

# **Bioinorganic Chemistry for Medical Students**

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**Note to users:** This is the beta version of the bioinorganic chemistry handout. All readers are kindly requested to report any typos, inconsistencies or errors to the following email address: [medchem@med.unideb.hu](mailto:medchem@med.unideb.hu). Hungarian readers are encouraged to help authors and editors with Hungarian translation. Medical students may request credit for properly translating any 5 pages from the text.

# **Bioinorganic Chemistry for Medical Students**

## **Course Outline**

### **1. Co-ordination chemistry**

- 1.1. Introduction to bioinorganic chemistry: biological roles of elements
- 1.2. Coordination Complexes
- 1.3. Characteristics of coordination compounds
- 1.4. Bonding in Complexes
- 1.5. Coordination of metal ions in biological molecules

### **2. Alkali metal ions (**

- 2.1. Regulatory role of  $\text{Na}^+$  and  $\text{K}^+$  ions. Sodium-potassium ATP-ase
- 2.2. Natural and synthetic ligands for alkali metal ions
- 2.3. Lithium as anti-mania agent

### **3. Alkaline earth metal ions**

- 3.1. Calcium metabolism (absorption, excretion, hormonal control)
- 3.2. The calcium signal
- 3.3. Calcium binding proteins
- 3.4.  $\text{Mg}^{2+}$

### **4. Transition metal ions**

- 4.1. Iron metabolism (absorption, transport, storage, hemosiderosis, hemochromatosis)
- 4.2. Iron in hemoglobin
- 4.3. Heme proteins
- 4.4. Non-heme iron containing proteins. Iron-sulfur clusters.
- 4.5. Cu
- 4.6. Zn
- 4.7. Other transition metal ions: Mn, Ni, Au, Mo, Co, Cr

### **5. Toxic metals**

- 5.1. Pb
- 5.2. Hg
- 5.3. Cd

### **6. Non-metals**

- 6.1. O
- 6.2. Se
- 6.3. Si
- 6.4. F (
- 6.5. Cl
- 6.6. Br
- 6.7. I

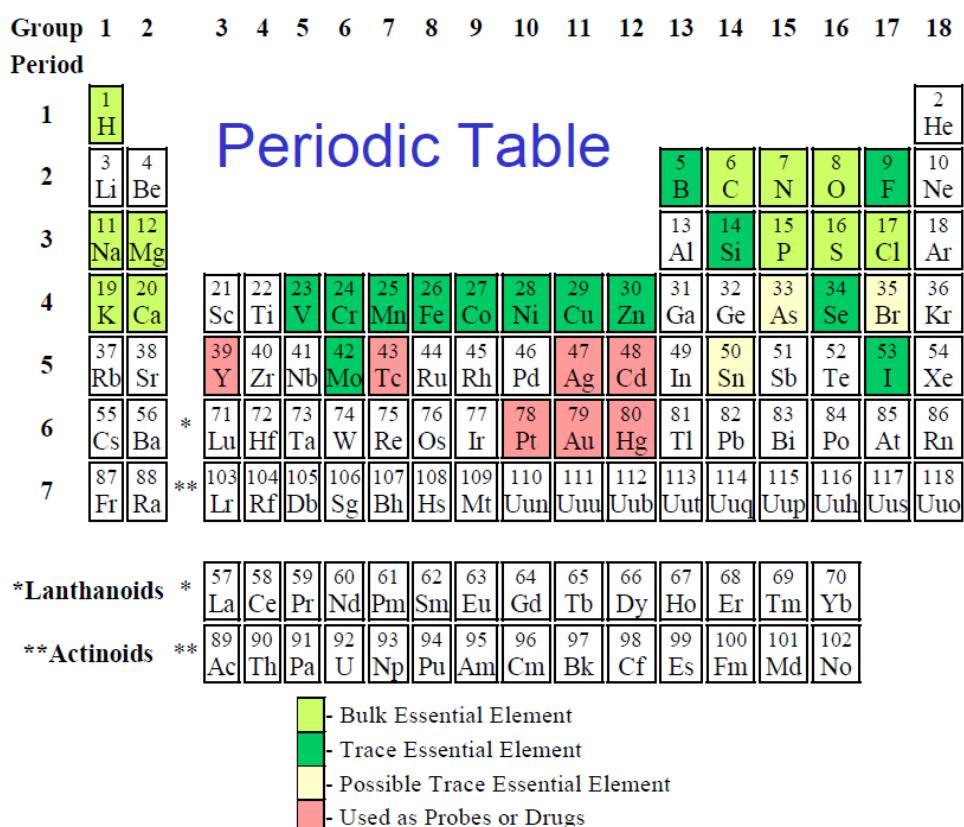
## COORDINATION CHEMISTRY

### 1. Coordination chemistry

#### 1.1. Introduction to bioinorganic chemistry: biological roles of elements

“Bioinorganic Chemistry” is at the gate-way of inorganic chemistry and biochemistry, i.e. it describes the mutual relationship between these two sub-disciplines, with focus upon the function of inorganic “substances” in living systems, including the transport, speciation and, mineralisation of inorganic materials, and including the use of inorganic elements and compounds in medicinal therapy and diagnosis. These inorganic “substances” can be metal ions (such as K<sup>+</sup>, Fe<sup>2+/3+</sup> etc.), composite ions (e.g. molybdate), coordination compounds (like cisplatin), or inorganic molecules such as CO, NO, O<sub>3</sub>.

**Fig. 1** classifies the bio-elements: along with the “organic elements” (C, H, O, N, S), many “inorganic elements” play important physiological roles building up bioorganic molecules, or „bio-mass”. Some of these inorganic elements, such as Fe, Cu and Zn, are present in (practically) all organisms, others are important for a restricted number of organisms only. An additional group of elements are used for diagnostic or therapeutic applications.



**Fig. 1.** Bio-elements in the periodic table: classification of the elements as bulk, trace and possible trace elements, and elements used as probes or as components of drugs.

## **Significance of biologically important elements**

Many of the inorganic elements and their derivatives (i. e. ions, composite ions) have well-defined biological and medical significance. Below is a selection of the most important biological functions of some of the elements.

**Na<sup>+</sup> and K<sup>+</sup>:** Most important „free“ intra- and extracellular cations. They regulate osmotic pressure, membrane potentials, enzyme activity, signalling.

**Mg<sup>2+</sup>:** Chlorophyll; anaerobic energy metabolism (ATP → ADP).

**Ca<sup>2+</sup>:** Signalling, muscle contraction, enzyme regulation. Main inorganic part of the endoskeletons (bones, teeth, enamel: hydroxyapatite; Ca<sub>5</sub>(PO<sub>4</sub>)<sub>3</sub>(OH)). Exoskeletons of mussels, shells, corals, sea urchins etc: aragonite or calcite; CaCO<sub>3</sub>).

**V(IV/V), Mo(IV/VI), W(IV/VI), Mn(II/III/IV), Fe(II/III), Ni(I/II/III), Cu(I/II):** active centres in electron-transport, (redox) enzymes, oxygenases, dismutases.

**Fe<sup>2+</sup> and Cu<sup>2+</sup>:** Transport of oxygen.

**Fe<sup>3+</sup>:** Iron-storage proteins (ferritins).

**Fe<sup>2+</sup> /Fe<sup>3+</sup>:** in magnetite (Fe<sub>3</sub>O<sub>4</sub>): orientation of magnetobacteria, pigeons, bees in Earth's magnetic field.

**Co:** Synthases and isomerases (cobalamines, e.g. vitamin-B<sub>12</sub>).

**Zn<sup>2+</sup>:** In the active centre of hydrolases, carboanhydrase, alcohol dehydrogenase, synthases; gene transcription (zinc fingers), stabilisation of tertiary and quartary structures of proteins; repair enzymes.

**Si(IV) ("silicate"):** Involved in the built-up of bones. In the form of SiO<sub>2</sub>/silica-gels as support in monocotyledonous plants (like grass) and the shells of diatoms.

**P(V) (phosphate):** Constituent in hydroxi- and fluorapatite (Ca<sub>5</sub>(PO<sub>4</sub>)<sub>3</sub>(OH/F)); energy metabolism (ATP), NADPH, activation of organic substrate; phospholipids in cell membranes; phosphate esters (DNA, RNA,...).

**Se(II):** Selenocystein in special enzymes (e.g. glutathionperoxidase)

**F:** Fluorapatite (Ca<sub>5</sub>(PO<sub>4</sub>)<sub>3</sub>F) in dental enamel

**Cl<sup>-</sup>:** Along with hydrogencarbonate (HCO<sub>3</sub><sup>-</sup>) the most important free anion.

**I:** Constituent of thyroid hormones (such as thyroxine).

## **Elements with medicinal relevance**

Besides the biological occurrence and functions some of the elements or their compounds may have therapeutic significance, since they are used as drugs to treat certain diseases.

**Li<sup>+</sup>:** Treatment of bipolar disorder (maniac depression) and hypertension.

**Gd<sup>3+</sup>:** Contrast agent in magnetic resonance tomography of soft tissues.

**BaSO<sub>4</sub>:** Contrast agent for X-ray tomography. Sun protection.

**<sup>99m</sup>Tc** (a metastable γ-emitter; t<sub>1/2</sub> = 6 h): Radio diagnostics (bone cancer, infarct risk, etc.)

**Pt(II):** Chemotherapy (e.g. with cisplatin *cis*-[Pt(NH<sub>3</sub>)<sub>2</sub>Cl<sub>2</sub>]) of cancer (ovaria, testes)

**Au(I):** Therapy of rheumatic arthritis.

**Sb(III):** Treatment of inflammatory skin pimples like acne.

**Bi(III):** Treatment of gastritis.

## Biological functions of metals

### **Structural roles**

-Ca<sup>2+</sup> and Mg<sup>2+</sup>

Components of

- Hard material –bone and teeth
- Cell membranes
- DNA and RNA structure
- Proteins, including enzymes, influence on conformations

### **Charge carriers**

-Na<sup>+</sup>, K<sup>+</sup>, Ca<sup>2+</sup>

### **Electron transfer (redox reactions)**

-Fe, Cu, Mn, Mo, Ni, Co

### **Metabolism**

-Degradation of organic molecules

### **Activation of small molecules**

-O<sub>2</sub>, N<sub>2</sub>, CO<sub>2</sub>

### **Mass of Metals in an Average 70 kg human**

Metal	Mass (g)	Biological Roles
Na	65-115	Regulatory action
K	155-195	Osmotic control and nerve action
Ca	1100	Bones, teeth, muscle triggering actions
V	1.1 x 10 <sup>-4</sup>	Enzymes (nitrogenases, haloperoxidases)
Cr	1.2 x 10 <sup>-2</sup>	Claimed (not yet proven) to be essential for glucose metabolism
Mn	1.2 x 10 <sup>-2</sup>	Enzyme (phosphatase, mitochondrial super oxide dismutase, glycosal transferase) photoredox activity in Photosystem II
Fe	4.20	Electron transfer (Fe-S proteins, cytochromes), O <sub>2</sub> storage and transport, enzymes (nitrogenases, hydrogenases, oxidases, reductases)
Co	3.0 x 10 <sup>-3</sup>	Vitamin-B <sub>12</sub> coenzyme
Ni	1.5 x 10 <sup>-2</sup>	Enzymes (ureases, some hydrogenases)
Cu	7.2 x 10 <sup>-2</sup>	Electron transfer (blue copper proteins), O <sub>2</sub> storage and transport (hemocyanins), Cu transport proteins (ceruloplasmin)
Zn	2.30	Lewis acid (Carboxypeptidase, carbonic anhydrase, alcohol dehydrogenase), structural roles
Mo	5.0 x 10 <sup>-3</sup>	Enzymes (nitrogenases, reductases, hydroxylases)

### **Daily allowances of metals in adults and infants**

inorganic constituents	recommended daily allowances (in mg)	
	adult	infant
K	2000-5500	530
Na	1100-3300	260
Ca	800-1200	420
Mg	300-400	60
Zn	15	5
Fe	10-20	7.0
Mn	2.0-5	1.3
Cu	1.5-3	1.0
Mo	0.075-0.250	0.06
Cr	0.05-0.2	0.04
Co	ca. 0.2 (vitamin B <sub>12</sub> )	0.001

## Metal deficiency syndroms

Metal	Function	Typical deficiency symptoms
Sodium	Charge carrier; osmotic balance	
Potassium	Charge carrier; osmotic balance	
Magnesium	Structure; hydrolase; isomerase	muscle cramps
Calcium	Structure; trigger; charge carrier	retarded skeletal growth
Vanadium	Nitrogen fixation; oxidase	
Chromium	Unknown, possible involvement in	diabetes symptoms, glucose tolerance
Molybdenum	Nitrogen fixation; oxidase; oxo transfer	retardation of cellular growth;
Tungsten	Dehydrogenase	
Manganese	Photosynthesis; oxidase; structure	infertility; impaired skeletal growth
Iron	Oxidase; dioxygen transport and storage;	anemia; electron transfer; nitrogen fixation
Cobalt	Oxidase; alkyl group transfer	disorders of the immune system
Nickel	Hydrogenase; hydrolase growth	pernicious anemia
Copper	Oxidase; dioxygen transport; e <sup>-</sup> transfer	depression; dermatitis
Zinc	Structure; hydrolase	artery weakness; liver disorders; anemia
		skin damage; stunted growth; sexual retardation

### 1.2. Coordination Complexes

In biological systems metal ions are found coordinated to biological macromolecules (e.g. in hemoglobin, chlorophyll, and enzymes. Therefore, to elucidate the physiological roles of most of the metals, it is essential to understand the basic principles of coordination chemistry. **Coordination compounds** represent a class of substances with chemical structures in which a central metal ion is surrounded by nonmetal atoms or groups of atoms, called ligands, joined to the central ion by chemical bonds.

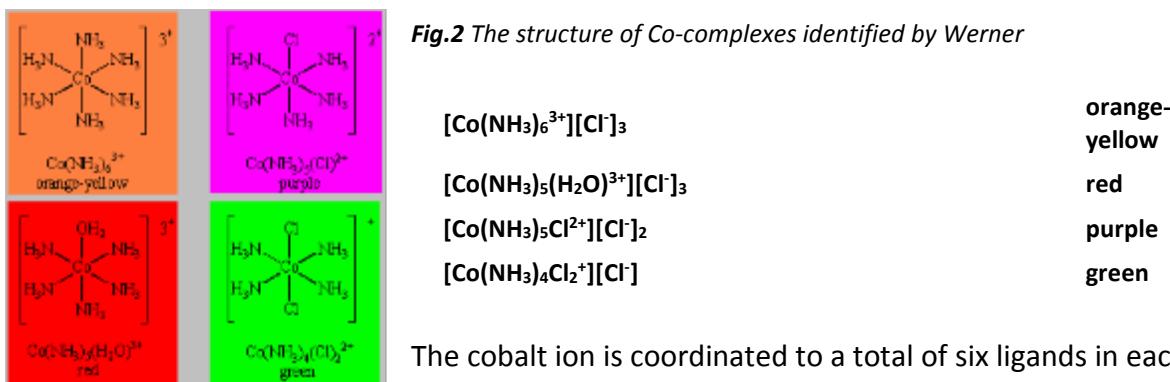
**Coordination compounds** are called such because they contain ions or molecules linked, or coordinated to a metal, most preferably to transition metals.

**Coordination compounds** are also known as **complex ions** or **coordination complexes**. They are **Lewis acid-base complexes**. The ions or molecules that bind to transition-metal ions to form these complexes are **called ligands** (from Latin *ligare*, "to tie or bind"). The **number of ligands** bound to the transition metal ion is called the **coordination number**.

#### 1.2.1. Historical aspects: Werner's Theory of Coordination Complexes



Werner (1866-1919; Nobel prize for Chemistry, 1913) developed a model of coordination complexes which explains the following observations: at least three different cobalt(III) complexes can be isolated when  $\text{CoCl}_2$  is dissolved in aqueous ammonia and then oxidized by air to the +3 oxidation state of cobalt. A fourth complex can be made by slightly different techniques. These complexes have different colors and different empirical formulas.



The cobalt ion is coordinated to a total of six ligands in each complex, which satisfies the **secondary valence** of this ion.

Each complex also has a total of three chloride ions that satisfy the **primary valence**. Some of the  $\text{Cl}^-$  ions are free to dissociate when the complex dissolves in water. Others are bound to the  $\text{Co}^{3+}$  ion and neither dissociates from nor reacts with  $\text{Ag}^+$ .

Ammonia reacts rapidly with hydrochloric acid to form ammonium chloride but in these complexes the reactivity of ammonia has been drastically reduced. In addition, the moles of  $\text{Cl}^-$  available to form precipitate with  $\text{Ag}^+$  are also different in the distinct complexes. Werner explained these observations by suggesting that transition-metal ions such as the  $\text{Co}^{3+}$  ion have a **primary valence** and a **secondary valence**. The **primary valence** is the number of negative ions needed to satisfy the charge on the metal ion. In each of the cobalt(III) complexes previously described, three  $\text{Cl}^-$  ions are needed to satisfy the primary valence of the  $\text{Co}^{3+}$  ion.

The **secondary valence** is the number of ions or molecules that are coordinated to the metal ion. Werner assumed that the secondary valence of the transition metal in these cobalt(III) complexes is six. The formulas of these compounds can therefore be written as follows.

Measurements of the conductivity of aqueous solutions of these complexes suggest also that the different forms dissociate to different number of ions. Werner assumed that transition-metal complexes had definite shapes. According to his theory, the ligands in six-coordinate cobalt(III) complexes are oriented toward the corners of an octahedron (**Fig. 2**).

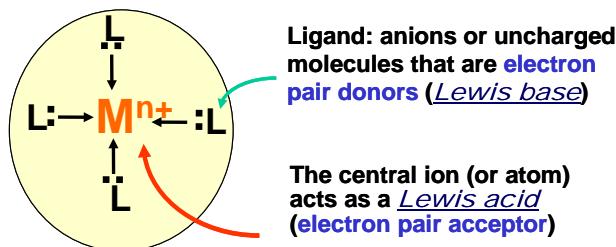
Werner also established the **configuration** (the spatial arrangement of ligands around the metal ion) of complexes by comparing the number and type of **isomers** (see below **Isomerism**) that he actually prepared for various series of compounds with the number and type theoretically predicted for various configurations. In this way he was able not only to refute the rival chain theory but also to demonstrate unequivocally that hexacoordinate cobalt(+3) possesses an octahedral configuration. Shortly after he and his American student Victor L. King resolved (split)  $[\text{CoCl}(\text{NH}_3)(\text{en})_2]\text{Cl}_2$  into its optical isomers in 1911, Werner received the 1913 Nobel Prize for Chemistry.

### 1.3. Characteristics of coordination compounds

#### 1.3.1. Formation of coordination compounds (complexes): a Lewis acid-base reaction

**G. N. Lewis** was the first to recognize that the reaction between a transition-metal ion and ligands to form a coordination complex was analogous to the reaction between the  $\text{H}^+$  and  $\text{OH}^-$  ions to form water. The reaction between  $\text{H}^+$  and  $\text{OH}^-$  ions involves the donation of a pair

of electrons from the  $\text{OH}^-$  ion to the  $\text{H}^+$  ion to form a covalent bond. The  $\text{H}^+$  ion can be described as an electron-pair acceptor. The  $\text{OH}^-$  ion, on the other hand, is an electron-pair donor. Lewis argued that any ion or molecule that behaves like the  $\text{H}^+$  ion should be an acid. Conversely, any ion or molecule that behaves like the  $\text{OH}^-$  ion should be a base. A Lewis acid is therefore any ion or molecule that can accept a pair of electrons. A Lewis base is an ion or molecule that can donate a pair of electrons.



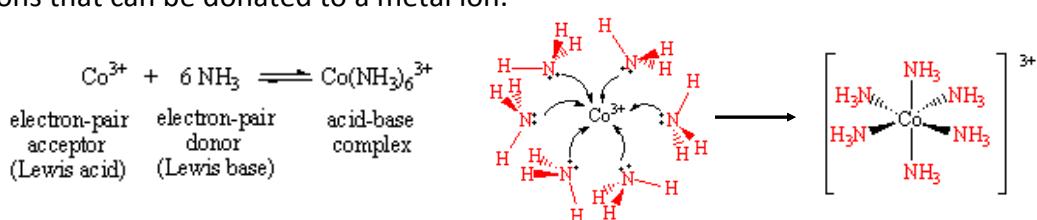
In a *coordination complex*, a central atom or ion forms coordinate bonds with ligands.

- Ligand is derived from Latin verb 'ligare' meaning 'to bind'.
- When a Lewis base donates a pair of electrons to a Lewis acid, a coordinate bond is formed and the resulting species is an adduct.

**Fig. 3.** Formation of coordination compounds (complexes): a Lewis acid-base reaction

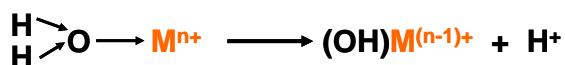
Following Lewis's ideas, the suggestion was made that the bonds between metals and ligands were of this same type, with the ligands acting as electron donors and the metal ions as electron acceptors (**Fig. 3**). This suggestion provided the first electronic interpretation of bonding in coordination compounds. The coordination reaction between cobalt ion and ammonia illustrates this acid base-reaction (**Fig. 4**)

According to this model, transition-metal ions form coordination complexes because they have empty valence-shell orbitals that can accept pairs of electrons from a Lewis base. Ligands must therefore be Lewis bases: They must contain at least one pair of nonbonding electrons that can be donated to a metal ion.



**Fig. 4.** Example of formation of complexes as a Lewis acid and-base reaction

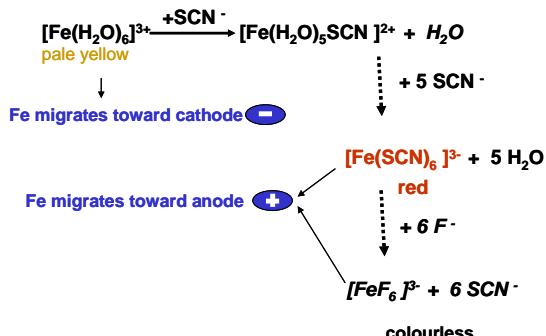
Complexes are formed in aqueous solution between metal ions and water molecules. When  $\text{H}_2\text{O}$  coordinates to  $\text{M}^{n+}$ , a general metal ion with  $n$  positive charges, the electrons in oxygen are withdrawn towards the metal center, leaving the H atoms more positively charged than in bulk water, and this charge separation will lead to dissociation of  $\text{H}^+$ :



For this reason the aqueous solution of some of the metal ions is acidic. A typical example of the aqueous solution of iron ions:



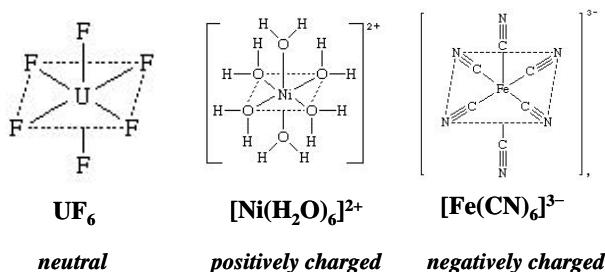
Hydrated cations can act as Bronsted acids by loss of H<sup>+</sup> from a coordinated water molecule. In aqueous solution stepwise complex formation occurs by substitution of the molecules bound in the first coordination sphere by other ligands binding stronger as illustrated by the following scheme:



During these complex formations the color, charge and conductivity of the components were changed similarly to Werner's predictions.

### 1.3.2. Charge of the complexes, oxidation number of the central metal ion/atom

**Coordination compounds** have been studied extensively to reveal the **molecular structure** and **chemical bonding** as well as to uncover their **unusual chemical nature** and **useful properties**. The substances in this class of compounds may be composed of **electrically neutral molecules** or of positively or negatively **charged species (ions)**



**Fig.5. Formation and structure of neutral and charged complexes**

Crystalline salts containing complex ions include potassium hexacyanoferrate (potassium ferricyanide) K<sub>3</sub>[Fe(CN)<sub>6</sub>], and the hexahydrate of nickel chloride, hexaaquanickel(II) chloride, [Ni(H<sub>2</sub>O)<sub>6</sub>]Cl<sub>2</sub>. In each case the charge on the complex ion is balanced by ions of opposite charge. In the case of potassium ferricyanide, three positively charged potassium ions (K<sup>+</sup>), balance the negative charge on the complex, and in the nickel complex the positive charges are balanced by two negative chloride ions, Cl<sup>-</sup>. The oxidation state of the central metal is determined from the charges on the ligands and the overall charge on the complex. For example, in hexaaquanickel(II), water is electrically neutral and the charge on the complex ion is +2; thus, the **oxidation state of Ni is +2**. In hexacyanoferrate(3-), all six cyano ligands have a charge of -1; thus, the overall charge of -3 dictates that the **oxidation state of Fe is +3**.

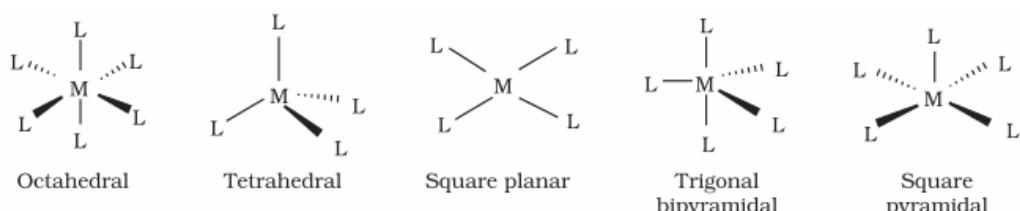
### 1.3.3. Coordination number

**Coordination number** is the term proposed by Werner to denote the **total number of bonds from the ligands to the metal atom**. Coordination numbers generally range between 2 and 12, with 4 (tetracoordinate) and 6 (hexacoordinate) being the most common. Werner referred to the central atom and the ligands surrounding it as the **coordination sphere**. Coordination number should be **distinguished from oxidation number** (defined in the previous paragraph). For the hexaamminecobalt(III) ion,  $[\text{Co}(\text{NH}_3)_6]^{3+}$ , and the neutral molecule triaminetrinitrocobalt (III),  $[\text{Co}(\text{NO}_2)_3(\text{NH}_3)_3]$ , the coordination number of cobalt is 6 while its oxidation number is +3.

The number of bonds depends on the size, charge, and electron configuration of the metal ion and the ligands. The chemistry of complexes is dominated by interactions between **s** and **p** molecular orbitals of the ligands and the **d** orbitals of the metal ions. The **s**, **p**, and **d** orbitals of the metal can accommodate 18 electrons. The **maximum coordination number for a certain metal** is thus **related to the electronic configuration of the metal ion** (to be more specific, **the number of empty orbitals**) and to the ratio of the size of the ligands and the metal ion. Large metals and small ligands lead to **high coordination numbers**, e.g.  $[\text{Mo}(\text{CN})_8]^{4-}$ . Small metals with large ligands lead to **low coordination numbers** (e.g.,  $\text{Pt}[\text{P}(\text{CMe}_3)]_2$ ).

### 1.3.4. Geometry of complexes

Different arrangements of ligands around the central ion (or atom) may result from the coordination number. Most structures follow the pattern as if the central atom were in the middle of a polyhedron where the corners of that shape are the locations of the ligands. These shapes are defined by orbital overlap between ligand and metal orbitals and ligand-ligand repulsions, which tend to lead to certain regular geometries. The most observed geometries are shown in **Fig. 6**.



**Fig.6.** Possible geometrical arrangements of ligands around the central atoms (Taken from School Textbooks Online: 9. Coordination Chemistry <http://textbook.s-anand.net/>)

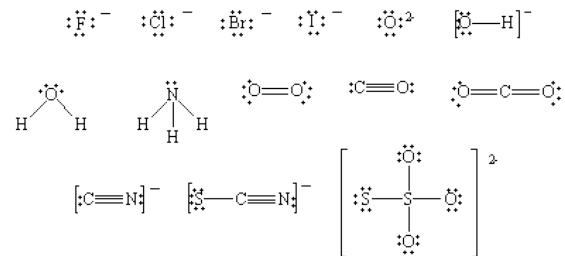
However, there are many cases that deviate from regular geometry. For example, ligands of different sizes and with different electronic effects often result in irregular bond lengths.

### 1.3.5. Classification of ligands

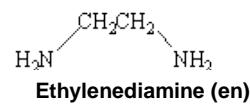
The **ions or molecules bound to the central atom/ion** in the coordination entity are called **ligands**. These may be simple ions such as  $\text{Cl}^-$ , small molecules such as  $\text{H}_2\text{O}$  or  $\text{NH}_3$ , larger molecules such as  $\text{H}_2\text{NCH}_2\text{CH}_2\text{NH}_2$  or  $\text{N}(\text{CH}_2\text{CH}_2\text{NH}_2)_3$  or even macromolecules, such as proteins. When a ligand is bound to a metal ion through a single donor atom, as with  $\text{Cl}^-$ ,  $\text{H}_2\text{O}$  or  $\text{NH}_3$ , the ligand is said to be **monodentate (or unidentate)**. When a ligand can bind through two donor atoms as in  $\text{H}_2\text{NCH}_2\text{CH}_2\text{NH}_2$  (ethane-1,2-diamine, or ethylenediamine) or  $\text{C}_2\text{O}_4^{2-}$

(oxalate), the ligand is said to be **bidentate (or didentate)** and when several donor atoms are present in a single ligand as in  $\text{N}(\text{CH}_2\text{CH}_2\text{NH}_2)_3$ , the ligand is said to be **polydeterminate**.

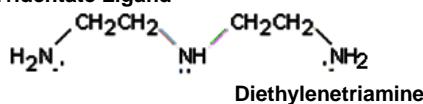
#### Monodentate Ligands



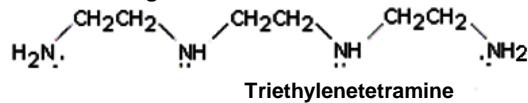
#### Bidentate Ligand



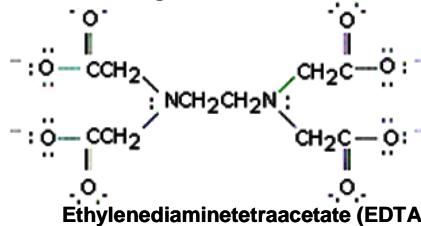
#### Tridentate Ligand



#### Tetradeinate Ligand



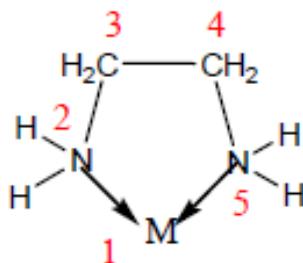
#### Hexadentate Ligand



**Fig. 7.** Structure of some mono- and polydeterminate ligands

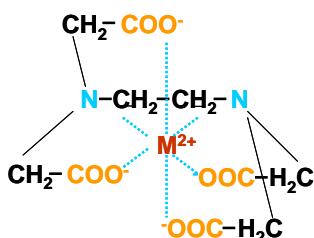
Ethylenediaminetetraacetate ion (EDTA) is an important hexadentate ligand. It can bind through two nitrogen and four oxygen atoms to a central metal ion.

When a di- or polydeterminate ligand uses its two or more donor atoms to bind a single metal ion, it is said to be a **chelate ligand**. The number of such ligating groups is called the denticity of the ligand. Such complexes, called chelate complexes tend to be more stable than similar complexes containing monodentate ligands. Chelate originates from the Greek word "Chelos" meaning "crab" claws, referring to the way crabs grab their food. Ethylenediamine,  $\text{H}_2\text{NCH}_2\text{CH}_2\text{NH}_2$  (en) forms a 5-membered chelate ring; you can think of it having "2 claws" coming in to grab the metal as indicated in **Fig. 8**.



**Fig. 8.** Chelate formation with the involvement of a metal ion and ethylenediamine

One very important chelating agent is ethylenediaminetetraacetate (EDTA<sup>4-</sup>) which has six donor groups/atoms and forms a complex with octahedral geometry (**Fig. 9**).



**Fig. 9.** Chelate formation between metal ion ( $M^{2+}$ ) and EDTA

EDTA is used to sequester unwanted metal ions from the organisms. In medicine sequestering agents are used to selectively remove toxic metal ions (e.g.  $Hg^{2+}$  and  $Pb^{2+}$ ) while leaving biologically important metals.

Ligand which can ligate through two different atoms is called **ambidentate ligand**. Examples of such ligands are the  $NO_2^-$  and  $SCN^-$  ions.  $NO_2^-$  ion can coordinate either through nitrogen or through oxygen to a central metal atom/ion. Similarly,  $SCN^-$  ion can coordinate through the sulphur or nitrogen atom.

### 1.3.6. Nomenclature of coordination compounds

Generally, the systematic naming of coordination compounds is carried out by rules recommended by the **International Union of Pure and Applied Chemistry (IUPAC)**. Among the more important of these are the following rules.

**1. Name the ligands first, in alphabetical order, then the metal atom or ion.** Note: The metal atom or ion is written before the ligands in the chemical formula. The names of some common ligands are listed in Table 1.

(i) For anionic ligands end in "-o"; for anions that end in "-ide" (e.g. chloride), "-ate" (e.g. sulfate, nitrate), and "-ite" (e.g. nitrite), change the endings as follows: -ide  $\rightarrow$  -o; -ate  $\rightarrow$  -ato; -ite  $\rightarrow$  -ito.

(ii) For neutral ligands, the common name of the molecule is used e.g.  $H_2NCH_2CH_2NH_2$  (ethylenediamine). **Important exceptions:** water is called 'aqua', ammonia is called 'ammine', carbon monoxide is called 'carbonyl', and the  $N_2$  and  $O_2$  are called 'dinitrogen' and 'dioxygen'.

**2. Greek prefixes** are used to designate the number of each type of ligand in the complex ion, e.g. di-, tri- and tetra-. If the ligand already contains a Greek prefix (e.g. ethylenediamine) or if it is polydentate ligands (ie. can attach at more than one binding site) the prefixes bis-, tris-, tetrakis-, pentakis-, are used instead. (See examples 3 and 4.)

**Table 1.** Names of Some Common Ligands

Anionic Ligands	Names		Neutral Ligands	Names
$Br^-$	bromo		$NH_3$	ammine
$F^-$	fluoro		$H_2O$	aqua
$O^{2-}$	oxo		NO	Nitrosyl

$\text{OH}^-$	Hydroxo	CO	Carbonyl
$\text{CN}^-$	cyano	$\text{O}_2$	dioxygen
$\text{C}_2\text{O}_4^{2-}$	oxalato	$\text{N}_2$	dinitrogen
$\text{CO}_3^{2-}$	carbonato	$\text{C}_5\text{H}_5\text{N}$	pyridine
$\text{CH}_3\text{COO}^-$	acetato	$\text{H}_2\text{NCH}_2\text{CH}_2\text{NH}_2$	ethylenediamine

3. After naming the ligands, name the central metal. If the complex ion is a cation, the metal is named same as the element. For example, Co in a complex cation is called cobalt and Pt is called platinum. (See examples 1-4). If the complex ion is an anion, the name of the metal ends with the suffix -ate. (See examples 5 and 6.). For example, Co in a complex anion is called cobaltate and Pt is called platinate. For some metals, the Latin names are used in the complex anions e.g. Fe is called ferrate (not ironate).

**Table 2:** Name of Metals in Anionic Complexes

Name of Metal	Name in an Anionic Complex
Iron	Ferrate
Copper	Cuprate
Lead	Plumbate
Silver	Argenate
Gold	Aurate

4. Following the name of the metal, the oxidation state of the metal in the complex is given as a Roman numeral in parentheses.

5. To name a neutral complex molecule, follow the rules of naming a complex cation. Name the (possibly complex) cation BEFORE the (possibly complex) anion. See examples 7 and 8.

For historic reasons, some coordination compounds are called by their common names. For example,  $\text{Fe}(\text{CN})_6^{3-}$  and  $\text{Fe}(\text{CN})_6^{4-}$  are named ferricyanide and ferrocyanide respectively, and  $\text{Fe}(\text{CO})_5$  is called iron carbonyl.

#### Examples:

1.  $[\text{Cr}(\text{NH}_3)_3(\text{H}_2\text{O})_3]\text{Cl}_3$  : **triaminetriaquachromium(III) chloride**

(i) The complex ion is inside the parentheses, which is a cation.

(ii) The ammine ligands are named before the aqua ligands according to alphabetical order.

(iii) Since there are three chlorides binding with the complex ion, the charge on the complex ion must be +3 (since the compound is electrically neutral).

From the charge on the complex ion and the charge on the ligands, we can calculate the oxidation number of the metal. In this example, all the ligands are neutral molecules. Therefore, the oxidation number of chromium must be same as the charge of the complex ion, +3.

2.  $[\text{Pt}(\text{NH}_3)_5\text{Cl}]\text{Br}_3$  : **pentaamminechloroplatinum(IV) bromide**

3.  $[\text{Pt}(\text{H}_2\text{NCH}_2\text{CH}_2\text{NH}_2)_2\text{Cl}_2]\text{Cl}_2$  : **dichlorobis(ethylenediamine)platinum(IV) chloride**

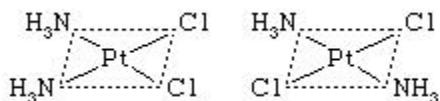
4.  $[\text{Co}(\text{H}_2\text{NCH}_2\text{CH}_2\text{NH}_2)_3]_2(\text{SO}_4)_3$  : tris(ethylenediamine)cobalt(III) sulfate
5.  $\text{K}_4[\text{Fe}(\text{CN})_6]$  : potassium hexacyanoferrate(II)
6.  $\text{Na}_2[\text{NiCl}_4]$  : sodium tetrachloronickelate(II)
7.  $\text{Pt}(\text{NH}_3)_2\text{Cl}_4$  : diamminetetrachloroplatinum(IV)
8.  $\text{Fe}(\text{CO})_5$  : pentacarbonyliron(0)

### 1.3 7. Isomerism of complexes

Coordination compounds often exist as isomers—i.e., as compounds with the same chemical composition but different structure. Many different kinds of isomerism occur among coordination compounds. The following section overviews some of the more common types of isomerism of complexes.

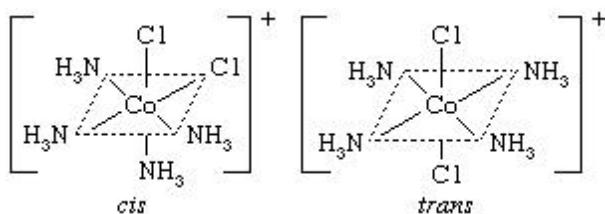
#### **Cis-trans isomerism**

**Cis-trans** (geometric) isomers of coordination compounds differ from one another only in the manner in which the ligands are distributed spatially; for example, in the isomeric pair of diamminedichloroplatinum compounds the two ammonia molecules and the two chlorine atoms are situated next to one another in one isomer, called the *cis* (Latin for “on this side”) isomer, and across from one another in the other, the *trans* (Latin for “on the other side”) isomer (**Fig. 10**).



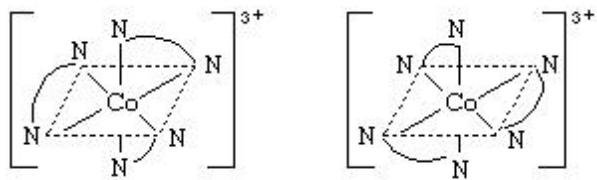
**Fig.10.** Structure of *cis-trans* isomers of diamminedichloroplatinum complex

A similar relationship exists between the *cis* and *trans* forms of the tetraammine-dichlorocobalt(I) ion (**Fig. 11**):



**Fig.11.** Structure of *cis-trans* isomers of tetraammine-dichlorocobalt(III) ion diamminedichloroplatinum complex

So-called **optical isomers (or enantiomers)** have the ability to **rotate plane-polarized light in opposite directions**. Enantiomers exist when the molecules of the substances are mirror images but are not superimposable upon one another. In coordination compounds, enantiomers can arise either from the presence of an asymmetric ligand, or from an asymmetric arrangement of the ligands. Examples of the latter variety are octahedral complexes carrying three bidentate ligands, such as ethylenediamine,  $\text{NH}_2\text{CH}_2\text{CH}_2\text{NH}_2$ . The two enantiomers are depicted by the structures below (**Fig. 12**).



**Fig.12.** Structure of enantiomers of  $\text{Co}(\text{en})_3$  complex. The ethylenediamine(en) ligands above are indicated by a curved line between the symbols for the nitrogen atoms

### **Ionization isomerism**

Certain isomeric pairs occur that differ only in that **two ionic groups exchange positions** within (and without) the **primary coordination sphere**. These are called ionization isomers and are exemplified by the two compounds, pentaamminebromocobalt sulfate and pentaammine-sulfatocobalt bromide:



In the former the bromide ion is coordinated to the cobalt(III) ion, and the sulfate ion is outside the coordination sphere; in the latter the sulfate ion occurs within the coordination sphere, and the bromide ion is outside it.

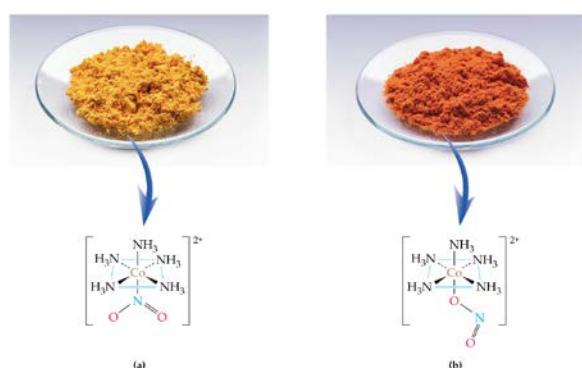
### **Linkage isomerism**

Isomerism also results when a given ligand is joined to the central atom through different atoms of the ligand. Such isomerism is called linkage isomerism. A pair of linkage isomers are the following complex ions:



in which the anionic ligand is joined to the cobalt atom through nitrogen or oxygen, as shown by designating it with the formulas  $\text{NO}_2^-$ (nitro) and  $\text{ONO}^-$ (nitrito), respectively. **Fig. 13** illustrates the different color and structure of these complexes.

**Fig. 13.** The different appearance and structure of linkage isomers



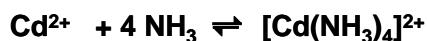
Another example of this variety of isomerism is given by the pair of ions



in which an isothiocyanate ( $\text{NCS}^-$ ) and a thiocyanate group ( $\text{SCN}^-$ ) are bonded to the cobalt(III) ion through a nitrogen or sulfur atom, respectively.

### 1.3.8. The stability of complexes

The complex formation is an equilibrium reaction: the ratios of the concentrations of the product(s) and the reactants at equilibrium raised to the power of the corresponding stoichiometric coefficient, that is an equilibrium constant ( $K_c$ ), characterizes the stability of coordination compounds and termed as stability constant ( $K_{st}$ ). For the formation of tetraamminecadmium(II) ion the reaction and the stability constant expression is given below.



$$K_{st} = \frac{[(\text{Cd}(\text{NH}_3)_4)^{2+}]}{[\text{Cd}^{2+}] [\text{NH}_3]^4}$$

$$K_{st} = 10^7$$

It is assumed that the larger is the  $K_{st}$ , the more stable is the complex. The stability of the complexes depends on the features of both the metal ion and the ligand (see Fig. 14). The polarization force of the metal ion influences the stability of aquacompounds as it is shown by the example of transition metal ions (Fig. 14a).

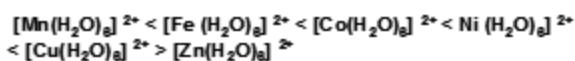
#### The stability of the complexes

##### Dependence on the properties of the central ion:

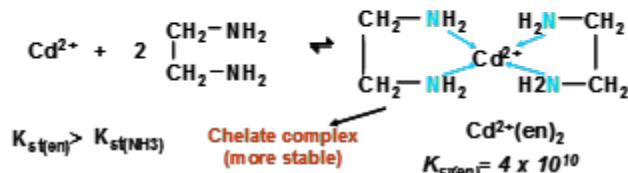
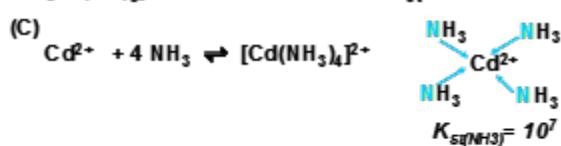
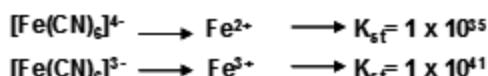
(a) ionic radii:  
 $\text{Mn}^{2+} > \text{Fe}^{2+} > \text{Co}^{2+} > \text{Ni}^{2+} > \text{Cu}^{2+} < \text{Zn}^{2+}$



Stability order of aquacompounds:



(b) oxidation number:



**Fig. 14.** The dependence of the stability of complexes on the central ion and ligands

The size of the  $\text{M}^{2+}$  metal ions decreases left to right in the periodic table from  $\text{Mn}^{2+}$  to  $\text{Cu}^{2+}$  complexes, the exception is  $\text{Zn}^{2+}$  where the complete  $d^{10}$  configuration results in shielding of the positive charge of the nucleus resulting in a relative increase of ion size. It is apparent that as the polarization force of the metal ions increases with increasing charge/size ratio the

aquacomplexes become more stable. The stability of complexes is influenced by the oxidation number of the metal ions too as illustrated by the  $[\text{Fe}(\text{CN})_6]^{4-}$  and  $[\text{Fe}(\text{CN})_6]^{3-}$  complexes: the higher is the oxidation number the more stable is the complex (*Fig. 14b*).

On the other hand the size of the ligand is also a determinant in the relative stability which is exemplified by the complexes of  $\text{Cd}^{2+}$  with different halogen anions and their stability constants:



Here the increasing size of the anion results in more stable complex (for explanation see the next section on “hard and soft acids and bases”).

Mono and polydentate ligands form complexes with the same metal ion with different stability even if the same donor atoms are involved in the bonding. This is shown by the different stability of the  $[\text{Cd}(\text{NH}_3)_4]^{2+}$  and  $[\text{Cd}(\text{H}_2\text{NCH}_2\text{CH}_2\text{NH}_2)_2]^{2+}$  complexes (see *Fig. 14*, lower part) confirming also that chelate complexes formed with polydentate ligands are more stable than the one formed with monodentate ligands. This is due to several reasons among which three are dominant: (i) kinetic effect: once one group was bound within the same molecule the other one is close and more favourable to bind, (ii) five membered ring is formed that also stabilizes the molecule, (iii) the stabilization effect due to increase of entropy by the replacing monodentate ligands with the chelate forming agent.

### 1.3.9. Pearson's Hard and Soft acids and bases: application to predict the stability of complexes

**Hard and Soft Acids and Bases Theory** is a qualitative concept introduced by Ralph Pearson to explain the stability of metal complexes and the mechanisms of their reactions. According to this theory, the Lewis acids and bases can be further divided into hard or soft or border line types (*Table 3*). **Hard Lewis acids** are characterized by small ionic radii, high positive charge, strongly solvated, empty orbitals in the valence shell. **Soft Lewis acids** are characterized by large ionic radii, low positive charge, completely filled atomic orbitals. **Hard Lewis bases** are characterized by small ionic radii, strongly solvated, highly electronegative, weakly polarizable. **Soft Lewis bases** are characterized by large ionic radii, intermediate electronegativity, highly polarizable. The **Border line** Lewis acids and bases have intermediate properties. It is not necessary for a Lewis acid or base to possess all the properties to be classified as hard or soft or borderline. In short, hard acids and bases are small and non-polarizable, whereas soft acids and bases are larger and more polarizable.

**Hard-soft acid-base principle:** according to this concept, hard acids prefer binding to the hard bases to give ionic complexes, whereas the soft acids prefer binding to soft bases to give covalent complexes.

- The large electronegativity differences between hard acids and hard bases give rise to strong ionic interactions.
- The electronegativities of soft acids and soft bases are almost same and hence have less ionic interactions. i.e., the interactions between them are more covalent.
- The interactions between hard acid - soft base or soft acid - hard base are mostly polar covalent and tend to be more reactive or less stable. The polar covalent compounds readily form either more ionic or more covalent compounds if they are allowed to react.

**Table 3.** Characteristics of hard, soft and borderline acids and bases

Type of Acid/Base	CHARACTERISTICS	EXAMPLES
Hard acids	<ul style="list-style-type: none"> <li>* Atomic centres of small ionic radii (&lt;90 pm).</li> <li>* High positive charge.</li> <li>* Empty orbitals in their valence shells.</li> <li>* Low electronegativity (0.7-1.6) and low electron affinity.</li> <li>* Likely to be strongly solvated.</li> </ul>	H <sup>+</sup> , Li <sup>+</sup> , Na <sup>+</sup> , K <sup>+</sup> , Be <sup>2+</sup> , Mg <sup>2+</sup> , Ca <sup>2+</sup> , Sr <sup>2+</sup> , Sn <sup>2+</sup>  Al <sup>3+</sup> , Ga <sup>3+</sup> , In <sup>3+</sup> , Cr <sup>3+</sup> , Co <sup>3+</sup> , Fe <sup>3+</sup> , Ir <sup>3+</sup> , La <sup>3+</sup> , Si <sup>4+</sup> , Ti <sup>4+</sup> , Zr <sup>4+</sup> , Th <sup>4+</sup> , VO <sup>2+</sup> , UO <sub>2</sub> <sup>2+</sup>  BeMe <sub>2</sub> , BF <sub>3</sub> , BCl <sub>3</sub> , B(OR) <sub>3</sub> , AlMe <sub>3</sub>
Soft acids	<ul style="list-style-type: none"> <li>* Large radii (&gt;90 pm).</li> <li>* Low or partial positive charge.</li> <li>* Completely filled orbitals in their valence shells.</li> <li>* Intermediate electronegativities (1.9-2.5)</li> </ul>	Cu <sup>+</sup> , Ag <sup>+</sup> , Au <sup>+</sup> , Hg <sup>+</sup> , Cs <sup>+</sup> , Tl <sup>+</sup> , Hg <sup>2+</sup> , Pd <sup>2+</sup> , Cd <sup>2+</sup> , Pt <sup>2+</sup>  Metal atoms in zero oxidation states
Border line acids		Fe <sup>2+</sup> , Co <sup>2+</sup> , Ni <sup>2+</sup> , Cu <sup>2+</sup> , Zn <sup>2+</sup> , Pb <sup>2+</sup> , B(CH <sub>3</sub> ) <sub>3</sub> , SO <sub>2</sub> , NO <sup>+</sup>
Hard bases	<ul style="list-style-type: none"> <li>* Small radii (around 120pm) &amp; highly solvated .</li> <li>* electronegative atomic centres (3.0-4.0).</li> <li>* Weakly polarizable.</li> <li>* Difficult to be oxidized.</li> </ul>	H <sub>2</sub> O, OH <sup>-</sup> , F <sup>-</sup> , Cl <sup>-</sup> , CH <sub>3</sub> CO <sub>2</sub> <sup>-</sup> , PO <sub>4</sub> <sup>3-</sup> , SO <sub>4</sub> <sup>2-</sup> , CO <sub>3</sub> <sup>2-</sup> , NO <sub>3</sub> <sup>-</sup> , ClO <sub>4</sub> <sup>-</sup> , ROH, RO <sup>-</sup> , R <sub>2</sub> O, NH <sub>3</sub> , RNH <sub>2</sub> , N <sub>2</sub> H <sub>4</sub>
Soft bases	<ul style="list-style-type: none"> <li>* Large atoms (&gt;170 pm) with intermediate electronegativity (2.5-3.0).</li> <li>* High polarizability</li> <li>* Easily undergo oxidation.</li> </ul>	RSH, RS <sup>-</sup> , R <sub>2</sub> S, I <sup>-</sup> , CN <sup>-</sup> , SCN <sup>-</sup> , S <sub>2</sub> O <sub>3</sub> <sup>-</sup> , R <sub>3</sub> P, R <sub>3</sub> As (RO) <sub>3</sub> P, RNC, CO, C <sub>2</sub> H <sub>4</sub> , C <sub>6</sub> H <sub>6</sub> , R <sup>-</sup> , H <sup>-</sup>
Border line bases		pyridine, N <sub>3</sub> <sup>-</sup> , Br <sup>-</sup> , NO <sub>2</sub> <sup>-</sup> , SO <sub>3</sub> <sup>2-</sup> , N <sub>2</sub>

**Examples:** in chemistry and coordination chemistry.

**In hydrogen bonding:** The strong hydrogen bond is possible in cases of H<sub>2</sub>O, NH<sub>3</sub> and HF, since the donor atoms (F, O and N) are hard Lewis bases and their interactions with partially positively charged H, which is a hard acid, are stronger.

**Linkage of ambidentate ligands to metal atoms:** The ambidentate ligand, SCN<sup>-</sup> can bind either by S end or N end. The bonding mode can be determined by using hard-soft acid-base principle: it bonds through sulfur atom (soft base) when bonded to Pt<sup>2+</sup>, a soft acid, however it bonds through nitrogen atom (a hard base) when linked to Cr<sup>3+</sup>, a hard acid.

**Stability of complexes with different sizes of ligands and central ions.** The distinct stability of Cd<sup>2+</sup>-halogen complexes such as [CdCl<sub>4</sub>]<sup>2-</sup> < [CdBr<sub>4</sub>]<sup>2-</sup> < [CdI<sub>4</sub>]<sup>2-</sup> could be easily explained by the fact that Cd<sup>2+</sup> is a soft metal ion and as the “softness” of the ligand is increases from Cl<sup>-</sup> to I<sup>-</sup>, the stability of the complexes increase, too.

#### 1.4. Bonding in Complexes

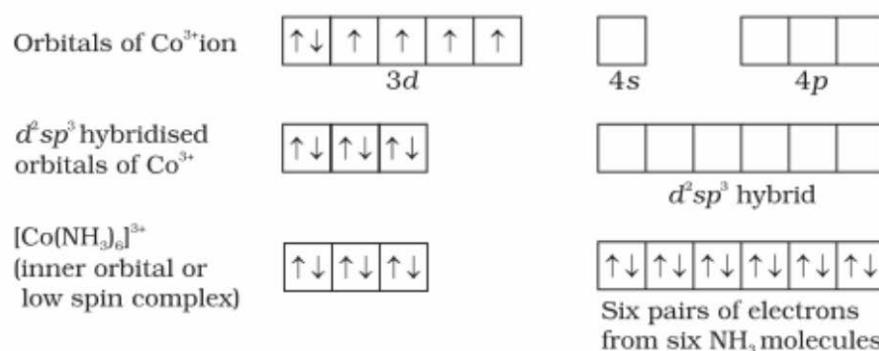
The decription of chemical bonding in coordination compounds should be in agreements with the structure and geometry of these molecules and shall explain specific features like the color and magnetic properties of complexes.

### 1.4.1. The Valence-Bond Approach to Bonding in Complexes

The idea that atoms form covalent bonds by sharing pairs of electrons was first proposed by G. N. Lewis in 1902. It was not until 1927, however, that Walter Heitler and Fritz London showed how the sharing of pairs of electrons holds a covalent molecule together. The Heitler-London model of covalent bonds was the basis of the valence-bond theory. The last major step in the evolution of this theory was the suggestion by Linus Pauling that atomic orbitals mix to form hybrid orbitals, such as the  $sp$ ,  $sp^2$ ,  $sp^3$ ,  $dsp^3$ , and  $d^2sp^3$  orbitals.

The valence-bond theory formally distinguishes between "inner-shell" complexes, which use  $3d$ ,  $4s$  and  $4p$  orbitals to form a set of  $d^2sp^3$  hybrids, and "outer-shell" complexes, which use  $4s$ ,  $4p$  and  $4d$  orbitals to form  $sp^3d^2$  hybrid orbitals.

It is easy to apply the valence-bond theory to some coordination complexes, such as the  $\text{Co}(\text{NH}_3)_6^{3+}$  and  $[\text{CoF}_6]^{3-}$  ions. These two complexes, however, are distinct with respect to the magnetic properties since  $\text{Co}(\text{NH}_3)_6^{3+}$  is diamagnetic, while  $[\text{CoF}_6]^{3-}$  is paramagnetic. Compounds in which all of the electrons are paired are diamagnetic — they are repelled by both poles of a magnet. Compounds that contain one or more unpaired electrons are paramagnetic — they are attracted to the poles of a magnet. **Paramagnetic complexes** could also be **high-spin** or **low-spin depending on the number of unpaired electrons** in the molecules. The force of attraction between paramagnetic complexes and a magnetic field is proportional to the number of unpaired electrons in the complex. We can therefore determine whether a complex is high-spin or low-spin by measuring the strength of the interaction between the complex and a magnetic field.



**Fig.15.** Formation of diamagnetic  $\text{Co}^{3+}$  complexes (Taken from School Textbooks Online: 9. Coordination Chemistry <http://textbook.s-anand.net/>)

Starting with the electron configuration ( $\text{Co}^{3+}$ : [Ar]  $3d^6$ ) and looking at the valence-shell orbitals ([Ar]  $3d^6 4s^0 4p^0$ ) note that the  $4s$  and  $4p$  orbitals are empty (**Fig. 15**). Concentrating and pairing the  $3d$  electrons in three  $3d$  orbitals frees the electrons from two  $3d$  orbitals. The  $3d$  freed from electrons with the  $4s$ ,  $4p_x$ ,  $4p_y$  and  $4p_z$  orbitals are then mixed to form a set of empty  $d^2sp^3$  orbitals that point toward the corners of an octahedron. Each of these orbitals can accept a pair of nonbonding electrons from a neutral  $\text{NH}_3$  molecule to form a complex in which the cobalt atom has a filled shell of valence electrons. Six pairs of electrons, one from each  $\text{NH}_3$  molecule, occupy the six hybrid orbitals. Thus, the complex has octahedral geometry and is diamagnetic because of the absence of unpaired electron. In the formation of this complex, since the inner  $d$  orbital ( $3d$ ) is used in hybridisation, the complex,  $[\text{Co}(\text{NH}_3)_6]^{3+}$  is called a **low spin, or spin paired complex**.

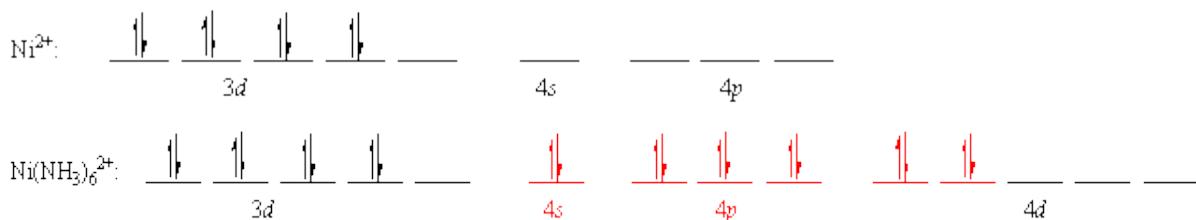
The paramagnetic octahedral complex,  $[\text{CoF}_6]^{3-}$  uses outer orbitals ( $4d$ ) in an  $sp^3d^2$  hybridisation scheme (Fig. 16). It is thus called **outer orbital, or high spin, or spin free complex**. Thus:

Orbitals of $\text{Co}^{3+}$ ion			
$sp^3d^2$ hybridised orbitals of $\text{Co}^{3+}$			
$[\text{CoF}_6]^{3-}$ (outer orbital or high spin complex)			

Six pairs of electrons from six  $\text{F}^-$  ions

**Fig. 16.** Formation of paramagnetic  $\text{Co}^{3+}$  complexes (Taken from School Textbooks Online: 9. Coordination Chemistry <http://textbook.s-anand.net/>)

The usage of outer  $d$  orbitals in coordination bonding is also useful to explain bonding in complexes, which are at first glance, such as the  $\text{Ni}(\text{NH}_3)_6^{2+}$  ion, seem hard to explain with the valence-bond theory. The configuration of this transition-metal ion is  $\text{Ni}^{2+}$ :  $[\text{Ar}] 3d^8$  (Fig. 17). This configuration creates a problem, because there are eight electrons in the  $3d$  orbitals. Even if we invest the energy necessary to pair the  $3d$  electrons, we can't find two empty  $3d$  orbitals to use to form a set of  $d^2sp^3$  hybrids. There is a way around this problem. The five  $4d$  orbitals on nickel are empty, so we can form a set of empty  $sp^3d^2$  hybrid orbitals by mixing two  $4d$  and the  $4s$ ,  $4p_x$ ,  $4p_y$  and  $4p_z$  orbitals. These hybrid orbitals then accept pairs of nonbonding electrons from six ammonia molecules to form a complex ion.



**Fig. 17.** Bonding in  $\text{Ni}(\text{NH}_3)_6^{2+}$  complex ion (Taken from School Textbooks Online: 9. Coordination Chemistry <http://textbook.s-anand.net/>)

While the VB theory, to a larger extent, explains the formation, structures and magnetic behaviour of coordination compounds, however, it suffers from the following shortcomings:

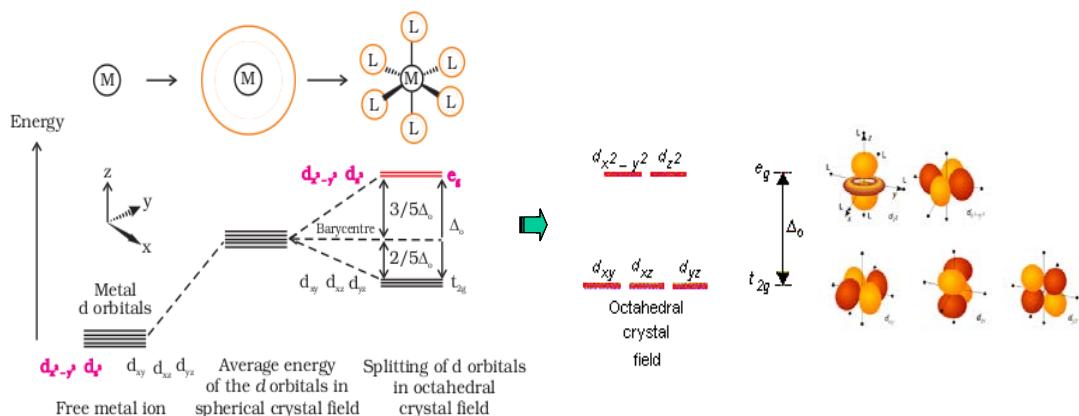
- (i) it involves a number of assumptions.
- (ii) it does not give quantitative interpretation of magnetic data.
- (iii) it does not explain the colour exhibited by coordination compounds.
- (iv) it does not give a quantitative interpretation of the thermodynamic or kinetic stabilities of coordination compounds.
- (v) It does not distinguish between weak and strong ligands.

#### 1.4.2. The Crystal Field Theory (CFT) Approach to Bonding in Complexes

At almost exactly the same time that chemists were developing the valence-bond model for coordination complexes, an alternative known as crystal field theory were developed. This theory tried to describe the effect of the electrical field of neighboring ions on the energies of the valence orbitals of an ion in a crystal and this is applied to complexes. The Crystal Field

Theory (CFT) is a model for the bonding interaction between transition metals and ligands. It describes the effect of the attraction between the positive charge of the metal cation and negative charge on the non-bonding electrons of the ligand. The key points are as follows.

When the ligands approach the central metal ion, ***d shell degeneracy is broken*** due to the static electric field. CFT successfully accounts for some magnetic properties, colors, and hydration energies of transition metal complexes, but it does not attempt to describe bonding. This assumes only electrostatic interactions and does not consider any covalent bonding ( $\sigma$  or  $\pi$  bonds) or orbital overlap. Ionic interactions between the metal ions and ligands is assumed.

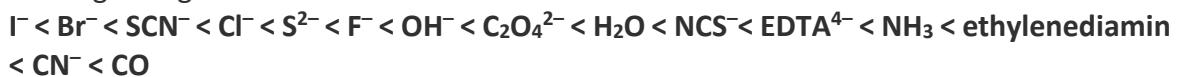


**Fig. 18.** Splitting of ***d*** orbitals in an octahedral crystal field (left and middle part) and interaction of the different ***d*** orbitals with the ligand (L). (Taken from School Textbooks Online: 9. Coordination Chemistry <http://textbook.s-anand.net/> and C. M. Kozak – Chemistry 3211)

All of the *d* orbitals are raised in energy due to repulsive interactions with the electrons of the ligand (L) (Fig. 18). However, degeneracy (equal energy) of the *d* orbitalson at this raised energy level would only be kept up in case of a spherical crystal field.

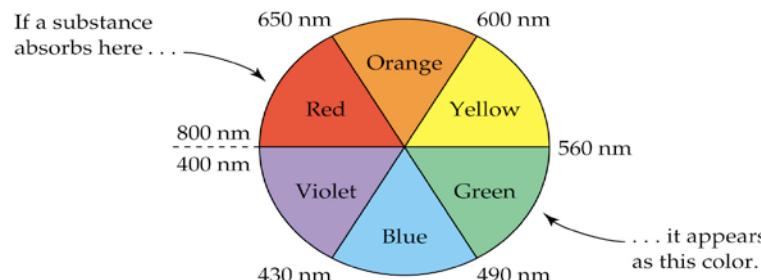
- Because electrons repel each other, the ***d electrons closer to the ligands will have a higher energy than those further away, resulting in the d orbitals splitting***. The splitting is affected by the nature and oxidation state of the metal ion and the arrangement and nature of the ligands.
- In *d* orbitals, the  $d_{x^2-y^2}$  and  $d_{z^2}$  all point directly along the x, y, and z axes and form an  $e_g$  set. The lobes of the  $d_{xy}$ ,  $d_{xz}$ , and  $d_{yz}$  all line up in the quadrants and form the  $t_{2g}$  set.
- The ***crystal field stabilization energy (CFSE)*** is the stability that ***results from ligand binding***.

The ***crystal field splitting***,  $\Delta_o$ , depends upon the field *d* orbitals produced by the ligand and charge on the metal ion. Some ligands are able to produce strong fields in which case, the splitting will be large whereas others produce weak fields and consequently result in small splitting of *d* orbitals. In general, ligands can be arranged in a series in the order of increasing field strength as given below:



Such a series is termed as ***spectrochemical series***. It is an experimentally determined series ***based on the absorption of light by complexes*** with different ligands. The most distinctive properties of transition metal complexes are their wide range of colours. This means that

some of the visible spectrum is being removed from white light (absorbed by the substance) as it passes through the sample, so the light that emerges is no longer white. The colour of the complex is complementary to that which is absorbed. The complementary colour is the colour generated from the wavelength left over; if green light is absorbed by the complex, it appears red. **Fig. 19** gives the relationship of the different wavelength absorbed and the colour observed.



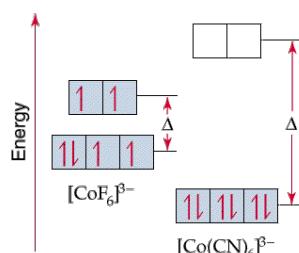
**Relationship between the Wavelength of Light absorbed and the Colour observed in some Coordination Entities**

Coordination entity	Wavelength of light absorbed (nm)	Colour of light absorbed	Colour of coordination entity
$[\text{CoCl}(\text{NH}_3)_5]^{2+}$	535	Yellow	Violet
$[\text{Co}(\text{NH}_3)_5(\text{H}_2\text{O})]^{3+}$	500	Blue Green	Red
$[\text{Co}(\text{NH}_3)_6]^{3-}$	475	Blue	Yellow Orange
$[\text{Co}(\text{CN})_6]^{3-}$	310	Ultraviolet	Pale Yellow
$[\text{Cu}(\text{H}_2\text{O})_4]^{2+}$	600	Red	Blue
$[\text{Ti}(\text{H}_2\text{O})_6]^{3+}$	510	Blue Green	Purple

**Fig. 19.** The light absorbtion and color of different complexes (Taken from School Textbooks Online: 9. Coordination Chemistry <http://textbook.s-anand.net/>)

The colour in the coordination compounds can be readily explained in terms of the crystal field theory. Consider, for example, the complex  $[\text{Ti}(\text{H}_2\text{O})_6]^{3+}$ , which is violet in colour. This is an octahedral complex where the single electron ( $\text{Ti}^{3+}$  is a  $3d^1$  system) in the metal  $d$  orbital is in the  $t_{2g}$  level in the ground state of the complex. The next higher state available for the electron is the empty  $e_g$  level. If light corresponding to the energy of yellow-green region is absorbed by the complex, it would excite the electron from  $t_{2g}$  level to the  $e_g$  level ( $t_{2g}^1 e_g^0 \rightarrow t_{2g}^0 e_g^1$ ). Consequently, the complex appears violet in colour (**Fig. 19**). **The crystal field theory attributes the colour of the coordination compounds to d-d transition of the electron. Therefore, different crystal field splitting ( $\Delta_o$ ), values due to the distinct ligands in the spectrochemical series determine the color of complexes** (see differences for changing  $\text{Cl}^-$  for  $\text{H}_2\text{O}$  or  $\text{NH}_3$  in the  $\text{Co}^{3+}$  complexes in **Fig. 2**).

CFS gives also explanation for the magnetic properties of the complexes as shown by the example of  $[\text{CoF}_6]^{3-}$  and  $[\text{Co}(\text{CN})_6]^{3-}$  complexes (**Fig. 20**)

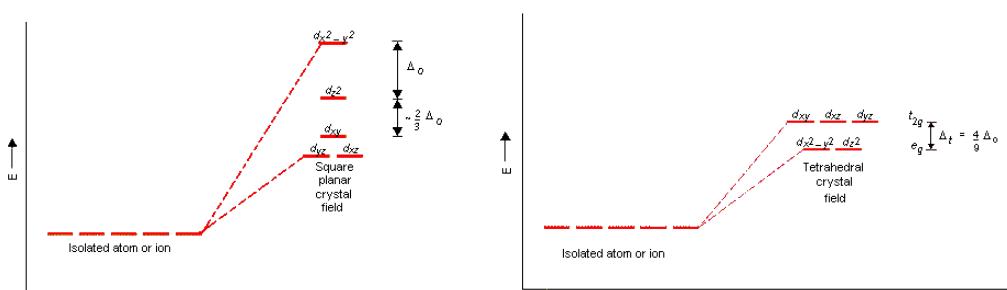


**Fig. 20.** Formation of low-spin and high spin complexes according to the Crystal Field Theory

How electrons occupy the splitted d orbitals depends on the requirement of energy to overcome **crystal field splitting ( $\Delta_o$ ), that is to occupy higher energy orbitals unpaired, or the energy needed to pair electrons (P) at the lower energy level.** Which of these possibilities occurs, depends on the relative magnitude of the crystal field splitting,  $\Delta_o$  and the pairing energy, P. The two options are:

- (i) If  $\Delta_o < P$ , as in  $[\text{CoF}_6]^{3-}$  electrons enter of the  $e_g$  orbitals giving the configuration  $t_{2g}^4e_g^2$ . Ligands for which  $\Delta_o < P$  are known as **weak field ligands** and form **high spin complexes**.
- (ii) If  $\Delta_o > P$ , as in  $[\text{Co}(\text{CN})_6]^{3-}$ , it becomes energetically more favourable for the electrons to occupy a  $t_{2g}$  orbital with configuration  $t_{2g}^6e_g^0$ . Ligands which produce this effect are known as **strong field ligands** and form **low spin complexes**.

It is to note that further splitting of d orbitals occur in square planar complexes since the energy of  $d_{z2}$  orbital, since ligand is not bound from that direction, is lower than the  $d_{x^2-y^2}$  orbital (**Fig.21**) and there is a slight increase in the energy of  $d_{xy}$  orbital, too.



**Fig. 21** Splitting of d orbitals in square planar and tetrahedral fields (taken from C. M. Kozak – Chemistry 3211)

These multiple splitting of orbitals creates novel possibilities for electron transitions between the distinct d levels. In tetrahedral field the energy levels of splitted d orbitals are the opposite of that found in octahedral field, i. e. energy levels of the  $d_{xy}$ ,  $d_{xz}$ , and  $d_{yz}$  orbitals are higher than the  $d_{x^2-y^2}$  and  $d_{z2}$  orbitals.

The crystal field model is successful in explaining the formation, structures, colour and magnetic properties of coordination compounds to a large extent. However, from the assumptions that the ligands are point charges, it follows that anionic ligands should exert the greatest splitting effect. The anionic ligands actually are found at the low end of the spectrochemical series. Further, it does not take into account the covalent character of bonding between the ligand and the central atom. These are some of the weaknesses of CFT, which are explained by ligand field theory (LFT) and molecular orbital theory which are beyond the scope of the present study.

## 1.5. Coordination of metal ions in biological molecules

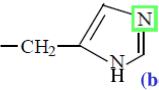
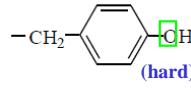
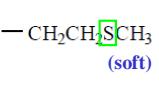
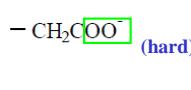
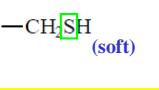
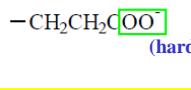
Metal ions are not usually present as free metal ions, but instead as complexes in biological system. The ligands involved in coordination may be more complicated than the ones discussed within coordination compounds, but the donor atoms are the usual ones (O, N, S) and the principles learnt in coordination chemistry can be equally applied to them such as matching the hard/soft properties of donor to metal, the effects of various geometries on orbital energies, formation of high- and low spin complexes.

### 1.5.1. Biological ligands

Coordination complexes constituted by **metal ions** and **bioligands** are often **biologically relevant**: metal ions are Lewis acids able to accept lone pairs coming from the ligand which acts as a Lewis base. Bioligands can be grouped in three main classes: (i) peptides (proteins) with amino acid side chains for coordination (ii) macrocyclic chelate ligands (iii) nucleobases (nucleic acids).

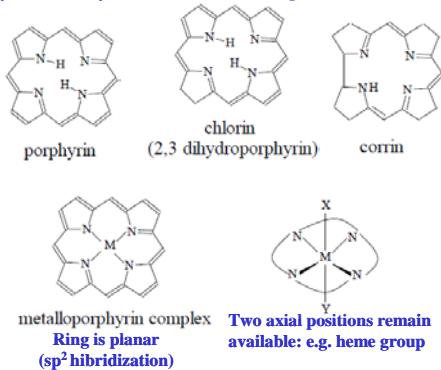
The role of proteins are numerous: (i) act as multidentate chelate ligand (via amino acid side chains) (ii) provide spatial fixation for the metal ion. **Table 4** lists the most important amino acids which could be involved in coordination of metal ions. It is also indicated in Table 4 if the donor atom features in the aminoacid side chain classifies the ligand as hard, soft or borderline base according to the criteria of Pearson's principles.

**Table 4 Aminoacid side chains involved in coordination of metal ions**

Aminoacid	R	Aminoacid	R
histidine		tyrosine	
methionine		aspartate	
cysteine		glutamate	

Typical coordination numbers are 4 and 6: it often occurs that coordination with amino acidic residues is not complete. This is fundamental for the catalytic activity of enzymes because an open site remains available for coordination of the substrate. On the contrary, this situation does not occur if the protein function is exclusively that of transferring electrons.

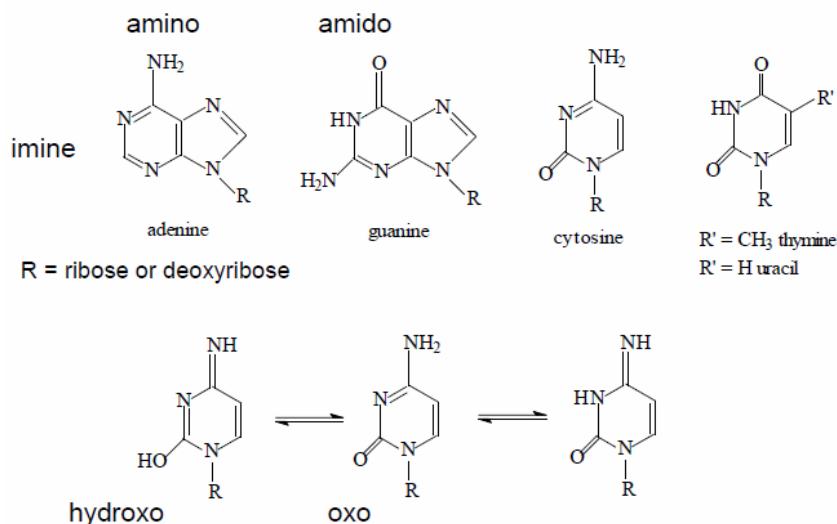
**Tetrapyrrol macrocycles are selective with regard to size of metal ion**



Metals in biological systems are often bound to ligands called *macrocycles*- these are cyclic compounds that contain several donor atoms. Two of the most common examples are the porphyrin ring and the corrin ring (**Fig. 22**). The most well known example of a porphyrin complex is the haem in haemoglobin, the oxygen-transporting protein in red blood cells.

**Fig.22. Tetradentate macrocycles biological ligands for metal ions**

Nucleobases can exist in different tautomeric forms and can be mono or multidentate ligands (**Fig. 23**).

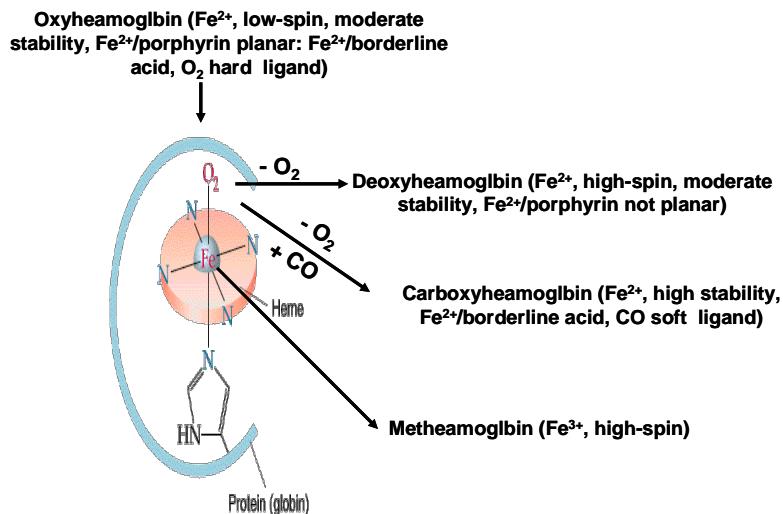


**Fig. 23.** Nucleobases are biological ligands for metal ions

Positively charged metal ions can affect the normal hydrogen-bond interactions that are the basis of base pairing in DNA. This can be exploited for the development of chemotherapeutic drugs (see later the mechanism how cisplatin acts as a chemotherapeutic agent).

### 1.5.2. Application of the principles of coordination chemistry for biological complexes

The most well known example of a porphyrin complex is the haem in haemoglobin, the oxygen-transporting protein in red blood cells. The coordination of Fe<sup>2+</sup>-ion in haemoglobin is shown in **Fig. 24**. Haemoglobin basically consists of two parts, the haem group (the iron with its porphyrin ligand), and a protein, globin. In its resting state with no oxygen bound, the iron is high spin Fe(II). When oxygen binds to form oxyhaemoglobin, it changes to low spin Fe(II).



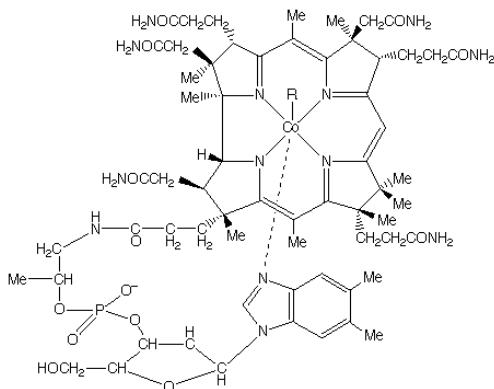
**Fig. 24.** Different oxidation state, magnetic features and coordination of Fe-ion in haemoglobin

This is a reversible process, so that when the red blood cell gets to an oxygen-deficient area, equilibrium gets reversed and O<sub>2</sub> is released. Therefore, bonding of O<sub>2</sub> could not be very

stable since needed to be given off easily at oxygen-deficient area. Notice that this is an example of *oxygenation* (adding oxygen) rather than oxidation, because the iron doesn't change oxidation state. If it is oxidised to Fe(III) (and to a very small proportion it occurs for every oxygenation/deoxygenation cycle) a substance called methaemoglobin forms, which is inactive for oxygen transport.

That change from high spin to low spin on oxygenation has far reaching consequences. In its high spin state, the radius of the iron atom is just too large to allow it to fit into the plane of the porphyrin ring. But when it changes to low spin, the radius decreases a bit and this allows the iron to move into the plane - dragging with it its sixth ligand, a histidine group, and thus triggering off a cascade of conformational changes which affect other subunits of the haemoglobin. This results in cooperative binding of O<sub>2</sub>: once an oxygen is bound to one subunit, the others pick one up more easily.

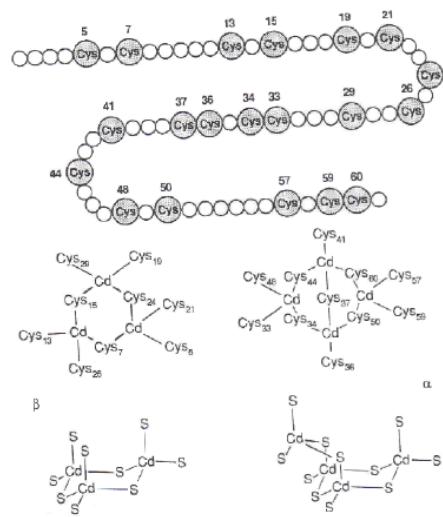
Another example of a corrin ring is in Vitamin B12 cofactor, which contains a cobalt corrin complex (**Fig. 25**).



**Fig. 25.** Coordination of cobalt in Vitamin B12.

The unusual thing about this species is that in the natural state the R group attached to cobalt is a methyl: this means that it is a rare example of a naturally-occurring organometallic compound (one with a direct metal-carbon bond).

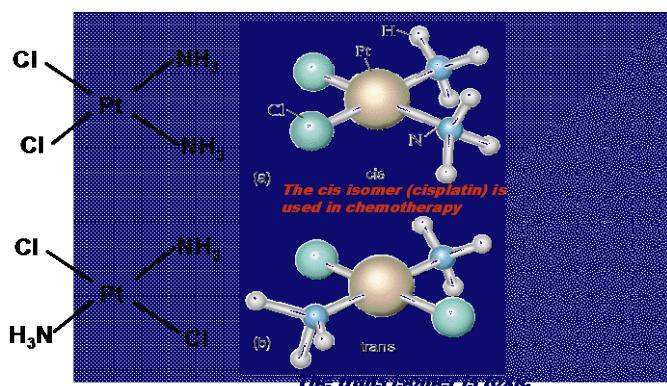
An example of the application of the hard-soft concept for biological complexes is the action of metallothioneins (**Fig. 26**).



**Fig. 26.** Coordination of Cd<sup>2+</sup> by -SH residues of metallothionein

In metallothioneins 30-35% of amino acids are cysteins with soft -SH groups and they prefer to coordinate of soft heavy metal ions such as Cd<sup>2+</sup>, Hg<sup>2+</sup>, Pb<sup>2+</sup>, Zn<sup>2+</sup>. Thus the major biological function of metallothioneins is to protect cells from toxic heavy metals by complexing them preventing the poisoning heavy metals to bind and inactivate enzymes and other functional proteins.

As well as occurring in biological systems, metal complexes can actually be used as drugs. The best example here is cis-platin, the name given to *cis*-[PtCl<sub>2</sub>(NH<sub>3</sub>)<sub>2</sub>], which is used in cancer chemotherapy (**Fig. 27**).



**Fig. 27.** The structure of *cis*- a *trans*- diamminedichloro-platinum (II)

This is a very simple complex, though there is one drawback: it has a certain degree of toxicity itself. The corresponding *trans* isomer has no beneficial (anti-cancer) effect, but is also toxic. This example emphasizes the importance of isomerism both in complex formation and in the biological effects of isomers.

In summary, the major principles of coordination chemistry (i. e. chemical bonding, geometry, magnetic features, hard-soft concept, isomerism) could be applied to explain the above characteristics of biologically important complex biomolecules and they may help understand the biological functions of metal ions.

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## **2. Alkali metal ions**

### **2.1. Regulatory role of Na<sup>+</sup> and K<sup>+</sup> ions. Sodium-potassium ATPase.**

#### **2.1.1. Basic facts**

In biological systems sodium (Na) and potassium (K) exist in the +1 oxidation state. Na<sup>+</sup> and K<sup>+</sup> ions form complexes mostly with O-containing ether or hydroxyl ligands. Complexes of Na<sup>+</sup> and K<sup>+</sup> are characterized by small stability constants, they are rather mobile, easily exchangeable and mostly function as charge carriers in biological processes.

#### **2.1.2. Biological importance and transport in the body**

Sodium and potassium are essential elements. Sodium is the predominant *extracellular* cation in animals and man. An adult human has approximately 90 g Na, about 30% is located in the bones, 10% is inside the cells and about 60% is in the extracellular fluid. On the other hand, potassium is the predominant cation in *intracellular* fluid. An average adult has approximately 170 g of K of which more than 90% is found in the intracellular space.

Na<sup>+</sup> and K<sup>+</sup> ions participate in the transmission of nerve signals, maintaining osmotic pressure, and regulating acid-base processes. They control the transport of sugar and amino acids into the cells. In addition, they are involved in the regulation of glycogen synthesis.

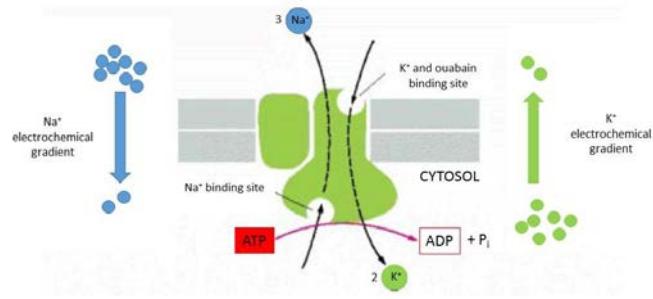
The distribution of Na<sup>+</sup> and K<sup>+</sup> ions across the cell membrane is unequal: Na<sup>+</sup> ions are found primarily on the outside of the cells, in the blood plasma and in the interstitial fluid which surrounds the cells, whereas the concentration of K<sup>+</sup> is higher inside the cells than outside. For example, in blood plasma, Na<sup>+</sup> is present at a concentration of 143 mM, while the K<sup>+</sup> level is only 5 mM. Within the red blood cells, the concentration of Na<sup>+</sup> is 10 mM, whereas the concentration of K<sup>+</sup> is near 105 mM.

Sodium is very soluble and therefore is easily absorbed from the stomach and small intestine. It goes into the blood and is circulated through the kidneys, which can reabsorb or eliminate it in order to maintain stable blood sodium levels. About 90% of the sodium consumed in the average diet is in excess of the body's needs and must therefore be eliminated in the urine. Aldosterone, a hormone made and secreted by the adrenal cortex, acts on the kidneys to regulate sodium metabolism. Some sodium is stored in the bones and is available if needed. Absorption of potassium from the diet is passive and does not require any specific mechanism. Absorption takes place in the small intestine as long as the concentration in gut contents is higher than that in the blood. Its excretion occurs through the kidney, and is linked to sodium excretion. Aldosterone hormone tends to promote potassium excretion.

#### **2.1.3. The sodium-potassium ATPase**

Low concentration of Na<sup>+</sup> and high concentration of K<sup>+</sup> inside the cell is maintained by a Na<sup>+</sup>-K<sup>+</sup> pump, found in the plasma membrane of most animal cells. The pump operates as an antiporter, moving Na<sup>+</sup> and K<sup>+</sup> simultaneously, both against their concentration gradients. Because the pump hydrolyzes ATP, it is also known as a **Na<sup>+</sup>-K<sup>+</sup> ATPase**. For every molecule of ATP hydrolyzed inside the cell, three Na<sup>+</sup> ions are pumped out and two K<sup>+</sup> are pumped in. It creates a net separation of charge across the membrane, making the inside of the cell negative relative to the outside. The resulting transmembrane potential of -50 to -70 mV is essential

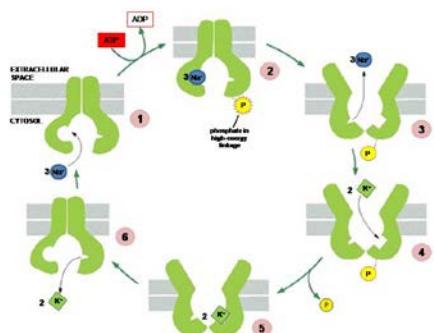
for neurons in the electrical signaling, and is also characteristic of many non-neuronal animal cells.



**Fig. 28. The  $\text{Na}^+ - \text{K}^+$  ATPase**

The  $\text{Na}^+ - \text{K}^+$  ATPase is a tetrameric integral membrane protein consisting of two dimers, each composed of a small and a large subunit.

Phosphorylation of the large subunit is an important step in the pumping cycle of  $\text{Na}^+ - \text{K}^+$  ATPase, during which the pump alternates between two conformational states. (1) The binding of  $\text{Na}^+$  and the (2) subsequent phosphorylation by ATP of the cytoplasmic face of the pump induce the protein to undergo a conformational change that (3) transfers the  $\text{Na}^+$  across the membrane and releases it on the outside. (4) Then, the binding of  $\text{K}^+$  on the extracellular surface and the (5) subsequent dephosphorylation return the protein to its original conformation, which (6) transfers the  $\text{K}^+$  across the membrane and releases it into the cytosol.



**Fig. 29. The pumping cycle of  $\text{Na}^+ - \text{K}^+$  ATPase**

Ouabain, the specific inhibitor of the pump is only effective when applied on the outer surface of the membrane, since ouabain and  $\text{K}^+$  compete for the same site on the extracellular side of the pump.

$\text{Na}^+$  gradient produced by the pump drives the transport of metabolite and nutrient molecules, e.g. glucose and amino acids. It is involved in the transport of ions, such as proton, calcium, chloride, and phosphate, and also has a crucial role in regulating cytosolic pH. The pump also regulates cell volume through its osmotic effects and it keeps many animal cells from bursting. Animal cells consume 5-30 % of their energy requirement in fueling the pump, while in nerve cells this value approaches 70%.

## 2.1.4. Examples for the regulatory role of $\text{Na}^+ - \text{K}^+$ ATPase

### 2.1.4.1. Importance of $\text{Na}^+ - \text{K}^+$ ATPase in controlling cell volume

Cells contain high concentration of solutes, including numerous negatively charged proteins and other organic molecules that are confined inside the cell as well as their accompanying cations that are required for charge balance. This tends to create a large osmotic gradient that, unless balanced, would tend to “pull” water into the cell. For animal cells this effect is counteracted by an opposite osmotic gradient due to a high concentration of inorganic ions -

chiefly  $\text{Na}^+$  and  $\text{Cl}^-$  - in the extracellular fluid. The  $\text{Na}^+ - \text{K}^+$  pump maintains osmotic balance by pumping out the  $\text{Na}^+$  that leaks in down its steep electrochemical gradient. The  $\text{Cl}^-$  is kept out by the membrane potential.

#### *2.1.4.2. Regulation of cytosolic pH*

Keeping the extracellular pH at about 7.2 requires the removal of excess  $\text{H}^+$ , which either leaks in or produced by the cell during acid forming reactions. For that,  $\text{H}^+$  can be directly transported out of the cell or  $\text{HCO}_3^-$  is brought into the cell to neutralize  $\text{H}^+$  in the cytosol. Carrier proteins transporting  $\text{H}^+$  and  $\text{HCO}_3^-$  ions are mostly  $\text{Na}^+$ -dependent, that is, they use the energy stored in the  $\text{Na}^+$  gradient to facilitate the exchange of these ions across the plasma membrane.

#### *2.1.4.3. Transport of nutrients driven by $\text{Na}^+$ gradient*

In the plasma membrane of animal cells the transport of nutrients or metabolites is controlled by carrier proteins, which usually co-transport their cargo molecules with  $\text{Na}^+$  (either in the same or in the opposite direction). The free energy released during the movement of  $\text{Na}^+$  ion down its electrochemical gradient is used as the driving force to pump the other molecule uphill, against its electrochemical gradient. The  $\text{Na}^+$  that enters the cell during transport is subsequently pumped out by  $\text{Na}^+ - \text{K}^+$  ATPase, which, by maintaining the  $\text{Na}^+$  gradient, indirectly drives the transport. Intestinal and kidney epithelial cells, for instance, transport sugar and amino acids into the cell with the help of symport systems (co-transport in the same direction) that are driven by the  $\text{Na}^+$  gradient across the plasma membrane: because  $\text{Na}^+$  tends to move into the cell the sugar or amino acid is “dragged” into the cell with it. The greater the electrochemical gradient, the greater the rate of the nutrient entry, conversely, if  $\text{Na}^+$  concentration in the extracellular fluid is reduced, the transport of the nutrient molecules is decreased.

#### *2.1.4.4. Impact of $\text{Na}^+ - \text{K}^+$ ATPase in health and disease states*

Alteration in  $\text{Na}^+ - \text{K}^+$  ATPase activity disturbs salt and water homeostasis, resulting in cardiovascular disorders, heart disease and hypertension, as well as diabetes and other metabolic diseases. Dysfunction or deficiency of  $\text{Na}^+ - \text{K}^+$  ATPase has also been identified in chronic neurodegenerative diseases, such as Alzheimer disease.

$\text{Na}^+ - \text{K}^+$  ATPase is inhibited by the so-called cardiac glycosides, which are plant-derived drugs such as ouabain, digoxin, and digitoxin. These drugs are used in the treatment of congestive heart failure or cardiac arrhythmia. Cardiac glycosides inhibit the pump by stabilizing it in the phosphorylated transition state, so that  $\text{Na}^+$  cannot be extruded: intracellular  $\text{Na}^+$  concentration therefore increases. Rise in the intracellular  $\text{Na}^+$  concentration leads to an increase of the intracellular  $\text{Ca}^{2+}$  concentration in cardiac myocytes, which increases cardiac contractility.

Sources:

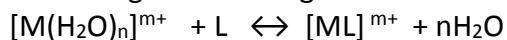
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## 2.2. Natural and synthetic ligands for alkali metal ions (L.B.)

Alkali metal cations generally do not form stable coordination complexes with simple Lewis bases due to their low charge of just  $+1$  and their relatively large size. Thus the  $\text{Li}^+$  ion forms the most complexes and the heavier alkali metal ions such as  $\text{Na}^+$  and  $\text{K}^+$  form less and less. Alkali metal ions exist in aqueous solution as very labile octahedral hexahydrate complexes  $[\text{M}(\text{H}_2\text{O})_n]^{m+}$  with the exception of the lithium ion, which due to its small size forms tetrahedral tetrahydrate complexes ( $[\text{Li}(\text{H}_2\text{O})_4]^{+}$ ). These complexes undergo ligand exchange with water molecules from the surrounding solution within nanoseconds or less and the formation of stable complexes with multidentate chelate ligands (L) from aqueous solutions is thus always a substitution reaction involving the water ligands:



The alkali metals form complexes because their ions are attracted by electrostatic forces of attraction to the polar water molecules. Alkali metals also readily form complexes with natural macrocyclic antibiotics, synthetic macrocyclic polyethers (crown ethers) and synthetic macrobicyclic aminoethers (cryptands) due to electrostatic attraction. Bound to these ligands alkali metal ions can cross biological membrane barriers which otherwise are not permeable to ions.

### 2.2.1. Natural ionophores (macrocyclic antibiotics)

Natural ionophores are relatively small molecules excreted by microorganisms such as fungi, lichen and marine organisms. These ionophores selectively bind and transport alkali or alkaline earth metal ions across cell membranes and artificial lipid bilayers. On one hand, metalloionophores (e.g.: nonactins and valinomycin) can serve as antibiotics by perturbing the stationary ionic non-equilibrium and thus the membrane function of bacteria without affecting the more complex ion-transport of the host organisms. The transport of cations results in disturbance of the ionic balance across the membrane upon release of the bound metal ions. On the other hand, *ion-channelling antibiotics* (e.g.: gramicidins) can create pores in membranes resulting in leakage of cations through the pores and the cations are transported directly into the cells to result in antibiotic effect.

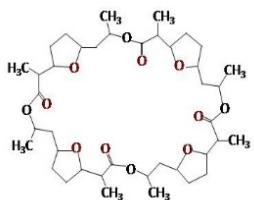
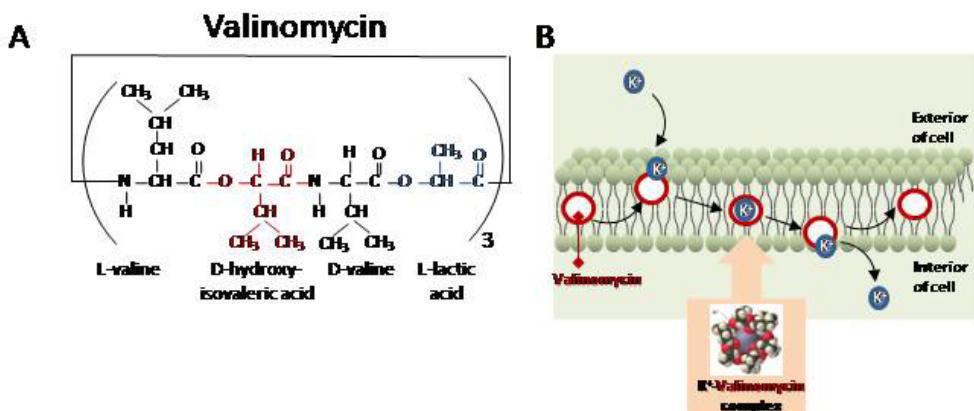


Fig. 30. Structure of Nonactin ionophore

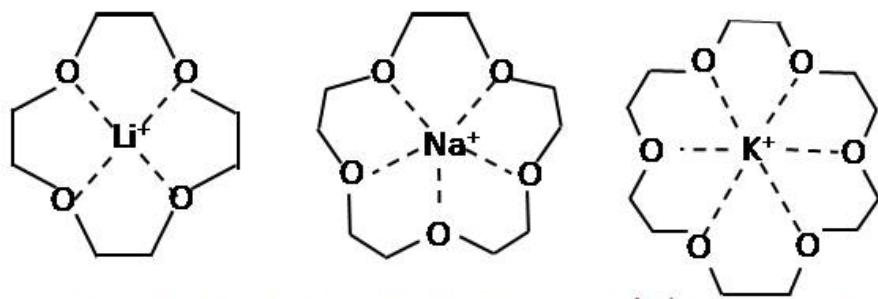
The two best-known examples, valinomycin and nonactin, illustrate that many of these ionophores are macrocyclic oligopeptides or esters with a number of chiral centers. Valinomycin is produced by several *Streptomyces* strains. It consists of enantiomers of D- and L-valine (Val), D-hydroxyvaleric acid and L-lactic acid. Its components are alternatively bound via amide and ester bridges and in complex with  $\text{K}^+$  forms an octahedral structure. (Valinomycin is highly selective for potassium ions over sodium ions.) These ionophores function as potassium-specific transporters and facilitate the movement of potassium ions through lipid membranes "down" an electrochemical potential gradient. The stability constant for the potassium-valinomycin complex is  $10^6$  and for the sodium-valinomycin complex only 10. This difference is important for maintaining the selectivity of valinomycin for the transport of potassium ions over sodium ions in biological systems.



**Fig. 31.** Molecular structure of the  $K^+$ /valinomycin complex (A-B). Transport of  $K^+$  across a membrane by Valinomycin (C)

### 2.2.2. Synthetic macrocyclic polyethers (crown ethers)

**Crown ethers** are cyclic polyethers discovered by Pederson in 1967. Efficient synthetic complex ligands for the alkali metal cations have become available since the 1970s with the design of multidentate macrocyclic ligands such as crown ethers, cryptands and related molecules.



**Fig. 32.** Structure of the complexes of 12-crown-4 with lithium, the 15-crown-5 with sodium and the 18-crown-6 with potassium

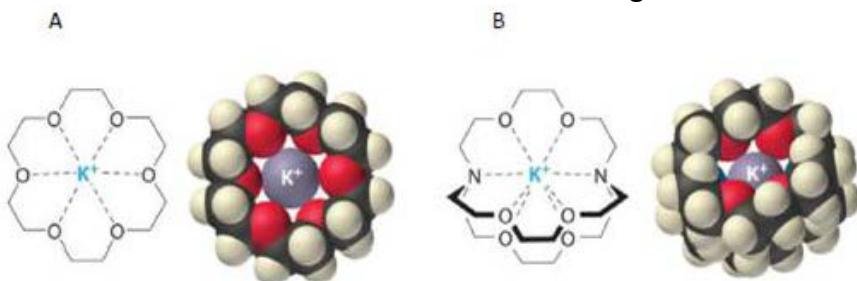
Crown ethers are cyclic compounds that consist of a ring containing several ether groups. The term "crown" refers to the resemblance between the structure of a crown ether in complex with a cation. The oxygen atoms are well situated to coordinate with an alkali metal ion located at the interior of the ring, whereas the exterior of the ring is hydrophobic. The resulting cations often form salts that are soluble in nonpolar solvents. Structures of three typical ethers are given above (Fig 32.). The common names of these ethers include a number as a prefix to designate the total number of atoms in the ring and a number as a suffix to designate the number of oxygen atoms in the ring. Thus, 15-crown-5 is comprised of 15 atoms in the ring, 5 of which are O and 10 of which are C. Macrocyclic polyethers have the capacity to recognise and bind molecules and ions with high stability and in a selective way. These characteristics are relevant on the selective transport of amino acids or metal ions across membranes and can serve as models in important biological processes. They are exceptionally versatile in selectively binding a range of metal ions and a variety of organic neutral and ionic species and this interaction depends on the structural characteristics and dimensions of the cavity formed by the polyether ring, the nature and number of the metal ion and the orientation of the donor atoms of the ligand. The efficiency of interaction may also be affected by the ability of the solvent molecules to compete with the donor atoms of the ligand towards

the coordination sites of the cation. These results that 18-crown-6 has high affinity for potassium cation, 15-crown-5 for sodium cation, and 12-crown-4 for lithium cation. The high affinity of 18-crown-6 for potassium ions contributes to its toxicity.

Crown ethers are currently being studied and used in a variety of applications beyond their traditional place in chemistry as anticancer agents since crown compounds could either induce toxicities that are different from those of conventional antitumor drugs, or complement drugs in current use, thereby providing a valuable adjunct to therapy.

### 2.2.3. Synthetic macrobicyclic aminoethers (cryptands).

The family of synthetic bi- and polycyclic multidentate ligands for a variety of cations is called cryptands. The term cryptand implies that the ligand binds substrates in a crypt, featuring a three-dimensional encapsulation of the guest (cations, anions and neutral species). These molecules contain three or more binding sites held together by covalent bonds and are three dimensional analogues of crown ethers. For this reason, the polycyclic cryptands with their largely preformed cavity are superior to the monocyclic crown ethers and ionophores with regard to complex stability and ion selectivity. Once a suitable molecular architecture has been designed, the number of individually weak coordinative interactions can lead to a collectively strong and inert coordinative binding of the metal ion by macrocycle. The resulting complex is called cryptate and this term is usually restricted to bicyclic or oligocyclic molecular entities. The complexes are lipophilic. Cryptands are more expensive and difficult to prepare, but offer much better selectivity and strength of binding than other complexants for alkali metals. They are able to bind otherwise insoluble salts into organic solvents.



**Fig. 33.** Structural comparison of a crown ether (18-crown-6)(A) and a cryptand (B) encapsulating potassium ions

The Nobel Prize for Chemistry in 1987 was given to D. J. Cram, J. M. Lehn and, C. J. Pederson for discovering and determining uses of crown ethers and cryptands, thus launching the field of supramolecular chemistry. Supramolecular chemistry is also important to the development of new pharmaceutical therapies by understanding the interactions at a drug binding site. The area of drug delivery has also made critical advances as a result of supramolecular chemistry providing encapsulation and targeted release mechanisms. In addition, supramolecular systems have been designed to disrupt protein-protein interactions that are important to cellular function.

## 2.3. Lecture notes on Lithium (Li)

### 2.3.1. Basic facts

Atomic number:	3
Electron configuration:	[He] $2s^1$

### 2.3.2. Biological importance

Lithium has no well-defined biological role. It is not known to be required for any biological function.

2.3.3. Lithium salts have been used effectively for the treatment of bipolar disease [a mental illness characterized by alternation of two phases: mania (frenzied mood) and depression]. In fact, lithium is the standard treatment for bipolar disease and is more effective in the maniac than in the depression phase of the disease.

The mechanism of action of lithium salts in bipolar disease is unknown. The following hypotheses have been put forward to explain its beneficial effects in mania:

- A.  $\text{Li}^+$  enhances the effect of the neurotransmitter serotonin, inhibits excitatory neurotransmission (dopamine and glutamate) and promotes inhibitory neurotransmission ( $\text{GABA} = \gamma\text{-amino butyric acid}$ )
- B.  $\text{Li}^+$  targets second-messenger systems and signal transduction pathways: phosphoinositide pathway, adenylate cyclase, glycogen synthase kinase 3  $\beta$  (GSK3  $\beta$ )
- C.  $\text{Li}^+$  interferes with the transport of  $\text{Na}^+$  and  $\text{K}^+$  ions

### 3. Alkaline earth metal ions

#### 3.1. Calcium (Ca)

##### 3.1.1. Basic facts

Atomic number: 20

Electron configuration: [Ar] 4s<sup>2</sup>

Oxidation state: Exists as 2+ ions.

Solubility: In the body calcium is counterbalanced by phosphates. Calcium phosphates become insoluble above a certain concentration, therefore changes in calcium or phosphate ion concentrations affect the concentration of the other ion. Therefore, 1) increases in phosphate ion concentration enhance calcium deposition (bones/teeth or ectopically) or 2) decrease in phosphate ion concentration increase calcium solubility and calcium concentrations.

##### 3.1.2. Calcium homeostasis

Calcium is ingested from foodstuffs (e.g. milk and its derivatives, egg or hard water) and daily need for calcium is ~1 g/day. Calcium requirement of the body increases in pregnancy, normally that is balanced by more efficient uptake and reuptake mechanisms.

The site of dietary calcium uptake is the GI tract. In the GI tract equilibrium forms between calcium secretion and calcium uptake (through a channel termed TRPV6; transient receptor potential cation channel, subfamily V, member 6) that leads to a net uptake.

Roughly 20% of the ingested calcium is taken up and enter the bloodstream that serves the needs of tissues and organs.

The calcium left in the intestine is excreted in feces. This circulation of calcium is termed external calcium trafficking.

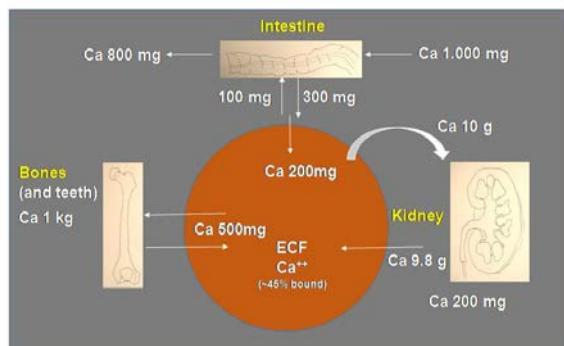


Fig. 34. Overview of calcium homeostasis

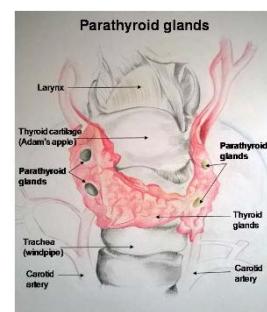
Total serum calcium concentrations fall between 2.1-2.6 mmol/L of which free (ionized) calcium is 1.1 – 1.4 mmol/L and the rest is mostly protein (albumin) bound calcium. The concentration of the latter varies as a function of serum albumin levels. Serum calcium levels

lower than normal is termed *hypocalcaemia*, while levels higher than normal are called *hypercalcaemia*. Both conditions are pathological. Calcium levels fine tune the response of  $\text{Na}^+$  channels, hence regulate the excitability of nerves. Hypocalcaemia leads to increased sodium passage through sodium channels and thus it enhances the excitability of muscles resulting in neuromuscular irritability, arrhythmias and tetany (involuntary contraction of muscles). Hypercalcaemia also lead to arrhythmias (serum calcium concentrations above 3.75-4 mmol/L leads to cardiac arrest), bone and intestinal pain, polyuria and the occurrence of stones (biliary or renal).

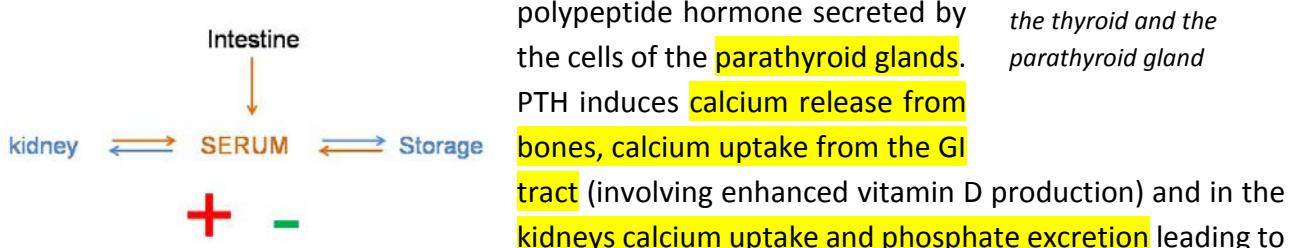
Two organs have privileged role in calcium homeostasis. In the kidney calcium and phosphate can be excreted allowing the regulation of serum calcium and phosphate levels. Daily 10 g of calcium is filtered through the kidney of which 9.8 g is reabsorbed, while  $\sim$ 0.2 g is excreted.

Bones and teeth contain the majority of the organismal calcium ( $\sim$ 1 kg in an adult human). Calcium is present in the form of calcium phosphates or apatites (teeth) and strengthens these tissues. Furthermore, bone is the largest reservoir of calcium in the body. Calcium is deposited to bones in the process called mineralization. Osteoblasts (cells that differentiate to osteocytes and build up bones) synthesize and secrete the extracellular matrix of the bone. Consecutive calcium deposition is organized by the fibers of the extracellular matrix. Calcium deposition is enhanced by increases in local phosphate concentrations and in pH. Osteoblasts secrete an enzyme called alkaline phosphatase that cleaves pyrophosphate into phosphate units, increasing local phosphate concentrations and local pH. Bone resorption is driven by osteoclast cells. Osteoclasts express  $\text{H}^+$  ATPase that lowers pH and proteases that cleave the extracellular matrix leading to calcium mobilization. The dynamic balance between bone mineralization and resorption on one hand regulate bone mass and strength, while on the other represent a mode to regulate serum calcium levels (add or remove calcium from blood).

Calcium homeostasis is regulated by the endocrine system with three hormones (parathyroid hormone, calcitonin, vitamin D) playing central roles described below. (Of note, other hormones (e.g. estrogens) also impact on calcium homeostasis.



**Fig. 35.** The location of the thyroid and the parathyroid gland

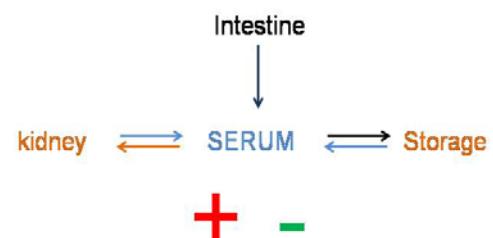


**Fig. 36.** Overview of PTH action on calcium homeostasis. Processes in green and induced,

**Calcitonin** is a polypeptide hormone synthesized in the clear (C) cells of the **thyroid gland**. Calcitonin blocks osteoclast activity (but does not directly enhance bone mineralization!), blocks the intestinal calcium uptake and in the kidney enhance phosphate reuptake and calcium excretion that culminate in **decreased serum calcium levels**.

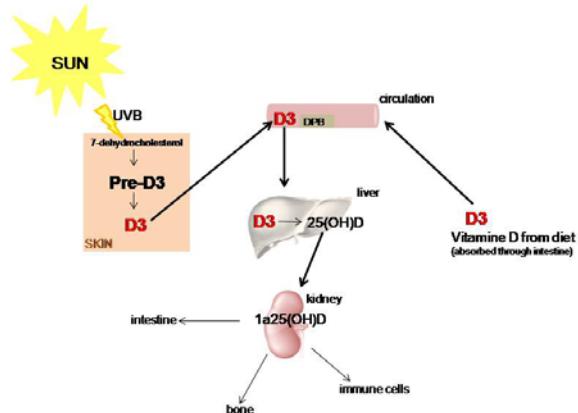
**Vitamin D (1,25-dihydroxycholecalciferol)** is a cholesterol derivative synthesized in multiple steps by different organs.

The B ring of 7-dehydrocholesterol is cleaved in the **skin** upon UV irradiation resulting in (pre)vitamin D<sub>3</sub> that is subsequently hydroxylated at position 25 in the **liver** (cholecalciferol) and then in the kidney at position 1 resulting in 1,25-dihydroxycholecalciferol (calcitriol, vitamin D<sub>2</sub>) that is the active form of vitamin D.



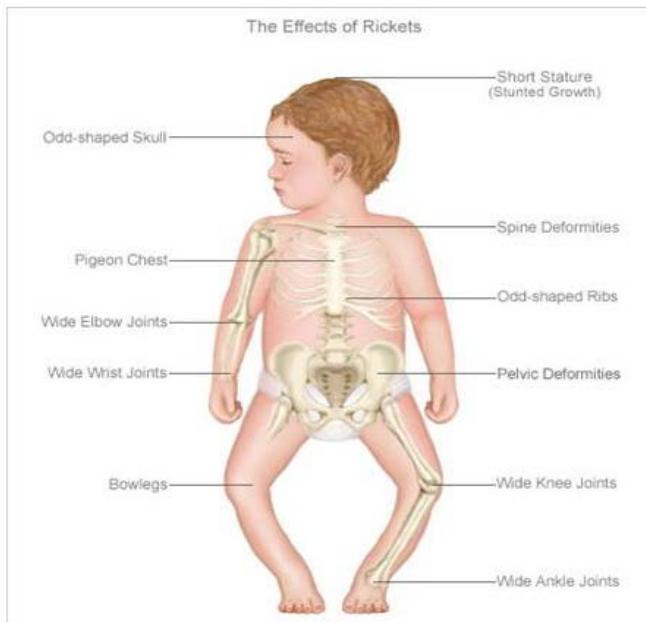
**Fig. 37.** Overview of calcitonin action on calcium homeostasis. Processes in green are induced, while those in red are inhibited

**Fig. 38.** The overview of vitamin D synthesis



Vitamin D induces osteoblast activity and bone mineralization, calcium resorption in the kidney, intestinal calcium uptake and enhance PTH secretion that altogether drive calcium uptake and bone calcium deposition.

Low calcium levels in the bones lead to the softening of bones. **Rickets** is a softening of bones in infants due to deficiency or impaired metabolism of vitamin D, phosphorus or calcium, potentially leading to fractures and deformity. The typical symptoms of rickets is illustrated on Fig. 39. Similar conditions in adults is termed **osteomalacia**.



**Fig. 39.** Typical signs of rickets

(<http://www.doctortipster.com/5762-deficiency-rickets-causes-risk-factors-symptoms-diagnosis-treatment-and-prophylaxis.html>)

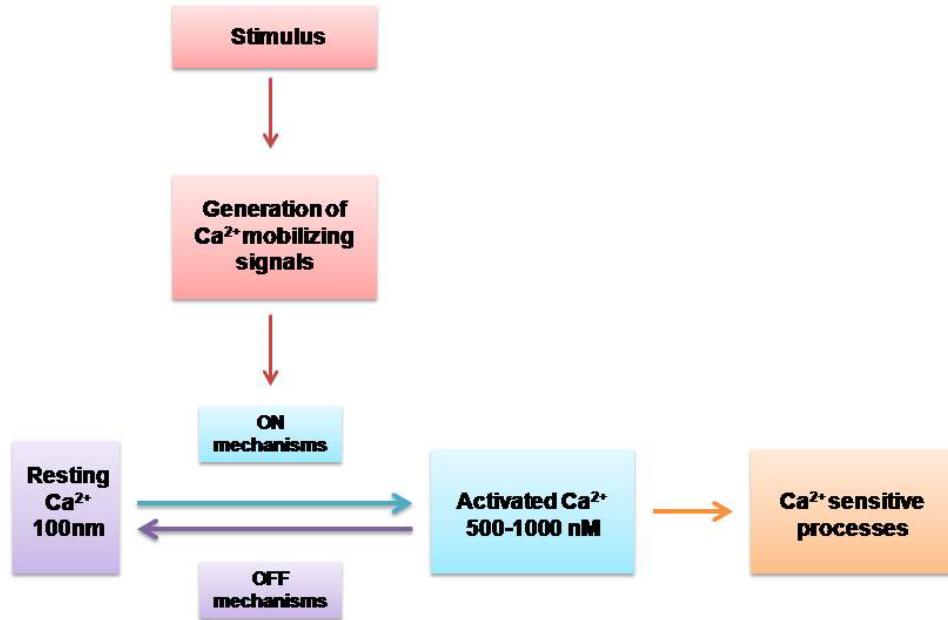
### 3.2. The calcium signal

Outside signal molecules (first messengers) such as most hormones act on plasma membrane receptors inducing the intracellular synthesis/liberation of second messengers, or activate the enzymatic function of the intracellular portion of the receptors. Both forward the message from outside to inside of the cell, and magnify the effect of the original signal as part of various signal transduction pathways.

The intracellular  $\text{Ca}^{2+}$  concentration is in the nM- $\mu\text{M}$  range, while it is in the mM range in the extracellular space. Furthermore,  $\text{Ca}^{2+}$  concentration is high in the ER/SR lumen (about 100  $\mu\text{M}$ ) and  $\text{Ca}^{2+}$  is also reserved in the mitochondria.

#### *The calcium signaling network*

$\text{Ca}^{2+}$  concentration in the cytosol may change rapidly, therefore,  $\text{Ca}^{2+}$  may act as a *second messenger* in a so called  $\text{Ca}^{2+}$ -signaling network (Fig. 40.) and may modulate various proteins directly or indirectly. Eventually calcium ions regulate diverse processes, such as muscle contraction, cell motility, transcription, exocytosis.



**Fig. 40.** The four units of the  $\text{Ca}^{2+}$ -signaling network. Stimuli act by generating  $\text{Ca}^{2+}$ -mobilizing signals that act on various ON mechanisms to trigger an increase in the intracellular concentration of  $\text{Ca}^{2+}$ . The increased level of  $\text{Ca}^{2+}$  stimulates various  $\text{Ca}^{2+}$ -sensitive processes to trigger many different cellular pathways. The response is terminated by OFF mechanisms that restore  $\text{Ca}^{2+}$  to its resting level. (Nature Reviews. Mol. Cell Biol., 1, 12-21 (2000))

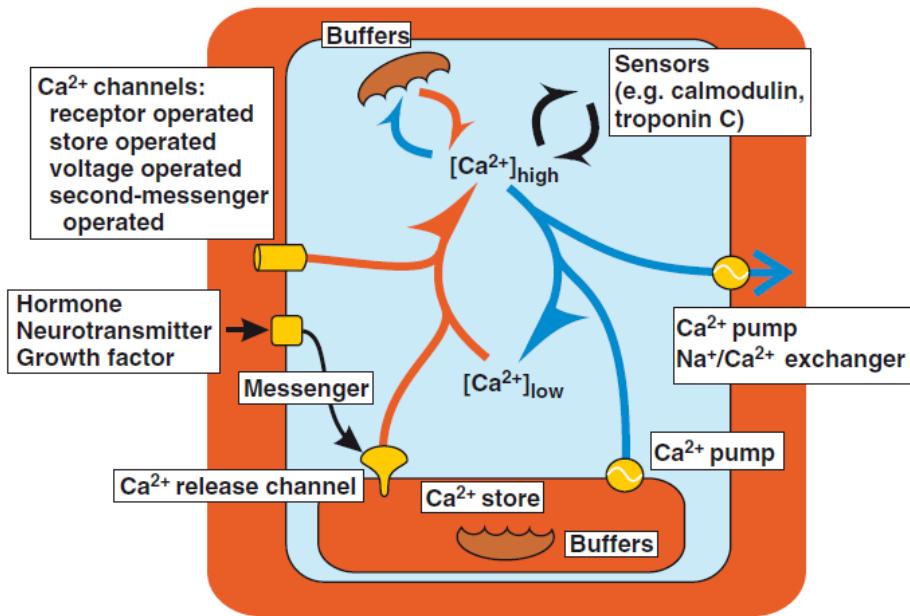
Cytosolic  $\text{Ca}^{2+}$  mobilizing signals can be evoked by:

- chemical stimuli (such as hormones, growth factors and toxins)
- environmental changes (such as changes in pH or temperature shifts)
- mechanical deformation
- depolarisation

These signals activate  $\text{Ca}^{2+}$  channels to increase  $\text{Ca}^{2+}$  in the cytoplasm/ to produce  $\text{Ca}^{2+}$  signals. Cells can access  $\text{Ca}^{2+}$

- via influx from the extracellular space (direct or indirect activation)
- from a variety of intracellular stores (including the sarcoplasmic or endoplasmic reticulum) (indirect activation)

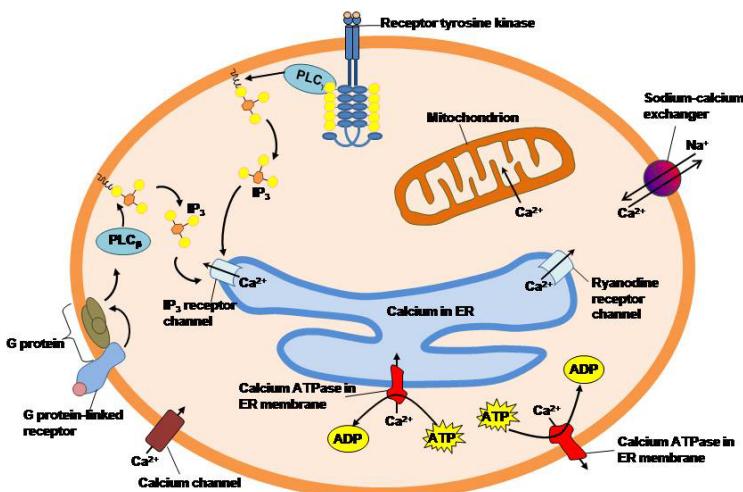
Depending on the cell type, the character of the stimulus and the extent of stimulation  $\text{Ca}^{2+}$  signals can be transient, oscillatory or sustained and can occur globally or as subcellular events. To generate such a diverse spectrum of signals, there is a wide range of messengers and mechanisms in the cell to evoke  $\text{Ca}^{2+}$  fluxes between various cellular compartments.



**Fig. 41.** The basic  $Ca^{2+}$  cycle present in all mammalian cell types. The pathways that increase cytosolic  $Ca^{2+}$  levels are shown with red arrows and the mechanisms that reduce  $Ca^{2+}$  levels are depicted with blue arrows. (*J. Cell Sci.*, 122, 2337-2350 (2009))

$Ca^{2+}$  influx can occur via several different types of channels that have diverse activation mechanisms. The characteristics of the  $Ca^{2+}$  signals evoked by these channels depend on their biophysical properties, their expression levels and their locations within the plasma membrane of the cells.

Release of  $Ca^{2+}$  from intracellular stores is also mediated by several different types of  $Ca^{2+}$  channels which are turned on by specific messenger molecules (like inositol triphosphate, IP<sub>3</sub>) produced due to the stimulation of plasma membrane receptors, like G-protein-linked receptor or receptor tyrosine kinase (Fig. 42.).



**Fig. 42.** A simplified overview of  $Ca^{2+}$  uptake and remission  
([http://www.mun.ca/biology/desmid/brian/BIOL2060/BIOL2060-14/14\\_12.jpg](http://www.mun.ca/biology/desmid/brian/BIOL2060/BIOL2060-14/14_12.jpg))

Elevation of cytosolic  $\text{Ca}^{2+}$  concentration is transduced into functional changes in cellular activity by numerous sensors that bind  $\text{Ca}^{2+}$  (e.g. calcium-binding proteins). Cellular  $\text{Ca}^{2+}$  sensors have varying affinities and locations, allowing them to specifically respond to different  $\text{Ca}^{2+}$  patterns. Many proteins involved in calcium signaling are tissue-specific ensuring unique signals that suit the physiology of the tissue.

The magnitude of  $\text{Ca}^{2+}$  signals can be limited by various buffers inside cells, such as  $\text{Ca}^{2+}$ -binding proteins and mitochondria.

$\text{Ca}^{2+}$  concentration is low in the cytoplasm of resting cells. How is the low concentration achieved and maintained?

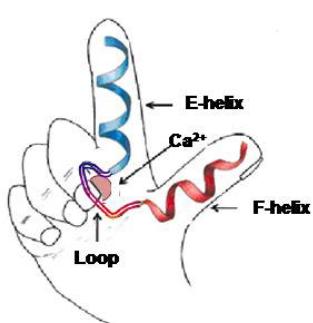
- plasma membrane  $\text{Ca}^{2+}$ -ATPase (PMCA) pumps and  $\text{Na}^+/\text{Ca}^{2+}$  exchangers extrude  $\text{Ca}^{2+}$  to the outside
- sarko/endoplasmic reticulum ATPase (SERCA) pumps  $\text{Ca}^{2+}$  to internal stores (the lumen of ER or SR)
- most of the cytoplasmic  $\text{Ca}^{2+}$  is bound to calcium binding proteins

Defects in the genes for the various isoforms of the PMCA pumps have been described and connected to hereditary hearing defects. Muscle disturbances resulting in prolonged  $\text{Ca}^{2+}$  overload can be linked to the SERCA pump.

### 3.3. Calcium binding proteins (CBP)

The function of  $\text{Ca}^{2+}$  binding proteins

- control of  $\text{Ca}^{2+}$  concentration in the cytosol or in the lumen of ER/SR or mitochondria
- $\text{Ca}^{2+}$  transport across cell membranes
- $\text{Ca}^{2+}$  modulated sensoring/switching on signaling pathways

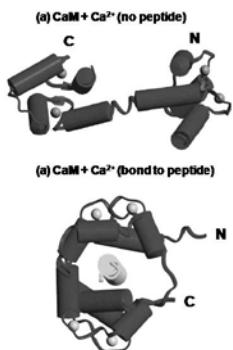


Oxygen atoms of carboxyl and carbonyl groups coordinate binding of  $\text{Ca}^{2+}$  to CBPs.

Many of CBPs share one or more helix-loop-helix motif, so-called EF-hand (Fig. 43) where the  $\text{Ca}^{2+}$  may bind (parvalbumin, calmodulin, calcineurin). Others, like calsequestrin and calreticulin (calcium buffer proteins in the lumen of SR and ER), bind  $\text{Ca}^{2+}$  via different binding sites.

**Fig. 43.** Representation of EF-hand domain. The classical EF-hand is a helix-loop-helix motif characterized by a sequence of, usually, 12 residues flanked with two alpha helices positioned perpendicular to one another in a spatial arrangement that mimics the spread thumb and the index finger of a human hand. The loop integrated in this sequence can accommodate  $\text{Ca}^{2+}$ . (in Adv. Exp. Medicine and Sign., Calcium Signaling, Editor S. Islam, Springer, 2012)

**Calmodulin** (calcium modulated protein) has two EF-hand pairs that cooperatively bind 4 Ca<sup>2+</sup>. Calmodulin exists in at least two different configurations: one which lacks Ca<sup>2+</sup> and the other one which has four Ca<sup>2+</sup> bound per molecule (Ca<sup>2+</sup>-calmodulin).



**Fig. 44.** Structure of Ca<sup>2+</sup>-calmodulin and its peptide binding  
<http://openwetware.org/wiki/Image:Calmodulin2.jpg>

In the absence of Ca<sup>2+</sup>, calmodulin may assume a dumbbell shape with 2 helix-loop-helix motifs at each end. When activated by Ca<sup>2+</sup> binding, calmodulin may wrap around the target domain of a sensitive protein, altering that protein's activity (Fig.44).

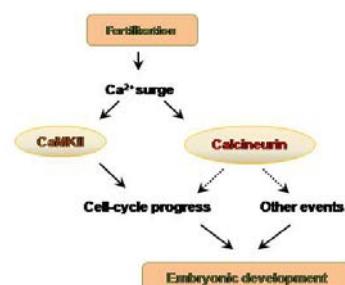
Calmodulin mediates many signaling pathways and processes of the cell, such as cell division and differentiation, gene transcription, muscle contraction. Protein targets regulated by calmodulin include several cytoskeleton proteins and ion channels, adenylate cyclases etc. Some protein kinases that transfer phosphate from ATP to hydroxyl residues on enzymes to be regulated are activated by Ca<sup>2+</sup>-calmodulin.

Calmodulin may be involved in many pathological processes, like Parkinson disease, Alzheimer disease or rheumatoid arthritis.

**Troponin C** is also an EF-hand containing Ca<sup>2+</sup>-sensing protein present in all striated muscle. Troponin C has a very specific function in the initiation of the cascade of conformational changes through the component proteins of the thin filament, leading to the formation of cross bridges between actin and myosin and the generation of force by the myocyte.

**Calcineurin** is a **calmodulin-dependent protein phosphatase** which removes phosphate groups from seryl or threonyl side chains of proteins. Calcineurin plays a pivotal role in the information flow from local or global calcium signals to effectors that control immediate cellular responses and alter gene transcription. Calcineurin can dephosphorylate side chains of transcription factors [e.g. **nuclear factor of activated T cells (NFAT)**], initiating a cascade of transcriptional events involved in physiological and development processes. A malfunction in calcineurin-NFAT signaling can engender several pathologies, such as cardiac hypertrophy, autoimmune diseases, osteoporosis, Alzheimer's disease, Down's syndrome and cancer.

Calcineurin and a calmodulin dependent protein kinase are important in early embryonic development (Fig.45). Fertilization results in cytoplasmic Ca<sup>2+</sup> release that independently activates CaMKII (Ca<sup>2+</sup>/calmodulin-dependent protein kinase II) and calcineurin. They regulate cell-cycle and control chromatin decondensation and other events.



**Fig. 45.** Role of calcineurin in early embryonic development (Nature 449, 297-298, 2007)

**Neuronal Ca<sup>2+</sup>Sensor (NCS) proteins** have four EF-hand motifs, but differ from other four EF-hand motifs proteins, like calmodulin, as they are more compact and globular even when Ca<sup>2+</sup> is bound. Dysregulation of NCS proteins have been observed in several CNS disorders, such as Alzheimer's disease, schizophrenia, and cancer.

## 3.4. Magnesium (Mg)

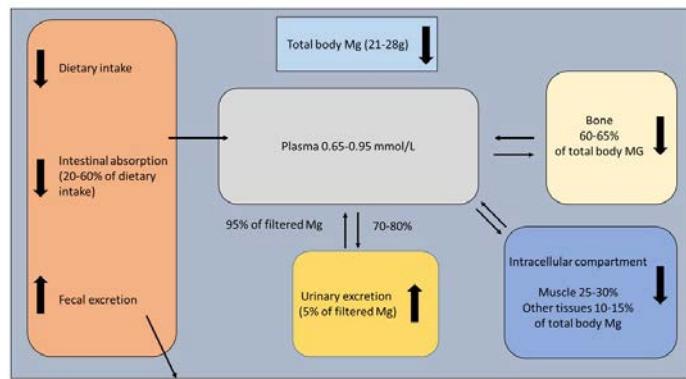
### 3.4.1. Basic facts

Atomic number:	12
Electron configuration:	[Ne] 3s <sup>2</sup>
Oxidation state:	Exists as 2+ ions.

### 3.4.2. Magnesium homeostasis

The homeostasis and biological behavior of magnesium in many respect resembles that of calcium. Magnesium is less abundant in the body (21-28 g in an average adult) than calcium (>1 kg in an average adult). Magnesium is ingested from foodstuffs (green vegetables) and daily need for magnesium in adults is ~350-400 mg/day.

**Fig. 46. Overview of magnesium homeostasis.**

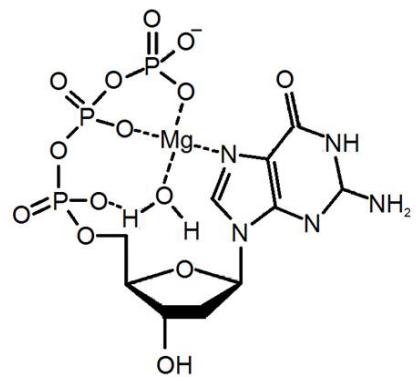


The site of dietary magnesium uptake is the GI tract. Magnesium is excreted through the kidneys. The major site of reabsorption is the loop of Henle. Similarly to calcium, the major reservoir of magnesium lies in the bones. It must be noted that a very small portion of total magnesium can be found in the blood, wherein magnesium is mostly (>90%) bound to chelators such as ATP, ADP or citrate. Total serum magnesium concentrations fall between 0.60-1.10 mmol/L. Serum magnesium level lower than normal is termed **hypomagnesemia**, while levels higher than normal are called **hypermagnesemia**. Both conditions are pathological. Hypomagnesemia is characterized by weakness, muscle cramps, cardiac arrhythmia and increased irritability of the nervous system. Hypermagnesemia is often associated with kidney hypofunction and it is characterized by hypotension, bradycardia and decreased or absent tendon reflexes.

### 3.4.3. Magnesium in cellular physiology

Magnesium easily forms complexes (e.g. Mg-chlorophyll). For biomedical aspects most important complexes are the ones that magnesium forms with the phospho groups in nucleotides. The binding of magnesium to di or triphosphate forms of nucleotides facilitate the cleavage of phosphodiester bonds. Therefore, in cells nucleotides (incl. ATP) form complex with magnesium.

Magnesium can act on several calcium channels as blocker (e.g. during endoplasmic or sarcoplasmic reticulum-dependent calcium release) therefore magnesium fine tunes calcium release and calcium levels, hence variations in magnesium levels provoke physiological changes associated with hypo or hypercalcemia.



**Fig. 47. The magnesium complex**

#### 3.4.4. Use of magnesium-oxide

Magnesium-oxide can be used as an antacid and can be used for oral magnesium supplementation.

## 4. Transition metal ions

### 4.1. Iron (Fe) metabolism

(absorption, transport, storage, hemosiderosis, hemochromatosis)

#### 4.1.1. Basic facts

Atomic number: 26

Electron configuration: [Ar] 3d<sup>6</sup> 4s<sup>2</sup>

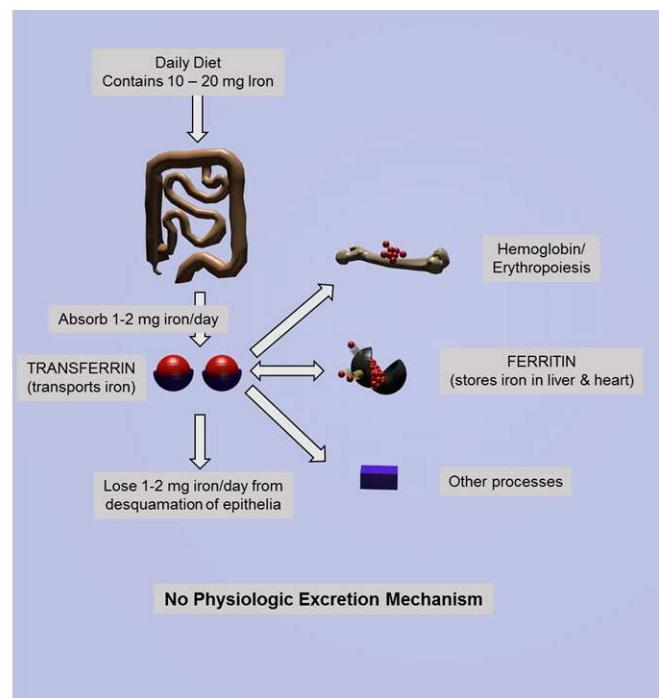
Iron is mostly in +2 (ferrous) or +3 (ferric) oxidation state.

#### 4.1.2. Biological importance

Iron proteins are very abundant in all life forms. Due to its redox activity, free iron is toxic and therefore it occurs in protein bound form. In the bloodstream, **transferrin** serves as the transport protein for iron (in the Fe<sup>3+</sup> form) whereas in storage compartments iron (Fe<sup>3+</sup>) is part of **ferritin** granules. Other important proteins of iron metabolism include **ferroportin** (the membrane transport protein of iron) and **hepcidin** (the master regulator of iron metabolism).

#### Overview of the body's iron turnover

Our body contains 3-4 grams of iron. To cover the **daily requirement (1-2 mg)** for iron, our diet needs to contain 10-20 mg iron. Absorption takes place in the duodenum (proximal small bowel). **Amount of absorbed iron is strictly controlled and is determined by the amount of iron lost** (mostly by desquamation of epithelial cells on mucosal surfaces and skin). Importantly, **there is no physiological excretion mechanism of iron**, therefore iron metabolism is absorption-controlled.



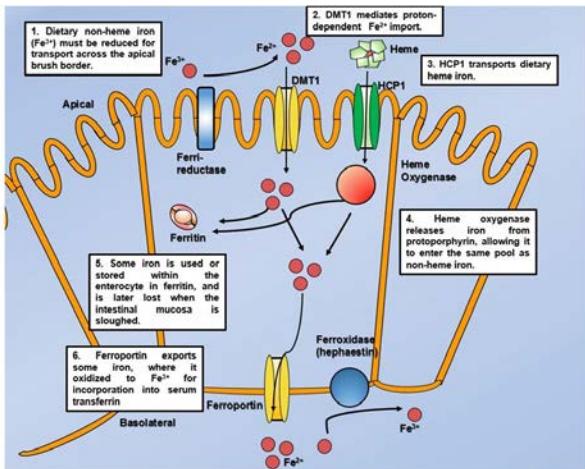
F

**Fig. 48. Iron turnover**

Key events of iron metabolism are

- absorption of iron from the GI tract
- export of iron from the enterocytes (gut epithelial cells) into the bloodstream
- transport of iron in the bloodstream
- uptake of iron by iron utilizing cells (basically all cells in the body take up iron)
- release of iron from macrophages (having engulfed aged red blood cells)

Absorption of dietary iron in the duodenum



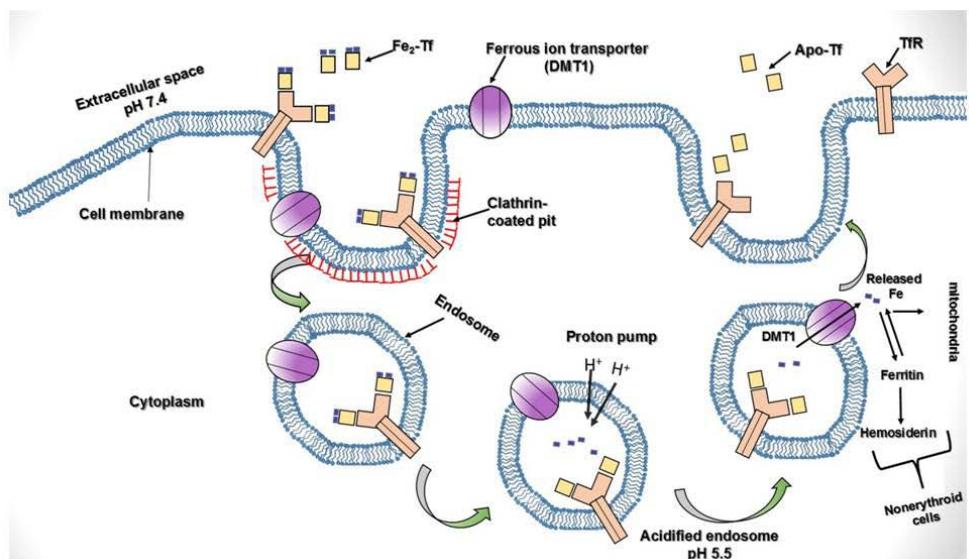
**Fig. 49.** Uptake of dietary iron in the duodenum and its release into the bloodstream.

Enterocytes take up iron in two ways: while heme is taken up via hem carrier protein 1 (HCP-1), free iron enters cells via the divalent metal ion transporter 1 (DMT1). Once inside the cell, the enzyme heme oxygenase 1 (HO-1) liberates iron ion from heme and ferric ions bind to ferritin. At the basal surface of the cell, iron ion is transported via ferroportin into the blood where it is loaded onto the transport protein transferrin. Note that iron ion must be in the oxidation state +2 when passing through membranes but for transport and storage it must be in the ferric ( $\text{Fe}^{3+}$ ) form. These redox changes are mediated at the apical and basal surfaces by ferrireductase and ferroxidase enzymes, respectively. (Hephaestin is a copper-containing ferroxidase localized to the basolateral side of enterocytes).

### Iron transport and uptake

In the blood, transferrin carries iron to the iron utilizing cells with the red blood cell producing bone marrow cells being the most active “users”. Transferrin is a glycoprotein\* with two binding sites for  $\text{Fe}^{3+}$  ions. In the absence or iron the protein is called apoferritin. Apoferritin is synthesized in the liver and binds iron ion very tightly but reversibly. Iron binding affinity of transferrin decreases sharply with decreasing pH.

\*Did you know that blood level of carbohydrate-deficient transferrin is determined in laboratory test to detect ethanol consumption? In particular sialic acid content of transferrin is decreased in people who consume significant quantities of alcohol.



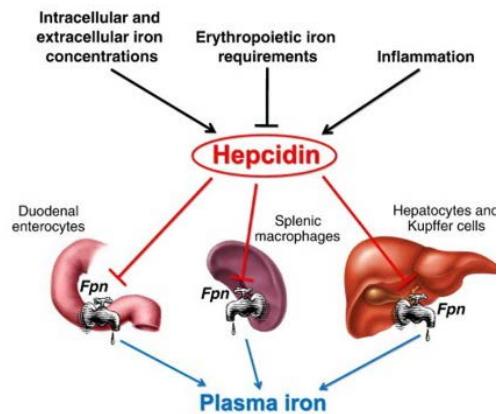
**Fig. 50.** The Transferrin Cycle

Iron-laden transferrin ( $\text{Fe}_2\text{-Tf}$ ) binds to transferrin receptors (TfR) on the surface of erythroid precursors. These complexes localize to clathrin-coated pits, which invaginate to form specialized endosomes. A proton pump decreases the pH within the endosomes, leading to conformational changes in proteins that result in the release of iron from transferrin. The iron transporter DMT1 moves iron across the endosomal membrane, to enter the cytoplasm. Meanwhile, transferrin (Apo-Tf) and transferrin receptor are recycled to the cell surface, where each can be used for further cycles of iron binding and iron uptake. In erythroid cells, most iron moves into

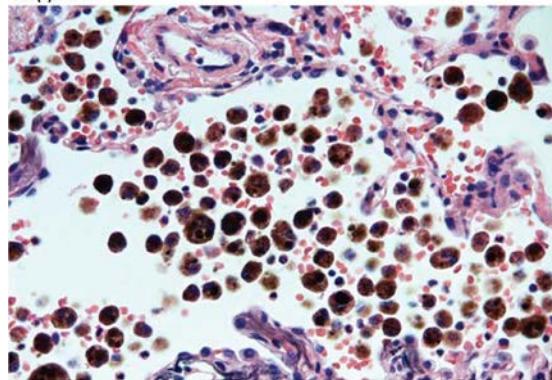
mitochondria, where it is incorporated into protoporphyrin to make heme. In nonerythroid cells, iron is stored as ferritin and hemosiderin. (source: Andrews N 1999 NEJM)

### Hepcidin: the master regulator of iron homeostasis

Hepcidin is a 25 amino acid peptide synthesized in the liver. It inhibits iron entry into the blood from the main sources: absorption in the duodenum; release from macrophages and release from hepatic stores. It acts by inhibiting ferroportin (Fpn) on the surface of enterocytes, macrophages and liver cells.



**Fig.51.** Center role of hepcidin in iron metabolism  
(source: Ganz and Nemeth (BBA MCR 2012))



### Hemosiderosis

Hemosiderosis means an increased deposition of the iron in various tissues in the form of the iron-containing pigment hemosiderin.

**Fig. 52.** Hemosiderin secondary to alveolar hemorrhage typically consists of large, coarse granules (Beasley MB (2010) The pathologist's approach to acute lung injury. Arch Pathol Lab Med. 2010; 134(5):719-27).

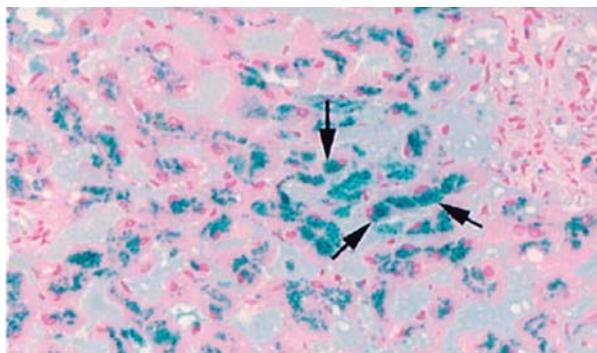
Hemosiderin is a yellowish-brown granular intracellular pigment that is formed in some phagocytic cells (macrophages) by the breakdown of hemoglobin. The exact chemical composition of hemosiderin is not precisely known but it likely contains ferric hydroxide, polysaccharides and proteins. Its iron content may reach 30-37%.

Examples for situations that can lead to hemosiderosis

- Patients receiving multiple blood transfusions (e.g. in thalassemia or sickle cell anemia) develop hemosiderosis due to iron overload
- Diseases associated with extensive lung hemorrhage e.g. in different autoimmune inflammatory disorders (hemosiderin deposited in the lungs)
- Idiopathic pulmonary hemosiderosis (idiopathic = cause unknown)

### Hemochromatosis

Hemochromatosis is a hereditary disorder of iron metabolism characterized by excessive accumulation of iron in tissues causing diabetes mellitus, liver dysfunction, and a bronze skin pigmentation. Hereditary hemochromatosis is the most common autosomal recessive disorder (affects 1:200 people) with six times higher incidence in white people than in blacks.

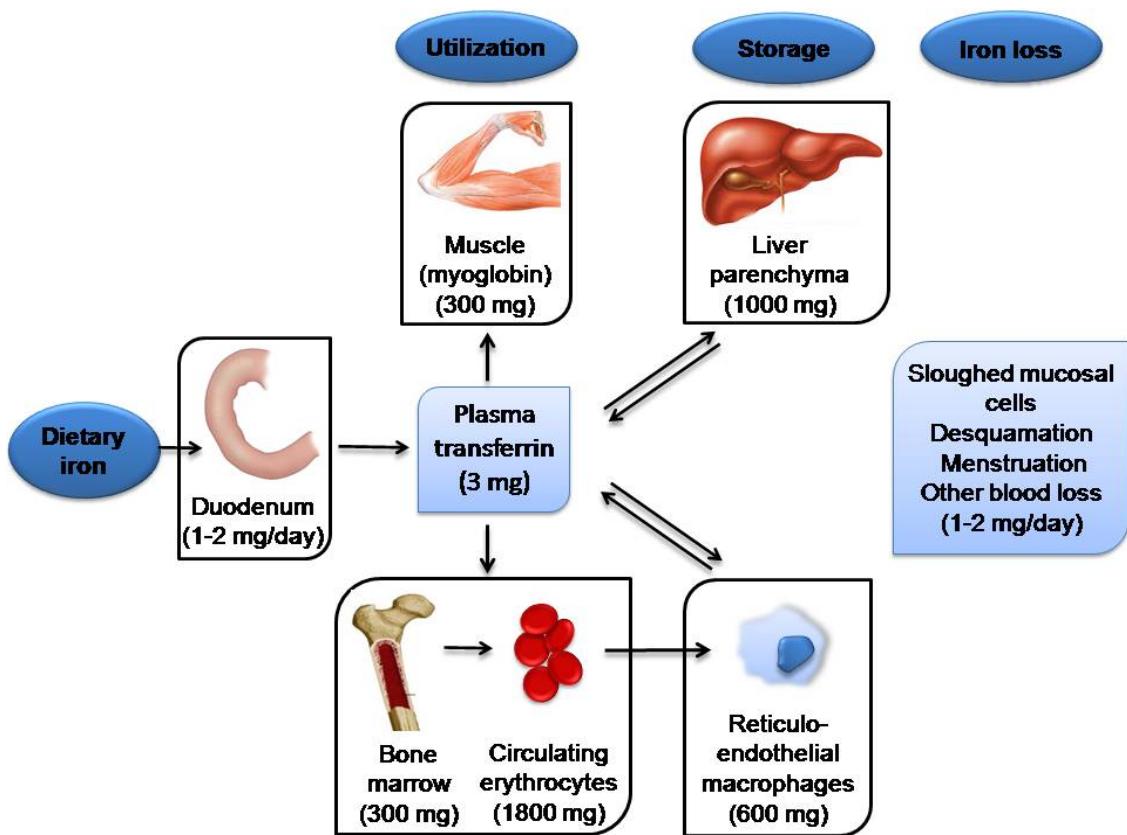


**Fig. 53.** Section from patient with hemochromatosis. The iron stain (Prussian blue), which displays iron granules in hepatocytes (arrowheads, x400) (Hussain SP, Raja K, Amstad PA, Sawyer M, Trudel LJ, Wogan GN, Hofseth LJ, Shields PG, Billiar TR, Trautwein C, Hohler T, Galle PR, Phillips DH, Markin R, Marrogi AJ, Harris CC

(2000). Increased p53 mutation load in nontumorous human liver of wilson disease and hemochromatosis: oxyradical overload diseases. *Proc Natl Acad Sci USA* 2000. 97(23):12770-5.

People with hemochromatosis can absorb four times more iron than normal. Organs commonly affected by haemochromatosis are the liver, heart, joints, pancreas and endocrine glands.

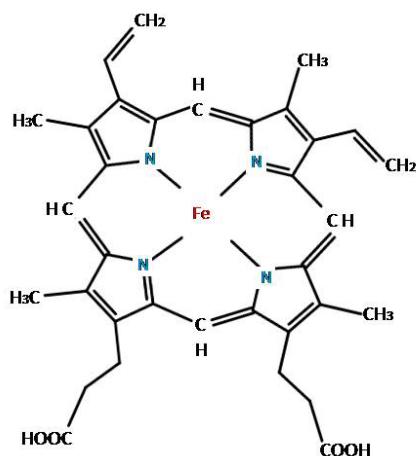
The most common type of hemochromatosis is caused by mutations in the HFE gene. The HFE protein encoded by the HFE gene acts in hepatocytes to regulate hepcidin expression. If HFE is mutated, the liver produces less hepcidin resulting in increased iron absorption.



**Fig. 54.** In the balanced state, 1 to 2 mg of iron enters and leaves the body each day. Dietary iron is absorbed by duodenal enterocytes. It circulates in plasma bound to transferrin. Most of the iron in the body is incorporated into hemoglobin in erythroid precursors and mature red cells. Approximately 10 to 15 percent is present in muscle fibers (in myoglobin) and other tissues (in enzymes and cytochromes). Iron is stored in parenchymal cells of the liver and reticuloendothelial macrophages. These macrophages provide most of the usable iron by degrading hemoglobin in senescent erythrocytes and reloading ferric iron onto transferrin for delivery to cells

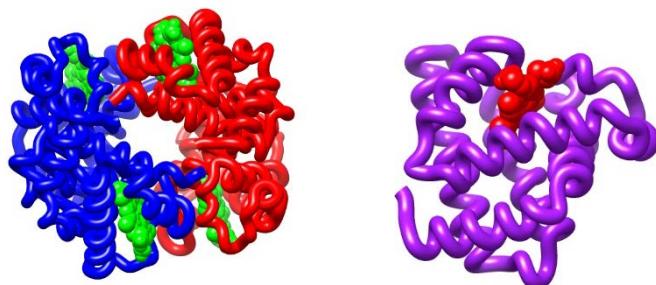
## 4.2. Hemoglobin

Oxygen has a low solubility in water, the major component of blood plasma. At physiological temperature and partial oxygen pressure, 1 L of plasma will dissolve only about 3 ml of O<sub>2</sub>. The oxygen transport protein hemoglobin greatly increases the amount of O<sub>2</sub> carried by blood. 1 L of whole blood, with its hemoglobin, can carry 200 mL of O<sub>2</sub>. Hemoglobin is the structural analog of myoglobin. The latter is found mainly in muscle tissue where it serves as an intracellular storage site for oxygen. Myoglobin and hemoglobin are both heme proteins. The heme group is the Fe<sup>2+</sup> complex of protoporphyrin IX.



**Fig. 55.** The structure of heme

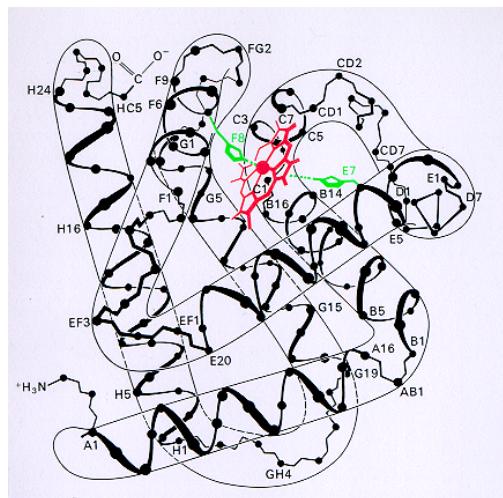
With a heme group and a single polypeptide chain, myoglobin is monomeric. Unlike myoglobin, hemoglobin molecules are tetramers composed of four subunits, each with a heme group plus a globin chain. Hemoglobin molecules consist of two different pairs of polypeptide chains.



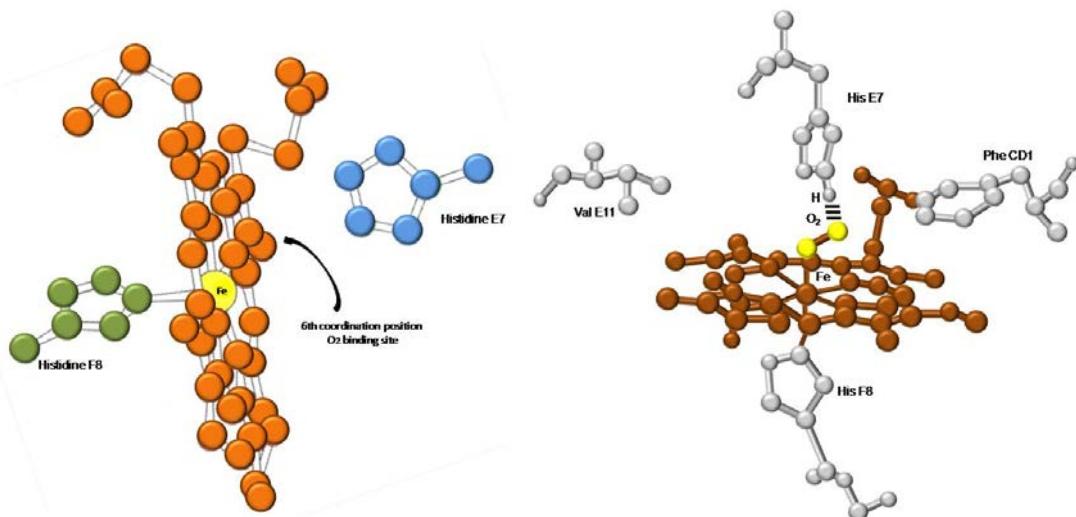
**Fig. 56.** Schematic structure of myoglobin (left) and hemoglobin (right) ([biologicalexceptions.blogspot.com](http://biologicalexceptions.blogspot.com))

The iron is the site of oxygen binding; each iron can bind one O<sub>2</sub> molecule. The iron in the heme is coordinated to the four nitrogen atoms of the porphyrin and also to a nitrogen atom from the proximal histidine (F8) in the globin protein (see Figs. 57-59). The distal histidine is near the heme but not bonded to it. The sixth coordination site around the iron of the heme is occupied by O<sub>2</sub> when myoglobin (or hemoglobin) is oxygenated and the complex has an

octahedral geometry. Oxygen binding occurs so that the Fe-O-O bond angle is about  $120^\circ$ , due to the lone electron pair on the coordinating oxygen atom. The distal histidine, with its ability to form hydrogen bonds, and additional residues (Val, Phe) are also involved in binding of  $O_2$  (Fig. 53).

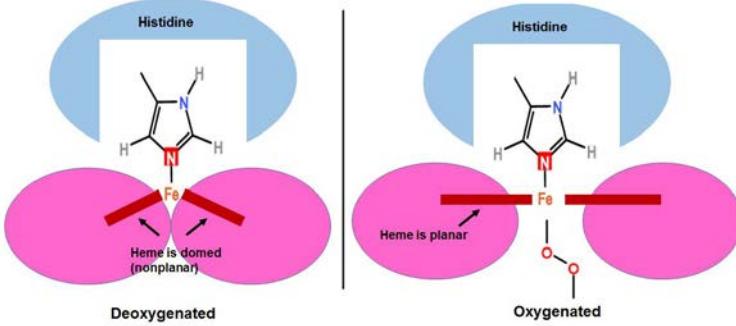


**Fig. 57.** Binding of heme in myoglobin. The heme group is shown in red, and two key histidines in green (left). Model of the oxygen-binding site in myoglobin showing the heme group, the proximal histidine (F8), and the distal histidine (E7) (Stryer, 1998)



**Fig. 58.** Oxygen binding in myoglobin (or hemoglobin). Oxygen binds to the sixth coordination site (left; Stryer, 1998). Oxygen binding involves the distal histidine (E7) and additional residues (right); [http://www.bio.miami.edu/tom/courses/bil255/bil255goods/03\\_proteins.html](http://www.bio.miami.edu/tom/courses/bil255/bil255goods/03_proteins.html))

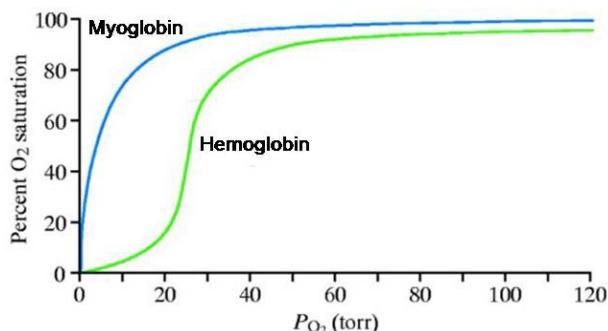
In the deoxy-heme protein (when  $O_2$  is not bound), the porphyrin adopts a domed (nonplanar) configuration and the Fe is out of the plane of the porphyrin ring (Fig.58, left). However, when the Fe in the heme group binds to an oxygen molecule, the porphyrin ring adopts a planar configuration and hence the Fe lies in the plane of the porphyrin ring (Fig.58, right).



**Fig. 59.** Representations of the electron density clouds of the heme group (pink) and the attached F8 histidine residue (light blue) for the deoxy-heme (left) and oxy-heme group

[http://www.chemistry.wustl.edu/~edudev/LabTutorials/CourseTutorials/Tutorials/Hemoglobin/151\\_T3\\_heme.pdf](http://www.chemistry.wustl.edu/~edudev/LabTutorials/CourseTutorials/Tutorials/Hemoglobin/151_T3_heme.pdf)

In the deoxy-heme protein,  $\text{Fe}^{2+}$  is in the high spin electron configuration and is larger whereas in the oxy-heme protein,  $\text{Fe}^{2+}$  is in the low spin electron configuration and has a smaller size. When a single heme group in the hemoglobin protein becomes oxygenated, the whole protein changes its shape. In the new shape, it is easier for the other three heme groups to become oxygenated. Thus, the binding of one molecule of  $\text{O}_2$  to hemoglobin enhances the ability of hemoglobin to bind more  $\text{O}_2$  molecules. This property of hemoglobin is known as "cooperative binding". Whereas the oxygen saturation curve of the monomeric myoglobin is hyperbolic, that of hemoglobin is sigmoidal (Fig.60.) Hemoglobin's oxygen affinity is relatively weak compared to myoglobin's affinity for oxygen. However, hemoglobin can effectively bind, and after transport, release oxygen.



**Fig. 60.** Oxygen saturation curve of myoglobin and hemoglobin

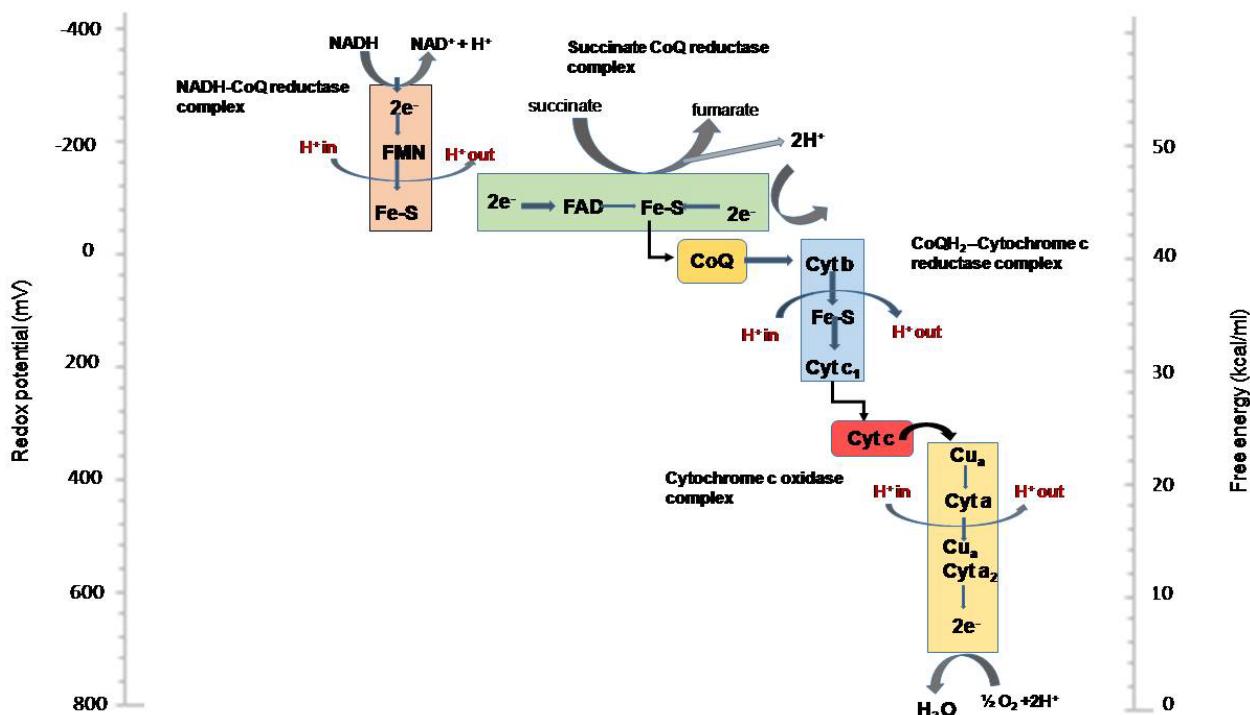
The extreme toxicity of carbon monoxide ( $\text{CO}$ ) is associated with its ability to coordinate to iron 200 times more strongly than does oxygen, thereby inhibiting hemoglobin's oxygen carrying function.

**Methemoglobinemia** is a life-threatening condition that can be congenital or acquired. It is characterized by the inability of hemoglobin to bind oxygen because  $\text{Fe}^{2+}$  of the heme molecule has been oxidized to  $\text{Fe}^{3+}$ .

### 4.3. Other heme proteins

Cytochromes are heme proteins involved in electron transfer reactions of the respiratory chain, photosynthesis, and other biological processes.

*Cytochrome c* is a small protein, the only one from the electron transport system not in a complex. It accepts electrons from complex III ( $\text{CoQH}_2$ - cytochrome reductase) and shuttles them to complex IV (cytochrome oxidase, Fig. 61).



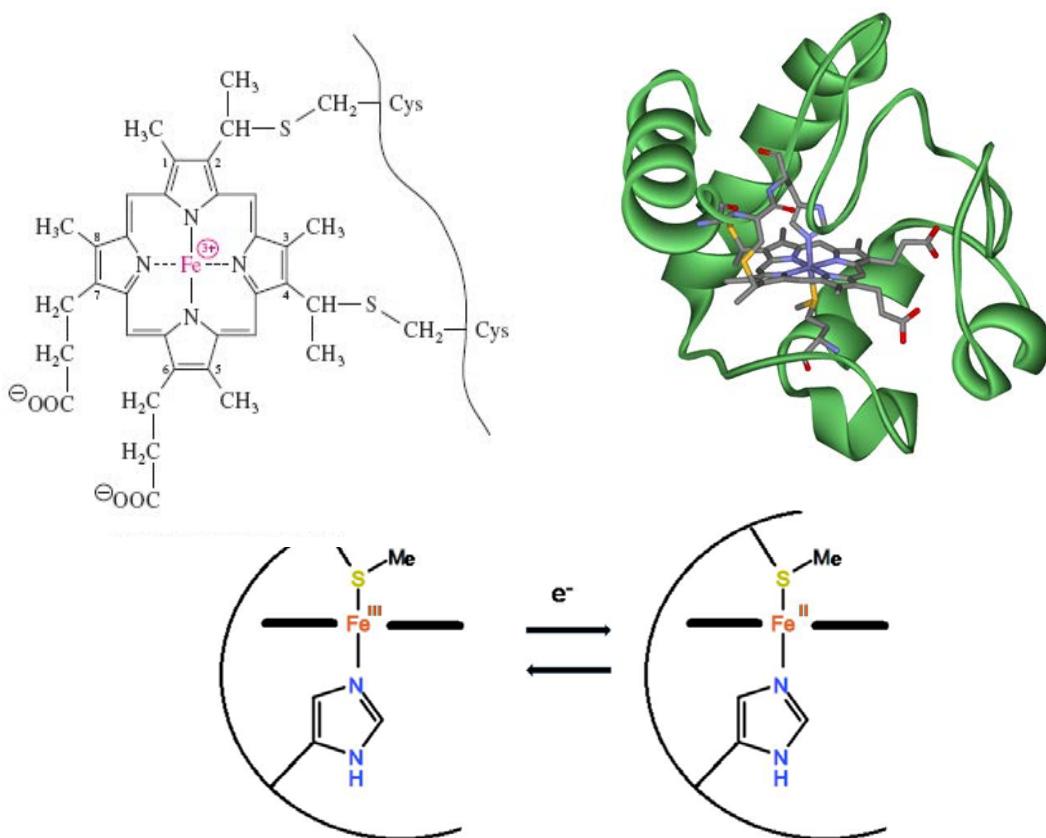
**Fig. 61.** Changes in the standard redox potential ( $E^\circ$ ) along the mitochondrial electron transport chain. Since  $\Delta E^\circ > 0$  and  $\Delta G^\circ = -nF\Delta E^\circ$ , free energy is liberated ( $\Delta G^\circ < 0$ )

Cytochrome c consists of a single polypeptide chain and a covalently attached heme group (Fig. 62) in which the oxidation number of Fe alternates due to the electron transfer.

The central iron ion is coordinated with the four nitrogen atoms of the porphyrin ring, furthermore, with the imidazole N of a histidine and the sulfur of a methionine in the polypeptide chain. Because of the two tightly bound axial ligands, cytochrome c iron cannot bind  $\text{O}_2$  (or CO).

Did you know? More recently, cytochrome c has been identified as an important mediator in apoptotic (programmed cell death) pathways. The release of mitochondrial cytochrome c into the cytoplasm stimulates apoptosis and is commonly used as an indicator of the apoptotic process in the cell.

For the structure and function of *cytochrome c oxidase*, see section 4.5 on copper.

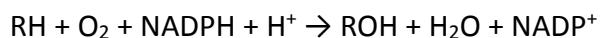


**Fig. 62.** Cytochrome c heme (left). The heme is covalently attached to two cysteine side chains of the polypeptide by thioether bonds. The iron atom alternates between a reduced (+2) state and an oxidized (+3) state during electron transport. Schematic representation of heme position, iron coordination, and protein folding in cytochrome c (right). The heme iron is bonded to two axial ligands, at a histidine imidazole and a methionine sulfur. It plays a role in the electron transport (down)

### Cytochrome P-450

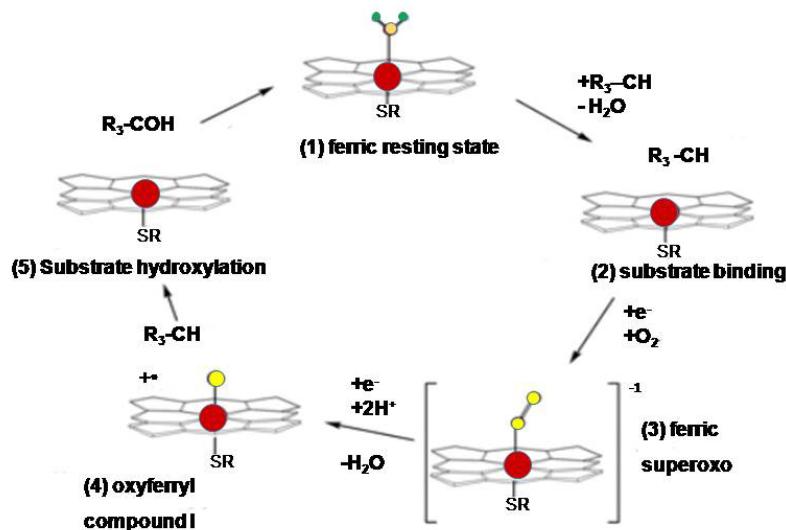
The cytochrome P450 superfamily (abbreviated as CYP) is a large and diverse group of ubiquitous enzymes that absorb light maximally at 450 nm when complexed to CO.

CYPs are monooxygenases, they catalyze the insertion of one atom of O<sub>2</sub> into an organic substrate (RH) while the other oxygen atom is reduced to water:



Human CYPs are primarily membrane-associated proteins located either in the inner membrane of mitochondria or in the endoplasmic reticulum of cells. They play essential role in the metabolism of endogenous substances (e.g., steroids). Furthermore, they are important in the detoxification of foreign substances. For example, the introduction of hydroxyl groups into aromatic compounds provides sites for conjugation with highly polar units (e.g., glucuronate). This mechanism highly increases the solubility of the modified molecule that will be excreted by the kidneys. On the other hand, hydroxylation of certain polycyclic aromatic hydrocarbons contributes to their bioactivation into cancerogenic intermediates.

CYPs are hemoproteins containing iron atom with alternating oxidation states (Fig. 63).



**Fig. 63.** Proposed catalytic cycle for hydroxylation by cytochrome P450.

1, Resting state with water coordinated to iron; 2, substrate binding displaces coordinated water and first electron-reduction results in  $O_2$  binding; 3, second electron reduction and distal oxygen protonation liberates a water molecule and the active compound I; 4, substrate hydroxylation and resting-state regeneration  
 (Guallar et al. PNAS 100:6998, 2003)

Catalase and peroxidases are enzymes which protect the body against uncontrolled oxidation by hydrogen peroxide.

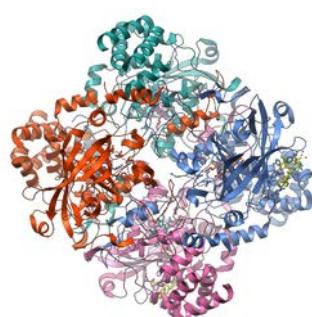
### Catalase

catalyzes the decomposition of hydrogen peroxide to water and oxygen:



Catalase has one of the highest turnover numbers of all enzymes; one catalase molecule can convert millions of molecules of hydrogen peroxide to water and oxygen each second. The protein exists as a tetramer of four identical subunits (Fig.64). Each monomer contains a heme prosthetic group at the catalytic center.

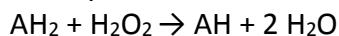
Hydrogen peroxide belongs to the reactive oxygen species (ROS) and is well known for its cytotoxicity. At low concentrations, however,  $H_2O_2$  acts as a cellular messenger (e.g. in insulin signaling). A catalase deficiency that causes elevated  $H_2O_2$  levels may increase the likelihood of developing type 2 diabetes.



**Fig. 64.** Crystal structure of catalase (<https://en.wikipedia.org/wiki/Catalase>)

### Peroxidases

are a large family of enzymes that catalyze the oxidation of various substrate molecules:



In the above reaction,  $\text{H}_2\text{O}_2$  is reduced whereas in the catalase reaction, it is disproportionated (reduced and oxidized). For the oxidation, many of the peroxidases use hydrogen peroxide preferentially. Some others are more active with organic hydroperoxides such as lipid peroxides.

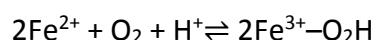
Peroxidases can contain a **heme cofactor** in their active sites (e.g. horseradish peroxidase, myeloperoxidase), or redox-active cysteine or selenocysteine residues (e.g., glutathione peroxidase, see chapters 6.1.4.2 and 6.2).

**Myeloperoxidase** is a hemoprotein that is abundantly expressed in neutrophil granulocytes. It catalyses the oxidation of chloride ions to the cytotoxic hypochlorous acid ( $\text{HClO}$ ) to be used by these cells as an antibacterial weapon".

### 4.4. Non-heme iron containing proteins. Iron-sulfur clusters.

#### Hemerythrin

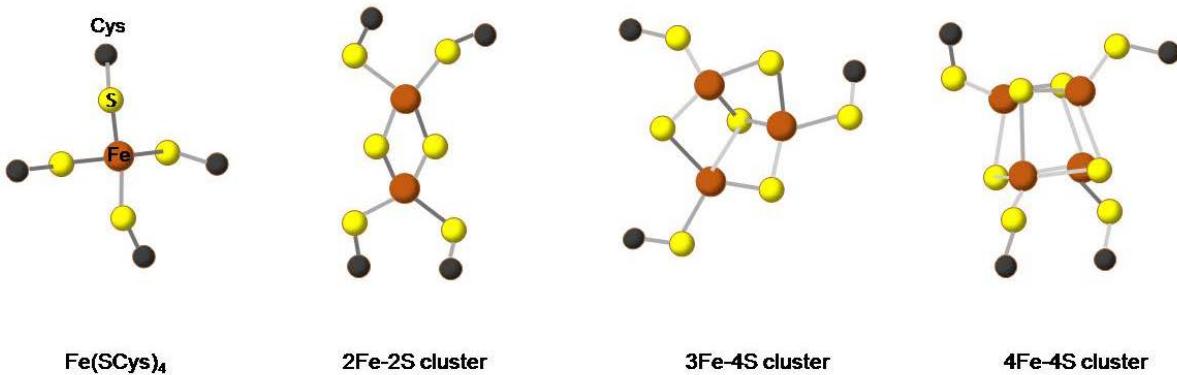
Hemerythrin is responsible for  $\text{O}_2$  transport in a variety of marine invertebrates. Hemerythrin is not a hem protein. The deoxygenated protein is essentially colorless but turns violet-pink upon  $\text{O}_2$  binding. Typically, it is composed of eight subunits, however, the monomeric form also exists. Each subunit contains two iron atoms and can bind one molecule of  $\text{O}_2$ . The binding of oxygen to hemerythrin and its release can be described by the following reaction, where the  $\text{HO}_2^-$  ligand is the hydroperoxide anion derived by the deprotonation of hydrogen peroxide:



For *transferrin*, *ferritin*, and *hemosiderin*, see chapter 4.1.

#### Iron-sulfur proteins

The majority of non-heme iron proteins are iron-sulfur proteins. They play a critical role in the electron transport of bacteria, plants, and animals. Fe-S centers have essential functions in photosynthesis, cellular respiration, nitrogen fixation, redox and nonredox catalysis. Fe-S centers are present in redox enzymes such as hydrogenases, sulfite reductase, and also in **aconitase** which catalyses a nonredox (isomerization) reaction. Iron-sulfur (FeS) clusters are important cofactors for numerous proteins involved in gene regulation, may stabilize the structure of certain proteins, and function as sensors of oxygen in bacteria and iron in bacteria or animal cells. Several types of iron-sulfur clusters are known (Fig.65). In almost all of them, the iron centers has a distorted tetrahedral arrangement. The sulfide groups are either two- or three-coordinated. Electron transport is accompanied by the  $\text{Fe}^{2+} \rightleftharpoons \text{Fe}^{3+} + \text{e}^-$  reaction.



**Fig. 65.** Molecular models of iron-sulfur centers.

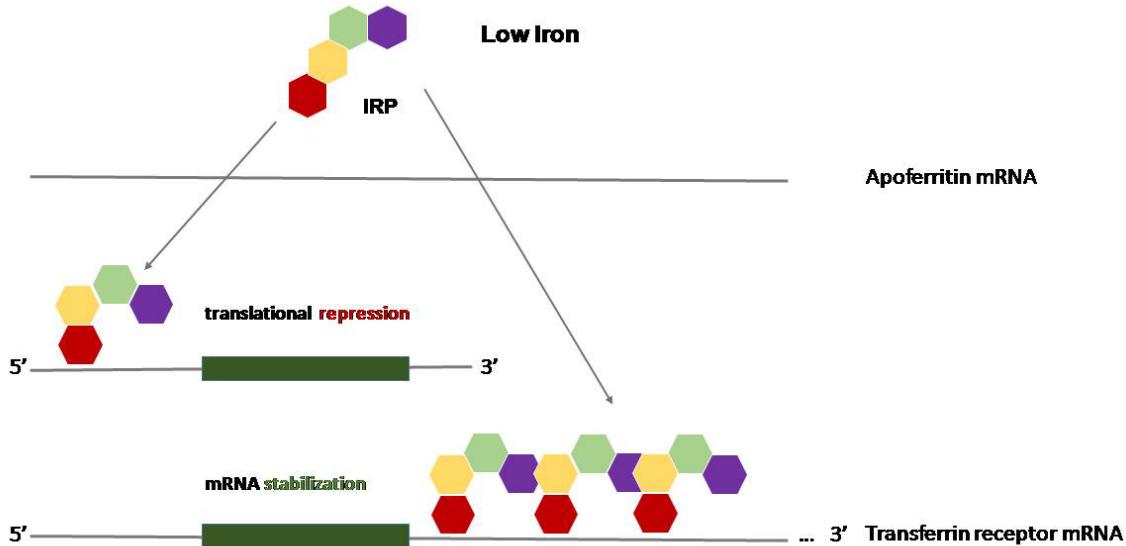
[http://2012books.lardbucket.org/books/general-chemistry-principles-patterns-and-applications-v1.0/section\\_27\\_06.html](http://2012books.lardbucket.org/books/general-chemistry-principles-patterns-and-applications-v1.0/section_27_06.html)

Rubredoxins contain only one iron center. In contrast to other iron-sulfur proteins, rubredoxins do not contain inorganic sulfide, iron is coordinated by the sulfurs of four conserved cysteine residues forming an almost regular tetrahedron. This is sometimes denoted as a 1Fe-0S center. Rubredoxins are electron carriers in sulfur-metabolizing bacteria and archaea.

In ferredoxins, the iron atoms are coordinated by both inorganic sulfur and sulfur of cysteine residues. They occur in nearly all organisms. Their amino acid composition, the oxygen sensitivity of the reduced species suggest that ferredoxins might have played an important role very early in evolution, in the absence of oxygen.

2Fe-2S ferredoxins occur in bacteria, plants, and mammals. The most common and most stable iron-sulfur centers are of the 4Fe-4S type. The majority of 4Fe-4S ferredoxins is involved in the electron transport. Some 4Fe-4S clusters bind substrates and are thus classified as enzyme cofactors. The active form of aconitase, required for the isomerization of citrate in the tricarboxylic acid cycle, has an 4Fe-4S cluster, which may convert to an inactive 3Fe-4S form. Cytosolic aconitase (also called iron regulatory protein, IRP) is a dual-function protein exhibiting either aconitase activity when cellular iron is abundant or RNA-binding activity when cellular iron is scarce. Aconitase contains a relatively unstable 4Fe-4S cluster at its center. When the iron-sulfur cluster is bound, it functions as an aconitase. Under conditions of low iron, the 4Fe-4S cluster dissociates and the protein will bind appropriate mRNAs by their stem-loop structures called iron responsive elements (IREs). The effect of IRP binding depends on the position of binding. An IRP bound to a 5' UTR (untranslated region) IRE represses translation, whereas an IRP bound to an IRE in the 3' end can indirectly activate translation through suppression of mRNA degradation (reviewed by Volz, Curr. Opin. Struct. Biol. 18, 2008). The mRNA of apoferritin has IRE in the 5'end whereas the mRNA of the transferrin receptor has IREs in the 3'end (Fig.66). When iron is abundant, the absence of IRP binding allows apoferritin mRNA to be freely translated and transferrin receptor mRNA to be degraded. As a result, ferritin will be made for iron storage in the cells but no transferrin

receptor. Under conditions of iron depletion, IRP stabilizes transferrin receptor mRNA to prolong its half-life, while inhibiting the translation of apoferritin mRNA. Thus, transferrin receptor will be made but no ferritin.



**Fig. 66.** Regulation of transferrin receptor and apoferritin expression under low iron conditions  
Modified from <http://flipper.diff.org/app/pathways/2341>

## 4.5. Copper (Cu)

### 4.5.1. Basic facts

Atomic number: 29

Electron configuration: [Ar]  $3d^{10} 4s^1$

Oxidation state: may exist in many oxidation states but in biological systems +1 and +2 are important. Copper undergoes redox cycling ( $Cu^+ \rightleftharpoons Cu^{2+}$ )

### 4.5.2. Biological importance and transport in the body

Copper is an essential element in living systems. Copper-containing proteins function in electron transfer reactions (e.g. cytochrome c oxidase), oxygen transport (e.g. hemocyanin), oxidase and oxygenase enzymes (e.g. tyrosinase) and in superoxide degradation (superoxide dismutase). Copper's essentiality was first discovered in 1928, when it was demonstrated that rats fed a copper-deficient milk diet were unable to produce sufficient red blood cells. The anemia was corrected by the addition of copper-containing ash from vegetable or animal sources. (Heart et al JBC 77:797, 1928).

Copper is absorbed in the stomach and small intestine. Copper absorption is inhibited by high intakes of iron, zinc or vitamin c reminding us of the fact that micronutrients need to be consumed as a balanced mixture. Once absorbed, copper binds to albumin and gets transported to the liver where it is incorporated into ceruloplasmin (a protein capable of binding 8 copper ions) for transport to all tissues. Excess copper can be excreted by the liver into the bile.

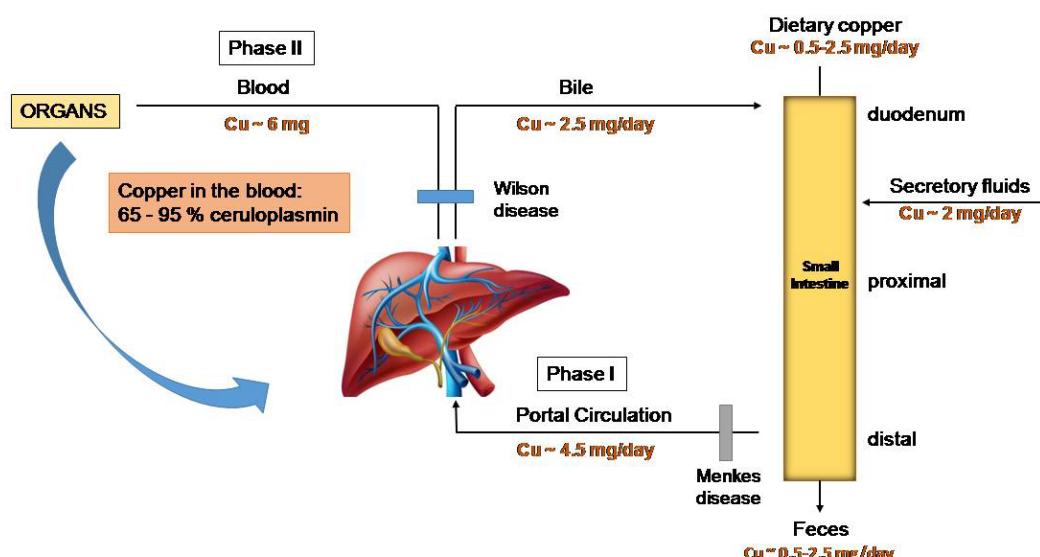
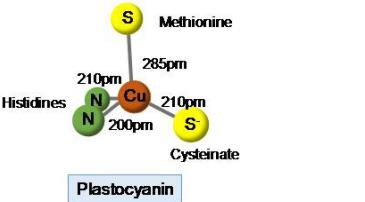
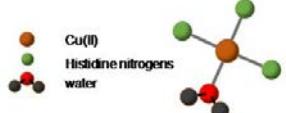
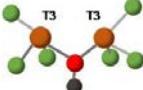


Fig. 67. Copper turnover and its disturbance in Wilson and Menkes diseases (source: studyblue.com )

#### 4.5.3. Types of cuproproteins

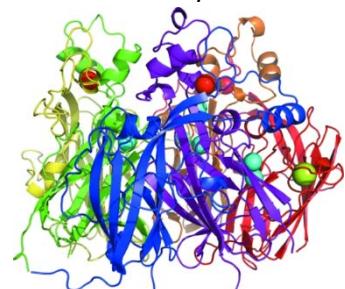
(source: [http://www.chm.bris.ac.uk/motm/caeruloplasmin/copper\\_proteins/](http://www.chm.bris.ac.uk/motm/caeruloplasmin/copper_proteins/))

In Type 1 (blue copper) proteins copper is coordinated by 2 N and 2 S atoms. These centers have an irregular tetrahedral geometry and function in electron transfer reactions	
In type 2 (non-blue) copper proteins copper is coordinated by 2-3 N and 1-2 O atoms. This is the most common type of copper coordination and has a planar geometry.	
Type 3 proteins contain copper dimers. This is the copper complex functioning as oxygen carrier in some arthropods and mollusks (horseshoe crab, lobsters) that have "blue blood".	

The nitrogens come from histidine groups, the sulphurs from methionine and cysteine, the oxygens from a carboxylic acid in the protein. Water, hydroxide and alkoxide oxygens are also used.

#### 4.5.4. Examples of Cu proteins

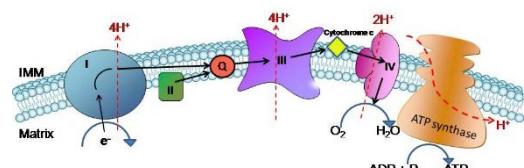
##### 4.5.4.1. Ceruloplasmin



Ceruloplasmin is a protein with dual function: on the one hand it is the major carrier of copper in the blood but it also functions as ferroxidase (catalyzing the oxidation of  $\text{Fe}^{2+}$  to  $\text{Fe}^{3+}$  and thus facilitating the loading of iron onto its transfer protein transferrin).

**Fig. 68.** The three-dimensional molecular structure of human serum ceruloplasmin (source: Bento et al. Acta Crystallogr D Biol Crystallogr. 2007; 63(Pt 2): 240)

##### 4.5.4.2. Cytochrome c oxidase



**Fig. 69.** The mitochondrial electron transport chain (source: Nat Rev Mol Cell Biol)

At the end of the mitochondrial electron transport chain  $\text{O}_2$  molecules normally undergo tetravalent reduction to form 2 water molecules. (The proton gradient formed in parallel to the electron transport is used by the ATP synthase for ATP generation.) Incomplete reduction of  $\text{O}_2$  may lead to the production of reactive oxygen species (ROS) such as superoxide anion radical ( $\text{O}_2^\cdot$ ) or peroxide ( $\text{O}_2^{2-}$ ) ion.



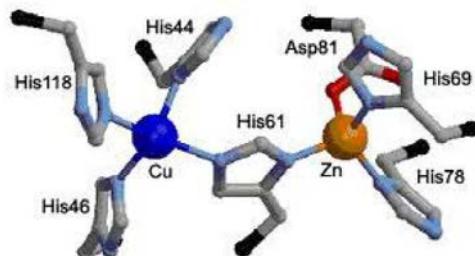
Cytochrome c oxidase (also known as complex IV of the mitochondrial electron transport system) is a multiprotein complex (consists of 14 protein subunits in mammals). It takes up electrons from cytochrome c (a mobile electron carrier) and gives the electrons to O<sub>2</sub> molecules which are the final electron acceptors of the mitochondrial electron transport chain.

**Fig. 70.** The path of electrons in cytochrome oxidase. Cytochrome oxidase contains two hemes, (cytochrome a and cytochrome a<sub>3</sub>) and two copper centers (the Cu<sub>A</sub> and Cu<sub>B</sub> centers)

Of note, toxicity of the potent respiratory toxin cyanide is due to inhibition of cytochrome oxidase.

#### 4.5.4.3. Cu-Zn-superoxide dismutase

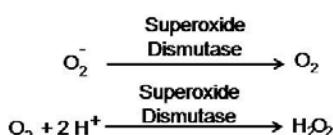
Superoxide dismutase (SOD) enzymes convert superoxide radicals into hydrogen peroxide and oxygen. The cytosolic form of the enzyme (known as copper-zinc SOD; CuZn-SOD) contains a Zn<sup>2+</sup> and a Cu<sup>2+</sup> ion. Whereas the **copper ion plays a catalytic role**, the **zinc ion stabilizes the structure of the active center of the enzyme** without being directly involved in the catalytic mechanism.



**Fig. 71.** Coordination of Cu and Zn in the active center of CuZn-SOD

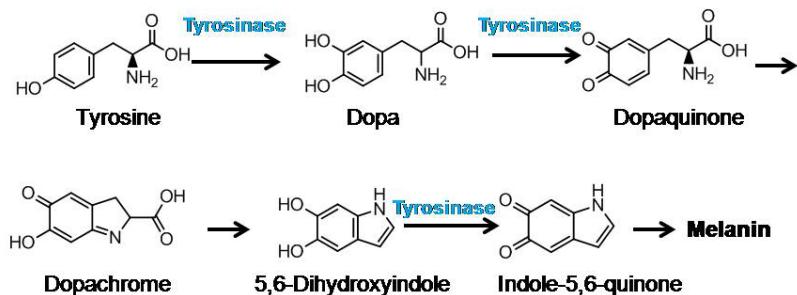
Coordination of Cu and Zn in the active center of CuZn-SOD (source: Osredkar J, Sustar N (2011) Copper and Zinc, Biological Role and Significance of Copper/Zinc Imbalance. J Clinic Toxicol S3:001)

In the first step Cu<sup>2+</sup> ions take 1 electron from superoxide (O<sub>2</sub><sup>-</sup>) and convert the radical to dioxygen molecule (O<sub>2</sub>). In the second step Cu<sup>+</sup> ions reduce another superoxide to peroxide ion (O<sub>2</sub><sup>2-</sup>) which forms hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) with 2 protons while Cu<sup>+</sup> ions get reoxidized to Cu<sup>2+</sup>.



#### 4.5.4.4. Tyrosinase

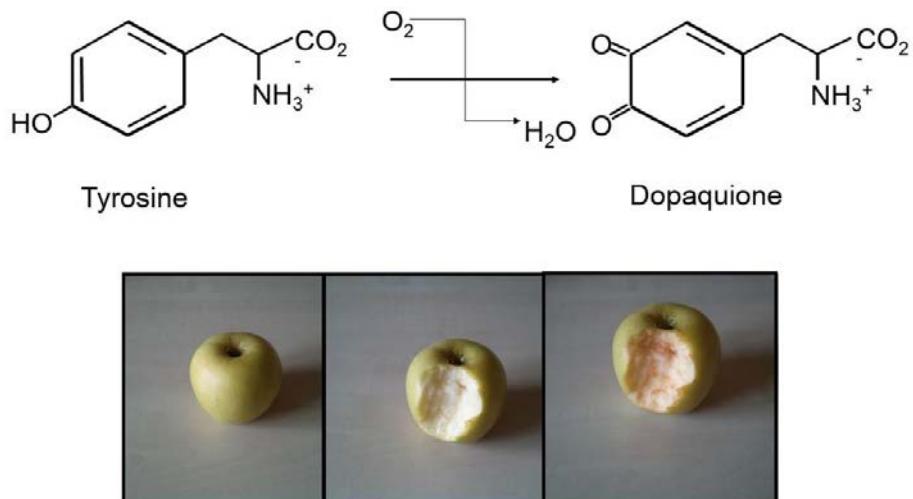
Tyrosinase is an oxidase enzyme catalyzing the key (rate limiting) step of melanin synthesis.



**Fig. 72.** Tyrosinase catalyzes the conversion of L-tyrosine to L-dopa (L-3,4-dihydroxyphenylalanine), and the oxidation of L-dopa to o-dopaquinone

Mutations in the gene of tyrosinase result in albinism due to lack of melanin pigmentation in the skin and the eye.

Tyrosinase is also responsible for enzymatic browning reactions in damaged fruits.



**Fig. 73.** Tyrosinase action

#### 4.5.4.5. Hemocyanin

The oxygen carrier protein in mollusks and some arthropods. This protein confers blue colour to the blood of these animals.

#### 4.5.5. Medical aspects

##### 4.5.5.1. Wilson's disease

Wilson's disease is hereditary (AR, autosomal recessive) disease. Symptoms usually appear between the ages of 6 and 20 years, but can begin as late as age 40.

Wilson's disease is caused by mutation in the gene encoding the intracellular copper transport protein named ATP7B. This protein is normally expressed in the liver and its main function is to pump out excess copper from the hepatocytes (liver cells) into the bile.

Hepatocytes of Wilson's disease patients cannot cope with excess copper which accumulates in the liver. Accumulating copper damages liver cells through the formation of reactive oxygen species via Fenton chemistry (for details see chapter 4.1. on iron metabolism). In turn copper is released from damaged hepatocytes and will reach other organs (kidney, eye, brain) causing secondary damage. In summary, tissue damage in Wilson disease is caused by copper overload.

The most characteristic sign is the Kayser-Fleischer ring (a rusty brown ring around the cornea of the eye) that can be seen at an eye exam. Other signs depend on whether the damage occurs in the liver, blood, central nervous system, urinary system, or musculoskeletal system.



**Fig. 74.** Kayser-Fleischer ring before (A) and after 5 years treatment with penicillamine  
(source: NEJM DOI: 10.1056/NEJMcm1101534)

The disease is treated with lifelong use of **D-penicillamine** (chelator) that helps remove copper from tissue, or **zinc** acetate, which stops the intestines from absorbing copper and promotes copper excretion. Patients will also need to take vitamin B<sub>6</sub> and follow a **low-copper diet**, which means avoiding mushrooms, nuts, chocolate, dried fruit, liver, and shellfish.

Wilson's disease requires lifelong treatment. If the disorder is detected early and treated correctly, a person with Wilson's disease can enjoy normal health.

#### 4.5.5.2. Menkes disease

Menkes disease (also known as kinky hair disease) is an X-linked neurodegenerative condition characterized by abnormal kinky hair, eyebrows, and eyelashes (short, sparse, coarse, twisted), often lightly or abnormally pigmented (can be white, silver, or gray) hair.

The disease is caused by **impaired copper transport**. Menkes et al. first described it in 1962 and in 1972 Danks et al. (Birth Defects Orig Artic Ser. 1974;10:132–7.) first noted that copper metabolism is abnormal in these



**Fig. 75.** Menkes disease

patients. In 1973, after noting the similarity of kinky hair to the brittle wool of Australian sheep raised in areas with copper-deficient soil, he demonstrated abnormal levels of copper in these patients.

Menkes disease is caused by mutation in the gene encoding for the intracellular copper transport protein named ATP7A. ATP7A Unlike the Wilson'S disease protein that is only

expressed in the liver, the Menkes disease-associated protein ATP7A is expressed in most tissues but is absent from the liver. This copper transporting ATP-ase protein normally assists absorption of copper from food and can also facilitate copper export from cells by shuttling between the Golgi apparatus and the plasma membrane. Mutations in ATP7A impair the absorption of copper from food, fail to supply copper to certain enzymes, or get stuck in the cell membrane, unable to shuttle back and forth from the Golgi. As a result of the disrupted activity of the ATP7A protein, copper is poorly distributed to cells in the body. In summary, most symptoms of Menkes disease are due to copper deficiency of organs.

## 4.6. Zinc (Zn)

### 4.6.1. Basic facts

Atomic number: 30

Electron configuration: [Ar] 3d<sup>10</sup> 4s<sup>2</sup>

The only common oxidation state: +2 (Unlike iron or copper ions, zinc is not redox active)

### 4.6.2. Biological importance

It is estimated that 10% of all proteins contain Zn. This means that humans have approximately 3000 Zn proteins. Zinc is coordinated in proteins by histidine (N), cysteine (S) glutamate (O) or aspartate (O) residues.

The role of zinc in proteins may be

- (a) **structural** (e.g. Zn-finger motif present in many transcription factors or the structure stabilizing role of Zn<sup>2+</sup> ions in CuZn-superoxide dismutase)
- (b) **catalytic** action in enzymes (e.g. Zn<sup>2+</sup> bound in the active center of carboanhydrase or carboxipeptidase A enzymes)
- (c) **regulatory** (e.g. temporary binding of Zn<sup>2+</sup> ions to zinc sensing or zinc transport proteins during signaling action of Zn<sup>2+</sup>)

### 4.6.3. Cellular zinc homeostasis

The cytosolic concentration of free Zn<sup>2+</sup> is low (in the **picomolar range**; 10-400 pM). Ten Zn transporter proteins of the ZnT family export zinc from the cytosol, whereas 14 Zip family proteins import zinc into the cytosol. These transporters control zinc trafficking between the cell and the extracellular space as well as between the cytosol and intracellular compartments. **Metallothionein** proteins are responsible for **storing and releasing zinc**.

Under certain conditions (e.g. oxidative stress, high glucose exposure, mercury exposure or electrical stimulation) the cytosolic Zn<sup>2+</sup> concentrations may temporarily rise to the low nanomolar range and thus it may play a signaling role similar to that of calcium ions.

### 4.6.4. Examples of Zn proteins

#### Insulin hexamer

Zn plays a role in the synthesis, storage and secretion of insulin as well as conformational integrity of insulin in the hexameric form. In the presence of zinc within the pancreatic beta cell, **insulin monomers assemble to a dimeric form for storage and secretion as the zinc crystal**. In vitro, in the presence of zinc and at neutral pH, dimeric insulin assembles further into a hexamer consisting of three dimeric units. This form of insulin is relatively stable and it is this **hexameric crystal** which is the **commonly used pharmacologic form** ([Chausmer AB J.Am.Coll Nutr 1998 Vol17 p109](#)).

### Zn-finger proteins

DNA binding proteins (e.g. transcription factors, DNA repair proteins) utilize specialized motives for binding to DNA. One such motif is the zinc finger motif that is a finger shaped structure stabilized by a  $Zn^{2+}$  ion.  $Zn^{2+}$  is coordinated by 4 Cys or 4 His residues or combinations of these two residues (e.g. 2 Cys and 2 His). (Of note, a zinc finger may also bind RNA or proteins not only DNA.)

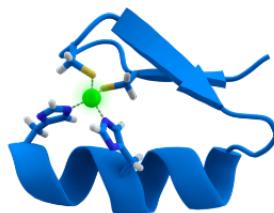


Fig. 76. Zn-finger motif

### Cu-Zn-superoxide dismutase

Superoxide dismutase (SOD) enzymes convert superoxide radicals into hydrogen peroxide and oxygen. The cytosolic form of the enzyme (known as copper-zinc SOD; CuZn-SOD) contains a  $Zn^{2+}$  and a  $Cu^{2+}$  ion. Whereas the copper ion plays a catalytic role, the zinc ion stabilizes the structure of the active center of the enzyme without being directly involved in the catalytic mechanism. (For more details see chapter on the bioinorganic chemistry of copper).

### Carboanhydrase

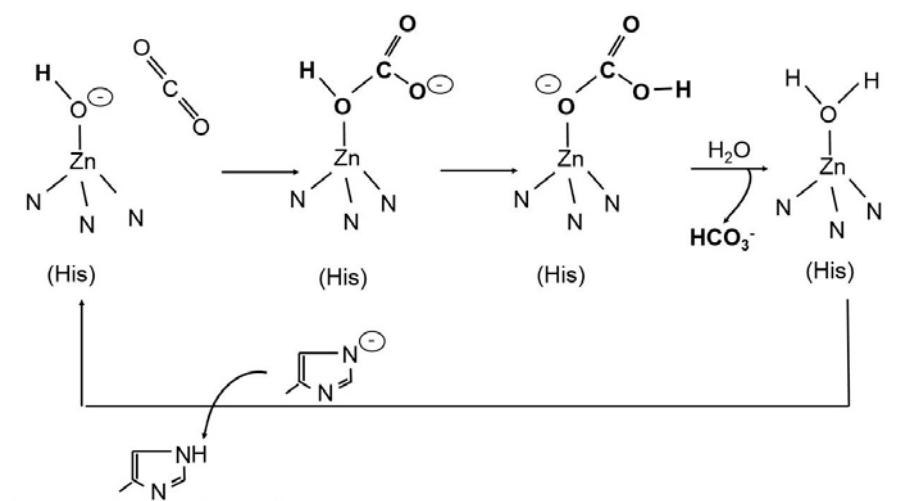
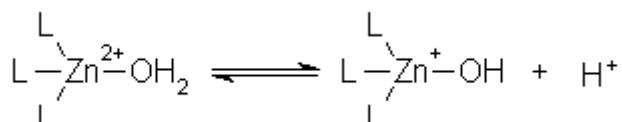


Fig. 77. Role of zinc in the active center of carboanhydrase

Carbonic anhydrases are enzymes that catalyze the hydration of carbon dioxide and the dehydration of bicarbonate:



The enzyme increases the rate of reaction by  $10^7$  fold (1 million catalytic events per second). The catalytic effect is based on  $Zn^{2+}$  acting as Lewis acid. Coordination of water molecules to Zn ion lowers the pKa of water and thus facilitates dissociation of the proton.



These carbonic anhydrase-driven reactions are of great importance in a number of tissues. Examples include:

1. Parietal cells in the stomach secrete massive amounts of acid (i.e. hydrogen ions or protons) into the lumen and a corresponding amount of bicarbonate ion into blood.

2. Pancreatic duct cells do essentially the opposite, with bicarbonate as their main secretory product.

3. Secretion of hydrogen ions by the renal tubules is a critical mechanism for maintaining acid-base and fluid balance.

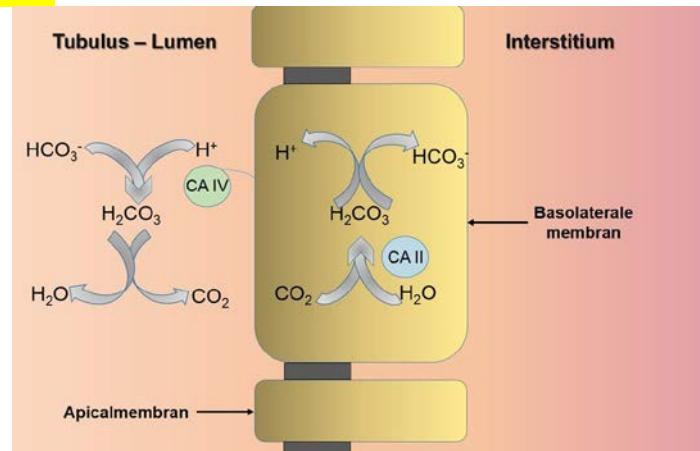


Fig. 78. Carboanhydrase in the proximal tubules of the kidney

4. Carbon dioxide generated by metabolism in all cells is removed from the body by red blood cells that convert most of it to bicarbonate for transport, then back to carbon dioxide to be exhaled from the lungs.

### Carboxypeptidase A

This enzyme is a digestive enzyme produced by the pancreas. It hydrolyses the peptide bond nearest to the terminal carbonyl group in polypeptide chains.

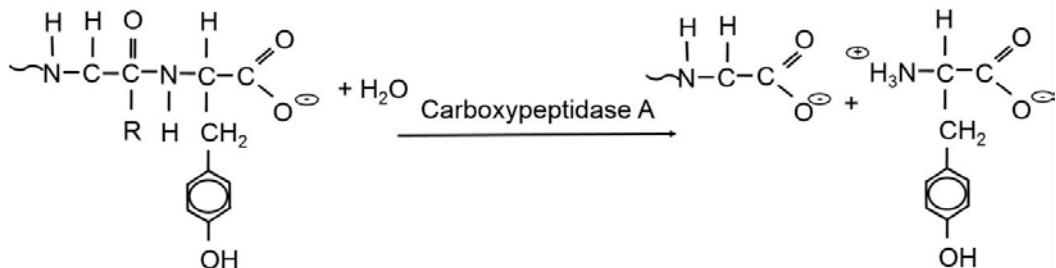
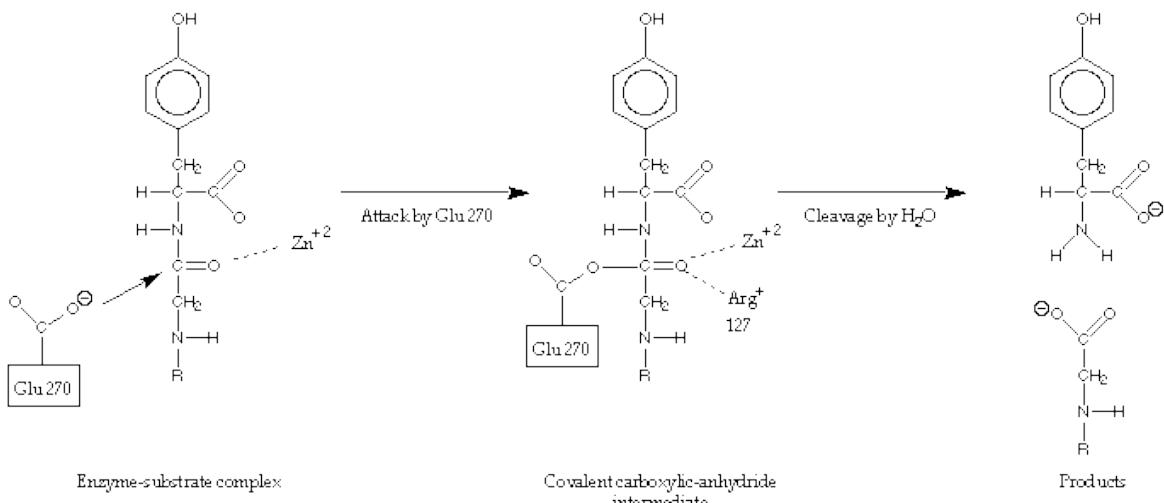


Fig. 79. Reaction catalyzed by carboxypeptidase A (source: Stryer L Biochemistry. 1988)

The reaction occurs most readily if the carboxyl-terminal residue contains a bulky aliphatic or an aromatic side chain (This is what “A” in the name of the enzyme refers to.).  $Zn^{2+}$  binds three enzymatic groups (His-196, Glu-72 and His-69) and water. The peptide carbonyl group is coordinated to zinc, and displaced water is delivered to the substrate when Glu 270 acts as general base. Tyr 248 also forms hydrogen bonds to the substrate and delivers another proton, so that cleavage occurs.



**Fig. 80.** Role of zinc in carboxypeptidase A (source: Stryer L Biochemistry. 1988)

#### 4.6.5. Medical aspects

##### Zinc deficiency

The World Health Organization considers zinc deficiency as the 5<sup>th</sup> leading cause of morbidity and mortality in developing countries (11<sup>th</sup> worldwide). The clinical symptoms of severe zinc deficiency in humans include dermatitis, diarrhea, alopecia, re-current infections (pneumonia) and neurological disturbances. In children, it is also a cause for stunted growth, including stunted sexual maturation in boys.

##### Zinc oxide treatment for skin diseases

Topical administration of zinc oxide paste/cream/ointment on the skin can protect against sunburn or diaper rash.

## **4.7. Manganese (Mn), Nickel (Ni), Gold (Au), Molybdenum (Mo), Cobalt (Co), Chromium (Cr)**

### *4.7.1. Manganese*

#### *4.7.1.1. Basic facts*

Atomic number: 25

Electron configuration: [Ar] 3d<sup>5</sup> 4s<sup>2</sup>

Oxidation state: exhibits oxidation state from -3 to +7, but the most common states

are +2, +3, +4, +6 and +7. The most stable oxidation state of manganese is +2.

#### *4.7.1.2. Biological importance*

The best known function of manganese is the one it plays in the oxygen-evolving complex (OEC). The OEC is responsible for the oxidation of water to dioxygen during photosynthesis in photosystem II. As a metalloenzyme OEC contains tetra-manganese penta-oxygen calcium ( $Mn_4O_5Ca$ ) in its active site. Moreover, manganese also occurs in a wide range of enzymes.

The enzyme families that contain manganese as a cofactor can be classified as oxidoreductases, transferases, hydrolases, lyases, isomerases, ligases, reverse transcriptases and glutamine synthetase. The most prominent example of manganese-containing polypeptide is manganese superoxide dismutase (Mn-SOD). The function of the antioxidant Mn-SOD enzyme is to neutralize the toxic effects of reactive oxygen species in the mitochondria.

Moreover, as cofactor of various enzymes, manganese plays an important role in a range of physiologic processes such as formation of connective tissue, bones, sex hormones, synovial fluid in the joints and it also plays a role in the clotting of blood. Moreover, manganese containing enzymes have important function in the regulation of carbohydrate and fat metabolism, and calcium absorption.

#### *4.7.1.3. Medical aspects*

Clinical studies demonstrated that people with diabetes or epilepsy have low levels of manganese in their blood serum. Other clinical trials showed that people with rheumatoid- or osteoarthritis express decreased level of Mn-SOD.

The elevated tissue concentration of manganese can exhibit cellular toxicity, particularly in the brain. Environmental exposure to high levels of manganese during fetal development can result in neurocognitive deficits, while in adults manganese accumulation in the basal ganglia can lead to decreased dopamine levels and cell death in a syndrome called manganism, which shares multiple features with Parkinson's disease. Specific cellular manganese importers and exporters are responsible for maintaining the optimal level of Mn.

### **4.7.2. Nickel**

#### *4.7.2.1. Basic facts*

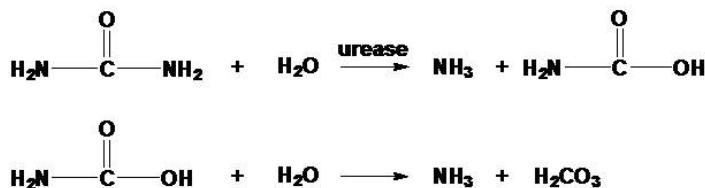
Atomic number: 28

Electron configuration: [Ar] 3d<sup>8</sup> 4s<sup>2</sup>

Oxidation state: in its compounds nickel exhibits oxidation states of -1, 0, +1, +2, +3 and +4, though the +2 state is by far the most common.

#### *4.7.2.2. Biological importance*

Nickel is not an essential element for humans and other higher-living organisms but it is a key metal for the gastric pathogen *Helicobacter pylori* the causative agent of gastric ulcer. This bacterium resists the very low pH (acidic environment) in the stomach by raising the local pH via the nickel enzyme urease that converts urea into carbon dioxide and ammonia.



**Fig. 81.** Urease-catalyzed reaction

Urease, a nickel-dependent metalloenzyme catalyzes the hydrolysis of urea to ammonia and carbamate. The carbamate then spontaneously hydrolyzes to form carbonic acid and a second molecule of ammonia. At physiological pH, the carbonic acid proton dissociates and the ammonia molecules equilibrate with water becoming protonated, resulting in a net increase in pH.

#### 4.7.2.3. Medical aspects

Nickel is one of the most important contact allergens. Metal allergy may result in allergic contact dermatitis.



**Fig. 82.** Patches of eczematous dermatitis on patient's face (left) in areas that came into contact with the headset of his cellphone (right). In some models, the area around the screen and the menu button tested positive for free nickel (source: Bercovitch I. and Luo J. Canadian Medical Association Journal 2008 February 12; 178(4): 440.)

#### 4.7.3. Gold

##### 4.7.3.1. Basic facts

Atomic number: 79

Electron configuration: [Xe] 4f<sup>14</sup> 5d<sup>10</sup> 6s<sup>1</sup>

Oxidation state: common oxidation states of gold are +1 in aurous and +3 in auric compounds

##### 4.7.3.2. Biological importance

The „noble” metal gold has no biological role in the living organisms.

##### 4.7.3.3. Medical aspects

Seifert O. and coworkers found that gold ions inhibit the lysosomal enzymes of phagocytic cells. The inhibitory effect leads to the decreased number of macrophages in the synovial membrane and reduces the production of pro-inflammatory cytokines in cell culture. Gold

salts can be used for the treatment of rheumatoid arthritis since the last century indeed the mechanisms behind the anti-inflammatory effect of metallic gold ions are still unknown.

#### 4.7.4. Molybdenum

##### 4.7.4.1. Basic facts

Atomic number: 42

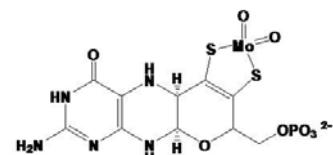
Electron configuration: [Kr] 4d<sup>5</sup> 5s<sup>1</sup>

Oxidation state: molybdenum has wide range of oxidation states: -2, 0, +1, +2, +3, +4, +5 and +6 but the most stable are +4 and +6.

##### 4.7.4.2. Biological importance

Molybdenum is an essential micronutrient for microorganisms, plants, and animals. Molybdenum itself is catalytically inactive in biological systems until it is complexed by a special cofactor molecule (pterin).

**Fig. 83.** Basic form of the molybdenum cofactor (molybdopterin)



The function of molybdenum enzymes is to catalyze the transfer of an oxo group or two electrons to or from the substrate. The transfer of two electrons changes the oxidation state of the molybdenum atom in the substrate-binding site from IV to VI. There are four known molybdenum cofactor containing enzymes in eukaryotes: sulfite oxidase, which catalyzes the final step in the degradation of sulfur-containing amino acid; xanthine dehydrogenase which is involved in the oxidation of hypoxanthine to xanthine and xanthine to uric acid (important reactions in nucleic acid metabolism); aldehyde oxidase which has a key function in the synthesis of abscisic acid in plants; and nitrate reductase, which plays an important role in biological nitrogen fixation.

##### 4.7.4.3. Medical aspects

In molybdenum cofactor deficiency the synthesis of molybdopterin cofactor is inhibited resulting in high levels of sulfite and urate and neurological damage. High levels of molybdenum can interfere with the body's uptake of copper (copper-molybdenum antagonism), producing copper deficiency. Molybdenum prevents plasma proteins from binding to copper, and it also increases the amount of copper that is excreted in urine.

#### 4.7.5. Cobalt

##### 4.7.5.1. Basic facts

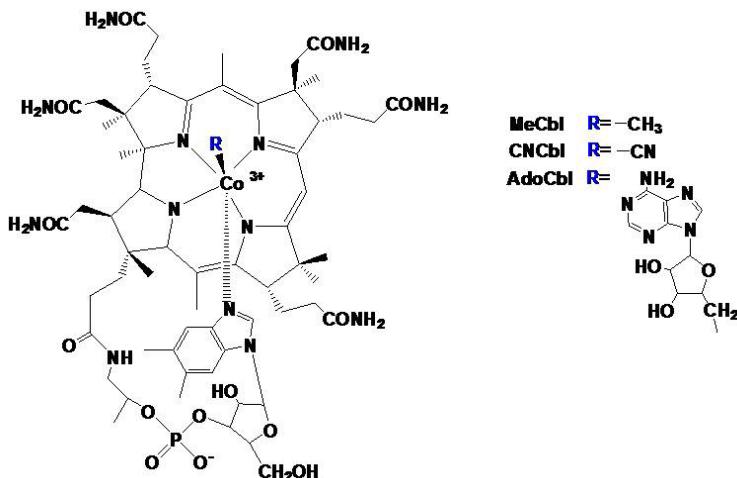
Atomic number: 27

Electron configuration: [Ar] 3d<sup>7</sup> 4s<sup>2</sup>

Oxidation state: common oxidation state of cobalt include +2 and +3, although compounds with oxidation states from -3 to +4 are known

##### 4.7.5.2. Biological importance

Cobalt is a key component of cobalamin that was discovered as the antipernicious anemia factor (vitamin B<sub>12</sub>). The cobalamin has two biological active forms: methylcobalamin and adenosylcobalamin and it serves as cofactor for the methyltransferase and isomerase families of B12 enzymes, respectively.



**Fig. 84.** Structure of cobalamine and its derivatives, including methylcobalamin (MeCbl), cyanocobalamin (CNCbl, vitamin B<sub>12</sub>) and adenosylcobalamin (AdoCbl) (source: Gherasim C et al. *J. Biol. Chem.* 2013;288:13186-13193)

In mammals there are two B<sub>12</sub>-dependent enzymes, methionine synthase and methylmalonyl-CoA mutase.

Methionine aminopeptidase 2 (MetAP2) enzyme also requires cobalt for its proper activity but in its structure the metal ions bind directly to the polypeptide. MetAP2 is responsible for the hydrolytic removal of N-terminal methionine from residues of proteins in humans and other mammals. MetAP2 has an important function in tissue repair, protein degradation and angiogenesis.

#### 4.7.5.3. Medical aspects

In chronic cobalt ingestion serious health problem were reported: cardiomyopathy (induced by cobalt-sulfate, the stabilizer of beer foam), vision or hearing impairment, reversible hypothyroidism and polycythemia.

#### 4.7.6. Chromium

##### 4.7.6.1. Basic facts

Atomic number: 24

Electron configuration: [Ar] 3d<sup>5</sup> 4s<sup>1</sup>

Oxidation state: under physiological conditions, the persistent oxidation states of chromium are +3 and +6

##### 4.7.6.2. Biological importance

Chromium(III) ion, found in most foods and nutrient supplements, is an essential nutrient with very low toxicity. Chromium was described earlier as part of organic complex, glucose tolerance factor (GTF) that was responsible for optimizing the effect of insulin. More recent studies suggested that chromium may function as part of the oligopeptide termed low-molecular weight chromium (LMWCr)-binding substance, which is composed of glycine, cysteine, glutamic acid, and aspartic acid. The principal carrier protein for chromium is transferrin, which also plays a critical role in the movement of chromium from blood to LMWCr. LMWCr can activate insulin receptor tyrosine kinase and potentiate the action of insulin.

Chromium(VI)-containing compounds are genotoxic (cause DNA damage) and can induce gene mutations, sister chromatid exchange and chromosomal aberrations.

#### 4.7.6.3. Medical aspects

Chromium(VI) ion is irritant to the skin and mucous membranes and its compounds also trigger allergic contact dermatitis characterized by eczema.

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## 5. Toxic metals

### 5.1. Lead (Pb)

#### 5.1.1. Basic facts

Atomic number: 82

Electron configuration: [Xe] 4f<sup>14</sup> 5d<sup>10</sup> 6s<sup>2</sup> 6p<sup>2</sup>

Oxidation state: can exist in +2 and +4 oxidation states but the lead(II) state is more stable, and there is a strong tendency for lead(IV) compounds to react to give lead(II) compounds.

#### 5.1.1.1. Biological importance and transport in the body

Lead is highly toxic metal. According to the recent estimation of World Health Organization (WHO) "lead is one of the most abundant heavy metals on earth considered as number one environmental persistent toxin and health hazard affecting 120 millions of people. Growing evidence of the effects of lead on the environment and on human health has resulted in efforts to restrict the use of Pb (widely used as a component of paint until 1970 and fuel additives until 1995). However, lead compounds are not biodegradable and therefore the current levels of environmental Pb contamination cannot be effectively reduced.

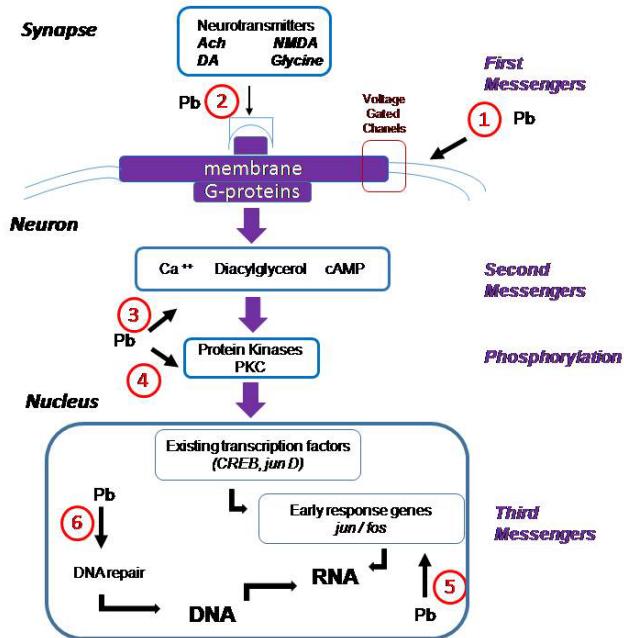
Lead competes with iron and calcium for absorption from the human gastrointestinal tract. Moreover it can be inhaled and organic (e.g. tetraethyl) lead can be absorbed via skin.



**Fig. 85. Source of Boy's Mysterious Lead Poisoning was in an unlikely place.**

"An 8-year-old hyperactive boy was admitted to the hospital after having been found to have elevated blood lead levels, ranging from 17.4 to 27.4 µg per deciliter (reference range, <10) over a period of 2 years. An abdominal radiograph revealed large numbers of small, hyperdense foci in the right lower quadrant. The entire family reported that they regularly ate geese that were killed with lead pellets from a shotgun, and the children reported that they had been eating the pellets as part of a game to make them disappear. The patient and his siblings underwent bowel washout. A follow-up radiograph of the patient revealed that pellets were still present in the gastrointestinal tract, in either the cecum or the appendix (left pane). Laparoscopic appendectomy was performed. The appendix weighed 27.5 g (normal weight, 4 to 5 g), measured 55 mm in length, and had an average external diameter of 7 mm with a wall thickness of 2 mm. A total of 57 lead pellets were recovered from the lumen (right panel)." (source: Zardawi I. and Siriweera E., N Engl J Med 2013; 369:e7)

After entering the bloodstream, 99% of Pb accumulates in erythrocytes. Lead has a high affinity for sulfhydryl groups. It is therefore particularly toxic to multiple enzyme systems. One important target protein for lead is an enzyme of heme biosynthesis: delta-aminolevulinic acid dehydratase. The major part of incorporated lead is stored in the bone and under certain conditions (e.g. pregnancy, osteoporosis, hormonal disorders) it is released into the bloodstream. The neurotoxicity of Pb has been shown to result from inhibition of multiple cellular targets.



**Fig. 86.** Potential cellular targets of Pb in neurons (1) voltage-gated channels; 2) neurotransmitters (first messenger); 3) second messengers; 4) protein kinases; 5) third messenger; 6) DNA repair. ACh – acetylcholine, NMDA – N-methyl-D-aspartic acid, DA – dopamine, Pb – lead, Ca<sup>2+</sup> – calcium, cAMP – cyclic adenosine monophosphate, PKC – Protein kinase C

(source: Neurologia i Neurochirurgia Polska 2012; 46, 6: 569-578 DOI: 10.5114/ninp.2012.31607)(based on Finkelstein et al. Brain Res Rev 1998; 27: 168-176.)

### 5.1.1.2. Medical aspects

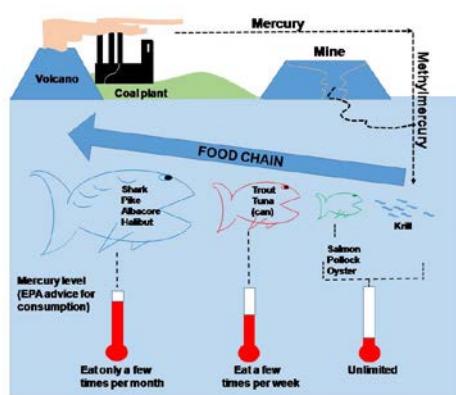
The cell membrane permeability of the organometallic triethyl lead cation ( $C_2H_5)_3Pb^+$  can result in **severe disorders of the central and peripheral nervous system**. One possible target of the neurotoxic effect of lead at the synapse level is the **N-methyl-D-aspartic acid (NMDA) receptor**. These receptors play a major role in fast synaptic transmission via their associated ion channel, which is permeable for  $Ca^{2+}$ ,  $Na^+$ ,  $K^+$ , which are key factors in neural network formation and synaptic plasticity (and therefore for learning and memory). A form of **anemia** results before the long-term neurotoxic symptoms, especially a mental retardation in children, because **Pb<sup>2+</sup> inhibits the incorporation of iron into the porphyrin molecule**. Characteristic indication of lead poisoning is the occurrence of protoporphyrins and unreacted delta-aminolevulinic acid in the urine. Detoxification of lead is based on the chelation therapy. **Chelation therapy** consists of receiving a chemical chelating agent, usually the combination of 2,3-dimercapto-1-propanol (BAL) with Ca(EDTA); sodium 2,3-dimercaptopropane-l-sulfonate (DMPS) or meso-2,3-dimercapto-succinic acid (DMSA).

## 5.2. Mercury (Hg)

### 5.2.1. Basic facts

Atomic number:	80
Electron configuration:	[Xe] 4f <sup>14</sup> 5d <sup>10</sup> 6s <sup>2</sup>
Oxidation state:	Mercury exists in two main oxidation states, I and II. Higher oxidation states are also detected (mercury(IV)fluoride [HgF <sub>4</sub> ]) but only under extraordinary conditions.

Mercury is a heavy, silvery d-block element, the only metal that is liquid at standard conditions. It is commonly known as quicksilver, occurs in deposits throughout the world mostly as cinnabar (mercuric sulfide, HgS). The origins of atmospheric mercury are volcanoes and human-generated sources, such as coal-fired power plants. Mercury is dissolved in natural waters in the form of methylmercury, which can be concentrated in the body of fish and shellfish (Fig. 87.)



**Fig. 87. The Mercury Cycle**

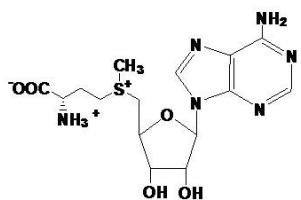
Human-generated sources, such as coal-fired power plants, emit about half of atmospheric mercury, with natural sources such as volcanoes responsible for the remainder. Fish and shellfish concentrate mercury in their bodies, often in the form of methylmercury, a highly toxic organic compound of mercury. Species of fish that are long-lived and high on the food chain, such as tuna, shark, or swordfish

### 5.2.2. Application

In medicine mercury alloys are utilized in amalgam dental fillings.

### 5.2.3. Toxicity

Mercury is toxic in any form, the most toxic forms of mercury are its organic compounds, such as dimethylmercury and methylmercury. Because of its toxicity, all above uses are being phased out or are under review. The clinical signs and symptoms, and the response to treatment depend on which way and what form mercury is absorbed. Mercury poisoning can result from vapor inhalation, ingestion, injection, or absorption through the skin. Nervous system, gastrointestinal tract, eyes, skin respiratory and renal systems are the most commonly affected organ systems in mercury exposure. Mercury can cause both chronic and acute poisoning. Mercury poisoning can result in different diseases, such as acrodynia (pink disease: pain and pink discoloration of the hands and feet in children), Hunter-Russell syndrome (paresthesias, visual field constriction, ataxia, impaired hearing, and speech impairment), and Minamata disease (neurological syndrome) caused by severe methyl mercury poisoning that produces ataxia, numbness in the hands and feet, general muscle weakness, narrowing of the field of vision, and damage to hearing and speech. In extreme cases, insanity, paralysis, coma and death). Mercury irreversibly inhibits selenium-dependent enzymes and may also inactivate S-adenosyl-methionine, which is necessary for catecholamine catabolism by catechol-o-methyl transferase (COMT).



**Fig. 88.** S-adenosyl methionine

Due to the body's inability to degrade catecholamines (e.g. epinephrine), a person suffering from mercury poisoning may experience profuse sweating, tachycardia (persistently faster-than-normal heart beat), increased salivation, and hypertension (high blood pressure). Another **selenoenzyme, thioredoxin reductase** restores a number of important antioxidant molecules, back into their reduced forms, enabling them to counteract oxidative damage within body cells. High exposures to mercury in its various forms are particularly toxic to fetuses and infants. Mercury inhibits the formation of myelin.

Currently available drugs for acute mercurial poisoning include chelators N-acetyl-D, L-penicillamine (NAP), British Anti-Lewisite (BAL), 2,3-dimercapto-1-propanesulfonic acid (DMPS), and dimercaptosuccinic acid (DMSA).

#### 5.2.4. Medical aspects

Mercury-containing "Blue Mass" was used to treat syphilis, tuberculosis, constipation, toothache, parasitic infestations, and the pains of childbirth in the 19th-century. Mercury is utilized in amalgam dental fillings [commonly consist of mercury (50%), silver (~22-32%), tin (~14%), copper (~8%), and other trace metals] since the 1800s. Thiomersal is an organomercury compound used as a preservative in vaccines since the 1930s to prevent bacterial and fungal contamination. Thiomersal was phased out, because it was hypothesized to elevate the risk of autism.

Mercury in any form is poisonous, with mercury toxicity most commonly affecting the neurologic, gastrointestinal (GI) and renal organ systems. Poisoning can result from mercury vapor inhalation, mercury ingestion, mercury injection, and absorption of mercury through the skin. (See Etiology and Prognosis.)

Mercury has 3 forms: (1) elemental mercury, (2) inorganic salts, and (3) organic compounds. Perhaps the most deadly form of mercury is methylmercury. Only 2-10% of the ingested mercury is absorbed from the gut, and ingested elemental mercury is not absorbed at all; however, 90% of any methylmercury ingested is absorbed into the bloodstream from the GI tract.

Minamata disease is an example of organic toxicity. In Minamata Bay, a factory discharged inorganic mercury into the water. The mercury was methylated by bacteria and subsequently ingested by fish. Local villagers ate the fish and began to exhibit signs of neurologic damage, such as visual loss, extremity numbness, hearing loss, and ataxia. Babies exposed to the methylmercury in utero were the most severely affected. Furthermore, because mercury was also discovered in the breast milk of the mothers, the babies' exposure continued after birth.<sup>[4]</sup> A very controversial source of organic mercury exposure is thimerosal, a preservative used in vaccines to prevent bacterial contamination. The most commonly used vaccines that contain thimerosal are for diphtheria-tetanus-whole cell pertussis (DTP), *Haemophilus influenzae* (HIB), and hepatitis B. However, no definite link between this small amount of mercury and any known disease has been found. Nonetheless, concerns over mercury content in vaccine have led to the increased availability of mercury-free vaccines

## 5.3. Cadmium (Cd)

### 5.3.1. Basic facts

Atomic number: 48

Electron configuration: [Kr] 4d<sup>10</sup> 5s<sup>2</sup>

Oxidation state: it prefers oxidation state +2 in most of its compounds; it also exists in the +1 state

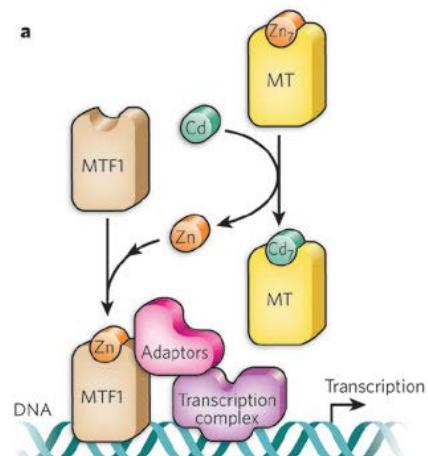
### 5.3.1.2. Biological importance and transport in the body

Cadmium has no known useful role in higher organisms. The most dangerous characteristic of cadmium is that it accumulates throughout lifetime. After uptake from the lung or the gastrointestinal tract, cadmium is transported in blood plasma initially bound to albumin. Cadmium bound to albumin is preferentially taken up by the liver. In the liver, cadmium induces the synthesis of metallothionein and a few days after exposure metallothionein-bound cadmium appears in the blood plasma. Because of its low molecular weight, cadmium-metallothionein is efficiently filtered through the glomeruli and thereafter taken up by the tubules. Cadmium accumulates in the human kidney over the entire lifetime. Average cadmium concentrations in the kidney are at birth near zero, and rise roughly linearly with age to a peak. Liver cadmium concentrations also begin near zero at birth. Most cadmium that is ingested or inhaled and transported to the gut via mucociliary clearance is excreted in the feces.

### 5.3.1.3. Metallothionein (MT)

MT is a family of cysteine-rich, low molecular weight (MW ranging from 500 to 14000 Da) proteins. They are localized to the membrane of the Golgi apparatus. MTs have the capacity to bind both physiological (such as zinc, copper) and xenobiotic (such as cadmium, mercury, silver, arsenic) heavy metals through the thiol group of its cysteine residues, which represents nearly the 30% of its amino acidic residues.

Metallothioneins likely participate in the uptake, transport, and regulation of zinc in biological systems. By binding and releasing zinc, metallothioneins (MTs) may regulate zinc levels within the body.



**Fig. 89.** Cadmium binds to MT more tightly than does zinc. Zinc released from metallothionein is taken up by zinc-fingers of MTF1 (metal-regulatory transcription factor), which in turn interacts with metal-response elements on DNA to effect the transcription of target genes through adaptor proteins (Kevin J. Waldron et al., *Nature*, 2009)

[41.media.tumblr.com/c1b1914709c9a6bb9701b92d0f8b908b/tumblr\\_ndeh4hq67d1spr9rqi1\\_1280.jpg](http://41.media.tumblr.com/c1b1914709c9a6bb9701b92d0f8b908b/tumblr_ndeh4hq67d1spr9rqi1_1280.jpg) ..

#### 5.3.1.4. Medical aspects

##### Cancer

Because MTs play an important role in transcription factor regulation, problems with MT function or expression may lead to malignant transformation of cells and ultimately cancer. Studies have found different expression level of MTs in some cancers of the breast, colon, kidney, liver, skin (melanoma), and lung.

##### *Itai-itai disease*

"Itai itai" (Japanese: "it hurts-it hurts" or "ouch ouch") disease was the documented case of mass cadmium poisoning in Toyama Prefecture, Japan. The cadmium poisoning caused softening of the bones and kidney failure. The disease is named for the severe pains caused in the joints and spine. The cadmium was released into rivers by mining companies in the mountains. This water was then used to irrigate the rice fields. The rice absorbed heavy metals, especially cadmium. The cadmium accumulated in the people eating contaminated rice.

One of the main effects of cadmium poisoning is weak and brittle bones. Spinal and leg pain is common, and a waddling gait often develops due to bone deformities caused by the cadmium. The pain eventually becomes debilitating, with fractures becoming more common as the bone weakens. Other complications include coughing, anemia, and kidney failure, leading to death. A marked prevalence in older, postmenopausal women has been observed.



**Fig. 90. Itai-itai disease**

<http://84d1f3.medialib.glogster.com/media/eb/eb55f068574bd88cec67cce836b63d329054ffa07d5c61996f4757dc76867adc/itai-itai-disease-jpg-214405-pixels-jpg.jpg>

#### 5.3.1.5. Applications

##### 5.3.1.5.1. Batteries

An important use for cadmium is in the nickel-cadmium storage battery. Nickel-cadmium cells have a nominal cell potential of 1.2 V. The cell consists of a positive nickel hydroxide electrode and a negative cadmium electrode plate separated by an alkaline electrolyte (potassium hydroxide).

## 6. Non-metals

### 6.1. Oxygen (O)

#### 6.1.1. Basic facts

Atomic number: 8

Stable isotopes:  $^{16}\text{O}$ , most abundant (99.762%)

$^{17}\text{O}$

$^{18}\text{O}$ , used for isotope labelling and in paleoclimatology to estimate climate changes

Electron configuration: [He] 2s<sup>2</sup>2p<sup>4</sup>

Oxidation state: Oxygen has the second highest electron negativity among elements. In most cases its oxidation number is -2 except when it combines with fluorine or with another oxygen atom.

Occurrence in Nature: Oxygen is the third most abundant element in the universe after hydrogen and helium. It constitutes 0.9% of the Sun where it is produced in nuclear reactions. O is the most abundant element in the Earth's crust (49.2% m/m) and in the oceans (88.8% m/m). It is the prototype of the chalcogen elements (group 16 in the periodic system) as it is found in many rocks, ores, and gems.

Oxygen reacts with all of the elements except He, and Ne. The reaction with oxygen is called oxidation. Oxygen has the second strongest oxidizing power after fluorine. The oxides of non-metallic elements are acid anhydrides (by the way, the name of oxygen means "acid producing"). Oxides with some metals are base anhydrides, while the oxide with other metals and hydrogen are amphoteric.

Oxygen is the component of complex inorganic anions like phosphate, sulphate, and carbonate and is the part of many important functional groups in organic compounds including alcohols, phenols, ethers, aldehydes, ketones, kinones, carboxylic acids, and their derivatives. In all of the biologically important macromolecules (proteins, carbohydrates, lipids, and nucleic acids) oxygen is a vital component.

As a free element it has two allotropes: dioxygen  $\text{O}_2$  (or triplet oxygen), and trioxygen  $\text{O}_3$  (ozone).  $\text{O}_2$  makes up 20.8% (v/v) of the atmosphere, while  $\text{O}_3$  is found in traces (2-8 ppm) in the stratosphere.

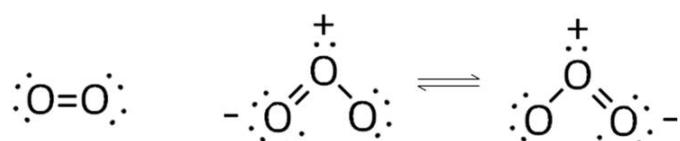


Fig. 91. The structure of  $\text{O}_2$  and the resonance structures of  $\text{O}_3$

#### 6.1.2. Biological importance

Note that in biology oxygen is a two-edged sword, it is essential for life (especially for the aerobic organisms) but at the same time it is the source of toxic reactive oxygen species (ROS). The primary biological function of  $\text{O}_2$  is cellular respiration but it is also needed for

**metabolism.** The oxygen used by the living organisms is absorbed directly from the air, or indirectly from water. The solubility of O<sub>2</sub> in water is 7.6 mg/L at 20 °C.

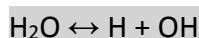
#### 6.1.2.1. Atmospheric oxygen

The Earth's atmosphere has changed dramatically during geological history. At the beginning, (about 3.8 billion years ago) oxygen was absent from the atmosphere that was dominated by hydrogen and helium. Under these reducing conditions most of the elements of biological significance were present in the reduced state (Fig. 92.).

Reducing atmosphere		Oxidizing atmosphere	
Fe	(+2)	→	Fe (+3)
Cu	(+1)	→	Cu (+2)
S	(-2)	→	SO <sub>4</sub> <sup>2-</sup> (+6)
Se	(-2)	→	SeO <sub>3</sub> <sup>2-</sup> (+4)
MoS <sub>x</sub>	(+2)	→	MoO <sub>4</sub> <sup>2-</sup> (+6)
CH <sub>4</sub>	(-4)	→	CO <sub>2</sub> (+4)
H <sub>2</sub>	(0)	→	H <sub>2</sub> O (+1)
NH <sub>3</sub>	(-3)	→	NO, NO <sub>2</sub> <sup>-</sup> , NO <sub>3</sub> <sup>-</sup> (+2, +3, +5)

**Fig. 92.** The effects of oxygen accumulation on the biologically important elements. Based on W. Kaim and B. Schwedersky (1991) *Bioinorganic Chemistry: Inorganic Elements in the Chemistry of Life*, publisher John Wiley and Sons, Chichester

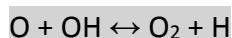
The oxidizing atmosphere evolved in 5 stages (Fig. 93). In stage 1 the only source of oxygen was the photolysis of water and carbon dioxide upon irradiation by high energy UV light and cosmic rays.



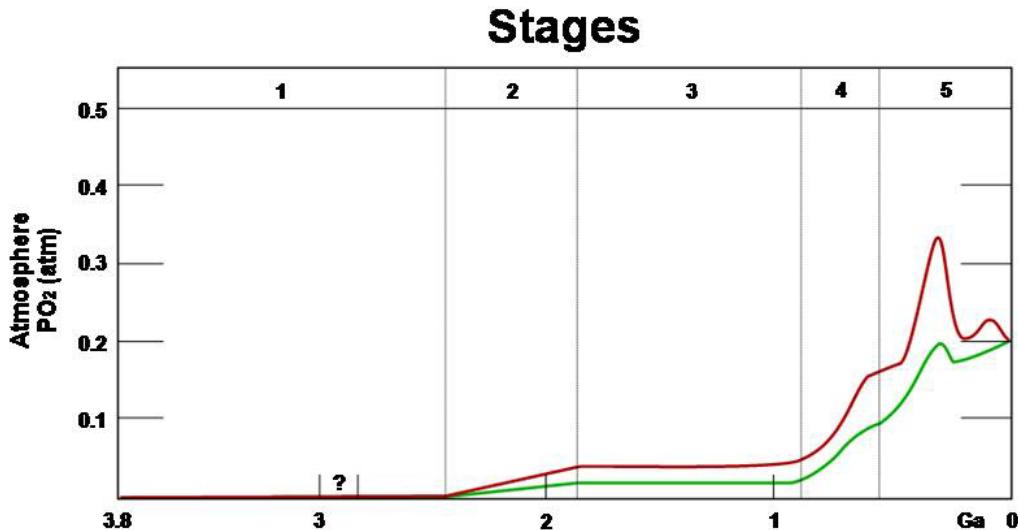
produces hydroxyl radical (OH) and



produces atomic oxygen (O). The OH is very reactive and combines with O



The hydrogen atoms are light and escape to space allowing the O<sub>2</sub> to accumulate at a very low concentration (less than 1% of the present atmospheric level). ([http://www.globalchange.umich.edu/globalchange1/current/lectures/Perry\\_Samson\\_lectures/evolution\\_atm/](http://www.globalchange.umich.edu/globalchange1/current/lectures/Perry_Samson_lectures/evolution_atm/)) The oxygen produced in this way was reacted or absorbed in the oceans.



**Fig. 93.** O<sub>2</sub> build-up in the atmosphere. Red and green lines represent the range of the estimates while time is measured in billions of years ago (Ga).

Stage 1. Practically no O<sub>2</sub> in the atmosphere.

Stage 2. O<sub>2</sub> produced, but absorbed in oceans & seabed rock.

Stage 3. O<sub>2</sub> starts to gas out of the oceans, but is absorbed by land surfaces.

Stages 4 & 5. O<sub>2</sub> sinks filled, the gas accumulates

Source: [http://en.wikipedia.org/wiki/Geological\\_history\\_of\\_oxygen](http://en.wikipedia.org/wiki/Geological_history_of_oxygen), for details see the original publication:<http://rstb.royalsocietypublishing.org/content/361/1470/903.full.pdf>

The oxygen concentration started to increase only before 3.5 billion years ago, when photosynthetic microorganisms (archaea and bacteria) appeared in the primordial oceans. These organisms used the energy of sunlight for the syntheses of organic molecules, first of all glucose from carbon dioxide and water:



The biogenetic oxygen was absorbed by the oceans in stage 2 and by the land surfaces in stage 3, than it started to build up steadily in the atmosphere (stage 4) and after some fluctuation reached a steady state level (stage 5).

The accumulation of oxygen in the air generated oxidising conditions and converted the biologically important elements into a higher oxidation state (Fig. 2). This process is called the Great Oxygenation Event (GOE) or oxygen catastrophe ([http://en.wikipedia.org/wiki/Great\\_Oxygenation\\_Event](http://en.wikipedia.org/wiki/Great_Oxygenation_Event)). It is supposed that most of the organisms which were unable to accommodate to changing conditions went extinct (only a small proportion of the archaea survived in exotic niches like in deep seas and volcanoes) and the development of the aerobic organisms gained great impetus. The latter learnt how to avoid the toxic effects of oxygen and how to utilize the energy of combustion. It was also suggested that the increased oxygen concentration of the air was a pre-condition for the evolution of more complex organisms, because only oxidation can provide sufficient energy needed for the synthesis of structural proteins e.g. collagen. To support cellular respiration of the cells inside the larger life forms intricate oxygen transport systems evolved. Metalloproteins containing

copper or iron ions evolved during the GOE for the metabolism, transportation and elimination of oxygen (see previous chapters).

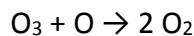
The current atmospheric level of oxygen is controlled by the biogeochemical cycle that includes the biosphere and lithosphere as well. Oxygen utilised for respiration in the biosphere and oxidation in the lithosphere is released into the atmosphere by the photosynthesizing organisms like plants and cyanobacteria. The present steady state is maintained by the balance between production and consumption.

#### 6.1.2.2. Stratospheric ozone

The appearance of oxygen in the higher levels of atmosphere (10-50 km) had one more important consequence, it lead to ozone formation that was initiated by short wave ultraviolet light (UVC band).



In this reaction M stands for a third helper molecule that is carrying away the excess energy of the reaction and makes the stabilisation of ozone possible. **O<sub>3</sub> forms a protective shield that filters out high energy UV radiation from sunlight.** The building up of stratospheric **ozone layer** resulted in reduced irradiation of Earth's surface by high energy rays and thus contributed to the spreading of terrestrial life. It should be noted that ozone decomposes in a chemical reaction with atomic oxygen.



The generation of O atom is catalysed by several reactive radicals like hydroxyl ( $\cdot\text{OH}$ ), nitric oxide ( $\text{NO}^\cdot$ ), atomic chloride (Cl) or atomic bromine (Br) thus the concentration of ozone is kept at a steady state level by the balance between synthesis and destruction. In the recent years the delicate natural balance has been upset by manmade organic chemicals first of all by fluoro-chloro-carbons (CFCs) that cause the seasonal ozone hole observed in the Southern hemisphere. Read more at [http://en.wikipedia.org/wiki/Ozone\\_layer](http://en.wikipedia.org/wiki/Ozone_layer).

#### 6.1.3. Oxygen metabolism

##### 6.1.3.1. Kinetic properties of dioxygen

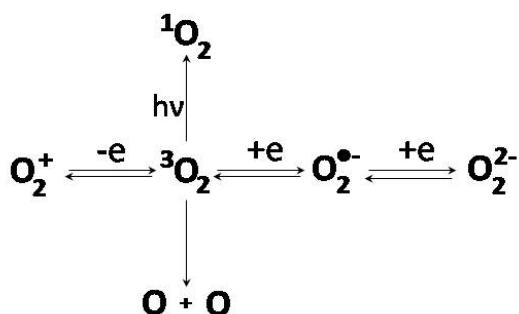
Despite of the high electron negativity of oxygen and the exergonic nature of oxydation the dioxygen molecule is kinetically inert. The low reactivity of oxygen molecule can be explained by its electronic structure (Fig. 94).

$\sigma^* 2p$	${}^3O_2$	${}^2O_2^\bullet$	${}^1O_2^{2-}$	${}^1O_2^{(1\Delta)}$	${}^1O_2^{(1\Sigma)}$
$\pi^* 2p$	$\uparrow \uparrow$	$\uparrow \downarrow \uparrow \downarrow$	$\uparrow \downarrow \uparrow \downarrow$	$\uparrow \downarrow$	$-$
$\pi 2p$	$\uparrow \downarrow \uparrow \uparrow$	$\uparrow \downarrow \uparrow \uparrow$	$\uparrow \downarrow \uparrow \uparrow$	$\uparrow \downarrow \uparrow \downarrow$	$\uparrow \downarrow \uparrow \downarrow$
$\sigma 2p$	$\uparrow \downarrow$	$\uparrow \downarrow$	$\uparrow \downarrow$	$\uparrow \downarrow$	$\uparrow \downarrow$
	Triplet oxygen	Superoxide anion	Peroxide anion	Singlet oxygen	
Bond order	2	1.5	1		
Bond length (pm)	121	$\approx 128$	$\approx 149$		

**Fig. 94.** The electronic structure of dioxygen and two of its reduced derivatives. Based on W. Kaim and B. Schwedersky (1991) *Bioinorganic Chemistry: Inorganic Elements in the Chemistry of Life*, publisher John Wiley and Sons, Chichester

According to the MO theory triplet oxygen is the most stable form of dioxygen. It is paramagnetic i.e. it has two unpaired electrons at the outermost shell according to the Hund's rule. This unusual structure of the molecule hinders many of the reactions because of forbidden spin transitions (the sum of spin quantum numbers should not change during a reaction). Thus oxygen has to be activated.

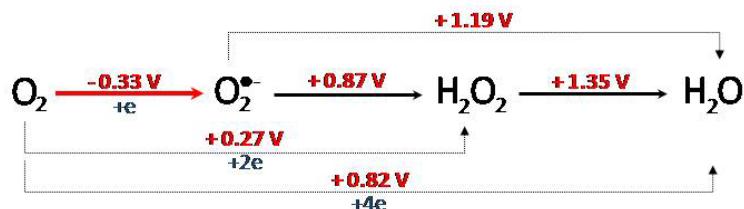
#### 6.1.3.2. Activation of molecular oxygen



**Fig. 95.** Possible ways for activation of triplet oxygen

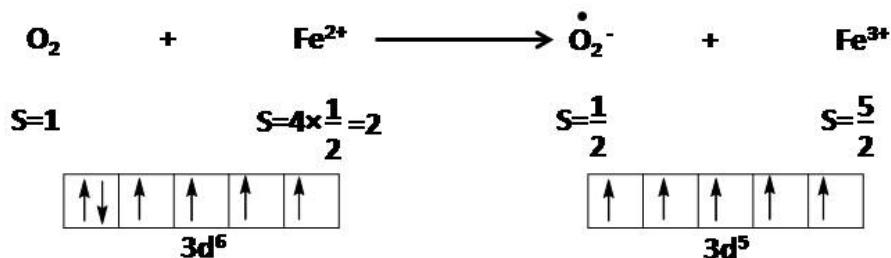
The oxidation of  $O_2$  (taking away one electron) is unlikely and the breaking of the covalent bonds between the atoms requires high energy that can be supplied only under specific conditions (see formation of ozone). The irradiation of triplet  $O_2$  with electromagnetic radiation of the appropriate wavelength can turn the spin of one electron and thus can generate one or the other form of singlet  $O_2$  (see Fig. 95). Singlet oxygen is diamagnetic because it has zero overall spin quantum number consequently restricted spin transitions do impede its chemical reactions. It is produced under very specific conditions in living organisms. The most common biological activation of  $O_2$  involves reduction (adding electrons). The products of reduction, superoxide anion (that is a free radical), and peroxide anion are reactive

oxygen species. However, it should be noted that the one-electron reduction of oxygen requires external energy, as the reduction potential of the reaction is negative (the free enthalpy change is positive). The investment of energy is regained in two- or four-electron reduction steps but for the initiation of the reduction a second partner with high reducing power is required (Fig. 96).



**Fig. 96.** Standard reduction potentials for the stepwise reduction of  $O_2$  are given in Volts. Based on B.G. Malmstörm (1982) Enzymology of oxygen. Ann Rev Biochem 51, 21-59

The problem of the forbidden spin transitions and the requirement for reducing power are both solved by flavin coenzymes (FMN and FAD) that produce flavin peroxides. An alternative way of circumventing forbidden transitions is provided by metal (iron or copper) ions (Fig. 97).



**Fig. 97.** Iron (II) assisted activation of triplet  $O_2$ . The sum of spin quantum numbers does not change (blue numbers) as the change in the metal's electronic structure compensates the change caused by the reduction of oxygen

#### 6.1.3.3. Enzymes of oxygen metabolisms

There are more than 200 enzymes which use oxygen as substrate. All of them belong to the enzyme family of oxidoreductases.

**Oxygenases** catalyse the incorporations of oxygen into organic molecules.

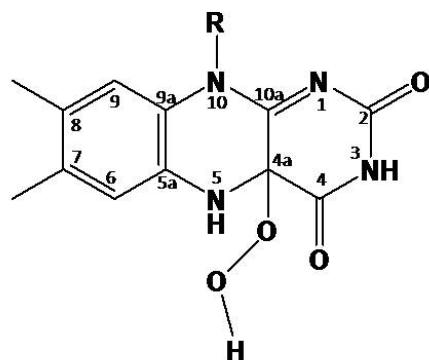
**dioxygenases** incorporate both atoms of the  $O_2$  into the organic substrate, while

**monooxygenases** insert only one oxygen atom and produce water as a side-product.

**Oxidases** use the  $O_2$  molecule as an electron acceptor, and produce superoxide anion, peroxide anion or water.

As pointed out in the previous chapter the activation of oxygen takes place in the presence of essential prosthetic groups according to two general mechanisms:

- Reduced flavin coenzymes ( $\text{FADH}_2$  or  $\text{FMNH}_2$ ) combine with  $\text{O}_2$  and generate flavin peroxide. The unusual structure of this intermediate is shown in Fig. 98.

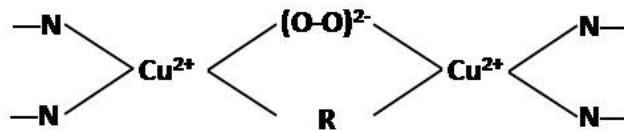


**Fig. 98.** Flavin C(4a) peroxide. Monooxygenases usually produce this intermediate, while oxidases generate the C(10a) product. Based on B.G. Malmstrom (1982) Enzymology of oxygen. Ann Rev Biochem 51, 21-59

Examples for the flavin- containing oxygenases are **amino acid oxidase** or **glucose oxidase**. The latter is used in combination with a peroxidase enzyme for the enzymatic determination of blood glucose in diagnostic laboratories (see practical classes).

- Reduced metal centers containing  $\text{Fe}^{2+}$  or  $\text{Cu}^{1+}$  reduce  $\text{O}_2$ . Both monometallic and bimetallic centers can bind  $\text{O}_2$ , but bimetallic centers are more effective as they catalyse a thermodynamically favoured two-electron reduction step (Fig. 99).

One of the well characterised metal containing monooxygenases, **cytochrome P450** contains a single iron center (it is discussed in the chapter: Iron). A typical example for the bimetallic monooxygenase enzymes is **tyrosinase** that has two copper ions in its active site. (The functions of this enzyme are described in chapter: Copper.) After  $\text{O}_2$  binding the two  $\text{Cu}^{1+}$  ions get oxidized to  $\text{Cu}^{2+}$ , and oxygen is reduced to peroxide ion in the enzyme substrate complex (Fig. 99).

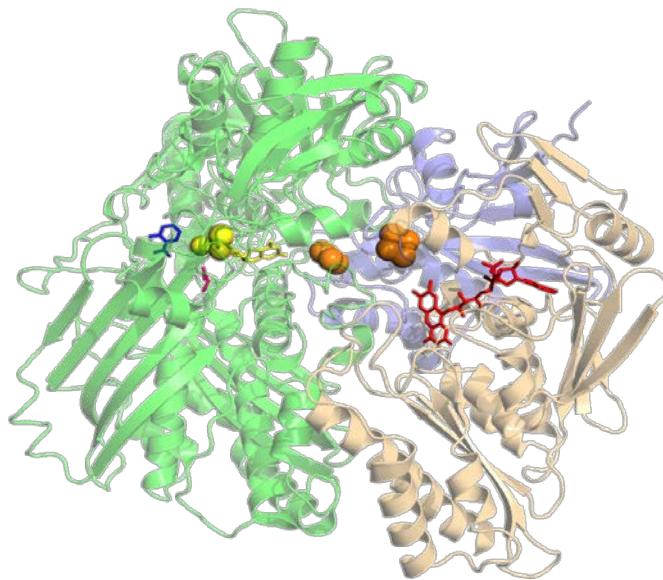


**Fig. 99.** The active centre of tyrosinase after the activation of oxygen in a two-electron reduction. Based on B.G. Malmstrom (1982) Enzymology of oxygen. Ann Rev Biochem 51, 21-59

The complex transmembrane enzyme **cytochrome oxidase** contains two Cu and two Fe ions. It generates energy (a proton gradient) at the final stage of terminal oxidation by the four-electron reduction of  $\text{O}_2$  according to the reaction:



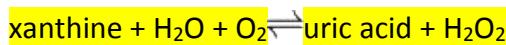
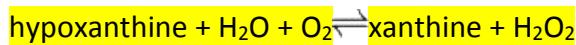
**Xanthine oxidase** is an exceptionally complex enzyme that combines metal centres (molybdenum and Fe-S clusters) with FAD coenzyme binding site in its structure (Fig. 100).



**Fig. 100.** Crystallographic structure of the bovine xanthine oxidase monomer. The bounded FAD (red), Fe-S-cluster (orange), the molybdopterin cofactor with molybdenum (yellow) and salicylate (blue) are indicated

Source:[http://en.wikipedia.org/wiki/Xanthine\\_oxidase](http://en.wikipedia.org/wiki/Xanthine_oxidase)

Within one subunit of the dimeric protein there are one Mo, one FAD, and two Fe-S centers. The enzyme normally catalyses the following reactions:



The reduction goes on step by step so the electrons are transmitted from one center to the next (Fig. 101).

Redox center	Electron transfer	Electrons
FADH <sub>2</sub>	2	$2e^-$ { 6 } ↓ { 4 }
Mo <sup>III</sup>	2	$2e^-$ { 4 } ↓ { 2 }
-Fe-S-	1	$2e^-$ { 2 } ↓ { 1 }
-Fe-S-	1	$1e^-$ { 1 } ↓ { 0 }

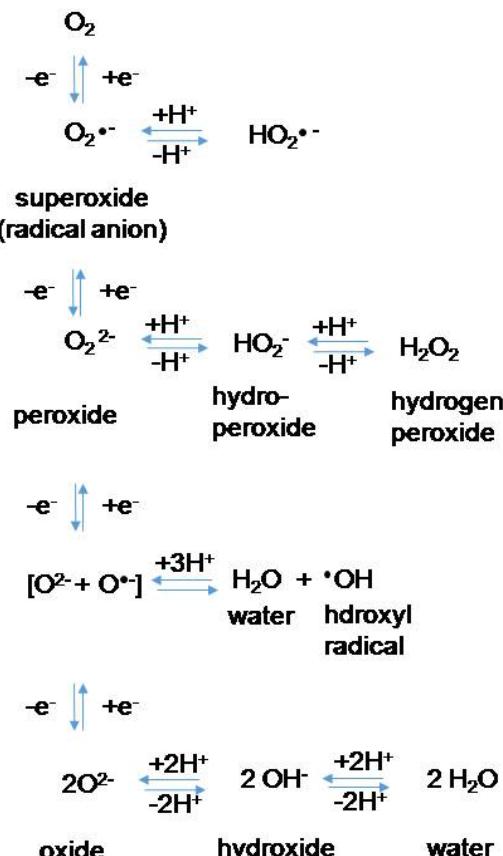
**Fig. 101.** Electron transfer steps in xanthine oxidase

The first two steps of reduction produce H<sub>2</sub>O<sub>2</sub> while the last two steps produce superoxide:



#### 6.1.4. Reactive oxygen species (ROS)

During the metabolism of oxygen many reactive molecules, ions, and radicals, are generated as intermediates or side products. These are called Reactive Oxygen Species and are abbreviated as ROS. ROS include singlet oxygen, superoxide anion (that is a radical too), hydroperoxide radical, hydroperoxide ion, peroxide ion, hydrogen peroxide, and hydroxyl radical (Fig. 102). Note, that radicals contain an unpaired electron and are very reactive.

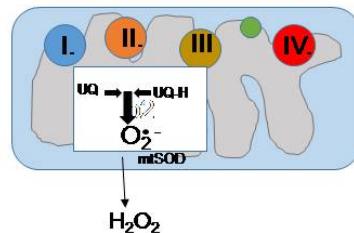
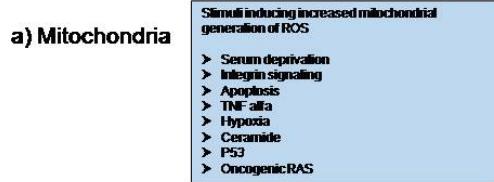


**Fig 102.** Reactive intermediates and side products of oxygen utilization. Source: W. Kaim and B. Schwedersky (1991) *Bioinorganic Chemistry: Inorganic Elements in the Chemistry of Life*, publisher John Wiley and Sons, Chichester

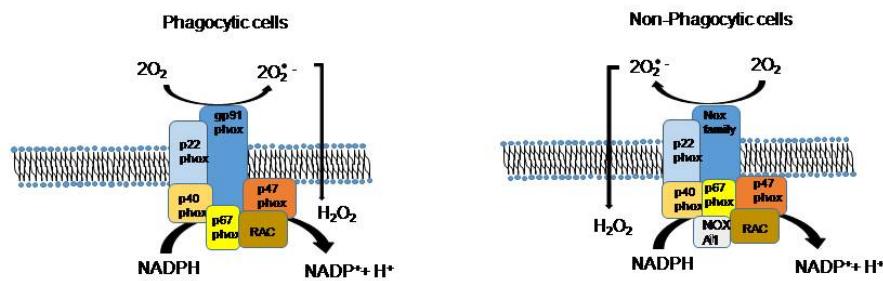
##### 6.1.4.1. Generation and elimination of ROS (reactive oxygen species)

In biological systems ROS are generated in the following ways:

1. Interaction of ionizing radiation with biological molecules
2. Incomplete cellular respiration. Some electrons leak away from the ubiquinon (UQ) in mitochondria and directly reduce oxygen molecules to superoxide anion (Fig 103)
3. Synthesis by dedicated enzymes (NADPH oxidase and 5-lipoxygenase) in defense mechanisms.



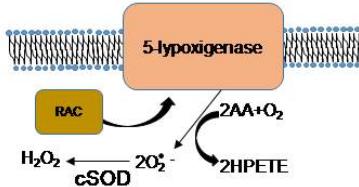
**b) NADPH oxidase**



Stimuli for activation NADPH oxidase and 5-lipoxygenase

- > Integrin signaling
- > Growth factors
- > Cytokines/hormones
- > Immunological stimuli
- > Hypoxia
- > Oncogenic RAS

**c) 5-lipoxygenase**

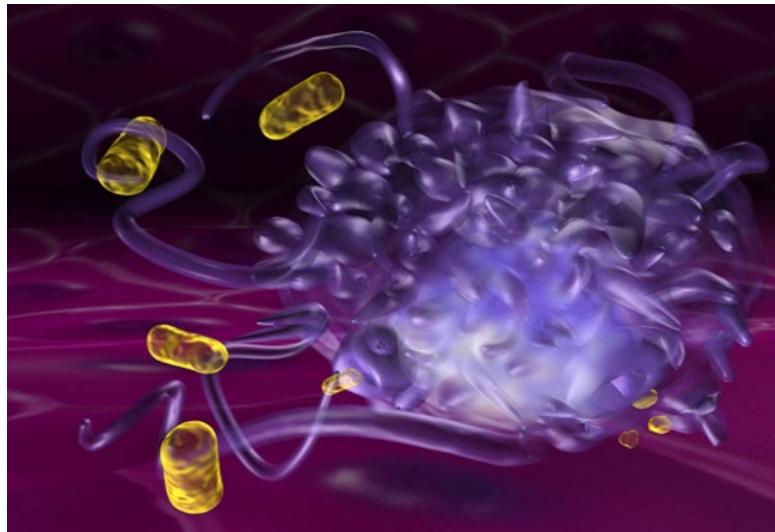


**Fig. 103. Physiological sources of ROS in animals.** Source:  
 (AA arachidonic acid, HPETE 5-hydroperoxyeicosatetraenoic acid, cSOD cytosolic superoxide dismutase)  
 (Novo and Parola, 2008)

**NADPH oxidase** is a membrane bound enzyme complex that produces superoxide from oxygen outside the cells and oxidizes the reduced coenzyme inside the cell (Fig. 103):



Superoxide kills bacteria and fungi, thus acts as a chemical weapon against the microbes invading the body (Fig.104).



**Fig. 104.** Phagocytosis by a neutrophyl granulocyte. Source: <http://med.mui.ac.ir/slides/immunu/immunu4.html>

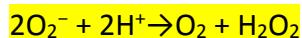
**5-lipoxygenase** or more precisely arachidonate 5-lipoxygenase catalyses the peroxydation of the arachidonic acid (AA) component of the membrane lipids and generates 5-hydroperoxyeicosatetraenoic acid (HPETE) together with superoxide (Fig. 103). HPETE is the precursor of leukotriene biosynthesis. Superoxide is eliminated by SOD (see the next chapter).

In general ROS are considered to be harmful agents as they damage essential biomolecules. First the unsaturated carboxylic acids of the lipids are oxidized (lipid peroxidation), then reduced coenzymes and the amino acid residues of proteins become oxidized, finally the DNA is damaged in the nucleus. The latter is especially serious defect as the mutations accumulate and propagate upon cell divisions. Oxidation by ROS has also been implicated in the mechanism of aging.

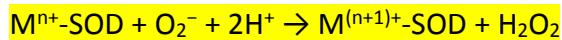
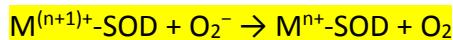
#### 6.1.4.2. Protection against ROS

To avoid the potentially damaging effects of unwanted oxidations, effective mechanisms evolved to protect cells from ROS. However, complete elimination of ROS is not desirable as they are involved in important processes like apoptosis (programmed cell death), induction of host defense systems, and activation of ion transport. Small organic molecules, e.g. vitamin C, vitamin E, uric acid, reduced glutathione and other **antioxidants** act as scavengers of the free radicals. More effective protection can be achieved by the following **enzymes**:

**Superoxide dismutase (SOD)** eliminates superoxide anion and produces the less harmful hydrogen peroxide and oxygen. The overall reaction



can be divided into an oxidation and a reduction step

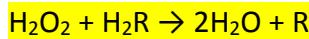


SOD enzymes contain a metal ion in the catalytic site M that can be Cu (n=1), Mn (n=2), Fe (n=2), or Ni (n=2). They are described in chapter: Copper.

**Catalase** converts hydrogen peroxide to water and oxygen:

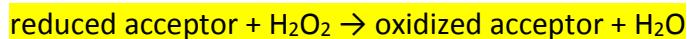


It can also catalyze the oxidation of several organic molecules:



This enzyme is an iron containing heme-protein that is discussed in chapter: Iron.

**Lactoperoxidase** is a special peroxidase that is secreted from mammary and salivary glands. It uses hydrogen peroxide to oxidize organic and inorganic substrates and has a potent antibacterial activity:



It contains a heme cofactor and iron ion in its active center (see chapter: Iron)

**Glutathione peroxidase** is a selenium containing peroxidase that oxidizes reduced glutathione (GSH) to glutathione (GS-SG):



The catalytic mechanism is unique, since it utilizes a selenocysteine residue (SeH) of the protein (R):



For more details see chapter: Selenium

**Peroxiredoxin (Prx)** utilizes an active Cys residue in its catalytic reaction and uses thioredoxin (Trx) for its regeneration:



### *6.1.5. Medical aspects*

The most important physiological role of O<sub>2</sub> is to support cellular respiration in order to provide chemical energy for cell via terminal oxidation. Our body adapted well to the normal atmospheric conditions, i.e. to around 20-21 kPa partial pressure (PO<sub>2</sub>) of oxygen at the sea level. Oxygen is adsorbed in the lungs, where the PO<sub>2</sub> is 14.2 kPa, and is transported by the blood. The arterial PO<sub>2</sub> is 11-13 kPa, while venous blood has a PO<sub>2</sub> 4-5.3 kPa value. Haemoglobin is the main transport protein and myoglobin stores oxygen inside the tissues (see chapter: Iron). Thus the optimal saturation of haemoglobin is the critical physiological question. The PO<sub>2</sub> changes together with the atmospheric pressure, it decreases at high altitudes and increases under the water. That is why oxygen rich air is supplied in high mountains for hikers, in the aircrafts, when the cabin pressure drops, and pure oxygen atmosphere (at around 30 kPa pressure) is provided in the spacecrafts.

#### **6.1.5.1. Oxygen therapy**

High-pressure (hyperbaric) oxygen treatment is used in medical conditions where oxygen transport is impaired by CO poisoning, gas gangrene (caused by Clostridium perfringens bacteria) or decompression sickness (due to fast decompression of divers). The same treatment is applied to overcome circulatory disorders caused by pneumonia or some heart diseases. Hyperbaric oxygen is often used in mechanical ventilation. However, the possibility of oxygen toxicity must be considered seriously whenever oxygen therapy is chosen.

#### **6.1.5.2. Oxygen toxicity**

The toxicity of oxygen was first proven by Lavoasier, who found that mice die in pure oxygen atmosphere. The toxic effects are related to the excessive production of ROS (see 6.1.4.). The toxic effects become significant when PO<sub>2</sub> exceeds 50 kPa. This may happen when mechanical ventilation is used in hospitals, or when divers descend deep under the sea. Some times ago premature babies were kept in an incubator containing oxygen rich air. Now blood oxygen level is tightly monitored during oxygen therapy of premature babies in order to prevent oxygen toxicity.

#### **6.1.5.3 Tropospheric ozone**

The ozone layer in the stratosphere protects us from high energy radiation (see 6.1.2.2.) The ozone hole that has developed recently, lead to increased exposure, mutation rates, and risk of skin cancer in the affected areas.

While ozone is beneficial high above our heads in the stratosphere, it is a harmful pollutant at the Earth's surface. It is generated in large cities due to the escape of volatile gasoline and other organic compounds into the air). In the presence of organic vapours CO is oxidised in a complicated set of photochemical reactions (including several reactive radicals and nitrogen oxides) that eventually produce ozone and carbon dioxide:



Ozone is a strong oxidizing agent that contributes to lung diseases caused by the metropolitan smog. It is denser than air, consequently accumulates in depressions and affects children first. Based on its oxidizing capacity ozone is used to sanitize uninhabited areas and to disinfect drinking water.

## 6.2. Selenium (Se)

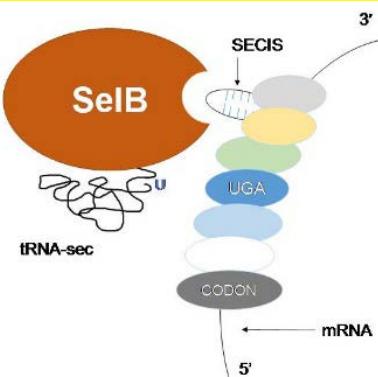
### 6.2.1. Basic facts

Atomic number:	34
Electron configuration:	[Ar] 3d <sup>10</sup> 4s <sup>2</sup> 4p <sup>4</sup>

### 6.2.2. Biomedical importance

#### 6.2.2.1. Selenocystein, selenomethionine

In selenoproteins a selenium atom is substituted for sulfur in a cysteine or methionine residue. In the 3' untranslated region (3' UTR) of their mRNA, selenoproteins contain a special sequence (called SECIS for SElenoCystein Insertion Element) which allows the STOP codon UGA to be recognized as a codon for selenocystein



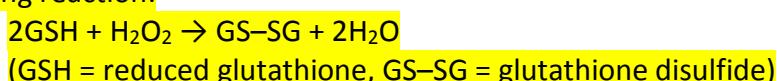
**Fig. 105. The selenocystein insertion machinery**

SelB is a key protein for the incorporation of selenocystein during protein translation. SelB specifically recognizes both SECIS (selenocystein insertion element) on the mRNA and the tRNA carrying selenocystein (tRNA<sub>Sec</sub>). The anticodon of tRNA<sub>Sec</sub> interacts with the UGA codon which signals selenocystein incorporation (In the absence of the SECIS sequence in the mRNA, UGA functions as a STOP codon)

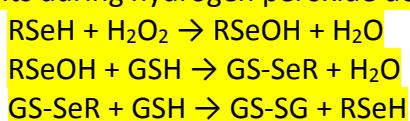
#### 6.2.2.2. Selenoproteins

##### 6.2.2.2.1. Glutathione peroxidase

Glutathione peroxidase (GPx) enzymes form an 8 member family (GPx1-8) and catalyze the following reaction:



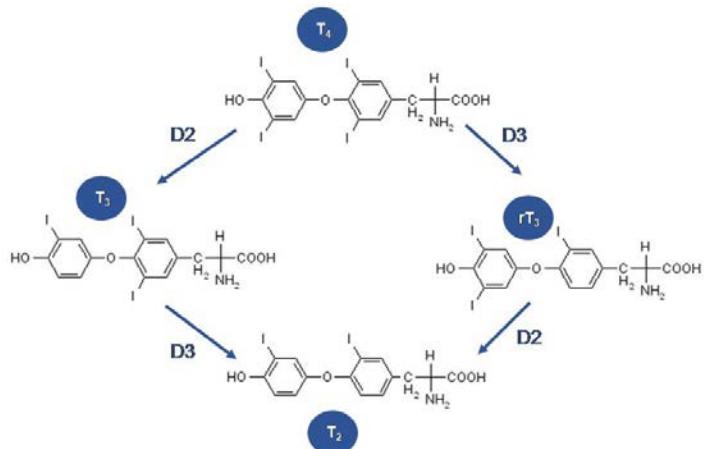
By decomposing the oxidant H<sub>2</sub>O<sub>2</sub> (and by reducing lipid hydroperoxides), GPx enzymes play important roles in the antioxidant defense of our body. The selenocystein residue in the active center of the enzyme plays an active role in the catalytic action. The simplified sequence of events during hydrogen peroxide decomposition is shown below:



(To complete the cycle, oxidized glutathione (GS-SG) is reduced back to GSH by the NADPH-dependent enzyme glutathione reductase.)

##### 6.2.2.2.2. Iodothyronine deiodinase

Iodothyronine deiodinase enzymes contain selenium in the form of a selenocysteine residue. D2 type enzymes remove iodine from the outer ring whereas D3 type enzymes remove iodine from the inner ring of the prohormone tyroxine ( $T_4$ ) into the active hormone triiodothyronine ( $T_3$ ) or to the inactive metabolite  $rT_3$  (reverse  $T_3$ ). Further removal of another iodine atom results in the formation of inactive  $T_2$  (see Fig. 106.).



**Fig. 106.** Actions of iodothyronine deiodinases. (D2 and D3 designate different types of iodothyronine deiodinases). Depending on the position from which these enzymes remove iodine, they may convert the prohormone  $T_4$  into the active hormone  $T_3$  or to the inactive metabolite reverse  $T_3$  ( $rT_3$ ). Removal of a further iodine results in  $T_2$  (also inactive)

#### 6.2.2.3. Selenium deficiency

Selenium deficiency occurs rarely and most cases of selenium deficiency were reported either in very old people, or in patients with compromised gut function or patients on parenteral nutrition.

Soil in certain regions of the world (e.g. New Zealand, Finland or Northeast China) contains lower amounts of selenium. In Keshan county (North-East China), selenium deficiency of soil led to severe *cardiomyopathy (heart failure)* called **Keshan disease**. For reasons not yet completely identified, selenium deficiency increased the sensitivity of people to Coxsackie B virus infections causing damage to the heart.

#### 6.2.2.4. Selenosis (selenium toxicity)

Excess intake of selenium (over 400 microgram/day) is toxic and leads to selenosis. Symptoms of selenosis include fatigue, irritability, neurological and gastrointestinal disorders, hair loss, sloughing of nails.

## 6.3. Silicon (Si)

### 6.3.1. Basic facts

Atomic number: 14

Electron configuration: [Ne] 3s<sup>2</sup> 3p<sup>2</sup>

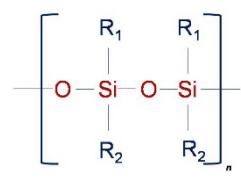
Oxidation state: 4, 3, 2, 1, -1, -2, -3, -4

Silicon the second most abundant element in the Earth's crust (about 28% by mass, exceeded only by oxygen), over 90% of the Earth's crust is composed of silicate minerals. It rarely occurs as the pure free element in nature, most widely distributed in dusts, sands as various forms of silicon dioxide (silica) or silicates.

### 6.3.2. Applications

Silicon minerals are used up in ceramic bricks, in porcelain and in traditional quartz-based soda-lime glass. Purified silicon is used in steel refining and in aluminum casting. A very highly purified silicone is used in the semiconductor industry.

**Silicones** (please note spelling: silicone vs. silicon) are a large group of synthetic, mixed inorganic-organic polymeric compounds with an **(Si-O-Si) backbone**. General chemical formula of silicones is  $[R_2SiO]_n$ , where R is an organic group such as methyl, ethyl, or phenyl which are attached to the silicon atoms.



**Fig. 107. The structure of silicones**

Silicones are a large group of synthetic mixed inorganic-organic polymeric compounds with an (Si-O-Si) backbone, with the chemical formula  $[R_2SiO]_n$ , where R is an organic group

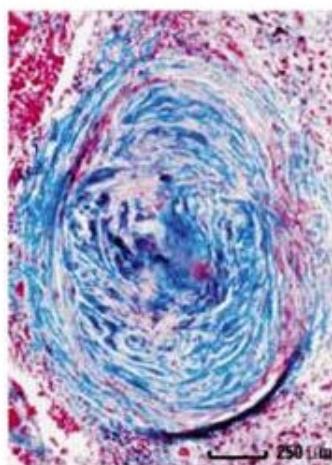
By varying the -Si-O- chain lengths, side groups, and crosslinking, silicones can be synthesized with a wide variety of properties and compositions. Silicones are inert compounds with a variety of forms and uses, typically heat-resistant and rubber-like. They are used in sealants, adhesives, lubricants, medical applications, cookware, insulation and as high temperature greases and waxes. Some common forms of silicones include oils, greases, rubbers and resins.

### 6.3.3. Silicone in biological systems

Diatoms, some protozoa, some sponges, and some plants use silicon dioxide ( $SiO_2$ ) as a structural material. Some plants (e.g. rice) need silicon for their growth. Si is present in all tissues. In the rat highest levels are found in bone and other connective tissues. The exact biological role(s) of silicon in bone health is still not clear, although a number of possible mechanisms have been suggested, including the synthesis of collagen and/or its stabilization, and matrix mineralization. Silicon is known to be required by chicks and rats for growth and skeletal development. The main route of entry of silicon into the body is from the gastrointestinal tract. The major source is from cereals, high levels of Si are found in unrefined ('whole') grains, although high fiber diet reduces the gastrointestinal uptake of minerals, including Si. Drinking water and other fluids (e.g. beer) provides the most readily bioavailable source of Si in the diet, since silicon is principally present as  $Si(OH)_4$ . Si-content of drinking water is dependent upon the surrounding geology. Seafood is also high in Si with mussels having the highest levels.

#### 6.3.4. Toxicity

The toxicity is associated with the inhalation of particulate crystalline silica and silicates, such as quartz, and man-made fibrous silicates (e.g. asbestos). Long-term exposure causes scarring of the lung, that may lead to reduced lung capacity, lung cancer, the increased risk of tuberculosis and heart complications. These crystalline silicates are phagocytized by macrophages, which then release cytokines that attract and stimulate other immune cells including fibroblasts, which are responsible for the excessive production of collagen (fibrotic tissue) that is characteristic of silicosis (Fig. 6.3.2).



**Fig. 108. Silicosis**

*Chronic silicotic nodule showing characteristic features of this lesion. The amorphous center is surrounded by concentrically organized hyalinized collagen fibers*

For much of the population with normal renal function, the normal intake of dietary silicon from food and water has not been associated with any known toxicity. Long-term use of high doses of silicate containing drugs, such as analgesics and antacids (magnesium trisilicates) could cause damage to the renal kidney tubules and lead to chronic interstitial nephritis. Silica in these drugs can lead to the formation of renal stones/calculi which are responsible for kidney damage.

#### 6.3.5. Medical aspects

Using silicon-containing bone implants and ceramics such as Si-substituted hydroxyapatites are beneficial, because such materials have been shown to bond much better to bone than their non-silicon-containing counterparts due to the spontaneous formation of a biologically active apatite-like layer on their surface. Silicone is used in contact lenses and in breast implants because silicones were thought to be very inert. Silicone breast implants may leak, and evidences show that leaked silicone may cause autoimmune disorders and increase the risk of cancer. In modern pharmaceuticals Si is present mainly in anti-diarrhoeals, antacids and in proprietary analgesics such as aspirin. In cosmetics, (toothpaste, creams, lipstick) silicones are used as viscosity-control agents, in hair care products silicones are used to increase hair shine and glossiness.

## 6.4. Fluorine (F)

### 6.4.1. Basic facts

Atomic number:	9
Electron configuration:	[He] 2s <sup>2</sup> , 2p <sup>5</sup>

### 6.4.2. Biomedical importance

#### 6.4.2.1. Fluorine in teeth and bones

About 2-3 grams of fluoride is present in our body (95% of it in the bones and teeth) and the daily requirement of fluorine is 3-4 mg/day. The only known function of fluoride in our body is to strengthen the teeth and no disease sign other than dental caries is associated with insufficient fluorine intake.

Fluorine can replace OH<sup>-</sup> in hydroxyapatite [Ca<sub>10</sub>(PO<sub>4</sub>)<sub>6</sub>(OH)<sub>2</sub>], a main component of the tooth enamel and bone mineral to form fluoroapatite [Ca<sub>10</sub>(PO<sub>4</sub>)<sub>6</sub>(F)<sub>2</sub>] conferring higher acid resistance for the teeth. According to the widely held view topical administration of fluoride (e.g. in tooth paste or mouthwash) strengthens the tooth enamel and helps prevent caries formation. Systemic administration of fluoride (e.g. mixed in drinking water), however, is highly debated and fluorination of drinking water (e.g. in many parts of the USA, Australia, Ireland or New Zealand) has been heavily criticized. Overdosing oral fluoride during tooth development may cause fluorosis (mottling of tooth enamel).



Fig. 109. Dental fluorosis

#### 6.4.2.2. Fluorine in pharmaceuticals

Since the carbon-fluorine bond is quite stable and the presence of a fluorine atom in a chemical structure greatly affects the chemical properties and biological effects of molecules (e.g. it may slow down its metabolism, increase bioavailability), pharmacological chemists often introduce F atom into drug candidate molecules. In fact, about one fifth of currently used medications contain F. Examples for fluorine-containing drugs include many antidepressants such as fluoxetine (Prozac) or the cholesterol lowering drug atorvastatin (Lipitor).

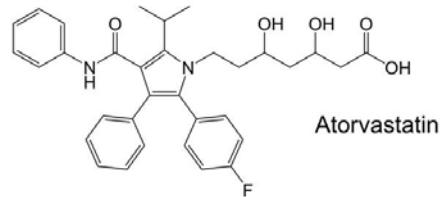
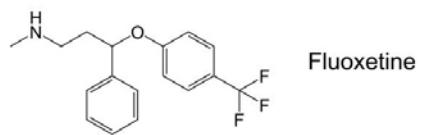


Fig. 110. Fluorine-containing drugs

## 6.5. Chlorine (Cl)

### 6.5.1. Basic facts

Atomic number: 17

Electron configuration: [Ne]3s<sup>2</sup>3p<sup>5</sup>

Oxidation state: may exist in many oxidation states but in biological systems -1 is important

### 6.5.2. Biological importance

It is distributed throughout the body fluids. It is absorbed in the intestines and secreted through urine, sweat, vomit and diarrhea. In biological systems, chlorine accounts for about 0.15% of the human body weight.

	Cl <sup>-</sup> concentration (mmol/dm <sup>3</sup> )
plasma	115 – 120
muscle	3.8
red blood cell	77
gastric juice	150

Fig. 111. Chloride concentration in the human body

#### 6.5.2.1. Secretion of HCl in stomach

Chlorine is primarily used in the production of hydrochloric acid which is secreted from the parietal cells in the stomach and is used in maintaining the acidic environment for the digestive enzyme pepsin.

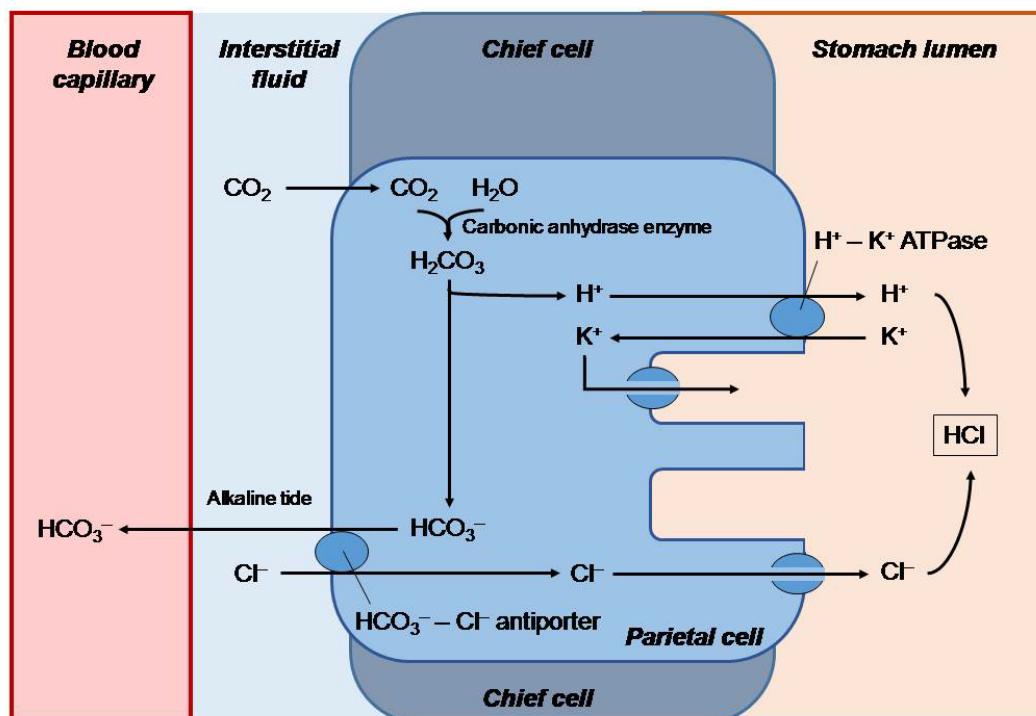


Fig. 112. Secretion of hydrochloric acid in stomach

### 6.5.2.2. Maintaining acid-base balance in red blood cells

It is required by the body to maintain the correct acid-base balance in the blood.

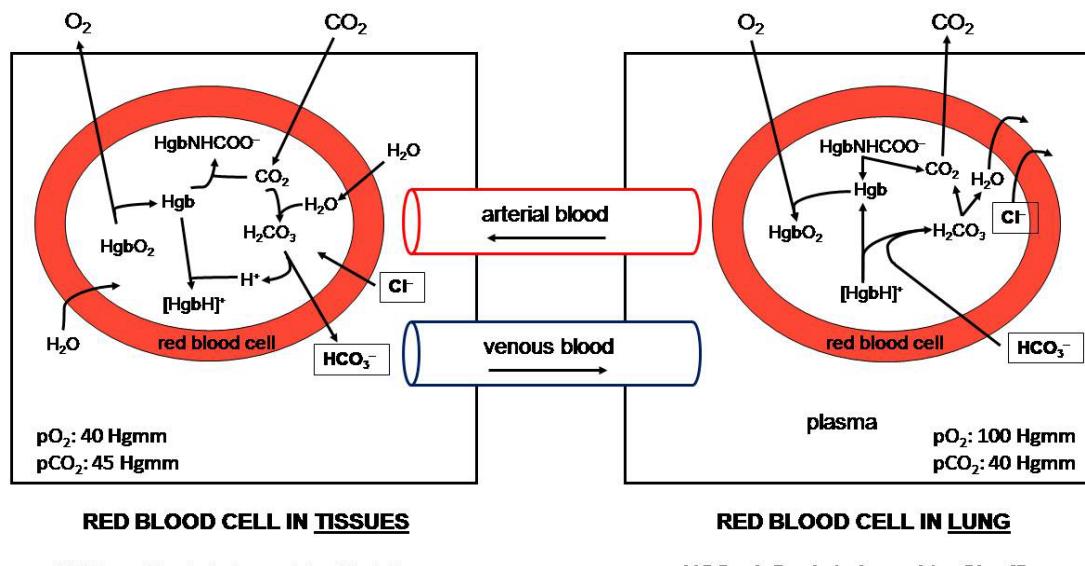


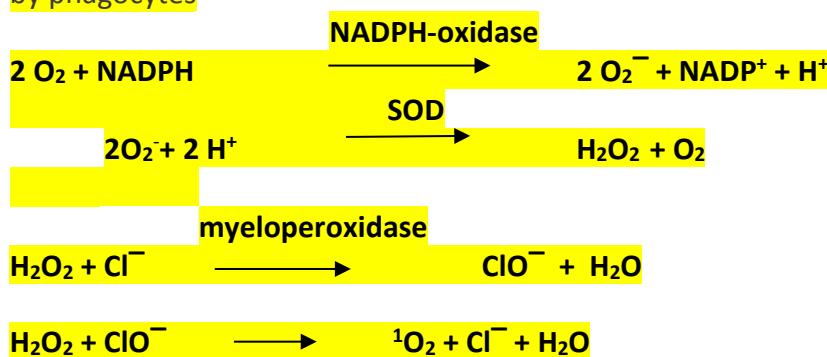
Fig. 113. Role of  $\text{Cl}^-$  ion in red blood cells

It plays a vital role in maintaining the proper acid-base balance of body fluids

A deficiency in chlorine can lead to a condition called metabolic alkalosis where the pH of the blood is higher than normal. Symptoms include: decrease in ventilation, acidic urine, and excessive excretion of potassium.

### 6.5.2.3. Role of chlorine in phagocytosis of bacteria by neutrophils

The generation of oxidants by neutrophils is critical to host defenses against microbial pathogens. Oxidant production begins with a cytoplasmic membrane-associated NADPH oxidase, which reduces molecular oxygen to superoxide. Dismutation of superoxide then yields hydrogen peroxide ( $\text{H}_2\text{O}_2$ ), but both superoxide and  $\text{H}_2\text{O}_2$  are relatively nontoxic to bacteria. However, activated neutrophils also secrete the heme protein myeloperoxidase, which constitutes ~5% of cellular protein. It is proposed that halogenating intermediates generated by this enzyme are of major importance in killing bacteria. At plasma concentrations of halide, the major action of myeloperoxidase is to convert chloride ( $\text{Cl}^-$ ) to hypochlorous acid ( $\text{HOCl}$ ), the most potent bacterial cytotoxin known to be generated by phagocytes



### **6.5.3. Risk to the environment**

Atomic chlorine can also be linked to the depletion of ozone. Chlorofluorocarbons (CFCs), which are originally used in refrigerators and air conditioners, escape and rise into the stratosphere are then decomposed by UV light from the sun to form atomic Cl. The newly formed atomic chloride is then able to react with ozone ( $O_3$ ), thus depleting the ozone concentration in the stratosphere and forming oxygen gas ( $O_2$ ).

## **6.6. Bromine (Br)**

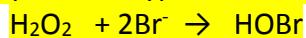
### *6.6.1. Basic facts*

Atomic number:	9
Electron configuration:	[Ar] $4s^2, 3d^{10}, 4p^5$

### *6.6.2. Biological importance*

Bromine has no essential function in mammals. It is not known to be required for any biological function. In the past bromides (e.g. potassium bromide) were used as sedative agents or anticonvulsive agent (treating seizures).

The peroxidase enzyme of eosinophil granulocyte (a subset of white blood cells instrumental in defense against parasites) named haloperoxidase preferentially uses bromide (if available) over chloride to produce hypobromite (hypobromous acid):



HOBr formed is toxic to parasites and intracellular bacteria.

## 6.7. Iodine (I)

### 6.7.1. Basic facts

Atomic number: 53

Electron configuration: [Kr]5s<sup>2</sup>5p<sup>5</sup>

Oxidation state: may exist in many oxidation states but in biological systems -1 and 0 are important

**Iodine** is an essential trace element for life, the heaviest element commonly needed by living organisms.

### 6.7.2. Biological importance

Iodine is an **essential mineral** for the body. It is heavily used in the **thyroid gland**, but also can be found in **breast tissue, salivary gland, and adrenal gland**.

Iodine is taken up as I<sup>-</sup> and is accumulated in the thyroid gland. Iodine is incorporated into thyreoglobulin.

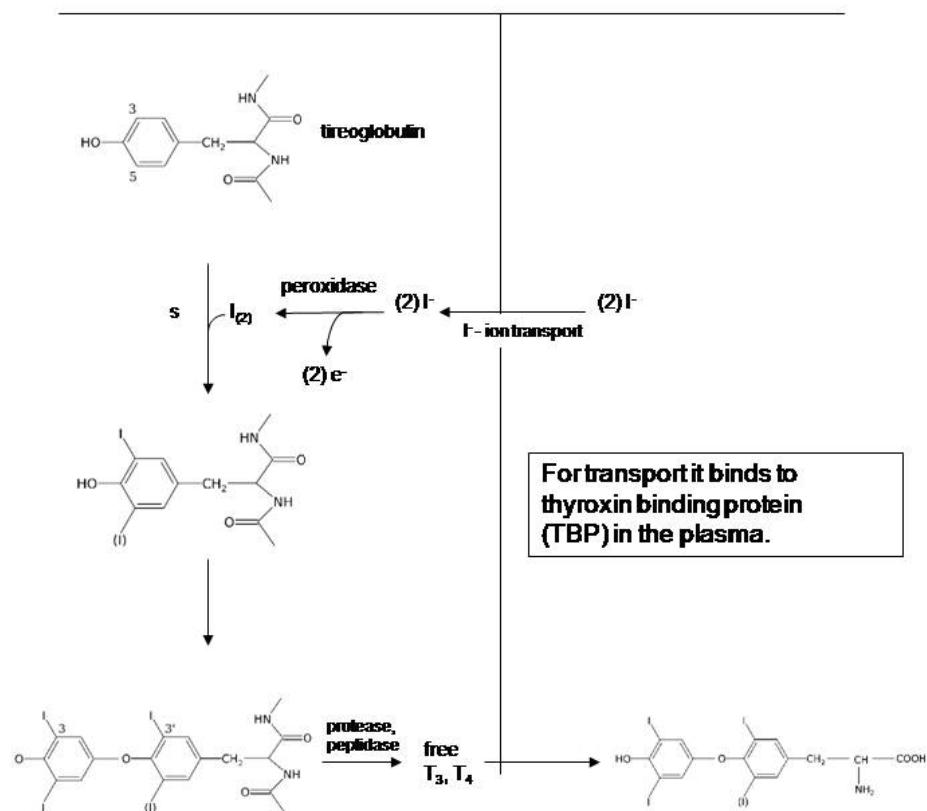
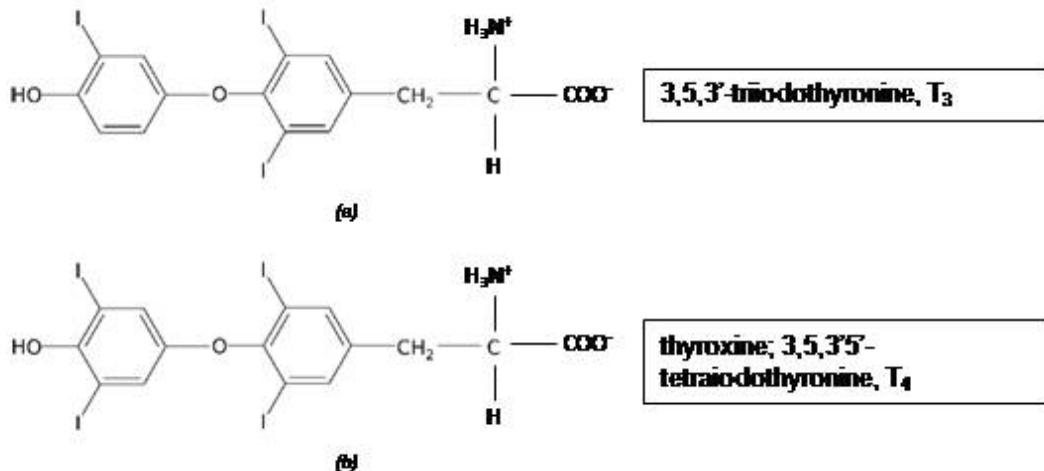


Fig. 114. Iodine traffic in the human body



**Fig. 115. Thyroid hormones**

Iodine's main role in animal biology is as constituents of the thyroid hormones, *thyroxine* (T4) and *triiodothyronine* (T3). These are made from addition condensation products of the amino acid tyrosine, and are stored prior to release in an iodine-containing protein called thyroglobulin. T4 and T3 contain four and three atoms of iodine per molecule, respectively. The thyroid gland actively absorbs iodine from the blood to make and release these hormones into the blood, actions which are regulated by a second hormone TSH from the pituitary. Thyroid hormones are phylogenetically very old molecules which are synthesized by most multicellular organisms, and which even have some effect on unicellular organisms.

#### 6.7.3. Medical aspects

Thyroid hormones play a basic role in biology, acting on gene transcription to regulate the basal metabolic rate. The total deficiency of thyroid hormones can reduce basal metabolic rate up to 50%, while in excessive production of thyroid hormones the basal metabolic rate can be increased by 100%. T4 acts largely as a precursor to T3, which is (with minor exceptions) the biologically active hormone.

Without iodine, thyroid hormones cannot be produced and this can lead to a condition called hypothyroidism. Without treatment, the thyroid gland will swell and produce a visible goiter. Children with hypothyroidism may develop mental retardation. In women, hypothyroidism can lead to infertility, miscarriages, breast, and ovarian cancer. Thyroid problems have been common issue for many years particularly in middle aged women. Hyperfunction: Graves-Basedow syndrome. The condition mainly seems to occur in people over forty, and the risk appears higher when iodine deficiency is severe and the initial rise in iodine intake is high.



**Fig. 116.** Iodine deficiency disorders (from left to right: goiter, mental retardation, cretinism)

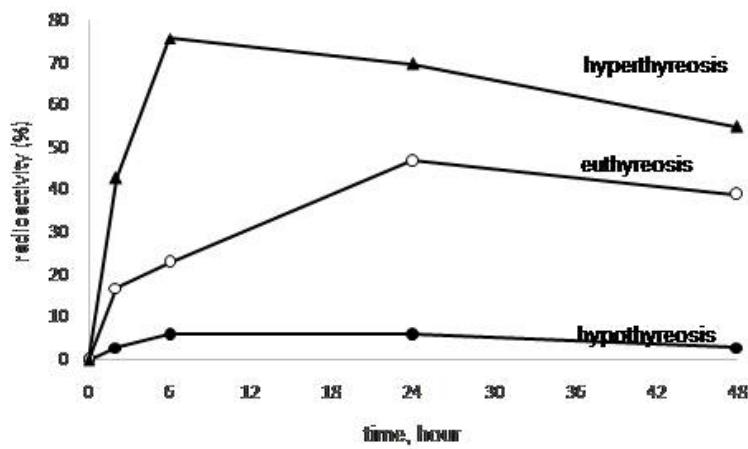
Correlated studies have shown that iodine levels in the general population have been significantly decreased in recent years. Most salt has iodine and with current health problems, many people have been consuming less salt, and therefore in-taking less iodine.

The US Food and Nutrition Board and Institute of Medicine recommended daily allowance of iodine ranges from 150 micrograms /day for adult humans to 290 micrograms /day for lactating mothers. However, the thyroid gland needs no more than 70 micrograms /day to synthesize the requisite daily amounts of T4 and T3. These higher recommended daily allowance levels of iodine seem necessary for optimal function of a number of body systems, including lactating breast, gastric mucosa, salivary glands, oral mucosa, arterial walls, thymus, epidermis, choroid plexus and cerebrospinal fluid , etc

Tolerable Upper Intake Level (UL) for adults is 1,100 µg/day (1.1 mg/day)

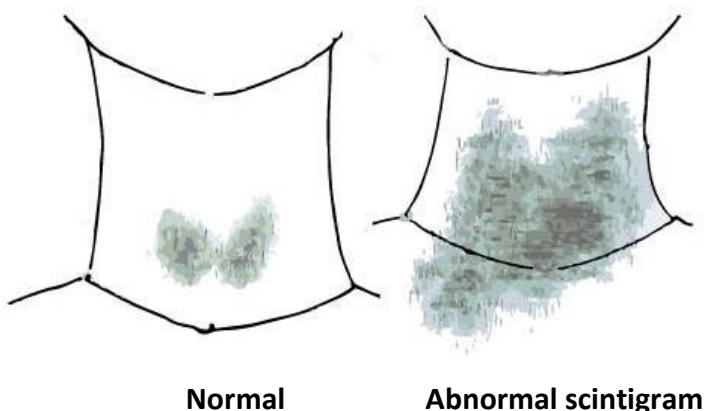
#### 6.7.4. Investigation of thyroid function

- Determination of serum iodine
  - a. Total iodine: 10 – 30 µg/ 100 cm<sup>3</sup>
  - b. Protein bound iodine (T3, T4): 4 – 8 µg/ 100 cm<sup>3</sup>
- Iodine storing curves



**Fig. 117. Iodine storing curves**

-Scintigraphy (mapping the distribution of radioactive iodine in the thyroid) Applied isotopes  $^{123}\text{I}$ ,  $^{131}\text{I}$



**Fig. 118. Images of thyroid scintigraphy**

### 6.7.5. Medical application of Iodine

Generation of certain thyroid hormones from iodine derivatives.

Disinfection

X-ray contrast material

Thyroid hormones such as thyroxine and triiodothyronine are dependent on a source of a small amount of iodine. A deficiency of iodine would usually involve in the development of "goitre" or a swelling in the thyroid gland and the larynx. In order to prevent such a swelling, it is recommended to consume more seafood seeing as how the ocean water naturally contains more iodine, the sea life would contain more percent iodine if consumed and absorbed in the body.

Due to its lower oxidizing power compared to fluorine and chlorine, there are not as many applications of this halogen as compared to the ones mentioned above, as most of the applications of halogens are derived from their oxidizing power. Nonetheless, iodine has its applications in the medical field. The solution of iodine and potassium iodide forms the

solution better known as "Lugol's iodine, which is present in emergency safety kits for the purpose of disinfecting wounds and disinfecting water by killing bacteria that may be present.

#### **6.7.6. Precautions and toxicity of iodine**

**Elemental iodine** is an oxidizing irritant and direct contact with skin can cause lesions, so iodine crystals should be handled with care. Solutions with high elemental iodine concentration such as tincture of iodine and Lugol's solution are capable of causing tissue damage if use for cleaning and antiseptics is prolonged. Some cases of reaction to Povidone-iodine (Betadine) have been documented to be a chemical burn

Elemental iodine ( $I_2$ ) is poisonous if taken orally in larger amounts; 2–3 grams of it is a lethal dose for an adult human.

Iodine vapor is very irritating to the eye, to mucous membranes, and in the respiratory tract. Concentration of iodine in the air should not exceed  $1\text{mg}/\text{m}^3$  (eight-hour time-weighted average).

Medical use of iodine (i.e. as a contrast agent, see above) can cause anaphylactic shock in highly iodine sensitive patients. Some cases of sensitivity to iodine can be formally classified as iodine allergies. Iodine sensitivity is rare but has a considerable effect given the extremely widespread use of iodine-based contrast.