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

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:

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

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.

V17

Effects of Lanthanide and Calcium Ions on the Polymerisation of Collagen

C. H. EVANS and B. J. COLLINGWOOD

Departments of Orthopaedic Surgery and Biochemistry, University of Pittsburgh, School of Medicine, Pittsburgh, Pa. 15261, U.S.A.

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).

TABLE I.

Metal Ion	Concentration	lag phase (min)	growth rate (ΔA/min)
–	–	16	0.050
Sm^{3+}	20 μM	9	0.085
	50 μM	4	0.130
	100 μM	0	0.150
La^{3+}	100 μM	8	0.155
Er^{3+}	100 μM	8	0.085
Lu^{3+}	100 μM	1	0.020
Ca^{2+}	2.5 mM	7	0.095

TABLE II.

Concentration of Sm^{3+} (μM)	Average fibril thickness (nm)
0	320 ± 62
25	210 ± 49
50	130 ± 31
100	78 ± 17

Arrhenius plots of the rates of polymerisation at different temperatures gave an activation energy (E_a) of 45.8 ± 5.6 kcal/mol for the nucleation phase and 57.2 ± 5.8 kcal/mol for the growth phase. Ca^{2+} (5 mM) and Sm^{3+} (25 μM) reduced the E_a of the growth phase to 34.8 ± 2.3 kcal/mol and 18.0 ± 1.1 kcal/mol respectively. However, the E_a for the nucleation phase was little changed by either 5 mM Ca^{2+} (E_a = 38.2 ± 4.3 kcal/mol) or 25 μM Sm^{3+} (E_a = 38.0 ± 2.3 kcal/mol).

The ability of physiological concentrations of Ca^{2+} to influence the rate of polymerisation of collagen suggests a modulating role for calcium *in vivo*. The ability of low concentrations of Ln^{3+} to mimic these effects suggests that they may prove useful probes of the interaction between Ca^{2+} and collagen.

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V18

The Effect of Silicon Compounds on Blood Cell Membranes

M. G. VORONKOV, V. B. KAZIMIROVSKAYA, L. I. KHOLDEEVA and T. V. SHELKOVA

Institute of Organic Chemistry, Siberian Division of the USSR Academy of Sciences, 664033 Irkutsk, U.S.S.R.

Silicon compounds play an essential role in metabolic processes, participate in oxidative reactions and are able to change the surface potential of blood cells, which is determined by the structural integration of cell membranes [1].

If found, the compounds of this element, increasing the structural stability of cell membranes would allow one to control the sensitivity of cells to external environmental effects and provide a remedy for curing cells and the organism as a whole.

We have established that in the development of haemolytic anaemia of different types the introduction of silatranes, $XSi(OCH_2CH_2)_3N$ with X = CH_3, $ClCH_2$, C_2H_5O, $(CH_3)_2CHO$ and HO which are efficient silicon donors [2, 3] increases the stability of blood red cell membranes manifested in a high osmotic and chemical resistance.

The haemolytic processes are always accompanied by the peroxidation of lipids. *In vivo* the silatranes studied inhibit free-radical and peroxidation reactions of erythrocytary membranes.

These data show that silatranes are worth studying as a promising means of prophylaxis of thrombosis. The use of $I\text{-}CH_2ClSi(OCH_2CH_2)_3N$ decreases or completely inhibits thrombocyte aggregation induced by ADP, adrenaline, thrombin and collagen. Such a combination of antioxidative and antiaggregate action is observed in neither of the known inhibitors of this process which are able to suppress the aggregation caused by only one specific inductor.

$I\text{-}CH_2ClSi(OCH_2CH_2)_3N$ increases almost two-fold the blood heparin level forming blood protein complexes which stimulate the anticoagulation system and non-enzymatic fibrinolysis in the organism.

All these effects are of great importance in natural prophylaxis of intravascular blood coagulation.

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