals and plants, a vital role is played by polyisoprene alcohol **dolichol**. It is contained in the phospholipid bilayer of membranes of endoplasmic reticulum and its polar alcohol group is involved in biosynthesis of the carbohydrate part of *N*-glycoproteins.
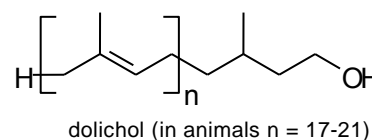
Natural rubber, a polyisoprene hydrocarbon, is present in the form of colloid dispersion in the milky sap (latex) of the rubber tree. Double bonds of polyene chains in rubber have the configuration *cis*. Elastic natural rubber extracted from the latex of certain rubber trees is the most important raw material for rubber production. The *trans* isomer of natural rubber, **gutta-percha**, is less elastic. It is used in dentistry as a filling material for filling root canals.

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41 Steroids

Steroids, widely spread in eukaryotic cells, originate from isoprenoids. Intermediates of their biosynthesis are aliphatic triterpene **squalene**, and tetracyclic triterpene alcohol **lanosterol**.

Steroids are mostly very hydrophobic. The 17 C skeleton is made from condensated alicyclic rings, perhydrocyclopenta[*a*]phenanthrene, called **sterane**. Rings of the steroid skeleton are indicated with letters A to D; their carbons are numbered in a specific manner.

sterane   5α-gonane (A/B trans)   5β-gonane (A/B) cis
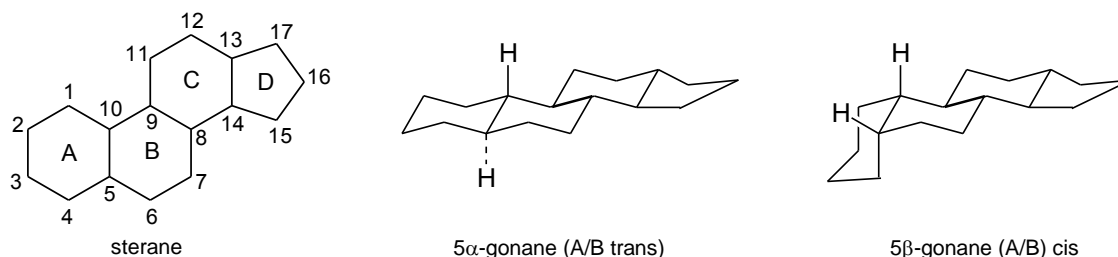
This planar presentation somewhat conceals the spatial arrangement. Three cyclohexane rings always make chair conformations, and even the forth cyclopentane ring is not planar due to the deformation of valence angles. Each of three connections can have the configuration *cis* or *trans*. In most natural steroids, the connection between rings B/C and C/D has a *trans* configuration and the bond from C10 aims above the plane. Steroids can be considered as derivatives of two stereoisomers with *trans* or *cis* A/B connection called **5α-gonane** and **5β-gonane**, respectively. If hydrogen atoms (or substituents) are placed *above* the ring level, they have a β-position (bond is marked with a full line); if they go *below* the ring plane, i.e. α-position, the bond is marked with a dashed line.

The initial substrate for biosynthesis of all animal steroids is cholesterol (27 C). They originate through gradual breakdown of alkyls bound to the steroid skeleton and other changes (see the table below).

No. of C	Steroid group	Principal representative
27	sterols	cholesterol
27	calciols	calciol
24	bile acids	cholic acid
21	gestagens	progesterone
21	corticoids	cortisol, aldosterone
19	androgens	testosterone
18	estrogens	estradiol

Sterols (C$_{27}$)

are steroid alcohols, mainly with a secondary hydroxyl in position 3; often with a double bond between C5 and C6 in ring B. The most common animal sterol is **cholesterol** (cholest-5-en-3β-ol).

cholesterol   coprostanol
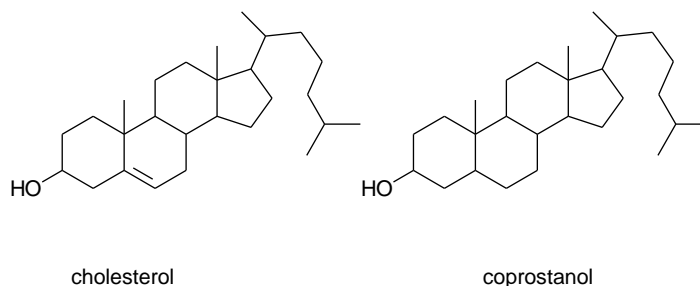
Cholesterol has 27 carbons, in the position 17β it has an eight-carbon branched alkyl. In all animal cells, it is a part phospholipid bilayer of cell membranes. Membranes with a larger amount of cholesterol have noticeably lower fluidity and permeability. In the adult human body, there are about 250 g of cholesterol, a considerable part of which occurs in the brain and spinal cord. Even though it has one hydroxyl group, it is rather hydrophobic, and insoluble in water. In blood plasma, it is transported together with other lipids as a part of lipoproteins. The total concentration of cholesterol in blood is about 5 mM, only a part of which is free cholesterol; about 2/3 are hydrophobic cholesteryl esters with unsaturated fatty acids.

Cholesterol is not only received in food, it is synthesised from acetyl-coenzyme A. It is remarkable that the steroid skeleton in mammal cells cannot be degraded into simple metabolites. The largest amount of cholesterol is excreted from the body as free cholesterol, or it is transformed into bile acids. A part of cholesterol from bile is reabsorbed again (enterohepatic circulation); the remaining cholesterol is discharged in stool, usually after reduction (hydrogenation of double bond by enzymes of intestinal bacteria) into saturated **coprostanol** (5β-cholestanol).

Calciols (C$_{27}$)

are a specific group of sterols, sekosterols, in which the ring B (C9-C10 bond) is broken. Considering its necessity for mammals they were named **vitamin D**.

Bile acids (C$_{24}$)

originate from cholesterol by hydroxylation at position 7 and/or 12, by reduction of the double bond, and by shortening and oxidation of the side chain at C24 to carboxyl. All hydroxyl groups are always in the α-position. In the liver, primary bile acids, **chenodeoxycholic** (3α,7α) and **cholic acid** (3α,7α,12α), are made.

cholic acid   glycocholate
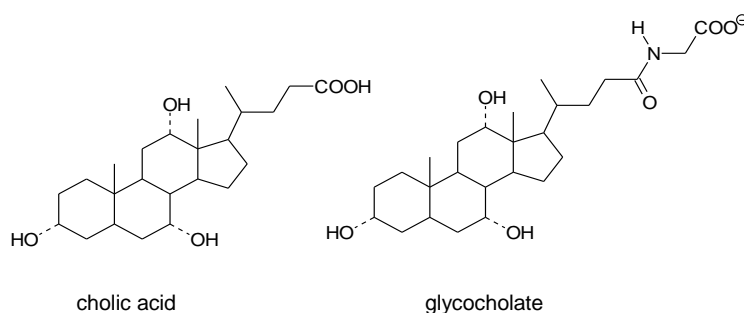
They are discharged into bile as **conjugated** bile acids, amides made by binding carboxyl to the -NH$_2$ group of glycine or taurine, e.g., cholate is converted into glycocholate or taurocholate. Conjugation increases the strength of bile acids, and therefore their degree of dissociation and solubility.

Bile acids are effective surfactants, even though their molecule is more or less flat: the hydrophilic side of the flat steroid skeleton exposes all hydroxyl groups, atoms of the amide bond, and the end group with a negative charge; the other side with methyl groups and a bare steroid skeleton is hydrophobic. Bile acids are needed for proper lipid digestion in the intestine; they emulsify fat into small micelles. They also activate pancreatic lipase, necessary for the hydrolytic breakdown of triacylglycerols. A major portion of bile acids is absorbed in the distal part of the ilea and colon and discharged again by the liver into bile (enterohepatic circulation). Dehydroxylation by microbial flora in the colon leads to **deoxycholic** (3α,12α) and **lithocholic** (3α) acid (secondary bile acids).

Steroid hormones (C$_{18}$-C$_{21}$)

According to the tissue of their main production steroid hormones are classified as the hormones of the adrenal cortex (corticoids) and sex hormones (male androgens, female estrogens and gestagens). All steroid hormones are nonpolar substances, in the blood transported bound to plasma proteins.

A common precursor of them all is cholesterol. Their effect is noticeable after penetration into cells. After binding to specific intracellular receptors, they affect the expression of some genes of nuclear DNA.

progesterone

corticosterone

testosterone

aldosterone

cortisol

estradiol
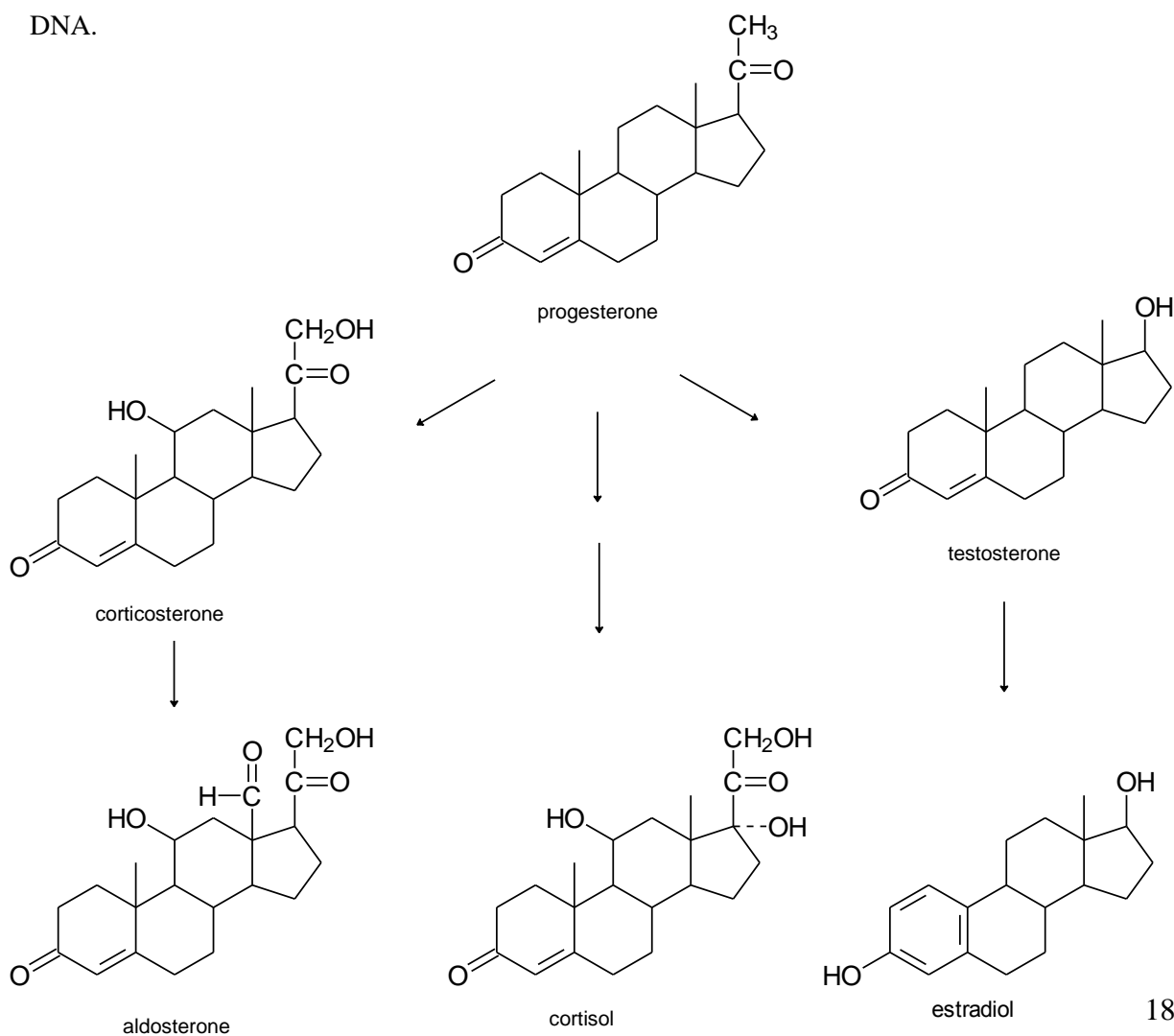
Biogenetic relations between key steroid hormones are shown in the scheme above. The common intermediate is the progesterone (C_{21}). Most steroid hormones, except oestrogens, have the same arrangement of atoms in the A ring – the oxo group at C3 and the C4-C5 double bond.

Corticoids (C_{21}) comprise two main groups of hormones produced by the adrenal cortex, glucocorticoids and mineralocorticoids. They are steroids with 21 carbons. They differ from progesterone by the presence of a hydroxyl – always on C-21, or 11β (17α).

Glucocorticoids play a crucial role in adaptation of the organism to stress. They increase glucose concentration in blood by stimulating liver gluconeogenesis, synthesis of glucose from some amino acids. They also make amino acids more easily available by suppressing proteosynthesis and supporting the breakdown of proteins, e.g., in muscles, bones, and the cells of the lymphoreticular system. They have a catabolic effect on protein metabolism, which also results in suppressing the immune reaction (synthesis of antibodies). As glucocorticoids suppress the synthesis of substances released during inflammation (eicosanoids), they are widely used as anti-inflammatory drugs.

The most important glucocorticoid is **cortisol** with three hydroxyl groups (11β,17α,21), in pharmacology known as hydrocortisone. Corticosterone has less pronounced glucocorticoid effects, but it also shows the clear effect of mineralocorticoids.

Mineralocorticoids play an important role in managing Na^+ and K^+ ions and therefore in osmolality and the volume of extracellular fluid regulation. In kidney tubules, reverse resorption of ions Na^+, thus their retention in the body and increased excretion of K^+ ions is supported. The most effective hormone of this group is **aldosterone**. It is unique by the fact that its carbon 18 (in other steroids it is methyl bound in position 13) is oxidised into the aldehyde group. The 11β-hydroxyl is easily added to this aldehyde group to make an intramolecular cyclic hemiacetal.

Sex hormones (C_{18}-C_{21})

influence reproduction and development of secondary sex features. Most of these hormones are produced by gonads. In males androgens, in females estrogens and gestagens are the prevailing hormones.

Androgens are 19-carbon male sex hormones. The typical representative is **testosterone**, from its precursor progesterone it differs by hydroxyl at C17. In target organs, it is partly transformed into the active hormone 5α-dihydrotestosterone. Androgens influence the development of external male genitals and secondary sex characteristics. Their metabolic effects are generally anabolic.

Estrogens are 18-carbon female sex hormones originating in the ovaries. Unlike other steroids their ring A is aromatic (aromatisation of ring A is conditioned by the elimination of carbon 19, i.e. the methyl at C10); they are slightly acidic, because the C3 hydroxyl became phenolic. The most active is **estradiol**, with C3 phenolic OH and C17 hydroxyl, like testosterone. Oestrogens initiate proliferation of uterine mucosa in the first half of the menstrual cycle, development of female secondary sex characteristics. **Gestagens** are 21-carbon steroids synthesised mainly in *corpus luteum* and the placenta. The most important one of them is **progesterone**, which is also the intermediate of biosynthesis of other steroid hormones. It prepares the uterine epithelium for nidation of a fertilised egg in the second half of the menstrual cycle and prevents further ovulation. During pregnancy the production of progesterone is many times increased.

42 Amino acids

This chapter describes a group of twenty amino acids, which are substrates of cell proteosynthesis regulated by the genetic code. They are known as **coded** amino acids.

Amino acids whose carbon skeleton animal organisms cannot synthesise are called **essential** amino acids. They are obtained from food. There are nine essential amino acids: valine, leucine, isoleucine, phenylalanine, tryptophan, threonine, methionine, lysine, and histidine.

All coded amino acids are carboxylic acids with the primary amino group bound to the α-carbon, i.e. carbon C2 neighbouring with carboxyl. An exception is proline, which has the secondary amino group -NH- as a part of the pyrrolidine ring. Except in glycine, the α-carbon of standard amino acids is **chiral**; isoleucine and threonine also have the second chirality centre; amino acids are therefore optically active. In proteosynthesis, only amino acids that have an α-carbon configuration L are built into polypeptide chains.

α-amino acid　　　　L-amino acid　　　　D-amino acid
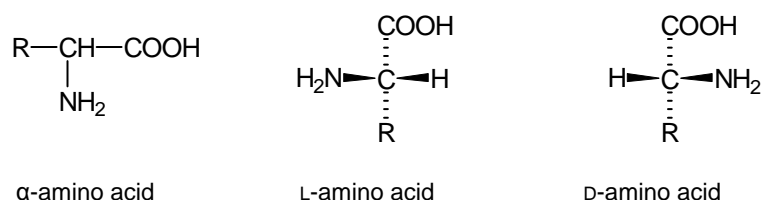
Standard amino acids differ from each other just by the substituent R on the α-carbon, called a **side chain**. Side chains of amino acids determine the secondary and tertiary structure of proteins. According to the polarity of the side chain and its behaviour under physiological pH ($\sim$ 7.4) standard amino acids are divided into following four groups:

> · side chain is **non-polar**
> · side chain is **polar unionised**
> · side chain is **positively charged (basic amino acids)**
> · side chain is **negatively charged (acidic amino acids)**

In proteins, non-polar side chains are mutually bound by hydrophobic interactions, polar groups create hydrogen bonds, acidic and basic groups stabilise the tertiary or quaternary structure of proteins by electrostatic interactions.

Amino acids with non-polar side chains (Gly, Ala, Val, Leu, Ile, Phe, Pro, Trp, Met)

Glycine (aminoacetic acid) is the simplest amino acid and the only one that is not chiral. Glycine is especially abundant in collagen.

Alanine (2-aminopropanoic acid) has a close relation to pyruvate by transamination.

glycine (Gly)　　　　alanine (Ala)
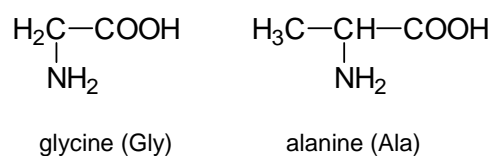
Valine (2-amino-3-methylbutanoic acid), **leucine** (2-amino-4-methylpentanoic acid), and **isoleucine** (2-amino-3-methylpentanoic acid) have quite similar characteristics, especially the isomers leucine and isoleucine. They are essential for animals, because they cannot synthesise their branched carbon chain.

$$H_3C-\underset{\underset{CH_3}{|}}{CH}-\underset{\underset{NH_2}{|}}{CH}-COOH \qquad H_3C-\underset{\underset{CH_3}{|}}{CH}-CH_2-\underset{\underset{NH_2}{|}}{CH}-COOH \qquad H_3C-CH_2-\underset{\underset{CH_3}{|}}{CH}-\underset{\underset{NH_2}{|}}{CH}-COOH$$

<div align="center">valine (Val)   leucine (Leu)   isoleucine (Ile)</div>
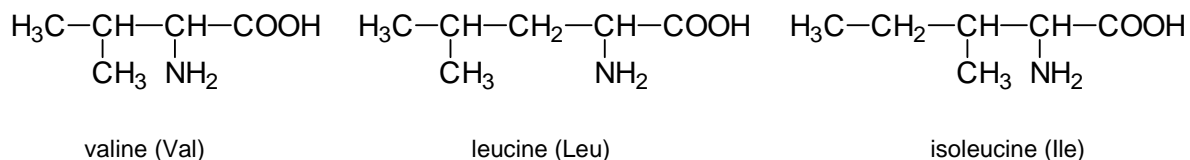
Phenylalanine (2-amino-3-phenylpropanoic acid) has in the side chain a very non-polar aromatic residue phenyl. It is essential acid; animal cells cannot synthesise the aromatic skeleton *de novo*.

Proline (pyrrolidine-2-carboxylic acid.) is a very unusual α-amino acid; it does not have a primary amino group -NH$_2$, but a secondary amino group -NH- as a part of the pyrrolidine ring. The carbon chain that closes the five-membered heterocycle is non-polar. A major amount of proline is bound in collagen, causing the specific secondary structure of tropocollagen chains. About half of proline in collagen is post-translationally hydroxylated to 4-hydroxyproline.

Tryptophan, 2-amino-3-(indol-3-yl)propanoic acid, contains a condensed aromatic system of indol (benzopyrrol); the heteroatom of nitrogen is not basic like in pyrrole. Tryptophan is essential, and is also the precursor of serotonine, melatonine, and nicotinamide (see Chapter 32).

<div align="center">phenylalanine (Phe)   proline (Pro)   tryptophan (Trp)</div>
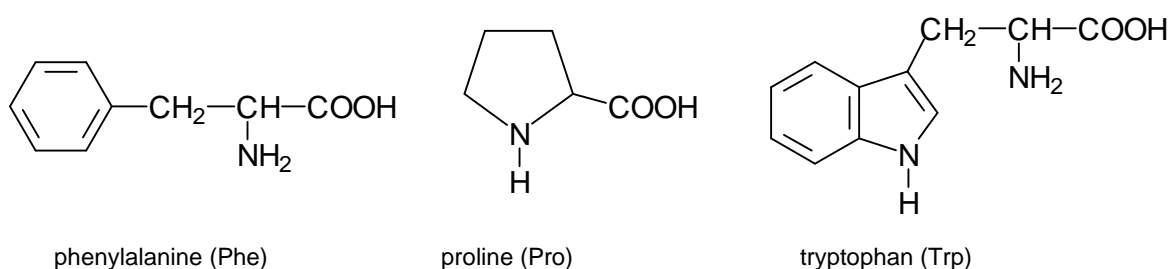
Methionine (2-amino-4-(methylsulfanyl)butanoic acid) has in its side chain a -S-CH$_3$ group (belongs among dialkylsulfides). It is a very valuable, essential amino acid. The methyl group bound to the S atom is used for methylations (active form is *S*-adenosylmethionine). During methylation reactions, **homocysteine** (cysteine homolog) is a by-product, from which methionine can be regenerated again.

<div align="center">methionine (Met)   homocysteine</div>

184
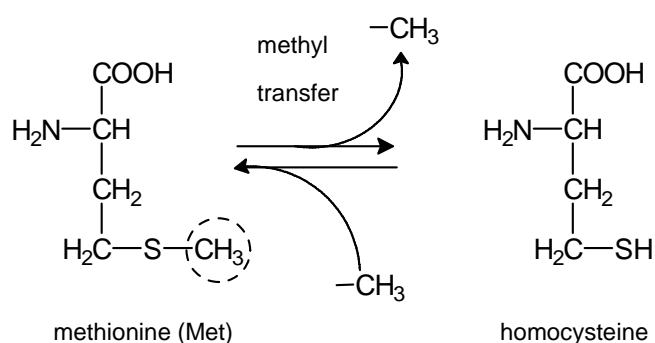
Amino acids with a polar side chain (Ser, Thr, Asn, Gln, Tyr, Cys)

Serine (2-amino-3-hydroxypropanoic acid) and serine homolog, four-carbon **threonine** (2-amino-3-hydroxybutanoic acid) can esterically bind phosphoric acid by their hydroxyl, which is important in the regulation of protein function. Essential threonine has two chiral centres, can make four stereoisomers ($2^2 = 4$); the standard amino acid is L-threonine.
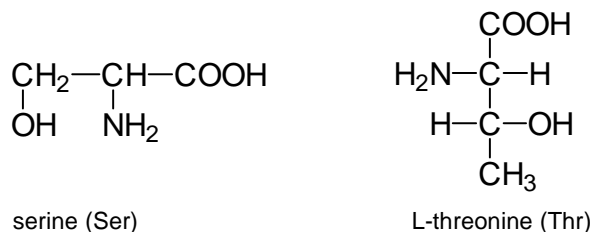
serine (Ser)   L-threonine (Thr)

Asparagine (2-amino-3-carbamoylpropanoic acid, β-amide of Asp) and **glutamine** (2-amino-3-carbamoylbutanoic acid, γ-amide of Glu) are neutral amino acids; the amide group -CO-NH$_2$ is neither basic nor acidic (amides are non-electrolytes). Glutamine is important for non-toxic transport of ammonia in the body. The amide bond is easily hydrolysed, and releases ammonia and aspartic (glutamic) acid. Both amides are important donors of the amino group (e.g. in synthesis of glucosamine).
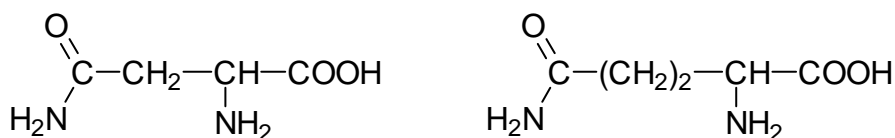
asparagine (Asn)   glutamine (Gln)

Tyrosine is 2-amino-3-(4-hydroxyphenyl)propanoic acid. Unlike phenylalanine it is not an essential amino acid, and is formed by hydroxylation of phenylalanine. Its phenolic hydroxyl releases a proton only if pH > 9; therefore in cells it does not show its very weak acidic properties ($pK_A = 10.1$). Tyrosine is the precursor of, e.g., melanine pigments, adrenaline hormones, and iodinated thyronines.
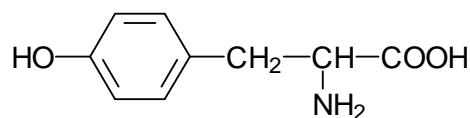
tyrosine (Tyr)

Cysteine (2-amino-3-sulfanylpropanoic acid) has a –SH group, whose very weak acidity ($pK_A = 8.3$) is not used in the range of physiological pH values. On the contrary, the -SH group easily undergoes dehydrogeneration. Two cysteine molecules are oxidised to a disulfide **cystine**. The reaction is reversible and important for all compounds that contain cysteine residues. In proteins, **disulfide bridges** are made this way. A tripeptide glutathione (GSH) contains cysteine and its reduced form works in cells as important antioxidant (see Peptides).
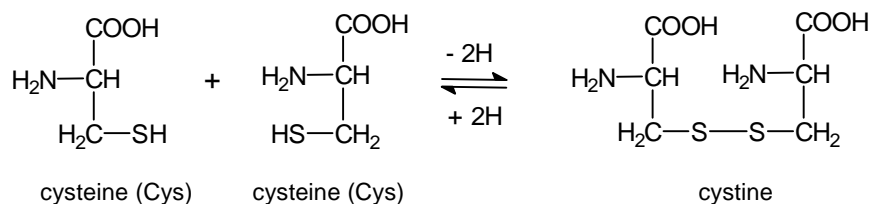
cysteine (Cys)   cysteine (Cys)   cystine

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Free cystine is barely soluble. In cystinuria, a metabolic disorder, cystine crystals are found in urine sediment and cystine urine stones are often created. Some organisms, including humans, use the amino acid **selenocysteine**, which has a selenium atom instead of sulfur, for the synthesis of a few proteins (e.g. glutathione peroxidase).

Basic amino acids (Lys, Arg, His)

Lysine (2,6-diaminohexanoic acid), essential amino acid, has the second amino group ($pK_B = 3.5$) in its side chain. Side chains of lysine are vitally important for making covalent inter-chain bonds in collagen (cross bridges) and elastin (desmosine).

Arginine (2-amino-5-guanidinepentanoic acid) contains a guanidine (iminourea) group, which contains three nitrogen atoms. Nitrogen bound by double bond (imino nitrogen) is with a value $pK_B = 1.5$ much more basic than the α-amino group of lysine. In the liver, arginine is an intermediate of ureosynthesis. Hydrolysis of the guanidine group creates urea and diamino acid ornithine. In some cells, the oxygenation of the imino group of arginine produces nitric oxide NO.

lysine (Lys)   arginine (Arg)
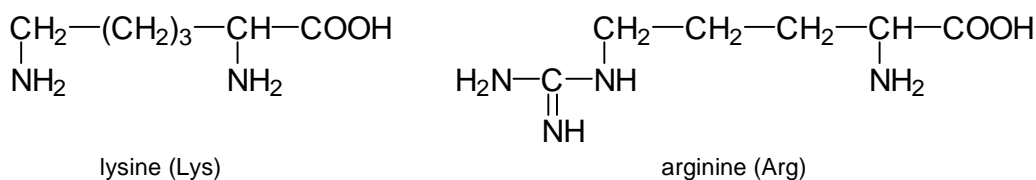
Histidine (2-amino-3-(imidazol-4-yl)propanoic acid) contains imidazole in the side chain. Heterocyclic nitrogen N3 causes that the imidazole ring is a weak base with a value $pK_B = 8.0$ (i.e. pK_A of protonated form is 6.0). Side chains of histidine in proteins are the only ones that act as donors or acceptors of protons (**protein buffers**) under physiological pH. Apart from this, in haemoglobin, the side chain of histidine provides the bond of heme iron to globin. Free histidine provides histamine by decarboxylation.
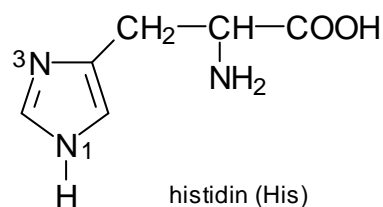
histidin (His)

Side groups of basic amino acids give positive charges to proteins under physiological pH values.

Acidic amino acids (Asp, Glu)

Aspartic acid (2-aminobutanoic acid, aspartate) and **glutamic acid** (2-aminopentanoic acid, glutamate) carry the second acidic (carboxyl) group in their side chain. Aspartate is a donor of nitrogen in the synthesis of urea; glutamate is involved in the transamination of amino acids. Under physiological pH values, ionised carboxyl groups provide proteins with negative charges.
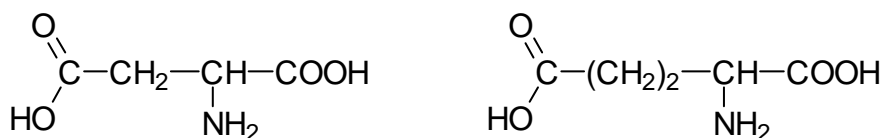
aspartic acid (Asp)   glutamic acid (Glu)

Properties and Biochemical Conversions of Amino Acids

Free amino acids are very polar structures; they have at least two ionisable groups, the α-carboxyl and α-amino group. They are crystalline; their solubility in water varies greatly, from not very well soluble cystine and tyrosine to well soluble proline, cysteine, and lysine. However, in low concentrations, in which they are found in blood plasma, they are all soluble. The standard amino acids have properties of usual carboxylic acids: they form salts with hydroxides, and they can create functional derivatives of acids (amides, esters). The amino group could be acylated to form amides, alkylated to betains, or form aldimines with aldehydes.

Deamination, the removal of an amino group from amino acid, is an important event in the catabolism of amino acids. Typically, it proceeds in two steps:

1) transamination
2) dehydrogenation and deamination of glutamate.

Transamination is the exchange of the amino group between amino acid and 2-oxoglutarate; the cofactor required is pyridoxal phosphate. The two products are oxo acid and glutamate.

$$R-\underset{\underset{NH_2}{|}}{CH}-COOH \quad + \quad HOOC-\underset{\underset{O}{||}}{C}-CH_2CH_2COOH$$

amino acid $\qquad\qquad$ 2-oxoglutarate

$$R-\underset{\underset{O}{||}}{C}-COOH \quad + \quad HOOC-\underset{\underset{NH_2}{|}}{CH}-CH_2CH_2COOH$$

2-oxo acid $\qquad\qquad$ glutamate
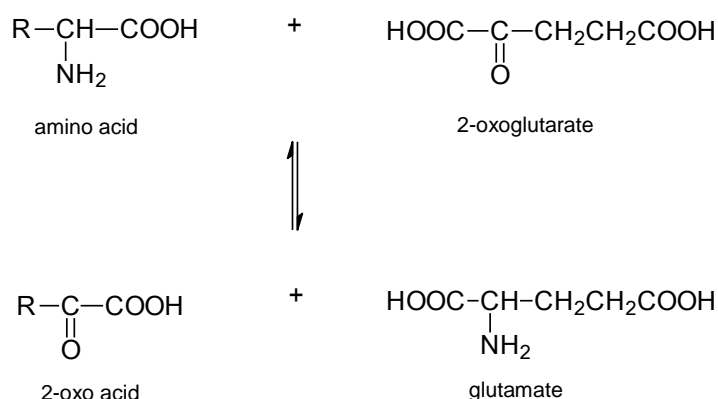
General scheme of transamination

The following dehydrogenation of glutamate produces 2-iminoglutarate, which is spontaneously hydrolysed to ammonia and 2-oxoglutarate. This **dehydrogenation and deamination of glutamate** (enzyme glutamate dehydrogenase) is the main producer of ammonia in the body.

$$HOOC-\underset{\underset{NH_2}{|}}{CH}-CH_2CH_2COOH \xrightarrow{\;-2H\;} HOOC-\underset{\underset{NH}{||}}{C}-CH_2CH_2COOH \xrightarrow[-\,NH_3]{H_2O} HOOC-\underset{\underset{O}{||}}{C}-CH_2CH_2COOH$$

glutamate $\qquad\qquad$ 2-iminoglutarate $\qquad\qquad$ 2-oxoglutarate
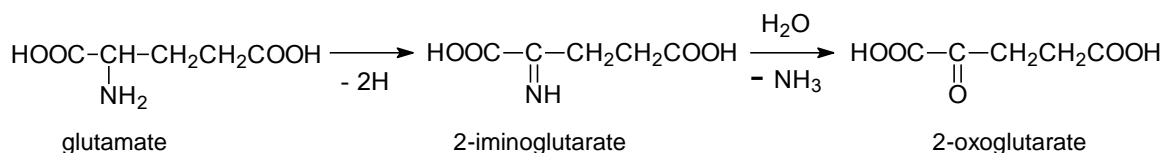
Scheme of dehydrogenation and deamination of glutamate

Oxidative deamination of amino acids with flavin coenzyme proceeds in a similar way as the oxidative deamination of biogenic amines (see Chapter 31).

Decarboxylation of amino acids produces biogenic amines (see Chapter 31), which have a lot of biological effects. Biogenic amines are components of structures of other compounds or their precursors.

Ionisation of Amino Acids

The molecule of an amino acid contains the acidic carboxyl group as well as the basic amino group. Therefore they are amphoteric, ampholytes with independent ionisation. It means that even if the degree of ionisation of all groups is determined by the pH of the environment, the degree of ionisation of individual groups is given by the value of pK_A and does not depend on the degree of ionisation of the other groups. Neutral amino acids are those which do not have any other ionisable group in their side chain, e.g., serine can exist in three ionic forms, depending on the pH of the environment:

· **cation** with a protonated amino group (ammonium) and non-dissociated carboxyl,
· **amphion** carrying one positive and one negative charge (both groups are ionised),
· **anion** (carboxylate) with a non-ionised amino group (trivalent nitrogen).
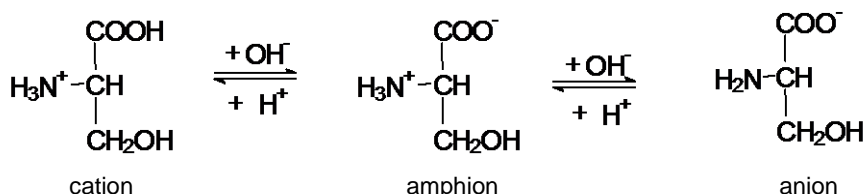
Amino acids do not occur in the form of non-ionised groups, -COOH and $-NH_2$, though, for simplicity, we usually write their formulas in this way. The ionisation of an amino acid is described by the titration curve, which can be obtained by the gradual alkalisation of the amino acid solution in the acidic environment (pH ~ 0).

Isoelectric point pI is the pH value of a solution at which the amino acid occurs only in the form of an amphion (the same number of positive and negative charges), an **electroneutral** species. In the range of the isoelectric point the amino acid has the lowest solubility in polar solvents. The value of pI can be derived from the titration curves of amino acids, it corresponds to the inflex point in the steep part, or can be calculated from the values of pK_A of the individual ionisable groups. They are usually expressed as pK_{A1} for α-COOH, pK_{A2} for α-NH_3^+, and pK_{A3} for the group in the side chain. The following table shows the values of pK_A of acidic groups and the degree of dissociation at physiological pH.

Acidic group	pK_A	Occurrence	Dissociation at pH 7.40
α-Carboxyl	1.8-2.6	all amino acids	complete
β-Carboxyl	3.9	aspartate	complete
γ-Carboxyl	4.3	glutamate	complete
Imidazolium	6.0	histidine	partial
Sulfanyl	8.3	cysteine	none
α-Ammonium	8.8-10.6	all amino acids	none
Phenolic hydroxyl	10.1	tyrosine	none
ε-Ammonium	10.5	lysine	none
Guanidinium	12.5	arginine	none

The isoelectric point of neutral amino acids is the mean of the values of pK_{A1} and pK_{A2}, thus $pI = (pK_{A1} + pK_{A2})/2$, and is found in the weakly acidic area, in the range of pH 5.0-6.2. The isoelectric point of acidic amino acids (aspartate and glutamate) does not depend on the value of pK_{A2} (α-NH_3^+). It is the mean of the values of pK_{A1} and pK_{A3}, thus both acidic groups. It lies in the acidic area, pH 3.0-3.2. In case of basic amino acids (arginine, histidine, and lysine) pI is the average of the values of pK_{A2} (α-NH_3^+) and pK_{A3} (guanidinium, imidazolium, ϵ-NH_3^+, respectively). It is found in the alkaline region, pH 7.6-10.8.

Less Frequent Amino Acids

Some derivatives of the standard amino acids are found free in the organism, as intermediate products of metabolism; they are not components of proteins.

Methylation on the glycine nitrogen atom gives **sarcosine**, its further methylation gives **betaine** (*N*-trimethylglycine), which is found only in the form of amphion; according to it the similar amphions of tetraalkylammonium salts are called betaines.

Glycine forms amides with the benzenecarboxylic acids in the organism, e.g., *N*-benzoylglycine is called **hippuric acid**; it is the product of the biotransformation of benzoate and thus the regular component of urine. Similar derivatives are conjugated bile acids, e.g., glycocholic acid.
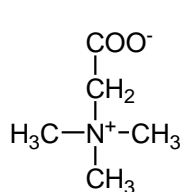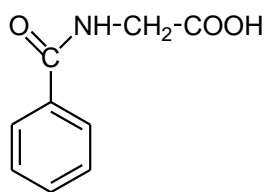
betaine   hippuric acid   taurine
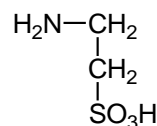
Cysteic acid is formed by the oxygenation of the -SH group of cysteine; **taurine** is a product of cysteic acid decarboxylation. Taurine is similarly to glycine the component of conjugated bile acids, e.g., taurocholic acid. Methionine is transformed to **homocysteine** by giving its methyl group. Its increased concentration in blood plasma is the risk factor of atherosclerosis, independent of the concentration of cholesterol. **Homoserine** results from the breakdown of methionine.

The hydrolytic cleavage of urea from arginine gives **ornithine**. Aspartate creates **β-alanine**, which is the component of pantothenic acid (and coenzyme A), Glutamate creates **γ-aminobutyric acid (GABA)** through the removal of α-carboxyl, the important inhibition mediator in the CNS.
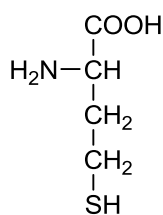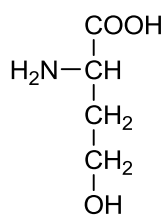
homocysteine   homoserine   ornithine   GABA
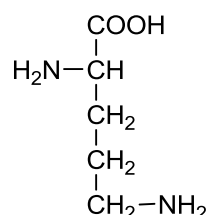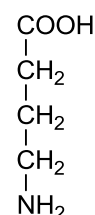
43 Peptides

From a biochemical point of view, the most important characteristic of amino acids is their ability to link together in peptides and proteins. The **peptide bond** -CO-NH- is the special type of an amide bond between the α-carboxyl group of one amino acid and the α-amino group of another amino acid; water is excluded in the reaction. A peptide bond can undergo hydrolysis and the initial reactants are released.
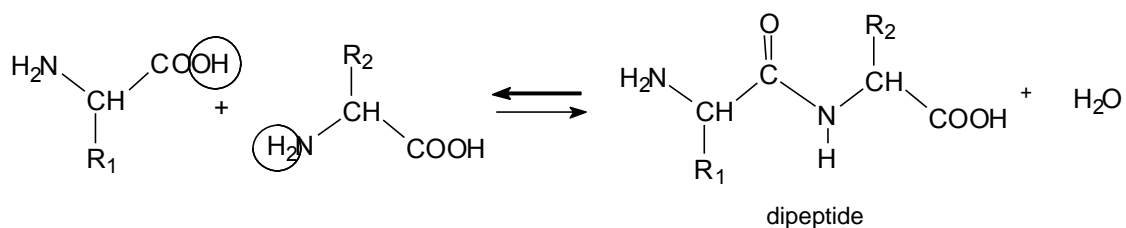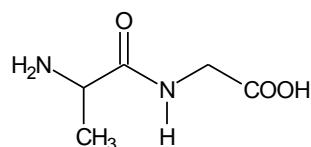

dipeptide

While peptide bond hydrolysis is an exergonic reaction, the synthesis of a peptide bond is endergonic. *In vivo*, the reaction of amino acids with ATP results in high-energy intermediates (aminoacyl adenylates), from which the aminoacyls are transferred to specific tRNA (see Chapter 38). The proteosynthetic cell apparatus (ribosomes) provides the synthesis of peptide bonds.

The peptide bond has its specific features caused by the delocalisation of π-electrons of carbonyl and the unshared electron pair of the nitrogen atom. The peptide bond -CO-NH- partly has the character of a double bond: it is slightly stronger than a single bond and slightly weaker than a double bond; the rotation about the bond C-N is restricted. The atoms of the group -NH-CO- have a planar geometry, which is the consequence of resonance. This geometry and restricted rotation of the peptide bond help to impart secondary structures of proteins. By linking amino acids through peptide bonds, a **linear peptide chain** originates. There is a free α-amino group at one end (N-terminal) and a free α-carboxyl group at the opposite end (C-terminal). The **main chain** of a peptide consists of the regularly alternating sequences -NH-C^{α}H-CO-. **Side chains** R of individual amino acid residues attached to C^{α}-atoms are branching off from the main chain.
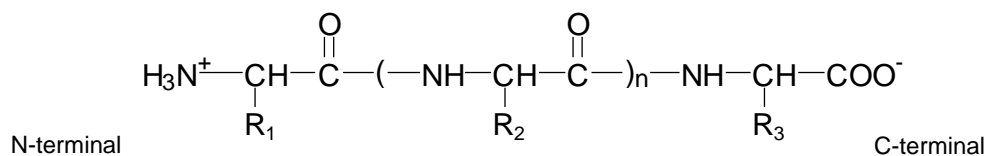
Peptides and proteins are polyamides that consist of α-amino acids joined by peptide bonds. Peptides usually contain less than 50 amino acid residues, proteins more than 50 amino acid residues, which corresponds approximately to a molecular weight higher than 6,000. However, the main trait, which differentiates proteins from peptides, is the existence of a defined secondary and tertiary structure of proteins.

In general, peptides are classified according to the number of amino acid residues as dipeptides with two amino acid residues, tripeptides with three, etc. If the peptide consists of no more than 10 amino acid residues, it is an oligopeptide, and if it binds more than that, then it is a polypeptide. Peptides are taken as N-aminoacyl derivatives of a C-terminal amino acid. The names of aminoacyls are derived from the names of amino acids by replacing –*ine* suffixes with the –*yl* suffix, e.g., Gly glycyl, His histidyl, Trp tryptophyl, etc.; however, there are exceptions: Asn asparaginyl, Asp α-aspartyl, Cys cysteinyl, Glu α-glutamyl, and Gln glutaminyl. The names of aminoacyls are inserted successively in the direction from the N-end to C-end; the C-terminal amino acid original name. The complete charac-

terisation of a peptide must include not only the aminoacyl components, but also their sequence. It is obvious that two amino acids, e.g., alanine and glycine, can create two different dipeptides: alanyl-glycine, glycyl-alanine.

alanyl-glycine
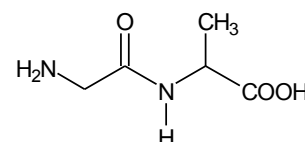
glycyl-alanine

Selected peptides

Glutathione (γ-glutamyl-cysteinyl-glycine, GSH) has an N-terminal glutamate linked to $-NH_2$ of cysteinyl by the γ-carboxyl group (less usual iso-peptide bond). The free sulfanyl -SH group of the

glutathione (the reduced form, GSH)

cysteinyl residue undergoes easily to oxidation creating oxidised glutathione (GSSG). In cells, glutathione is one of the antioxidants, keeping -SH groups of proteins in the reduced state. In erythrocytes, a high concentration of glutathione protects Fe^{2+} of haemoglobin from oxidation to methemoglobin. It takes part (in the presence of the enzyme glutathione peroxidase) in decomposition of hydrogen peroxide and organic hydroperoxides, which are produced in cells by the reactive oxygen species and other free radicals:

$$H\text{-}O\text{-}O\text{-}H + 2\,GSH \rightarrow 2\,H_2O + G\text{-}S\text{-}S\text{-}G.$$

Oxidised glutathione (GSSH) is reduced by glutathione reductase in the presence of NADPH. Glutathione also participates in the biotransformation of xenobiotics; it binds to some compounds and the resulting conjugates (dialkyl sulfides) are eliminated from the body more easily.

$$R\text{–}X + G\text{-}SH \rightarrow R\text{–}S\text{-}G + HX.$$
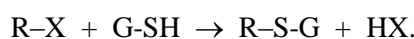
Peptide hormones. Most hormones produced by the hypothalamus are peptides regulating the secretion of hormones from adenohypophysis. Also **calcitonin** released from the thyroid gland and **atrial natriuretic peptides** from myocard belong to the peptide hormone.

Glucagon originates by hydrolysis of the precursor protein in α-cells of Langerhans islets of pancreas. It is secreted into the circulation when the blood concentration of glucose is low, and it increases it by stimulating the breakdown of liver glycogen.

Insulin is the hormone produced by β-cells of Langerhans islets, contains 51 amino acids. Because of its secondary and tertiary structure, insulin is classified as a small protein. It originates from the pre-cursor protein (proinsulin) by the enzyme-catalysed hydrolysis that breaks off the internal sequence (called C-peptide) of the protein; two peptide bonds between basic aminoacyl residues (Arg or Lys) are split. The monomeric molecule of insulin therefore consists of two polypeptide chains A and B (A with 21, B with 30 aminoacyl residues) linked together through two disulfide bridges. Insulin mon-omers circulate in blood, if the concentration of insulin is low. At higher concentrations, dimers of insulin occur. Insulin can also aggregate into insulin hexamers at a very high concentration and in the presence of Zn^{2+} ions. In the β-cells of the pancreas, insulin is deposited as a hexamer with two Zn^{2+} ions. An increase of blood glucose stimulates the secretion of insulin from the pancreas. Insulin sup-ports the transport of glucose into tissues and the breakdown of it (namely in skeletal muscle and fatty tissue), and the synthesis of glycogen in the liver and muscle. Thus, the concentration of glucose in blood decreases.

Peptide antibiotics. Bacterial peptide antibiotics such as bacitracins, gramicidins, are rather toxic, and mainly used externally in therapy. **Gramicidin S** is a cyclic decapeptide. **Actinomycin D** is the anti-biotic from *Streptomyces antibioticus.* Because it binds non-covalently to double-stranded DNA in the nuclei of cells and thus inhibits transcription of DNA, it is used as a cytostatic in oncology. **Cyclo-sporin A** is the cyclic peptide antibiotic from the fungus line *Tolypocladium.* It inhibits production of some cytokines and thereby supresses activation of lymphocytes. It is used as an effective immuno-suppressant.

Toxins. Numerous toxins are peptides. The most venomous mushroom in this country, the death cap mushroom (*Amanita phalloides*), contains extremely toxic dicyclic oligopeptides **amanitins** and **phal-loidins**. α-Amanitin inhibits eukaryotic RNA polymerase; phalloidin binds to actin and prevents de-polymerisation of actin microfilaments. The ingestion of even one such mushroom is usually lethal.

Biologically active peptides	Examples*
Gastrointestinal peptides	Gastrine (17), secretine (27)
Hormones	Calcitonin (32), glucagon (29), insulin (51), atrial natriuretic peptide (28)
Neuropeptides	β-endorfine (31)
Vasoactive peptides	Angiotensine II (31)
Toxins	Amatoxins (8), phallotoxins (7)
Antibiotics	Bacitracin (16), actinomycin D (10), valinomycin (6)

*Numbers in brackets mark the total number of amino acid residues in the molecule.

Synthetic peptides have sometimes interesting or useful properties. For example, **aspartam**, an ester of dipeptide (Asp-Phe-O-CH₃, methyl ester of aspartyl-phenylalanine), is about 180 times sweeter than sucrose. It is a non-sucrose sweetener in common use (not recommended for individuals with phenyl-ketonuria).

44 Proteins

Proteins are linear biopolymers built from proteinogenic L-α-amino acids linked by peptide bonds. The sequences and number of amino acids in the chains are unique for various proteins and specified by genes. Most proteins contain more than 50 amino acid residues, so the molecular mass is higher than 6,000. Proteins differ from polypeptides qualitatively. Polypeptide chains in different proteins have their specific spatial arrangement, stabilised mainly by non-covalent interactions. This characteristic structure is called a **native conformation** of the protein. Native conformation originates in the process called folding of a protein in the course of proteosynthesis on ribosomes.

Proteins have a lot of functions in the cells. They are components of cell structures (e.g. actin, collagen, fibronectin, laminin) and show crucial function in the metabolism, e.g., enzymes, transport proteins. They are responsible for maintaining oncotic pressure, and also form the buffer system of blood and participate in protection of the organism (immunoglobulines), etc.

Protein Structures

The primary structure is the **sequence** of amino acid residues in the polypeptide chains. The primary structure of proteins is genetically determined. By convention, the amino acid sequences are read from the N-terminal, in accordance with the direction of proteosynthesis. A polypeptide chain consists of a **main chain** with the recurrent sequences of atoms -NH-C$^{\alpha}$H-CO- (backbone atoms). The **side chains** R of individual amino acid residues are attached to the C$^{\alpha}$-carbon atoms.

Secondary structure of a certain segment is the **spatial arrangement of atoms of the main chain** without any regard to its side chains and to the interactions of the segment with other remote parts. Secondary structures of the segments can be described by the values of torsion angles. It is possible to view torsion angles while turning the model of a main chain around bonds that start from the α-carbon atoms (N-C$^{\alpha}$ and C$^{\alpha}$-C). The peptide bond C-N has a considerable double-bond character that prevents rotation, but the two rigid peptide units -CO-NH- adjacent to the C$^{\alpha}$-carbon may rotate freely around the bonds between the amino group and the C$^{\alpha}$-carbon and between the C$^{\alpha}$-carbon and the carbonyl group.

Regular secondary structures. Right-handed **α-helix** is an important structure in most globular proteins. There are 3.6 amino acid residues per turn. Intra-chain hydrogen bridges that are approximately parallel to the helix axis stabilise the helix. They bind oxygen atoms of carbonyl groups and the nitrogens of peptide bonds so that the C=O group of the amino acid residue *i* forms a hydrogen bond with the -NH- group of the amino acid residue *i* + 4, i.e. of the fourth that succeeds. Hydrogen bonds thus close "heterocycles" containing altogether, including the connecting hydrogen atom, 13 atoms; hence α-helix is also called **3.6$_{13}$ helix**. α-Helix can be imagined as a narrow cylinder on whose surface the peptide bond planes descend helically. In the places where those planes cross, side chains protrude from C$^{\alpha}$-atoms.

Side chains of aminoacyl residues represent an external covering of the helix. In globular proteins, the polar side chains are usually protruding from one side of the helix and the non-polar from the opposite side. In proline residues, free rotation about the bond N-C$^\alpha$ is not possible; a proline residue within the amino acid sequence of an α-helix "breaks" the regular helical structure or it finishes the helix.

An unusual type of helix structure is the left-handed **steep helix** (3.3 residues per turn) in **collagen**. It is formed, if the chain contains a high content of proline, which results in a chain bending. Such a steep helix cannot be stabilised by intra-chain H-bonds. However, three parallel chains wind around each other to form the right-handed **triple helix** that is stabilised by H-bonds between adjacent chains.

Another important secondary structure is the **β-strand** (β-structure). It is frequent in sequences with a high occurrence of glycine, alanine, and branched chain amino acid residues, in both globular and fibrous proteins. In β-strands, the main chains are considerably stretched. The peptide bond planes of the chain incline regularly up and down as if the chain ran on the surface of a folded sheet of paper. In places where the planes cross, the side chains are pointing from C$^\alpha$-carbons alternately in opposite directions, up and down. Hydrogen bonds cannot originate between atoms of only a single segment of β-strand. The stabilisation of β-strands is usually provided by

an antiparallel pleated sheet   a parallel pleated sheet

linking two or more β-strands by hydrogen bonds between the adjacent strands, which run in the opposite (antiparallel) direction or less often in the same (parallel) direction. These relatively flat structures, combined from two or more chains, are called **pleated sheets** (or **β-sheets**). Most of them adopt a somewhat twisted shape. In the main protein of silk, fibroin, these sheets are stacked in layers that are bound by hydrophobic interactions between side chains that protrude vertically from the plane sheets.

Reverse turns, loops, and other secondary structures. In regular secondary structures, various bends, twists, concaves or other small deformations of the ideal forms can be frequently found. A residue of proline within an α-helical segment represents a breaking of the helix, the direction of which changes significantly. The **β-bend** is the secondary structure of a short segment that changes the direction of the main chain by 180°; it consists of four residues, two of which are polar amino acids, while the other two are glycyl and prolyl. It often links two adjacent β-strands in an antiparallel β-sheet, mainly near the surface of globular proteins.

Irregular structures are present in proteins as the defined segments of characteristic native conformation stabilised by non-covalent interactions. They function as **connecting segments** that bind segments with regular secondary structures or as relatively free **N- or C-terminal segments** of the main chain.

Supersecondary structures consist of various α-helix or β-strand combinations. They should be classified as certain motifs of tertiary structures, because weak interactions between side chains of amino acid residues are mostly prevailing in their stabilisation, e.g., bunches of approximately parallel α-helical segments are very frequent mo-

4-α-helix β-meandr
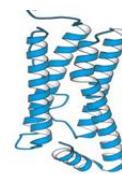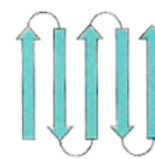
tifs. In **αα**-units, the effective interaction of two parallel α-helices occurs so that the ridge of side chains of one helix fits into the grooves between the side chains of the other; both helices can partly wind round each other. Similarly, four or even more helical segments are associated in large blocks in many membrane proteins, e.g., some hormone receptors. β-Strand segments, even when considerably remote in the chain, interact with each other to form motifs of β-meanders or "Greek keys" that participate in more complex twisted structures called β-barrels. The segments of β-strands and α-helices form βαβ and βαβαβ units.

βαβ

Tertiary structure of a protein molecule is the **spatial arrangement of all protein atoms,** the complete description of its unique native conformation and external shape. No account is given to the relations of the molecule to other molecules. Tertiary structure is a result of **stabilising interactions between side chains** R of segments with various secondary structures. The spatial arrangement of side chains is usually less fixed than the main chain arrangement; side chains are more flexible, which is important in interactions with other molecules. A general characteristic of the tertiary structure of globular proteins includes one important feature: the polar groups (especially those ionisable) occur on the molecular surface, exposed to water; this is why globular proteins are mostly soluble. Most hydrophobic groups are hidden within the compact inner of the molecules. If polar groups occur in the depth of tertiary structure, they all participate in hydrogen bonds. A number of regular building elements in the tertiary structures of globular proteins can even be relatively low. It is stated that those regular elements, such as α-helices, β-strands, and supersecondary structures, represent from 5% to 80% amino acid residues of polypeptide chains in various proteins. Because of this high occurrence, the classification of globular proteins according to the predominant elements exists.

In molecules of globular proteins with long polypeptide chains, relatively independent compact globular parts called **structural domains** occur. They are relatively separate compact globular parts, which may have their own partial tertiary structure that is, in fact, independent on the structure of other domains. Domains can be linked to each other by short connecting chains. Most globular proteins consisting of more than 200 amino acid residues contain two or more domains. In proteins, the domains show many specific functions, e.g., a domain for the binding of substrate and a domain for binding the coenzyme NAD$^+$ is described in pyridine dehydrogenases. In membrane receptors, a few domains are known. Namely, a domain for binding a signal molecule, a transmembrane region and intracellular (catalytic) domain, which is responsible for the production of the secondary messenger spreading a signal in the cell.

Quaternary structure. Oligomeric proteins also have a quaternary structure, i.e. they comprise several independent subunits. These subunits are bound only by non-covalent interactions (hydrogen bridges, electrostatic and hydrophobic bonds). The quaternary structure is then understood as the number and spatial arrangement of subunits in the oligomeric protein (assembly of contacts and interactions between subunits), no account is given to the internal structure of subunits.

Subunits can be identical or different; their number is various, usually even. By the influence of the external environment, conformation and spatial arrangement of subunits change to a certain extent, which usually becomes evident by a change of the biological function. Many controllable processes in cells are mediated by changes in the conformation of subunits of proteins with a quaternary structure (enzymes, transport proteins, membrane transporters or ionophores, specific receptors). An example of a hetero-tetrameric protein is hemoglobin. Some multi-enzyme complexes or viral proteins have very complicated quaternary structures.

Protein Classification

Nowadays, proteins are classified into three major types: globular, fibrous, and membrane proteins. The border is sometimes not very clear, e.g., some globular proteins also form fibrous aggregates, some fibrous proteins can have a rather spacious globular domain, and the structure of some membrane-bound proteins are very similar to that of globular proteins.

Examples of proteins are given in the table.

Protein type	Examples
Globular	hemoglobin, myoglobin, albumin, immunoglobulins, pepsin, lysozyme, amylase
Fibrous	myosin, F-actin, keratin, collagen, elastin, tubulin, spectrin
Membrane	glucose transporter (GLUT), acetylcholine receptor, Na^+/K^+-ATPase

Traditional group names according to non-protein components are also used, e.g., glycoproteins, metalloproteins, hemoproteins, lipoproteins, etc.

Globular proteins are usually soluble in water (unlike fibrous proteins); in solutions, they exist as an individual, on others as independent molecules. Protein molecules have an oval or even spherical shape. The main polypeptide chain and most non-polar side chains are in the compact inner of molecules; most polar side chains are exposed on the surface of molecules, in contact with water.

Hemoglobin is an important globular protein. It is tetramer occurring in erythrocytes. Its main function is the transport of oxygen. Hemoglobin HbA (97% of total hemoglobin) consists of two polypeptide chains α and two polypeptide chains β connecting through non-covalent bonds (hydrophobic and electrostatic). The structure of HbA is $\alpha_2\beta_2$. Each subunit of hemoglobin consists of a protein part (8 α-helices) and heme, which is placed in the hydrophobic pocket of the polypeptide chain.

Fibrous proteins are a relatively small group of proteins, which create microscopic fibres (fibrils). They function as internal scaffolding within cells (cytoskeleton), they give coherence, tension strength, and even elasticity to connective tissues, resistance to the skin, and they are components of contractile elements. Many fibrous proteins do not dissolve in water; a few of them are extraordinary resistant to chemicals. The structures of fibrous proteins differ from each other in a broad range. In some of them, an α-helical secondary structure prevails (e.g. in tropomyosin). Triple-helices consisting of steep-helical chains characterise collagen, β-pleated sheets prevail in fibroin. Actin filaments (F-actin in muscle or cytoskeleton) are polymers of the globular monomer (G-actin).

Keratin is a representative of α-helical fibrous proteins. In mammals, it is a primary component of the horny outer layer of the skin as well as of skin adnexa (hair and hairiness, fingernails, even horns and hooves). The basic structure of α keratins is a protofibril, which consists of two double-stranded α-helices coiled around one other in a left-handed superhelix; eight of these protofibrils are arrayed in a larger structure named a microfibril. Polypeptide chains of α-keratin are rich in cysteinyl residues, both protofibrils and microfibrils are cross-linked by many disulfide bonds. By breaking disulfide bonds through applying a reducing agent solution, the hair that becomes plastic is bent around a form of an appropriate shape; after that, an oxidation agent is applied to establish new disulfide bonds, but not between the same pairs of cysteinyl residues (the principle of "permanent waves").

Collagen is the major fibrous protein in the extracellular ground substance in all types of connective tissue (tenuous connective tissue, tendons, cartilages, and bones), and the most abundant protein in the body. It has great tensile strength. Due to numerous prolyl and glycyl residues, the polypeptide chains of collagen take the characteristic secondary steep-helical structure stabilised by H-bonds (**triple helix**) resulting in tropocollagen units. The rod-shaped tropocollagen units are arranged in parallel bundles – microfibrils. In these microfibrils, the adjacent tropocollagen units are arranged so that they are staggered by one quarter of their length (the cause of microfibril cross-striation viewed in the electron microscope). During collagen maturation, microfibrils are stabilised by formation of the intramolecular covalent cross-links that originate in the reactions of the side chains of **lysyl** and **allysyl** residues. A felt-like assembling of microfibrils forms the collagen fibrils, which can be observed, after a suitable staining, in a microscope. Boiling in water transforms insoluble collagen, by partial hydrolysis, into soluble **gelatin**; it creates gel (aspic) by cooling.

Elastin is the fibrous protein, which prevails over collagen in certain types of connective tissue (e.g., the elastic layer of large arteries, lungs, and skin). Elastin can be stretched reversibly in all directions like rubber. It is not soluble in any common solvent and does not denaturate in hot solutions. The precursor of mature elastin is soluble tropoelastin, the polypeptide chains of which contain parts rich in glycine and other hydrophobic amino acids separated by short regions rich in lysine. The elasticity of the mature elastin depends on firm covalent cross-linkages both between tropoelastin chains and inside them. Four side chains of lysyl (and allysyl) residues come together and react by condensation to give the residue of **desmosine** or a similar compound **isodesmosine**, residues of tetracarboxylic amino acids.

Membrane Proteins

Proteins embody phospholipid bilayers of biomembranes. Some of them only stabilise membranes, however many of them provide various biological functions – facilitated transport of polar substances across membranes, catalytic functions, specific recognition of certain signal molecules and transduction of the signal through the membrane, sites of antigenic determinants characteristic for given individuals of a species, etc. Membrane proteins differ in their association with the membrane structure – they are classified as integral and peripheral membrane proteins.

Integral membrane proteins (I_1, I_2, I_3 in the figure) are embedded more or less in the hydrophobic inner of the phospholipid bilayer and may even extend completely through the membrane (penetrating, transmembrane proteins). The hydrophobic side chains on their surfaces interact with non-polar chains of membrane phospholipids in the central portion of the bilayer. Hydrophobic interactions hold the proteins quite firmly in the membrane. Their isolation is rather difficult (only with surfactants or organic solvents) and after being isolated, they lose their biological activity. Many of them span biological membranes. Polar groups on the surfaces of integral proteins protrude from the membranes and make interactions with substances in surrounding solution. Many penetrating proteins of cytoplasmic membranes are glycoproteins with the saccharide component on their external part. Segments of polypeptide chains that penetrate through the membrane are very often **α-helical** segments, and there can be even a higher number (up to 24) of these. If one side of those helical segments is hydrophilic, the segment can make a transmembrane pore to transport polar species across the membrane.
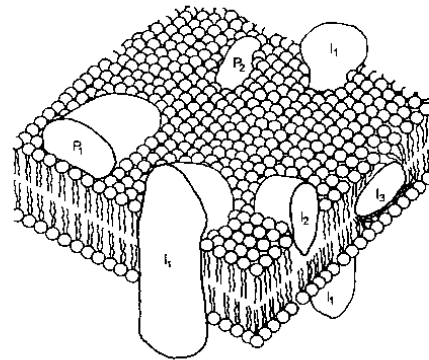
Peripheral membrane proteins (P_1, P_2 in the figure) are loosely attached to the membrane surface by electrostatic attractions to hydrophilic charged polar heads of the bilayer phospholipids or to the groups on the surfaces of integral proteins. They are often quite close to **globular** proteins. Mild procedures (e.g., an increase in ionic strength by adding a salt, changing the pH, chelating agents), which do not disrupt the phospholipid bilayer are effective in the isolation of peripheral proteins.

Protein Solutions

Solutions of proteins can be described as **molecular colloid solutions** (see Chapter 9). It should be noted that only proteins having a sufficient number of polar groups on their molecular surfaces are soluble in water. The **solubility of protein** depends on the **electric charge** on the molecule and on the existence of hydration shell. Therefore solubility of the protein increases with the net charge of its ionised groups, which depends on the pH; protein is less soluble at its isoelectric point where the protein is electro-neutral (equal number of positive and negative charge). The **hydration shell** of protein molecules is another stabilising factor. A very low concentration of salts (low value of ionic strength)

increases the solubility of some proteins in water; the presence of a small amount of salt stabilises the hydration shell resulting in the **"salting in"** effect.

Reversible precipitation of proteins. A high concentration of salts (high value of ionic strength) decreases the solubility of protein and the consequence is the precipitation of protein. This phenomenon is called the **"salting out"** effect. The cause of this salting-out is both the competition of salt ions with proteins over the solvent and the high concentration of ions that decreases the electrostatic repulsion of identically charged protein ions. Thus, if the salt stepwise is added to the solution of protein, the solubility of the protein solution increases up to reaching the certain maximal value (salting in), then the solubility begins to decrease resulting in the precipitation of protein (salting out). This process is reversible, if the solution of protein is diluted (the concentration of salt is decreased), the protein will dissolve again. Salting out is used in the gentle separation of proteins. Various concentrations of the solutions of $(NH_4)_2SO_4$, Na_2SO_4 or NaCl are most often used because proteins differ in amino acid arrangement (it means in ionised groups). Reversible precipitation also occurs due to organic solvents (effect on the hydration of proteins), for example by addition of ethanol or acetone, but only at low temperatures around 0 °C (at room temperatures they cause denaturation).

Irreversible precipitation of proteins occurs, e.g., in proteinuria tests by sulfosalicylic acid, or during deproteination of biological fluids by the action of acetonitrile, perchloric or trichloroacetic acid.

Denaturation

In denaturation, native conformation of protein (quaternary, tertiary, and secondary structure) is disrupted due to various physical and chemical factors, which partially or completely break up non-covalent interactions; the primary structure is kept in its original state. The highly-ordered native conformation of the protein is changed into the partly or completely unfolded polypeptide chain (random coil). The protein loses its specific biological properties, e.g., the enzyme can lose its catalytic activity. There are many known factors which can cause the irreversible denaturation of proteins, e.g., the heating of a protein solution above 43 – 55 °C (e.g. hard-boiled egg). Another physical effect is a mechanical agitation (e.g. whipped egg whites), high pressure, UV radiation, ultrasound etc. Irreversible denaturation is also caused by various chemical agents, for example, strong acids/hydroxides; or other reactive compounds introducing covalent changes in the protein structure, like heavy metal ions making complexes with –SH or –COO⁻ groups, or aldehydes forming aldimines.

If denaturation cause can be removed and the native protein conformation restored, we talk about **renaturation**, e.g after removing reducing agent that reduced disulfide -S-S- bridges in protein molecule.

45 Glycoproteins

Glycoproteins are proteins that contain saccharide components attached to amino acid side chains by glycosidic bond. Carbohydrate compounds are hexoses (Man, Gal), pentoses (arabinose, xylose), amino sugars (GlcNAc, GalNAc), deoxyhexoses (L-fucose), sialic acid (NeuAc). About 50% of all proteins in the human body and almost all proteins of blood plasma (except albumin) are glycoproteins.

The saccharide part increases the solubility of proteins, contributes to the surface charge of the molecule, if it contains sialic, or uronic acids, or sulfate groups. It protects the protein against the effect of proteinases, and in many cases it decides about the biological half-time of glycoprotein in the body. The saccharide component is often responsible for the biological function of protein. It contains antigen determinants, decides about binding bacteria and viruses to the surface of the cell, while it is often the condition for the specific binding hormone-receptor, etc.

A saccharide component is linked by ***O*-glycosidic bond** to the side chains of serine or threonine, or by ***N*-glycosidic bond** to the amide group of asparagine.
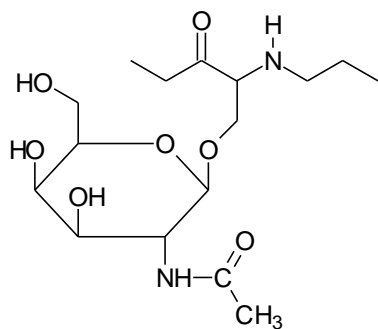
N-acetylgalactosamine (GalNAc) bound to serine
in the polypeptide chain (*O*-glycosidic bond)
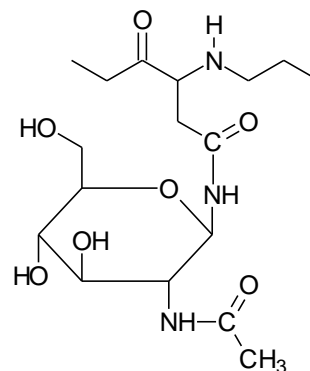
N-acetylglucosamine (GlcNAc) bound to asparagine
in the polypeptide chain (*N*-glycosidic bond)

A special example is **collagen**, the main protein of connective tissue. The saccharide component is bound as *O*-glycoside to the hydroxyl group of 5-hydroxylysine, which is formed by post-translational hydroxylation of lysine during collagen maturation.

Glycoproteins can be divided into three main types:
1. membrane-bound,
2. circulating in blood plasma,
3. mucin glycoproteins.

Membrane Glycoproteins
The saccharide components of membrane glycoproteins and sphingolipids form a layer on the outer surface of the cytoplasmic membrane called **glycocalyx**. It is highly important for the differentiation of cells during intercellular communication. Parts of the saccharide chains represent certain specific signals or antigenic determinants, recognisable by other cells, or macromolecules (binding ligands to

specific receptors, binding antibodies to antigen, binding proteins mediating the adhesion of cells, etc.). The diversity of monosaccharide units implies that the structure of the saccharide component of glycoproteins has a high information value, e.g., **the system of blood groups A-B-0** is based on the presence of an antigenic determinant, which is the component of the saccharide part of glycolipids and glycoproteins included in the membrane of erythrocytes. The antigenic determinant 0 is a very weak immunogen. A and B antigens have small modifications of the non-immunogenic residue 0 at the end of the saccharide branch (all chains contain L-fucose).

$$
\begin{array}{l}
\text{Gal}—\text{GlcNAc}—\text{Gal-}-\ - \qquad \text{antigenic determinant} \ \ 0 \\
\ \ | \\
\text{Fuc}
\end{array}
$$

$$
\begin{array}{l}
\text{GalNAc}—\text{Gal}—\text{GlcNAc}—\text{Gal-}-\ - \qquad \text{antigenic determinant} \ \ A \\
\qquad\qquad\ \ | \\
\qquad\qquad \text{Fuc}
\end{array}
$$

$$
\begin{array}{l}
\text{Gal}—\text{Gal}—\text{GlcNAc}—\text{Gal-}-\ - \qquad \text{antigenic determinant} \ \ B \\
\qquad\ \ | \\
\qquad\ \ \text{Fuc}
\end{array}
$$
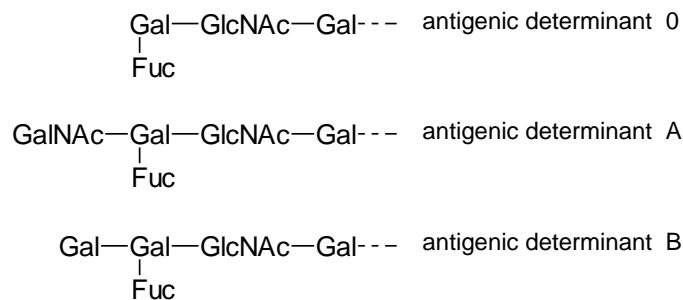
Glycoproteins of Blood Plasma

Glycoproteins circulating in plasma are secreted to circulation by hepatocytes (transferrin, fibrinogen, acidic α_1-glycoprotein). The glycoprotein immunoglobulins are secreted by plasmatic cells. All of them contain oligosaccharides linked by *N*-glycosidic bond. The saccharide component is connected by *N*-acetylglucosamine to the amide -NH_2 group of asparagine (*N*-glycosidic linkage). The oligosaccharide part also contains frequent branching, the branches (antennas).

There are three major types of *N*-oligosacharides: the complex type, hybrid type, and high-mannose type. All of them have a common pentasaccharide core $Man_3GlcNAc_2$ (two GlcNAc and three mannose units), some of them branching antennas, which bind oligosaccharides with variable composition. The various numbers of antennas are described, namely: bi-antennary, tri-antennary, tetra-antennary to penta-antennary structures of oligosaccharides.

High mannose oligosaccharides contains only mannose residues. The antennas have the structure $(Man)_n$ and the number of mannose residues is about 2-6. This type is the precursor of the other types of oligosaccharides during their biosynthesis.

Complex type consist of the GlcNac-Gal-NeuNAc sequence. It is typical for these oligosaccharides that NeuNAc is linked at the end of the antenna. The presence of sialic acids (NeuNAc) gives a negative charge to the oligosaccharide and thus the solubility of protein increases in the solution. Most complex oligosaccharides create di- to tetra-antennary structures.

Pentasaccharide core of *N*-linked glycoproteins
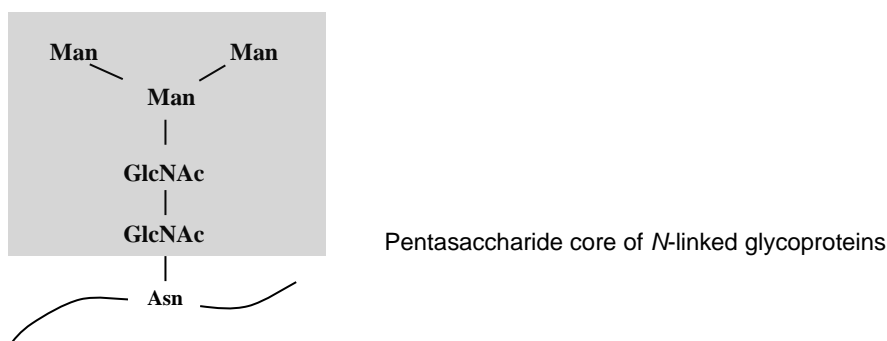
Hybrid type of oligosaccharides includes both types of antennas. It means both the high mannose type (containing only mannoses) and complex type (containing the sequence GlcNac-Gal-NeuNAc).

High-mannose type
(tri-antennary)

Complex type
(bi-antennary)

Hybrid type
(tri-antennary)
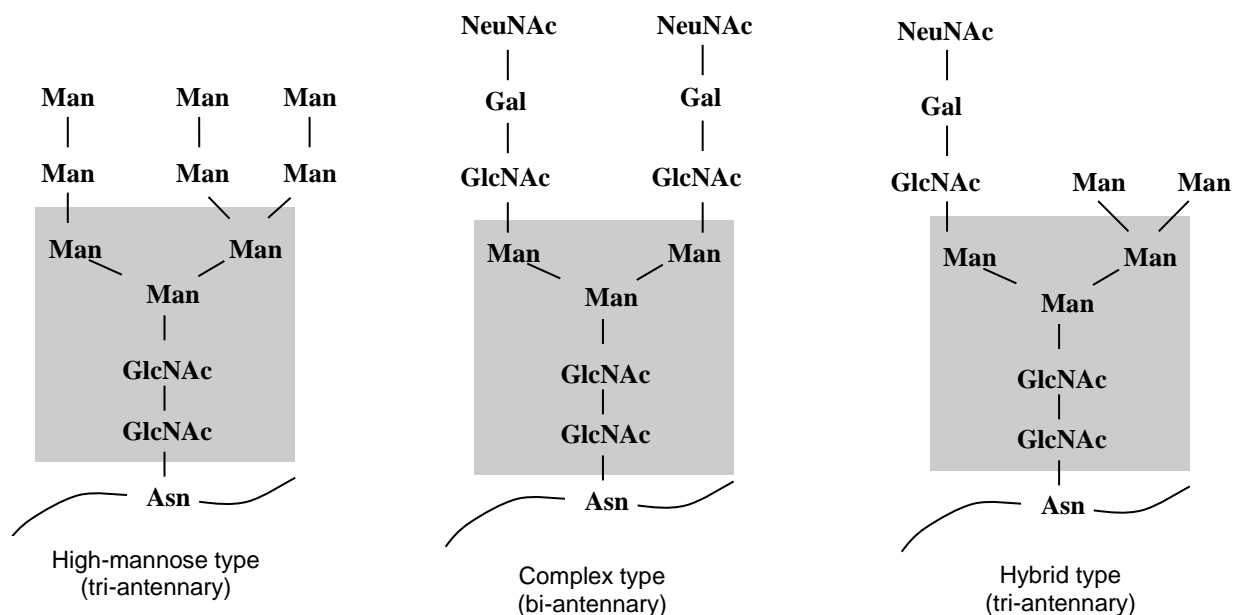
As it was noted, the structure of the saccharide component is responsible for the biological function of glycoprotein and its metabolic fate, e.g., if the terminal sialic acid is removed hydrolytically, the circulating glycoprotein (e.g. immunoglobulin, peptide hormone) is quickly bound to the membrane of the liver cell, removed from the circulation by endocytosis and decomposed. The examples of some glycoproteins of blood plasma are given in the table below.

Plasma glycoprotein	Function
Transferrin	binding and transport of Fe^{3+}
Ceruloplasmin	binding and transport of Cu^{2+}, oxidation of $Fe^{2+} \rightarrow Fe^{3+}$
RBP	retinol binding protein, transport of retinol (vitamin A)
Haptoglobin	binding free hemoglobin after degradation of erythrocytes
α_1-Antitrypsin	inhibits proteases secreted by some leukocytes
Fibrinogen	blood clotting, precursor of insoluble fibrin
CRP	C-reactive protein, indicator of inflammation

Mucin Glycoproteins

of the secretions of epithelial cells of mucosa have a protective and lubricating function. Mucins make up a wide group of glycoproteins; the saccharide components are connected via *N*-acetylgalactosamine by *O*-glycosidic bond to the side chain of serine or threonine. The saccharide component of mucins represents up to 75% of the mass of their large molecules. The number of saccharide chains bound to serine or threonine varies between 500 and 800; the length of chains strongly varies from short chains to 16-19 monosaccharide units, of which many are sulfated.

46 Vitamins

Humans obtain the main nutrients from food together with essential fatty acids, essential amino acids, minerals, and vitamins. Vitamins can be classified by their water/fat solubility. This classification can help us to guess in what type of food vitamins can be found. A balanced diet of fresh foods usually contains a sufficient amount of vitamins. A low intake of vitamins can lead to hypovitaminoses with unspecific symptoms or more serious disorders with characteristic symptoms (avitaminosis). Vitamin **deficit** can also happen due to intestinal malabsorption. **Excess** hydrophilic vitamins from food is usually excreted from the body in urine. On the other hand, soluble vitamins in fats can be deposited for a longer time – vitamin A and D in liver, partly vitamin K and tocopherols in fat tissue. Repeated high doses of retinol or calciol can be toxic (hypervitaminoses).

Fat Soluble Vitamins

They have a non-polar (hydrophobic) character because they are either isoprenoids (A and D) or they contain an isoprenoid chain in their molecule (E and K). In plant and animal food they are dissolved in fats. They are absorbed from the intestine together with dietary fats. During prolonged maldigestion, the absorption of vitamins is reduced; this results in hypovitaminosis, even though vitamin intake is sufficient. Lipophilic vitamins come into circulation as parts of chylomicrons.

Retinol (vitamin A) belongs to isoprenoids (see Chapter 40). It is formed by oxidative degradation of provitamin β-carotene, which is found in vegetables and fruits. There is a reserve of retinol (in ester form) in the liver. Aldehyde **retinal** is a component of rod cell rhodopsin in the retina. **Retinoic acid** takes part in cell regulation of gene expression. One of the first symptoms of retinol deficit is worse night sight – night-blindness followed by skin and mucosal membrane problems – dryness and keratinisation, damage of retina and conjunctiva (xerophthalmia). The recommended daily intake (RDI) of retinol is 800 mg. Repeated high doses of retinol are toxic because they can, besides others, endanger embryonic development during gravidity. The main sources of retinol in food are liver, fatty fish, egg yolk, and butter. Carotenes are found in yellow, red, and green vegetables and fruits.

retinol

retinal
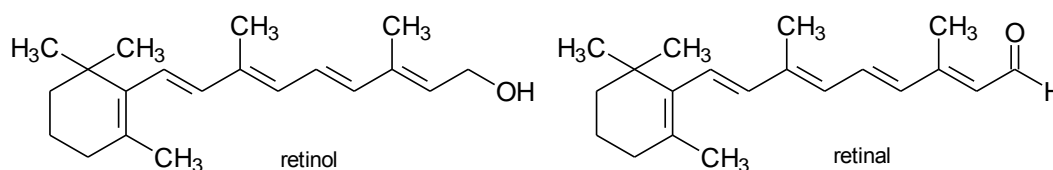
Calciol (vitamin D_3, cholecalciferol) and **ercalciol** (vitamin D_2, ergocalciferol) are 9,10-secosterols (see Chapter 41). Calciol is formed in the skin under UV light from 7-dehydrocholesterol; in plants ercalciol is similarly formed from ergosterol. Hormone **calcitrol** (1α,25-dihydroxycalciol) is formed in the liver and kidneys by hydroxylation. This hormone together with parathyrin and calcitonin has a central function in Ca^{2+} and phosphate regulation. Calcitriol induces the synthesis of proteins, which allows intestinal absorption of Ca^{2+} and regulates mineralisation of bone tissue. Calciol deficiency results in the insufficient mineralisation of bones. Serious deficiency in children causes rickets, in adults osteomalacia.

Repeated high doses of vitamin D lead to hypercalcemia or sometimes to adverse calcification of tissues or to calcium urolithiasis. The recommended daily intake of calciol is 15 µg. The main source of vitamin D is fish oil, cod liver, and also fortified margarines.

calciol   α-tocopherol

Vitamin E is a name for a group of natural antioxidants with qualitatively the same biological activity as **α-tocopherol** (5,7,8-trimethyltocol), which is the most powerful and the most abundant in nature. It was first isolated from wheat-seed oils.

All these antioxidants are derived from chroman with a 16-carbon isoprenoid residue attached in position 2. The phenolic hydroxyl in position 6, *para* to oxygen heteroatom, is important for its function. It reduces many oxygen radicals; the newly formed phenoxyl radical of tocopherol is either regenerated by ascorbate into original tocopherol or irreversibly destroyed by other radicals. Tocopherols inhibit peroxidation of unsaturated fatty acids of membrane lipids and lipoproteins. The daily intake of α-tocopherol is 12 mg and rises with a higher ingestion of polyunsaturated fats. Plant oils, nuts, seeds, and cereals are valuable sources of vitamin E. The amount of tocopherols in frozen or dehydrated foods is significantly reduced by autoxidation. Tocopherol deficit in humans has no specific symptoms; it can cause anaemia or various neurological manifestations.

Vitamin K is a name for a group of 2-methyl-1,4-naphthoquinone derivates, called anti-haemorrhagic vitamins. The natural ones are **phylloquinone** (K_1, 3-phytylmenadione) and **menaquinone** (K_2), which have a side polyisoprene chain and are therefore soluble in fats. Water soluble, synthetic **menadiol** or **menadione** have the same effect. They are essential for finishing the biosynthesis of prothrombin, blood coagulation factors VII, IX, X, and proteins necessary for bone mineralisation. These proteins take part in forming binding sites for Ca^{2+} (introduction of other carboxyl group into side chains of glutamate residues to give γ-carboxyglutamate). Vitamin K is therefore used for prevention or treatment of increased bleeding. Insufficiency in adults is not very common because vitamin K is commonly found in foods (mostly in leafy green vegetables), and besides this it is formed by **colon microflora**. Increased haemorrhage due to vitamin K deficiency can happen in newborns (their intestine is sterile), with fat absorption failure, typically due to insufficient bile acid formation, or with the deficit of colon microflora destroyed by wide-spectra antibiotics.

An important antagonist of vitamin K is **warfarin**. It is used in medicine to decrease blood coagulation, for example, during (possible) formation of thrombi.

phylloquinone

menadiol

menaquinone

menadione

Water Soluble Vitamins

are not stored in the body (with the exception of B_{12}); their excess is excreted by urine and therefore they have to be continuously supplemented with food. They are parts of **enzyme cofactors** in organisms.

L-Ascorbic acid (vitamin C, 2,3-didehydro-L-gulono-4-lactone) is derived from monosaccharides. It is not a carboxylic acid, but a lactone. The acidic character ($pK_{A1} = 4.2$; $pK_{A2} = 11.6$) is caused by two enol hydroxyls. It is synthesised by plants and most animals; humans, primates, and guinea-pigs are an exception. It is an important **reducing agent (antioxidant)**. It is easily oxidised (dehydrogenated) into biologically inactive L-dehydroascorbic acid. Vitamin C takes part in the hydroxylation reactions in the synthesis of collagen, bile acids, and adrenaline by keeping metal ions of metalloenzymes in reduced form. It increases iron absorption in GIT by reduction of Fe^{3+} (ferric) ions to Fe^{2+} (ferrous) ions. The daily intake is much higher in comparison to other vitamins; the recommended amount is about 80 mg for adults.

L-Ascorbate is found mainly in plants, especially in fresh fruits and vegetables. The richest sources are citrus fruits, kiwi, blackcurrant, pepper, potatoes. Damage of ascorbate by oxidation happens easily by air oxygen at increased temperatures, even more when traces of heavy metals are present – Cu and Fe.

ascorbic acid

dehydroascorbic acid
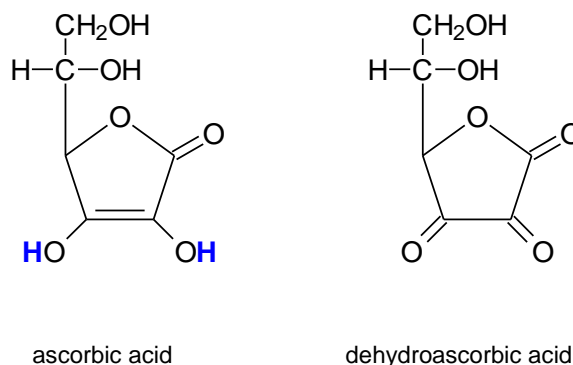
People with a low intake of vitamin C easily tire; they are more susceptible to small infections, swelling and bleeding of the gums. Extreme avitaminosis (scurvy) is very rare.

Prolonged intake of high doses of ascorbate (more than 0.5 g/day) is not beneficial. Excess is quickly excreted in urine; more ascorbate is degraded into oxalate (risk of urolithiasis) and acid-base balance

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can be disturbed. High ascorbate concentrations support the presence of reduced forms of metal ions, which catalyse the formation of aggressive oxygen radicals (Fenton reaction, see Chapter 21).

Thiamine (vitamin B_1) is derived from pyrimidine and thiazol. It is relatively unstable and thermolabile in solutions. Its biologically active form is the coenzyme **thiamine diphosphate**, which binds an intermediate of oxidative decarboxylation of pyruvate and 2-oxoglutarate. Thiamine is essential for degradation of all nutrients, mainly saccharides. The average daily amount for adults is 1-2 mg, which becomes higher when there is an excess of saccharides in food, during excessive sweating in heat, and when alcohol is ingested. Thiamine is found in various foods of plant and animal origin. The main sources are meat, especially pork and offal, yeast, and wholemeal cereals. White breads and white rice had been refined and are poor in thiamine. The first signs of frequent deficiency of thiamine are increased tiredness, muscle weakness, and a tendency to neuritis. Extreme avitaminosis is rare, but in Southeast Asia it is well known as **beri-beri** disease with various forms of circulatory, cardiac, and neural dysfunctions. Deficiency is common when only polished rice is used as a food source.

thiamine
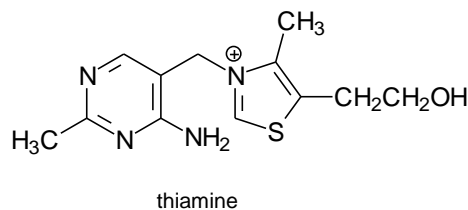
Riboflavin (vitamin B_2) is composed of heterocyclic isoalloxazine attached to ribitol (see Chapter 32). It is a component of flavin mononucleotide (FMN) or flavin adenine dinucleotide (FAD), cofactors of flavin dehydrogenases and other oxidoreductases. The daily amount of riboflavin for adults is 1.4 mg. Its food source is milk and dairy products, eggs, meat, and some plant products. It is usually bonded in the FMN or FAD form; free riboflavin is found only in goat's milk. B_2 avitaminosis is not known. Reduced intake is manifested by mucosal problems (inflammations of the lips, tongue, vascularisation of sclera) and facial skin problems.

riboflavin
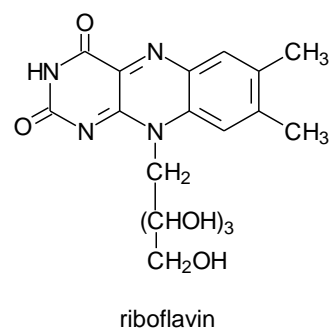
Vitamin B_6 is a name for the three related derivatives of 3-hydroxy-5-hydroxymethyl-2-methylpyridine. They all have the same biological effects and are distinguished by the group attached in position 4.
Pyridoxin (pyridoxol) has a primary alcoholic group (hydroxymethyl), **pyridoxal** an aldehyde group, **pyridoxamine** has a primary amine group (aminomethyl). The active form is **pyridoxal 5-phosphate**, a prosthetic group of enzymes which take part in amino acid transformation (aminotransferases, decarboxylases). The daily amount for adults is around 2 mg, which is covered by ordinary food but it becomes higher during lactation, catabolic states and when a high amount of ethanol is ingested (alcoholics). Absolute deficiency of vitamin B_6 is very rare, partial insufficiency with skin and mucosal problems or anaemia can be observed in alcoholics, pregnant or lactating women.

pyridoxal
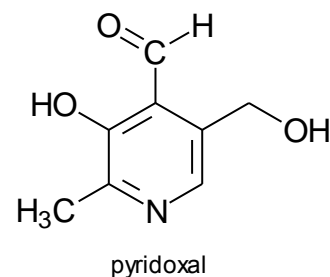
Niacin (vitamin B_3, vitamin PP, pellagra preventive factor) is a name for two derivatives of pyridine: **nicotinic acid** (pyridine-3-carboxylic acid) and its amide **nicotinamide**. Even though they can be formed in the human body, they belong to B type vitamins. Nicotinamide is a part of pyridine nucleotides, NAD^+ and $NADP^+$, coenzymes transferring hydrogen in reactions catalysed by many dehydrogenases. The daily intake of niacin for a healthy adult is 16 mg. Nicotinamide needs are covered by dietary proteins from milk or eggs, liver, meat, and yeast. Niacin is also formed in cells from tryptophan. Severe deficiency of niacin causes a disease called pellagra. The symptoms are, besides others, **d**iarrhoea, skin inflammation with pigmentations (**d**ermatitis), and psychical defects (**d**ementia) = **3D disease**; later neural and cardiac disorders are also observed. Pellagra occurs endemically in places where corn is the main component in a diet (it contains little tryptophan).
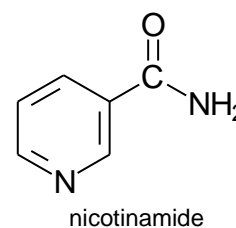
Cobalamin (vitamin B_{12}) has a very complex structure with a tetrapyrrole corrin system (see Chapter 32) and a central cobalt ion. It is only produced by bacteria, it is found in animal products, not in plant food. The pharmaceutical industry produces cyanocobalamin and hydroxocobalamin. A special glycoprotein, called internal factor, secreted in stomach mucosa is necessary for absorption of ingested cobalamin in the small intestine. Resorbed cobalamin is **stored in the liver**. In cells, it is transformed into the cofactors **methylcobalamin** and **deoxyadenosylcobalamin**. They are necessary for the degradation of some amino acids and mainly, together with folic acid, are needed for methylation reactions (remethylation of homocysteine into methionine, which is a determining factor for continuous synthesis of nucleic acid bases). The daily intake of B_{12} for adults is approximately 2 µg. Rich sources of cobalamin are meat, offal, dairy products, and eggs. Cobalamin is also formed by intestinal microflora, however, it is not absorbed and leaves the organism with stool. A deficiency of cobalamin is caused by insufficiency of the internal factor and malabsorption of cobalamin from food. Symptoms of deficiency are manifested as megaloblastic anaemia and specific neural disorders. It is treated by parenteral application of cobalamin. Hydroxocobalamin is used as an antidote in cyanide poisoning (see Chapter 33).

Folic acid (folate, acidum folicum) is a name used for pteroylglutamic acid. An active form in cells is **tetrahydrofolate**, a cofactor transferring one-carbon residues in various oxidation states. The daily intake of folate for adults is approximately 200 µg. The richest source is liver, green leafy vegetable (cabbage, spinach, sprouts), and yeast. A deficiency of folates is usually caused by insufficient absorption and results in abnormal blood count (megaloblastic anaemia, thrombocytopenia).

folic acid
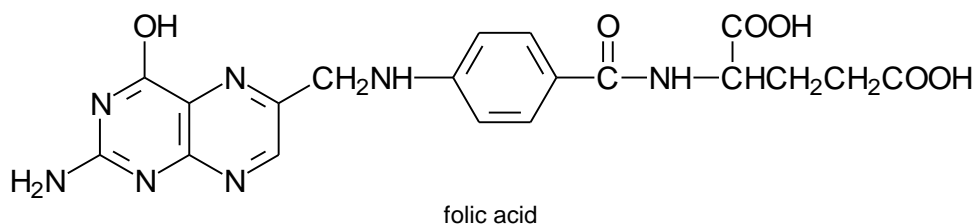
Pantothenic acid is an amide formed from β-alanine, to whose amino group the acyl of pantoic acid (2,4-dihydroxy-3,3-dimethylbutyric acid) is attached. It is very common in nature, therefore abundant in all foods. It is found in blood and in cells as a pantotheine (after attachment to cysteamine), which is a part of **coenzyme A** and the multienzyme complex synthesising fatty acids. The daily intake for adults is approximately 6-8 mg; a variety of foods covers it easily. A rich source is liver and egg yolk.

Supplementation of pantothenate is necessary while on complete parenteral nutrition. There are no characteristic symptoms of deficiency. Calcium salt (calcii pantothenas) or alcohol precursor **panthenol** (dexpanthenolum) can supplement and speed up healing of uninfected burns, surface scratches or catarrh of upper airways.

pantothenic acid
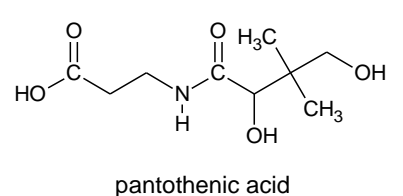
Biotin (vitamin H) has a condensed system formed by two five-membered rings, thiolane and imidazolidinone. It serves as a cofactor of carboxylases catalysing carboxylation reactions. Nitrogen heteroatom binds the CO_2 molecule and forms **carboxybiotin**, which is a donor of the carboxylic group for carboxylation, for example, pyruvate into oxaloacetate or acetyl-coenzyme A into malonyl-coenzyme A. It has an important role in gluconeogenesis (synthesis of glucose from lactate and amino acids), for the citric acid cycle (synthesis of oxaloacetate) and for synthesis of fatty acids.

The recommended daily intake (RDI) of biotin for adults is 50 μg. Biotin is found in common foods and the main part of the required amount is synthesized by **colon microflora**. A deficit of biotin in humans is quite rare. It could be caused by excessive consumption of uncooked egg whites. Those contain thermolabile glycoprotein avidin, which specifically binds biotin (complex cannot be digested by digestive enzymes) and therefore makes absorption of biotin impossible.

biotin

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