Various human metal intoxications have been treated efficiently by administration of a chelating agent. However, complexation reactions in the human body are influenced by a multitude of factors, including competing metals and ligands, dynamics of circulation, compartmentalization, and metabolism of the chelating agent. Accordingly, in vivo chelation reactions may differ extensively from what would be expected from our chemical knowledge about the metal and the chelating agent. Chelating agents can affect metal toxicity by mobilizing the toxic metal into (mainly) urine. However, an important effect of chelation is reduction of metal uptake and local toxicity at early times after oral intoxication. This was shown for the diethylenetriamine pentacetic acid (DTPA) complex of Cd²⁺ (6). Also orally administered dimercaptosuccinic acid (DMSA) reduced the intestinal uptake and toxicity of oral Cd²⁺ (2). Chelation of Ni²⁺ with EDTA and Hg²⁺ with DMSA or dimercaptosuccinic acid (DMPS) (7) reduced intestinal uptake. Accordingly, oral administration of chelating agents may in some cases offer both reduction of local toxicity and prevention of intestinal metal uptake (1,2,8).

Thermodynamic Considerations

In simple cases of formation of metal complexes with polydentate ligands, 

\[ M + L_i \rightarrow ML_i \] 

where \( M \) represents the solvated electron pair–accepting metal ion and \( L_i \) represents a chelator with \( i \) electron-pair–donating ligands (Lewis bases and acids), the overall stability constants is

\[ \beta_i = \frac{[ML_i]}{[M][L_i]} \]  

The stability of this complex depends on \( \Delta G = \Delta H - T \Delta S = RT \ln \beta_i \). For a complex with \( i \) ligands not associated in one molecule, the change in enthalpy related to bonding often contributes considerably to the free energy because the unfavorable entropy change associated with ordering \( i \) independent ligands around one ion counteracts the entropy effect of desolvation of the groups. Accordingly, multidentate ligands form more stable complexes than unidentate ligands because of the fully available entropy contribution from desolvation, and the stability in general increases with the number of rings formed. If one assumes that the enthalpy change due to complex formation does not depend on whether the donor groups are independent or joined in a multidentate ligand (which is not always true, however), the chelate effect should be entirely due to the entropy change.

The entropy contribution is indeed often the primary determinant of increased stability of metal complexes with multidentate ligands, but when mutual repulsive forces between charged groups are overcome by introducing them into one molecule, a considerable enthalpy effect may result. This may be illustrated by the thermodynamics of iminodiacetic acid (IDA) and EDTA complex formation with Cd²⁺ (Table 1, Figure 1). Even though the two complexes have the same number of groups (six) available for chelation, the number of rings is increased by one in the EDTA complex, increasing the entropy contribution to stability. Further, assembling the four negatively charged carboxyl groups in EDTA increases the enthalpy contribution. It can easily be calculated that the two contributions are of similar size.

The size of the chelate effect can be visualized from the change in log \( \beta \) for complexes with multidentate ligands with increasing numbers of identical donor groups. Thus, the stability of the Cd complexes with the polyaninopolyglycolamidoxylic acid increases in the following series: IDA with three donor groups and log \( \beta = 5.71 \); nitrilotriacetic acid with four donor groups and log \( \beta = 9.78 \); EDTA with six donor groups and log \( \beta = 16.36 \); and
DTPA with eight donor groups and \( \log \beta = 19.00 \) (9). Similar effects are seen with the series of homologous polyamines, where \( \log \beta \) for the Cd complexes increases from 5.45 to 16.10 when the number of donors increases from two to five (9). Steric conditions (e.g., ion size and ring size) considerably influence the stability, mainly through changes in \( \Delta H \).

**Hardness/Softness of Metal Ions and Ligands**

Determining factors for complex stability are the hardness/softness (HS) characteristics of electron donors and acceptors, discussed in the classical work by Schwarzenbach (10) and Ahland et al. (11) and further elaborated into the hard and soft acids and bases (HSAB) concept by Pearson (12). The HS characteristics of donor and acceptor atoms in complexation reactions determine not only stability of the formed complex but also the chelator’s degree of metal selectivity in relation to competing essential metals present in biological fluids. Further, the HS character determines the selectivity of the toxic metal for the chelator in relation to the competing biological ligands, often available at high concentrations compared with that of the chelating agent. Softness character is related to the ability of the empty frontier orbital of metal ions to accept electrons and to the deformability of the outermost occupied electron orbital of donor groups—that is, the propensity of metals and donors for forming covalent bonds.

The ionic index, \( Z/r \), where \( Z \) and \( r \) are the ion’s charge and radius, respectively, is positively related to degree of ionic bonding in an ion’s complexes. Conversely, the softness of an ion increases with the size of sum of the ionization energies divided by the ionic index \( (13), rE/Z^2 \). Nieboer and Richardson (14) described softness by the covalent index, \( \rho_{covalent} \), where \( \rho_{covalent} = \rho_{covalent}^{\text{atom}} \), where \( \rho_{covalent}^{\text{atom}} \) is the electron negativity. The rationale is that \( \rho_{covalent} \) is related to the ion’s empty frontier orbital energy and thereby to the ion’s ability to accept electrons and form covalent bonds. In brief, metal ions and donor groups prefer to form complexes with partners having similar HS character; however, the stability of complexes increases with the softness of both metal and donor. Thus, for a series of cadmium complexes with simple tridentate ligands, made by substituting the imino \( H \) in IDA with different functional groups, \( \log \beta \) increases from 5.71 (\( R = H \)) or 6.75 (\( R = \text{H}_2\text{N} \)), to 7.10 (\( R = \text{CH}_3 \)) to 9.78 (\( R = \text{COO}^- \)), 10.53 (\( R = \text{NH}_2 \)), or even 16.72 (\( R = \text{SH} \)) (9):

\[
\text{RN(COO}^-\text{)}_2 + \text{Cd}^{2+} \rightarrow \text{Cd}^{2+} \text{RN(COO}^-\text{)}_2.
\]

For another series of complexes, \( \log \beta \) varies between 12.43 (\( R = \text{CH}_3 \)) and 22.33 (\( R = \text{SH} \)) (9):

\[
2\text{RN(COO}^-\text{)}_2 + \text{Cd}^{2+} \rightarrow \text{Cd}^{2+}[\text{RN(COO}^-\text{)}_2]_2.
\]

**Competition, Rate Effects during Ligand Exchange, and Toxicokinetics**

The concentrations of “free” toxic metals are often very low in biological systems because of the availability of numerous small biological ligands forming mixed aquo-bioligand complexes with metals. Therefore, complexation reactions in vivo between toxic metals and “therapeutic” chelating agents most often occur as a series of ligand and/or metal exchange reactions. Even if the equilibrium constant is highly favorable, complex formation in vivo may be limited because of rate effects, competition by other ligands/metal, and systemic transport kinetics of the chelator. Under physiological conditions, numerous small mono- and bidentate ligands as well as functional groups in proteins participate in chelation reactions and compete for chelating agents. Ca\(^{2+}\), present at a concentration of about 1 mM, is the most important metal species competing for clinical chelating agents. Anticipating that equilibrium is achieved, and that the ML complex is quantitatively excreted in urine, the efficiency, \( E \), of a chelating agent for mobilizing a toxic metal can be described as

\[
E = \frac{[\text{ML}]}{[\text{M}]}.
\]

because the potential for mobilizing the metal depends on the degree of formation of the \( \text{ML} \) complex. In the simple situation of one major biological competing metal, Ca\(^{2+}\), and a total chelator concentration \( L_r \), the conditions for a large \( E \) can be visualized from the standard stability constants:

\[
E = \frac{[\text{ML}]}{[\text{M}]} = \beta_{\text{ML}} \times [L_r].
\]

By introducing the stability constants for the metal and calcium complexes into this expression and defining the concentration of \( L_r \) as the sum of all forms of the chelator in plasma, Schubert (15) derived

\[
E = \frac{\beta_{\text{ML}}}{\beta_{\text{Ca}}} \times \left[ \frac{L_r}{\text{Ca}^{2+}} \right].
\]

The mechanisms and kinetics of ligand exchange reactions have been extensively reviewed by Margerum et al. (16). They supply data for a range of divalent ions, that the rates of both solvent exchange and ligand exchange are related to the HS character of electron donors and acceptors. The rate of
complex formation depends on whether the chelator can easily get a grip on the metal ion by displacing a solvent molecule or a monodentate ligand to obtain the initial coordination site. The nature of this ligand exchange reaction determines whether the formed mixed complex is more or less stable than the disrupted complex. If a more stable complex is formed, further ligand exchange reactions are thermodynamically facilitated, sometimes even when subsequent ring opening is involved. The next step is formation of the first ring by coordinating a second donor group of the multidentate ligand to the metal ion, whereby the chelate effect decreases the rate of dissociation of the complex. Such processes may occur at a reasonable speed. If the initial complexation reaction involves breaking a preexisting chelate ring formed with a biological multidentate ligand, the process is often much slower. Besides the number of donor groups available for electron pair donation, that is, the maximum number of rings formed contributing to the chelate effect (the HS character of these donors), steric conditions for simultaneous access of ligands to coordination positions on the metal ion determine formation rate and overall stability. Also, lipophilicity, metabolic stability, and rate of (most often urinary) clearance are important.

Because of the complexity of biological systems, effects of antidotal chelators are often better described quantitatively from results of animal experiments or clinical treatments than by theoretical calculations of, for example, E. Increased mobilization of the toxic metal in experimental animals or humans, most often evaluated from urinary output, and decreased mortality or toxicity among exposed animals are major end points. The mobilizing effectiveness (ME) is expressed either as the factorial increase MEF in urinary and fecal excretion between treated and untreated animals or humans, or as the fractional retention MEQ of the metal in organs of treated animals relative to controls (17). The therapeutic effectiveness (TE) may be expressed for acute metal intoxication by the factorial change TEF in LD50 (the dose killing 50% of exposed animals) due to the chelation treatment (17). Similarly, two chelators may be compared from results of animal experiments by their relative potency, which is the ratio between equally effective doses, or by their relative efficiency (RE) the ratio of effects at equimolar doses (17). Because the efficiency of different chelators toward acute metal toxicity may vary extensively in some combinations allowing 100% survival even after doses considerably higher than LD50 (1,2), the RE method has limited applicability.

New Paradigms in Clinical Chelation Treatment: The Exit of BAL

EDTA, d-penicillamine, and British antilewisite [2,3-dimercaptopropanol (BAL)] came into clinical use after World War II to treat lead and mercury intoxication, and copper intoxication in Wilson disease (18), which is today treated with triethylene-diamine (19,20). In 1962, DFOA was shown to increase urinary iron excretion in patients with thalassemia (21). Today, DFOA is also used to treat aluminum intoxication and iron storage toxicity in sickle cell anemia patients. During the 1950s DMSA and DMPS came into use in China (22–24) and the Soviet Union (25,26). Since the 1970s these drugs have been available as experimental drugs in the Western countries. DMSA and DMPS are efficient antidotes for intoxications with several divalent metals besides lead and mercury as well as some organometallic and metalloid compounds (8,27). Both chelators are available as tablets for oral administration, which are stable for long periods at room temperature, and DMPS also as a dry preparation for parenteral administration after hydration. In China, DMSA has been administered parenterally to hundreds of patients (22), BAL is unstable, susceptible to oxidation, and difficult to store as a ready-for-use preparation. It has a low therapeutic efficacy in most cases, and because of high toxicity, BAL is suited only for brief treatment of acute intoxications. It can be administered only by intramuscular injection, normally in paratone oil. Administration of local anesthesia beforehand is necessary because the injection is very painful. Presently available experience indicates that DMSA or DMPS can substitute for BAL in most clinical situations, resulting in safer and more efficacious treatment.

Side Effects and Toxicity of BAL, DMSA, and DMPS

A considerable fraction of individuals treated with BAL experience unpleasant side effects, including nausea, vomiting, sweating, high fever, hypertension, and tachycardia. BAL administration increased the brain deposition of arsenite (28) and organic mercury compounds (29) and increased the toxicity of cadmium (30) and lead (31) in animal experiments. DMPS does not redistribute arsenic, lead, or inorganic mercury to the brain (28,32), and DMSA chelation decreases the brain deposition of lead (33) and methylmercury (34). BAL is significantly more toxic than DMPS, which is slightly more toxic than DMSA. Representative LD50 values selected from the large number of published toxicity studies are given in Table 2.

In the only reported case of a DMSA overdose, a 3-year-old girl ingested approximately 2.4 g DMSA or 185 mg/kg body weight without clinical signs of intoxication (40). During the last two decades, many patients have been treated with DMSA in the United States and with DMPS in Europe, with a very low frequency of toxic side effects necessitating discontinue treatment. Adverse reactions during treatment with DMSA or DMPS include gastrointestinal discomfort, skin reactions, mild neutropenia, and elevated liver enzymes. For both compounds, symptoms may subside, allowing continued therapy. DMPS seems to be better tolerated than is DMSA with respect to gastrointestinal symptoms but may cause hypotension, especially after rapid intravenous infusion. Some patients, especially those with allergic asthma symptoms, may develop hypersensitivity to DMPS (41,42).

For DMSA two serious reactions to therapy have been reported: DMSA chelation of a man with chronic lead intoxication was discontinued because of a strong mucocutaneous reaction to the drug (43). A 45-year-old African-American man developed hemolytic anemia during DMSA chelation for occupational lead intoxication. After cessation of treatment, the hematological values normalized. The patient was glucose 6-phosphate dehydrogenase deficient, a genetic trait known to contraindicate BAL chelation because of risk of hemolysis (44). For DMPS, severe toxicity has not been reported in peer-reviewed literature except for a case of Stevens-Johnson syndrome in a lead-intoxicated patient after eight daily oral doses of 200 mg/m2 DMPS (45). DMPS is registered in the United States as a drug for treatment of lead intoxication. DMPS is registered in Germany for treatment of mercury intoxication; however, it is not approved in the United States, so unless special permission is given by the U.S. Food and Drug Administration, it is not lawful for physicians to use it in the United States, nor is it lawful for pharmacies to compound it. Still, DMPS is being illegally used by members of the alternative health industry to treat people allegedly suffering from mercury intoxication, most often claimed to be due to amalgam.

Table 2. Representative LD50 values for clinically relevant dimericatopoloxyating compounds.

| Compound | Species | Administration route | LD50 | Reference |
|----------|---------|----------------------|------|-----------|
| BAL      | Mouse   | Intraperitoneally     | 90–180 mg/kg | (25,39) |
| DMSA     | Mouse   | Orally               | 4.34 g/kg | (37)     |
| DMPS     | Mouse, rat | Intraperitoneally | 2.46 g/kg | (38)     |
| DMPS     | Mouse, rat | Orally | 1.1–1.4 g/kg | (26,39) |
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