terms of intramolecular structuring within the peptide molecule opposing metal coordination driven by deprotonation of the imidazole ring.

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Possible Applications of Coordination Compounds for Correcting Biometal Metabolism in Different Pathologies

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During the last two decades laboratory (with animals) and clinical researches have shown that many pathologic states of a body are accompanied by statistically reliable disturbances in the metabolism of metals at the molecular and body levels. Any chronic disease, the cause of which has not yet been established, can be due to abnormalities in metal metabolism. The determination of the amount of biometals in the body is suggested as the earliest diagnostic test of diseases.

The metals participating in metabolism can be divided into the following groups:

(1) inherent in a living body and involved in the sphere of essential biofunctions (Cu, Fe, Zn, Mn, Mo, Co, Mg, Ca, K, Na);

(2) introduced, often toxic, whose physiological role has not been fully elucidated and their presence in the body tissue and liquids is due to their abundance in nature and wide application by people (Al, Cr, Cd, Ni, Pb, *etc.*).

For the 1st group of metals both positive and negative balances were detected in different pathologies, and for the 2nd group, as a rule, only the positive balance was observed.

One of the reasons for the abnormal accumulation and removal of metals from a human body may be the wide application of drugs in clinics and which, by their chemical nature, are good ligand–complexing agents (up to 80% of all used drugs). Using non-steroidal antiinflammatory compounds (HL) and a copper-containing blood enzyme, ceruloplasmin (CuCPL), the ligands (drugs) were shown to take away competitively the metals from metal-containing and metal-activating enzymes:

$$CuCPL + HL = CuL + CPL$$

Such an interaction results in a 'discomfort' of an enzyme system in the body which is indicative of a side effect of drugs, *i.e.* complexing agents.

For some diseases the shifts in metal metabolism are specific: rheumatoid arthritis—(–) Fe, Zn; (+) Cu, Al, Mn, Mo, Cr; atherosclerosis—(–) Cr, Mn, Zn; cancerogenesis—(–) Cu, Fe, Mg; (+) Zn, Mn; diabetis—(–) Cu, Mn, Cr; (+) Zn; *etc.* The correction in the concentration of these metals results in a therapeutic effect.

The complex compounds of biometals with different types of drugs are the most promising tool for introducing the required metal into the body. It has been established that the application of antiinflammatory agents as complexes with some biometals decreases their toxicity and increases and prolongs their therapeutic effect (chemico-therapeutic synergism); antiulcerogenic, cytotoxic and other helpful properties, unusual to non-complexed agents, appear.

It has been shown that a probable form of storage and transport of cardiovascular biogenic amines is their highly stable complexes with essential biometals and ATP.

In all cases the ligands (drugs) in the composition of metallocomplexes cause no decomposition of endogenic metalloferments and have no side effects.

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Therapeutically Active Cu(II), Zn(II) and Fe(III) Complexes with N-phenylanthranilic Acid Derivatives and Their Effect on Redox Reactions Modeling Inflammation

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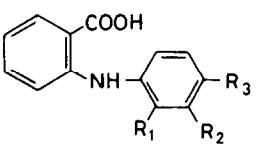
The amount of copper(II), zinc(II) and iron(III) involved in the majority of metalloferments during different diseases of inflammatory nature varies more than that of other metals.

The possibility of bonding the metal involved in ferments by drugs of acidic nature has been established by studying the interaction between ceruloplasmin (CP) and N-phenylanthranilic acid derivatives (HL), antiinflammatory agents. The reaction



HL

$$CP + HL \overset{K_{eq}}{\rightleftharpoons} apoCP + CuL_2$$

yields low-molecular complexes, CuL_2, as shown by the correlation between the equilibrium constants for the reaction, K_{eq}, and the stability constants of Cu(II) complexes, CuL_2. The latter are active metabolites of acids, *i.e.*, drugs.

Comparison of the therapeutic activities of HL and their Cu(II), Fe(III) or Zn(II) complexes has shown that the therapeutic properties inherent in drugs are improved in the complexes; the toxicity decreases, the action of agents is prolonged, and antiulcerogenic, cytotoxic and other effects, unusual to drugs, appear.

These complexes were found to affect the model reactions accompanying an inflammation at the molecular level: *i.e.*, the oxidation of ascorbic acid, biogenic amines and free radicals. The Cu(II) and Fe(III) complexes catalyze the oxidation of: a) ascorbic acid, b) *p*-aminophenol, the analog of serotonin, a biogenic amine. The Cu(II), Fe(III) and Zn(II) complexes interact with a free radical, triphenylverdazyl ($RN^{\bullet}$) to yield a non-radical cation (RN^{+}). The rate of reactions (a) and (b) proceeding via the steps of alternate oxidation–reduction of the catalyst are the highest for coordinatively unsaturated complexes. For the Cu(II) complex the reaction rate exceeds that for CP and is lower for the Fe(III) complexes than for the Cu(II) ones. The Fe(III) complexes, under otherwise equal conditions, were shown to be more apt to form polynuclear species with a lower oxidation potential compared with the Cu(II) complexes. The Cu(II) and Fe(III) complexes oxidize the $RN^{\bullet}$ to yield RN^{+} after dissociation to solvated metal ions via the scheme:

$$M^{n+} + RN^{\bullet} \longrightarrow M^{(n-1)+} + RN^{+} \quad (1)$$

which is confirmed by the inverse relationship between the RN^{+} concentration and stability constants of the complexes and also by the absence of the radical–complex interaction when the dissociation of the complex is suppressed. The Fe(III) complex oxidizes the $RN^{\bullet}$ to a lower extent than the Cu(II) one due to its higher stability. The Zn(II) complex causes the disproportionation of $RN^{\bullet}$ by reaction (2) involving the formation of an intermediate metal complex. The bridge metal ion in this complex facilitates the electron transfer from one $RN^{\bullet}$ to another:

$$M^{n+} + 2RN^{\bullet} \rightleftharpoons [\overset{e}{\frown}RN^{\bullet}\text{–}M\text{–}RN^{\bullet}]^{n+} \longrightarrow [RN^{+}\text{–}M\text{–}RN^{-}]^{n+} \quad (2)$$

This process is followed by the RN^{+} escape from the inner coordination sphere of the intermediate complex to yield the products: RN^{+} and $M(RN^{-})$. According to (1) $[RN^{+}] = [RN^{\bullet}]_o - [RN^{\bullet}]$ and (2) $[RN^{+}] = 1/2([RN^{\bullet}]_o - [RN^{\bullet}])$ which was actually observed.

The above data indicate that the therapeutic activity of the complexes under study depends not only on their ability to act as low-molecular metabolites, active therapeutic species, but also on their ability to dissociate to the ionic species responsible for some catalytic and other effects.

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Effects of Lanthanide and Calcium Ions on the Polymerisation of Collagen

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The polymerisation of individual collagen molecules into ordered polymeric fibrils is a spontaneous, extracellular process, which helps determine the properties of connective tissues. Our previous work [1, 2] has suggested that lanthanide ions (Ln^{3+}) might interact with collagen in interesting ways. During experiments to measure the binding of Ln^{3+} to collagen, it was discovered that these cations greatly accelerated the rate of collagen polymerisation. Ca^{2+} also accelerated polymerisation, but concentrations about 100 times greater than those of Ln^{3+} were necessary.

For study of polymerisation, pepsin-solubilised calf-skin collagen (1.5 mg/ml) was incubated in 30 m*M* Tris-HCl, 0.2 *M* NaCl, pH 7.0 at 37 °C. The polymerisation reaction was followed as the increase in absorbance of the solution at a wavelength of 500 nm. As reported by others [*e.g.* 3, 4], the resulting curve was sigmoidal; a nucleation phase of 16 min, during which the A_{500} remained low, was followed by a growth phase during which the A_{500} increased at a maximum rate of 0.05 units/min. A maximum absorbance (A_{max}) of 1.1 units was finally reached. The nucleation phase is thought to represent the length-wise attachment of collagen molecules and the growth phase, their lateral accretion [5].

Sm^{3+} reduced the length of the nucleation phase, accelerated the growth phase (Table I) and lowered the A_{max}. These effects were maximal at 100 μM Sm^{3+}. Other Ln^{3+} also reduced the nucleation phase, but none were as effective as Sm^{3+}. Er^{3+} and La^{3+} also accelerated the growth phase, but Lu^{3+} was inhibitory. All four Ln^{3+} lowered the A_{max}. Ca^{2+} reduced the length of the nucleation phase and accelerated the growth phase (Table I), but unlike the Ln^{3+}, increased the A_{max}.

Electron microscopic examination of the fibrils demonstrated that those formed in the presence of increasing concentrations of Sm^{3+} were progressively thinner (Table II).