# Principles and Recent Developments in Chelation Treatment of Metal Intoxication

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## *1. Introduction*

For a chemist, it is intuitively obvious that human metal intoxications should be treated with chelating agents. Indeed, extensive clinical experience demonstrates that the prognosis in acute and chronic human intoxications with a range of metals can be improved considerably by administration of a suitable chelating agent. However, the intended antidotal complexation reaction in the human organism, like in that of an experimental animal used for optimization of chelation treatment, is influenced by a number of biological conditions, including competing metals and ligands, dynamics of circulation, compartmentalization, and metabolism of the chelating agent. Accordingly, the outcome of in vivo chelation may both qualitatively and quantitatively differ extensively from what would be expected from our knowledge about reactions between the metal and the chelating agent in the chemical laboratory.

Ole Andersen graduated in Biochemistry from Copenhagen University in 1973. He worked on in vitro genetic toxicology of metals and on in vivo metal toxicokinetics and toxicodynamics and effects of chelating agents and nutritional factors from 1973 at Odense University for about 20 years, before he became Professor of Environmetal Biology at Roskilde University in 1993.

The purpose of this review is to briefly summarize the historical background for treatment of metal-related disease with chelating agents, describe chemical and biological principles, advantages, and limitations in treatment of metal intoxications with chelating agents, and outline the recent development (during the last 10−15 years) in treatment of acute and chronic intoxication with divalent metal ions and some other metal and metalloid compounds. Also, the efficiency of the new chelating agents *meso*-2,3-dimercaptosuccinic acid (DMSA; **1**) and D,L-2,3-dimercapto-1-propanesulfonic acid (DMPS; **2**) will be compared with that of classical chelators, mainly 2,3-dimercaptopropanol (British Anti Lewisite, BAL; **3**) and ethylenediamminetetraacetic acid (EDTA; **4**),

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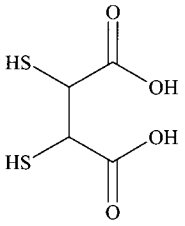
and important research needs and avenues for future development will be discussed.



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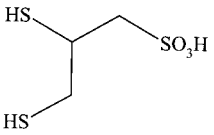

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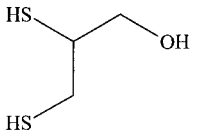

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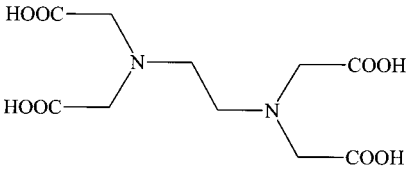

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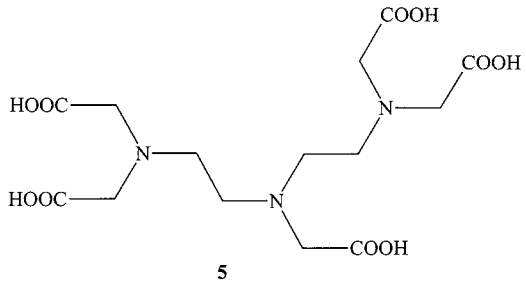
## 2. Origin of Chelation Treatment of Metal Toxicity

Historically, the use of chelating agents for decreasing metal or metalloid toxicity started about 100 years ago with the collaboration between Alfred Werner in Zürich and Paul Ehrlich in Frankfurt to find less toxic arsenic compounds for syphilis treatment. During 1920−1940, similar attempts were made to reduce the toxicity of arsenical drugs for trypanosomiasis by Voegtlin and antimony drugs for Schistosomiasis by Schmidt.

The more direct use of chelators to combat metal intoxication started in 1941 with Kety and Letonoff's[1] attempt to use citrate as an experimental antidote toward acute lead intoxication. Even though the success was limited due to the metabolic instability of citrate, this experiment started a new era in the treatment of acute and chronic metal intoxication, due to overexposures or genetic disturbances in metal metabolism.

During World War II, BAL was developed as an antidote to the war gas dichlorovinyl arsine (Lewisite).[2] Lewisite was, however, never used, so the first clinical use of BAL was in intoxications due to treatment of syphilis with organic arsenical drugs.[3−5] The next chelator to come into clinical use was EDTA, initially to combat lead intoxication and for decorporation of radionuclides,[6] the latter role presently played more efficiently by diethylenetriaminepentaacetic acid (DTPA; **5**).



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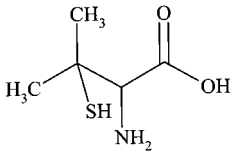
The value of EDTA as a clinical chelating agent is reduced by the need of slow intravenous administration due to a low intestinal uptake, the exclusive extracellular action of EDTA and its high stability constants with some essential metals; thus, care must be taken against induction of hypocalcemic tetani during intravenous infusion of EDTA in acute intoxication and against zinc depletion during prolonged use. Accordingly, in the text below, clinical use of EDTA and DTPA refer to pharmaceutical preparations of EDTA and DTPA in the form of Ca and/or zinc salts.

Treatment of metal storage diseases, a use of chelating agents of immense importance for afflicted individuals, was initiated in 1956 by Walshe,[7] who treated Wilson's disease patients with D-penicillamine (DPA; **6**), 3,3-dimethyl-substituted cysteine **7** to enhance copper excretion. He later used trieth-



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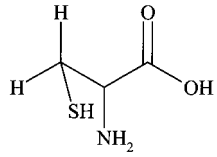

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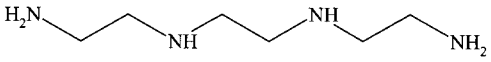
ylenetetramine (TETA; **8**) to treat patients developing penicillamine intolerance.[8,9] Today, TETA is consid-



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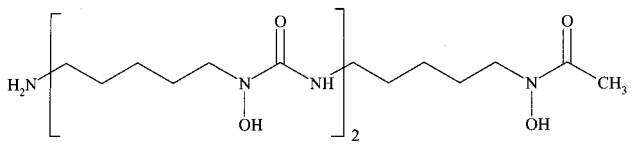
ered the drug of first choice in copper storage disease, even though a large number of Wilson's disease patients have been treated with DMSA in China, apparently with a good result.[10] Another important breakthrough was the demonstration[11] in 1962 of increased urinary iron excretion in Thalassaemia major patients treated with desferrioxamine (DFOA; **9**).



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## 3. Introduction of New Chelators

Already during the 1950s, DMSA and DMPS were used in China[10,12,13] and the Soviet Union,[14,15] and these drugs have been available as experimental drugs for decades, and DMSA is a registered drug in the United States and DMPS in Germany, respectively, for almost 10 years. Unfortunately, more than four decades have now passed since the original introduction of these chelators without western clinicians fully realizing their value. Today, they hold promise as antidotes in acute or chronic intoxications with many divalent metal salts and also some other metal or metalloid compounds. Their use in various intoxications was previously reviewed by Aposhian et al.[16]

Originally, BAL was considered a general antidote in metal poisoning due to its apparently high efficacy in human arsenic[5,17,18] and inorganic mercury[19] intoxications and the limited number of alternatives available. Thus, BAL has been used in a variety of

human metal intoxications. However, BAL is far from an ideal chelator due to the high toxicity and high frequency of various unpleasant side effects (see section 9 and Table 2, below) and because increased brain deposition due to BAL administration has been reported for arsenite[20] and organic mercury compounds.[21] Also, BAL increased the toxicity of cadmium[22] and lead[23] in animal experiments.

DMSA and DMPS are less toxic drugs suited for oral administration; however, parenteral administration is also an option. Also, DMPS does not redistribute arsenic, lead, or inorganic mercury to the brain,[20,24] and DMSA chelation decreases the brain deposition of lead[25] and methylmercury.[26] In cases of childhood and occupational lead exposure, EDTA has been and is still used for chelation therapy and for a challenge test to estimate lead burden, even though EDTA may redistribute lead to the brain both after chronic[27] and acute[28,29] lead exposure, and accordingly, this diagnostic use of chelation could endanger the patient.

Most chronic and acute metal intoxications are, fortunately, occurring at lower rates than formerly. Metal intoxications still occurring at some frequency are acute iron intoxications in children ingesting iron tablets, intoxications by bismuth, gold, and platinum compounds due to medical uses, intoxications with thallium- and arsenic-based pesticides, intoxications due to various forms of mercury, chronic cadmium intoxication due to environmental or occuptional exposure, and, being the most widely distributed metal poisonings, acute and chronic childhood or occupational lead intoxications.

Except for DFOA for iron storage diseases and possibly DMSA for lead and several other metal intoxications, drugs for treating metal intoxications have low commercial priority, because most metal intoxications are relatively rare. The expenses for developing and registering a new chelating agent are prohibitively high and expected worldwide sales moderate. Accordingly, it was only due to the "Orphan Drugs for Orphan Diseases" act in the United States (ODA; PL 97-414) signed into law in 1983 that Trientine was developed as a drug for treatment of Wilson's disease.[30] In Germany, DMPS is registered for treatment of mercury intoxication.

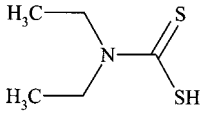
Since World War II, several thousand articles have been published on experimental animal studies on various forms of chelation treatments of acute or chronic metal exposure and hundreds of different compounds have been investigated. The number of chelating agents in actual use as experimental drugs or clinically established drugs is, however, surprisingly small (see section 9 and Table 2, below). This could indicate that such studies are futile. However, several important questions in chelation treatment of metal toxicity still remain and can be answered only by experimental studies. Also, even though the most important development in clinical treatment during recent years has been development of orally administrable chelators, oral chelation with efficient chelators of low toxicity is still not available or clinically accepted for some important intoxications to be discussed below.

## 4. Chemical-Biological Principles for in Vivo Chelation

The success of in vivo chelation depends on a range of characteristics of the metal, the chelator, and the organism. For the sake of clarity, metal-associated, chelator-associated, and organism-associated characteristics will to some degree be discussed separately from each other below. However, these three sets of determinants interplay to determine the degree of in vivo complexation of a metal by a chelator.

In most studies on the use of chelating agents to treat metal intoxication, focus has been on mobilization (mainly due to renal excretion) of the toxic metal. However, an important effect of chelating agents is reduction of metal toxicity. Thus, a chelating agent forming a stable complex with a toxic metal may shield the metal ion from biological targets, thereby reducing the toxicity, even at times after administration where mobilization has not yet occurred, or it may expose the metal to the biological environment and prevent the metal from being scavenged by biological protective mechanisms and thereby increase the toxicity of the metal. One example is chelation during acute iron toxicity: DFOA completely covers the surface of $Fe^{3+}$ during complex formation, thereby preventing iron-catalyzed free radical reactions.[31,32] However, EDTA is not able to shield the surface of the $Fe^{3+}$ ion but forms an open complex ("basket complex"), thereby increasing the catalytic capacity of $Fe^{3+}$ for generating oxidative stress by about 1 order of magnitude.[33]

Depending on the lipophilicity of a metal−chelator complex, chelating agents may change the metal's organ distribution, thereby potentially increasing the toxicity. Diethyldithiocarbamate (DDC; **10**) was originally suggested as an efficient chelator for acute Cd intoxication, as parenteral DDC administration decreased mortality induced by parenteral Cd, even at protracted time after Cd administration[34] and increased the rate of excretion of aged deposits of Cd;[35] however, DDC enhanced the brain deposition of Cd after parenteral Cd administration.[36,37] In general,

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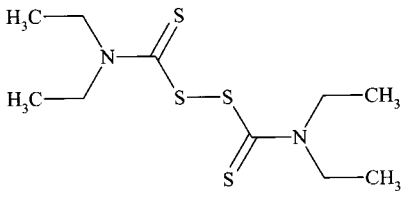
DDC forms highly lipophilic complexes with divalent metal ions,[38] and increased brain deposition due to DDC exposure has been reported also for inorganic and organic Hg,[39,40] Tl,[41] Pb,[42] Ni,[43] Cu, and Zn.[44,45]

The oral use of chelating agents is in general considered to require preceding removal from further exposure to circumvent chelator-mediated increased intestinal metal absorption. Thus, oral or parenteral administration of DDC enhanced the toxicity of orally administered Cd and extensively increased the brain deposition of Cd due to enhancement of intestinal uptake. Also, oral administration of DDC enhanced the local toxicity of Cd toward gastrointestinal tissues.[46,47] Enhancement by DDC of intestinal uptake has been reported also for several other metals.

Tetraethylthiuram disulfide (TTD, disulfiram, antabuse; **11**) is used for alcohol avoidance therapy in alcoholics. TTD is rapidly metabolized, most likely



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already in the gut, to two molecules of DDC and accordingly increases the intestinal uptake and brain deposition of orally administered Cd,[48] Ni,[49] and lead[50] in the same way as DDC. Also, various carbamate-based pesticides including Thiram, the tetramethyl analogue of TTD, enhance intestinal metal uptake and brain deposition of Ni.[51] The enhancing effect of dithiocarbamates on intestinal uptake and brain deposition of Cd is directly related to their lipophilicity; thus, more hydrophilic derivatives of DDC enhanced the intestinal uptake of Cd less efficiently than did DDC, and the degree of enhancement correlated positively with the octanol−water partitioning coefficient.[52]

As opposed to these effects of DDC and TTD on metal biokinetics, orally administered chelating agents forming hydrophilic metal complexes may efficiently reduce intestinal metal uptake and local toxicity at early times after oral intoxication, thereby improving prognosis. This has been demonstrated for the $Cd^{2+}$ complex with DTPA.[53] Also, orally administered DMSA reduced the intestinal uptake and toxicity of oral Cd.[54] Chelation of $Hg^{2+}$ with DMSA and DMPS[55] and $Ni^{2+}$ with EDTA (Nielsen and Andersen, unpublished data) 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.

In part of the literature describing effects of chelators on acute metal toxicity, metal excretion or organ distribution was not quantitated; accordingly, it is unclear to what extend increased excretion and decreased toxicity contributed to the observed alleviating effect of chelation treatment.

### 5. Thermodynamic Considerations

In simple cases of formation of metal complexes with monodentate or polydentate ligands:

$$\mathrm{M} + \mathrm{L} \rightarrow \mathrm{ML} \tag{1}$$

$$\mathrm{M} + i\mathrm{L} \rightarrow \mathrm{ML}_i \tag{2}$$

where M represents solvated electron pair accepting metal ions and L represents electron pair donating ligands (Lewis bases and acids), the expression of the stability constants is straightforward

$$K_{\mathrm{ML}} = \frac{[\mathrm{ML}]}{[\mathrm{M}][\mathrm{L}]} \tag{3}$$

and the overall stability constant

$$\beta_i = \frac{[\mathrm{ML}_i]}{[\mathrm{M}][\mathrm{L}]_i} \tag{4}$$

$K_{\mathrm{ML}}$ being equal to $\beta_1$. The stability of a complex depends on

$$\Delta G = \Delta H - T\Delta S = -RT \ln \beta_i \tag{5}$$

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 disfavorable 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, due to the fully available entropy contribution from desolvation and the stability in general increases with the number of rings formed.

Schwarzenbach[56] defined the chelate effect as the logarithm to the equilibrium constant for a displacement reaction where $i$ independent donors are exchanged by $i$ identical donors present in one ligand, thereby expressing the increased stability of the chelate as related to the free energy of the reaction. If one assumes (which is not always true, however) that the enthalpy change due to complex formation is not dependent on whether the donor groups are independent or joined in a multidentate ligand, the chelate effect should be entirely due to the entropy change. Comparison of two ligand exchange reaction series where the ligand L contains $i$ donors identical to A

$$\mathrm{M} + i\mathrm{A} \rightarrow \mathrm{MA}_i;\ \beta_{\mathrm{MA}} = \frac{[\mathrm{MA}_i]}{[\mathrm{M}][\mathrm{A}]^i};\ \Delta G_{\mathrm{MA}} = -RT \ln \beta_{\mathrm{MA}} \tag{6}$$

$$\mathrm{M} + \mathrm{L}_i \rightarrow \mathrm{ML}_i;\ \beta_{\mathrm{ML}} = \frac{[\mathrm{ML}_i]}{[\mathrm{M}][\mathrm{L}_i]};\ \Delta G_{\mathrm{ML}} = -RT \ln \beta_{\mathrm{ML}} \tag{7}$$

shows that formation of the MA complex depends much more on the concentration of the ligand (A is in the $i$th power in $\beta_{\mathrm{MA}}$) than does the formation of the ML complex (L is in the first power in $\beta_{\mathrm{ML}}$). Especially at low ligand concentration, chelates are far more stable than the corresponding complexes with unidentate ligands. For the combined ligand exchange reaction, see eq 8, the equilibrium is determined by the free energy for the combined reaction, see eq 9.

$$\mathrm{MA}_i = \mathrm{L}_i \rightarrow \mathrm{ML}_i + i\mathrm{A} \tag{8}$$

$$\Delta G_{\mathrm{MAL}} = -RT \ln\left(\frac{[\mathrm{ML}_i][\mathrm{A}]^i}{[\mathrm{MA}_i][\mathrm{L}_i]}\right) \tag{9}$$

The entropy contribution is often the primary determinant of increased stability of metal complexes with multidentate ligands; however, when mutual repulsive forces between charged groups are overcome by

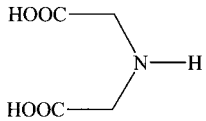
introducing them into one molecule, a considerable enthalpy effect may result.

Steric conditions, e.g., ion size and ring size, considerably influence the stability, mainly through changes in $\Delta H$. Thus, for the ligand exchange reaction

$$Cd^{2+}(CH_3N(COO^-)_2)_2 + (^-OOC)_2N(CH_2)_nN(COO^-)_2 \rightarrow Cd^{2+}(^-OOC)_2N(CH_2)_nN(COO^-)_2 + 2(CH_3N(COO^-)_2) \quad (10)$$

$\log \beta$ is 7.2, 2.4, −1, and −1.6 for $n = 2$ (EDTA), 3, 4, and 5, respectively, almost solely due to change in $\Delta H$.[57]

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 polyaminopolycarboxylic acids increases in the series iminodiacetic acid **12** with 3 donor groups and $\log \beta = 5.71$; Nitrilotriacetic acid **13** with 4 donor groups and $\log \beta = 9.78$; EDTA with six donor groups and $\log \beta = 16.36$; and DTPA with 8 donor groups and $\log \beta = 19.00$ (Data collected from Martell and Smith[58]). Similar effects are seen with the series of

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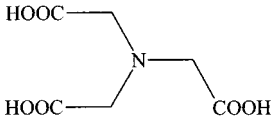


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homologous polyamines, where $\log \beta$ for the Cd complexes increases from 5.45 to 16.10 when the number of donors increase from 2 to 5 (Data from Martell and Smith[58]).

### 6. Hardness/Softness of Metal Ions and Ligands

Determining factors for complex formation immediately considered by the chemist are the hardness/softness characteristics of electron donors and acceptors, discussed in the classical work by Schwarzenbach[59] and Ahrland et al.[60] and further elaborated into the Hard and Soft Acids and Bases concept by Pearson.[61] The HS characteristics of donor and acceptor atoms in complexation reactions determine not only—speaking in general terms—the expected 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 selectivity for the chelator by the toxic metal in relation to the competing biological ligands, often available at high concentration compared to that of the chelating agent, is determined by HS character.

In popular terms, the softness character can be said to be related to the ability of the empty frontier orbital of metal ions for accepting electrons and to the deformability of the outermost occupied electron orbital of donor groups, i.e., the propensity of metals and donors for forming covalent bonds. Accordingly, the HS character of metal ions has been quantified in the literature by various descriptors related to the bonding preference of ions, which is a result of the ions' physical and chemical characteristics. The simpler descriptors are briefly mentioned below. Thus, the ionic index, $Z^2/r$ 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,[62] $rI/Z^2$. Nieboer and Richardson[63] described softness by the covalent index, $\Sigma X_m 2r$, where $X_m$ is the electronegativity. The rationale is that $X_m$ is related to the ion's empty frontier orbital energy and thereby to the ion's ability to accept electrons and form covalent bonds. For a more complete treatment, see the work of Andersen.[64] 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 degree softness of both metal and donor. For practical uses, metals and donors are divided into three groups, hard (H), intermediate (I), and soft (S). Examples from these classes of metal ions, biological ligands, and donor groups employed in clinically useful chelators are given in Table 1.

As shown above, the stability of complexes can be increased by increasing the number of rings formed, i.e., the number of donor groups in the chelating agent. The increase in $\log \beta$ has been experimentally determined to be about 2 per extra ring formed, only partially contributed by the chelate effect. Another way of increasing chelate stability is to increase the softness of the donor groups. Thus, for a series of cadmium complexes with simple tridentate ligands, made by substituting the imino H in iminodiacetic acid with different functional groups

$$RN(COO^-)_2 + Cd^{2+} \rightarrow Cd^{2+}RN(COO^-)_2 \quad (11)$$

$\log \beta$ increases from 5.71 (R = H) or 6.75 ($R = CH_3$) to 9.78 ($R = COO^-$), 10.53 ($R = NH_2$), or even 16.72 (R = SH). For another series of complexes

$$2RN(COO^-)_2 + Cd^{2+} \rightarrow Cd^{2+}(RN(COO^-)_2)_2 \quad (12)$$

$\log \beta$ varies between 12.43 ($R = CH_3$) and 22.33 (R = SH). These data are collected from Martell and Smith.[58]

Metal-related characteristics determining chelatability in vivo include ionic diameter and preferred coordination number, tendency for participating in redox reactions (e.g., transition metal ions), and preferred oxidation state, which besides contributing to HS character also determine bioligand preference and limitations for chelation. Further, chemical similarity to essential metal ions and rates of ligand exchange reactions in biological systems, e.g., with functional groups in proteins, are important. Together, these characteristics also influence the nature of the toxic reactions induced in the organism by the metal, i.e., induction of oxidative stress and lipid peroxidation by Fenton chemistry catalyzed by transition metals as Cu, Fe, and Ni, aggressive local reactions as induced by, e.g., chromate, irreversible enzyme inhibition as, e.g., binding of $Cd^{2+}$ or $Hg^{2+}$ to SH groups.

**Table 1. Representative Toxic and Essential Divalent Metal Ions and Molecular Structures Offering Electron Donors in Biological Molecules and Clinically Important Chelating Agents, Ordered According To Their HSAB Character**

| HS character | metal ion | donor group structure in biological molecules | donor group structure in clinically used chelators | examples |
|---|---|---|---|---|
| H | $Be^{2+}$ | R−COOH | R−COOH | DMSA, DPA, NAPA, EDTA, DTPA |
| | $Mg^{2+}$ | RR′C=O | RR′C=O | L1 |
| | $Ca^{2+}$ | RR′−CH−OH | RR′CH−OH, RR′=C−OH | BAL, L1 |
| | | $R-O-PO_3^{2-}$ | | |
| | | $R-O-SO_3^-$ | $R-O-SO_3^-$ | DMPS |
| I | $Ni^{2+}$ | $R-NH_2$ | $R-NH_2$ | DPA, TETA, DFOA |
| | $Fe^{2+}$ | RCO−N−R′ | RCO−N−R′ | NAPA |
| | $Co^{2+}$ | RN−CO−NR′ | RR′−NH | TETA, |
| | $Mn^{2+}$ | RN=CNR′N″ | RR′R″N | EDTA, DTPA, L1 |
| | $Zn^{2+}$ | | R−NOH−CO−NH−R′ | DFOA |
| S | $Cd^{2+}$ | R−SH | R−SH | DMSA, DMPS, BAL, DPA, NAPA |
| | $Cu^{2+}$ | R−SS−R* | $R_2N-CSSH$ | DDC |
| | $Pb^{2+}$ | | $R_2N-CSSR'$ | TTD |
| | $Hg^{2+}$ | | | |

## 7. Rate Effects during Ligand Exchange

Even if the equilibrium constant is highly favorable, complex formation in vivo may be limited due to rate effects and competition with kinetics of chelant transport in the organism. The mechanisms and kinetics of ligand exchange reactions have been extensively reviewed by Margerum et al.[65] 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 the ions.

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—step 1 in the process. The nature of this ligand exchange reaction (e.g., HS character of donor and ligand) determines whether the formed 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. Step 2 in the process 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.

Important characteristics of chelating agents are the number of donor groups available for electron pair donation, i.e., the maximum number of rings formed and contributing to the chelate effect stabilizing the complex with the metal, HS character of these donors, and steric possibilities for the donor groups' simultaneous access to coordination positions on the metal ion, especially during ligand exchange for metal ions chelated by proteins or other macromolecular chelators. Also, lipophilicity, metabolic stability, and rate of (most often urinary) clearance are important.

## 8. In Vivo Efficiency of Chelators

Organism-related characteristics include biological uptake mechanisms, i.e., whether the organism's active uptake mechanisms for essential metals can mistakenly transport the toxic metal or whether it is taken up passively, mechanisms of sequestering the metal, either by scavenging by specific proteins such as transferrin, ferritin, ceruloplasmin, or metallothionein (MT), or by deposition in depots such as bone (lead), kidney cortex (cadmium), brain ($Hg^{2+}$ after exposure to $CH_3Hg^+$ or $Hg^0$ vapor). Also, enterohepatic circulation and excretion mechanisms affect the organismal fate of toxic metals.

The concentrations of "free" toxic metals are often very low in biological systems due to the availability of numerous small biological ligands with which the metal forms mixed aquo−bioligand complexes. Therefore, the complexation reactions in vivo between the toxic metal and the "therapeutic" chelating agent most often occur as a series of ligand exchange reactions

$$A_jB_jM + L_{i+j} \rightarrow ML_{i+j} + A_i + B_j \qquad (13)$$

as well as by metal exchange reactions

$$LM' + M \rightarrow LM + M' \qquad (14)$$

Accordingly, an important factor determining chelatability in vivo is the nature of the metal's complexes formed with natural ligands: When a multidentate ligand reacts with a metal ion in a physiological solution, where, e.g., $Ca^{2+}$, $H^+$, and $OH^-$ and numerous small mono- and bidentate ligands as well as functional groups in proteins compete, the descriptions of reaction kinetics and thermodynamics of complex formation are complicated.

The extensive opportunity for competition reactions in biological systems indicated above made it clear early during theoretical considerations of which factors would govern the success of biological chelation, that "effective stability constants", $K'$ or $\beta'$, should be employed for description of complex formation in vivo.[66−68] Such constants may be several orders of magnitude smaller than standard stability constants. Mathematical descriptions of these multiple complexation reactions leading to simplified expressions of the effective stability constant and the effectiveness of chelation have been published.[66,68−70] For this treatment it suffices to mention that for most practical uses, only $Ca^{2+}$ needs to be taken into account for estimating the effective stability constant

in vivo, pH being about 7.4 and $Ca^{2+}$ about 1 mM in serum.

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

$$E = \frac{[ML]}{[M]} \tag{15}$$

since the potential for mobilizing the metal depends on the degree of formation of the ML complex. In the simple situation of one major biological competing metal, $Ca^{2+}$, and a total chelator concentration $L_t$, the conditions for a large *E* can be visualized in a rather naive but translucent way from the standard stability constants

$$\beta_{ML} = \frac{[ML]}{[M][L]}; \beta_{CaL} = \frac{[Ca^{2+}L]}{[Ca^{2+}][L]} \tag{16}$$

and thus

$$E = \frac{[ML]}{[M]} = \beta_{ML}[L] \tag{17}$$

As $[L] = [L_t] - [ML] - [Ca^{2+}L]$ and $[Ca^{2+}L]$ can be approximated as $\beta_{CaL}[Ca^{2+}][L]$, *E* can be expressed as

$$E = \beta_{ML}([L_t] - [ML] - \beta_{CaL}[Ca^{2+}][L]) \tag{18}$$

Accordingly, a large *E* requires $\beta_{ML} \gg \beta_{CaL}$ and $L_t \gg M$.

Using other terms, here changed in analogy with the treatment above, Schubert[67] similarly defined $E = [ML]/[M]$ and by introducing the stability constants for the metal and calcium complexes into this expression, he derived

$$E = \frac{\beta_{ML}}{\beta_{CaL}} \frac{[L_t]}{[Ca^{2+}]} \tag{19}$$

where $[L_t]$ is the sum of all forms of the chelator in plasma.

Concise—yet still approximative—treatments of this subject[68,71] offered by Heller and Catsch[71] and Catsch[68] lead to definition of α-values taking care of the effect of competition by protons

$$\alpha_L = 1 + \sum_{i=1}^{n} \beta_{ML}[H^+]^i \tag{20}$$

where *i* varies between 1 and the highest number of protons accepted by L, for estimation of the effective stability constant $\beta'$ as

$$\beta'_{ML} = \frac{\beta_{ML}}{\alpha_L} \tag{21}$$

Thus, eqs 20 and 21 allow *E* to be expressed as

$$E = \frac{\beta_{ML}[L_t]}{\alpha_L \beta_{ML}[Ca^{2+}L][Ca^{2+}]} \tag{22}$$

This expression underestimates *E* as it does not include $\beta'_{CaL}$ and it is valid if the concentration of L is considerably higher than the metal concentration. Estimation of *E* had practical significance in the early use of chelating agents, as the polyaminopolycarboxylic acids were major alternatives to BAL and DPA for, e.g., lead chelation and also used for several other intoxications with metals, including radioisotopes. These compounds efficiently chelate $Ca^{2+}$, leading both to reduced *E* and possibility for hypocalcemia.[72] The newer chelating agents, DMSA and DMPS, which during recent years have gained increasing use instead of the classical chelators, are considerably more selective

Due to the extensive 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, e.g., *E*. Two different endpoints are important, increased mobilization of the toxic metal in experimental animals or in metal-exposed humans, most often evaluated from urinary output, and decreased mortality or toxicity among exposed animals. Both of these endpoints were described by Catch and Harmuth-Hoehne:[72] The mobilizing effectiveness (ME) is expressed either as the factorial increase, MEF, in urinary and fecal excretion between treated and un- or pretreated animals or humans or as the fractional retention, MEQ, of the metal in organs of treated animals relative to controls. The therapeutic effectiveness, TE, may be expressed for acute metal intoxication by the factorial change, TEF, in $LD_{50}$ due to the chelation treatment. Similarly, two chelators may be compared from results of animal experiments by their relative potency RP, the ratio between equally effective doses, or by their relative efficiency, RE, the ratio of effects at equimolar doses. As the efficiency of different chelators toward acute metal toxicity may vary extensively, in some combinations allowing 100% survival even after doses considerably higher than $LD_{99}$ (see, e.g., Andersen[54,73]), the RE method has limited applicability.

### 9. Toxicity and Kinetics of Clinically Used Chelating Agents

This subject is important in relation to the intoxication situation: In acute life-threatening metal poisoning, some chelator toxicity is acceptable, granted the chelator is an efficient antidote. In chronic metal poisoning due to inborn errors of metabolism, the use of chelators is mandatory for continued health or even survival, and again the use of chelators with some toxicity is acceptable. The situation is different in less severe poisoning and chronic metal exposures, where the alternative to chelation is to offer the necessary support or environmental improvement while waiting for the "natural" excretion of the metal. A major problem in continued, often life-long, chelation treatment is induction of essential trace element imbalance, which may require correction.

## A. BAL

BAL has a lower $LD_{50}$ than the chelators available today as alternatives (Table 2). BAL is unstable,

**Table 2. Clinically Important Chelating Agents. Acute Toxicity Is Illustrated by Representative $LD_{50}$-Values Selected from the Large Published Database**

| compound | species | administration route | $LD_{50}$ | ref |
|---|---|---|---|---|
| $CaNa_2EDTA$ | mouse, rat | ip | 4−6 g/kg | 72, 105 |
| $CaNa_3DTPA$ | mouse, rat | ip | 2−4 g/kg | 72, 105 |
| BAL | mouse | ip | 90−180 mg/kg | 106, 107 |
| DPA | mouse | ip | 337 mg/kg | 105 |
| DPA | rat | oral | >1.2 g/kg | 108 |
| DMSA | mouse | oral | 4.34 g/kg | 109 |
| DMSA | mouse | ip | 2.48 g/kg | 110 |
| DMPS | mouse, rat | ip | 1.1−1.4 g/kg | 110, 111 |
| DFOA | rat | oral | >1 g/kg | 112 |
| DFOA | rat | iv | 520 mg/kg | 112 |
| L1 | mouse, rat | ip | 0.6−1 g/kg | 113, 114 |
| TETA | mouse, rat | oral | 1.6−2.5 g/kg | 115, 116 |
| PB (AFCF) | mouse | oral | >5 g/kg | 117 |
| PB (IPB) | rat | ip | 1.13 g/kg | 118 |

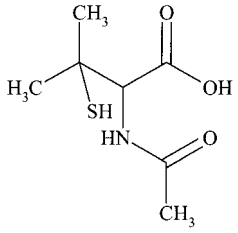
susceptible to oxidation, and therefore difficult to store as a ready-for-use preparation. It has a low therapeutic efficacy in most cases, and due to its high toxicity, BAL is suited only for brief treatment of acute intoxications.

It can be administered only by intramuscular injection. Due to BAL's lipophilicity, it is normally injected im in peanut oil. Preceding local anaesthesia is necessary, as the administration is very painful. A considerable fraction of treated individuals experience unpleasant side effects including nausea, vomiting, sweating, high fever, hypertension, and tachycardia. Due to the advent of the more efficient and safe drugs DMSA and DMPS, the clinical uses of BAL could now be phased out.

Extensive research in the pharmacokinetics of BAL took place in the decades after its first use, and a comprehensive description is given by Catsch and Harmuth-Hoene.[72] The absorption from the site of injection is rapid and complete, and BAL is apparently distributed into the intracellular space. The major part of the dose is rapidly excreted in urine. Studies using $^{35}S$-labeled BAL indicate that the major fraction of BAL-derived S is excreted as dithiols and glucuronides.

## B. DPA and *N*-Acetyl-D-penicillamine (NAPA)

DPA and NAPA (**14**) are both suited for oral chelation treatment. DPA can, however, also be administered by intravenous infusion. The intestinal




**14**

absorption of DPA in rats and humans is about 50%. The volume of distribution is close to that of extracellular water, and a major part of a systemic dose forms mixed disufides with serum albumin. The metabolism is insignificant, and the major part of a systemic dose is rapidly excreted in urine as free DPA or the oxidized dimer.[74−77] The metabolic behavior of NAPA is similar to that of DPA.[78]

The toxicity of oral and parenteral DPA is very low (Table 2). The clinical use of DPA is, however, limited by side effects which develop in a considerable fraction of patients during continued use, mainly hypertension, nephrotic syndrome, and various autoimmune reactions.

## C. EDTA and DTPA

The pharmacokinetics and toxicity of EDTA and DTPA were comprehensively reviewed by Catsch and Harmuth-Hoehne,[72] and below is a brief summary of pertinent and new data. EDTA and DTPA are poorly absorbed in the GI tract (<5%), and are most conveniently administered by slow iv infusion of their calcium or zinc complexes. Their volumes of distribution are close to that of extracellular water, and both chelators are rapidly excreted in the urine without significant metabolism. These chelators form stable complexes with many metal ions, including those of most essential metals. During continued exposure, trace element depletion may occur, especially for Zn, Cu, and Mn as shown for EDTA by Ibim et al.[79] Zn depletion is the cause for the teratogenicity of EDTA, which is readily reversed by coadministration of zinc.[80] In chelation treatment, the monocalcium salts of EDTA and DTPA are used to avoid hypocalcemic tetani. $ZnNa_3DTPA$ may alternatively be used. Extensive zinc binding is most likely involved in the acute toxicity of $CaNa_2EDTA$; thus $Zn_2EDTA$ is more than 1 order of magnitude less toxic than $Ca_2EDTA$, which is a factor 20× less toxic than the tetrasodium salt. Acute and subacute exposure to EDTA and DTPA induces dose- and time-dependent proximal tubular damage and degenerative changes in small intestinal mucosa. The nephrotic lesions are normally completely reversible. However, in the early clinical use of EDTA, deaths due to renal damage have occurred due to infusion of EDTA rather than the calcium complex.

## D. TETA

The normal administration route for TETA is oral. The absorption is, however, apparently poor, as less than 20% of an oral dose of $^{14}C$-labeled TETA to rats was recovered in carcass and urine. After iv administration, one-half the dose was rapidly excreted in urine and the cumulative fecal elimination was about 20%, indicating biliary excretion.[81] Kodama et al.[82] recovered only about 1% of an oral dose of TETA given to human volunteers as free TETA in the urine, the major part was excreted as a acid labile conjugate, subsequently identified as 1-acetyl-TETA.[83]

The acute toxicity of TETA is low (Table 2). In a study of the subacute and chronic toxicity of orally administered TETA, Yanagisawa et al.[84] calculated the threshold of toxicity to be close to 50 mg/kg/day in the female rat and less in the male rat. The recommended dosage to Wilson's disease patients is 0.75−2 g/day, which is quite close to a potentially

toxic dose. On the basis of experience with the long-term use of TETA in Wilson's disease patients, this chelator is remarkably free of side effects compared to DPA.[85]

## E. DMSA and DMPS

Both these chelators are available as tablets for oral administration and are stable for a long-time at room temperature, and DMPS is also available as a dry preparation for parenteral administration after hydration. In China, DMSA has been administered parenterally to hundreds of patients.[10] Both these drugs are absorbed to some degree in the intestinal tract (DMPS, 50−60% in dogs;[86] DMSA, Dart et al.[87] found up to 40% urinary excretion within 16 h of an oral dose of DMSA in humans). Our knowledge about the pharmakokinetics and metabolism of these two compounds is primarily due to the extensive work of Aposhian's group. The distribution volume for both drugs is predominantly extracellular; however, DMPS also has some intracellular distribution.[88,89] As the renal tubular excretion of DMPS is blocked by *p*-aminohippuric acid and probenicid, its excretion is conceivably mediated by carrier-mediated transport.[90]

The primary route of excretion is urinary with plasma and whole-blood half-lives and urinary elimination half-time of less than 4 h in humans for DMSA[91,87] and slower excretion of DMPS, with blood and plasma half-lives of 9−10 h.[92]

After an oral dose of DMSA to humans, more than 95% of the blood content is covalently bound to proteins, mainly to albumin.[91] More than 90% of urinary DMSA is excreted as the DMSA−cysteine mixed disulfide.[93] Also, DMPS is mainly bound to albumin in serum; however, as opposed to DMSA, the urinary excretion products after oral administration of DMSA to humans are various acyclic and cyclic homopolymers of DMSA, whereas a mixed disulfide with cysteine is almost completely absent.[94]

The acute toxicity of both compounds is low (Table 2). To date, only one case of DMSA overdose has been reported:[95] A 3-year-old girl ingested succimer capsules corresponding to 2.4 g of DMSA or 185 mg/kg of b.w. Extensive clinical evaluation failed to indicate signs of intoxication. During the last two decades, a large number of patients have been treated with DMSA, with a very low frequency of toxic side effects necessitating discontinued treatment. Adverse reactions during treatment with DMSA include gastrointestinal discomfort, skin reactions, mild neutropenia, and elevated liver enzymes.[96] Similar side effects have been noted for DMPS, which seems to be tolerated better in relation to gastrointestinal symptoms but may cause hypotension.[97] For both compounds, symptoms may subside, allowing continued therapy. For DMSA, two serious reactions to therapy have been reported: Grandjean et al.[98] discontinued DMSA chelation of a man with chronic lead intoxication due to a strong mucucutaneous reaction to the drug. A 45-year-old black male developed hemolytic anemia during DMSA chelation due to occupational lead intoxication. After cessation of treatment, hematological values normalized. The patient was glucose-6-phasphate dehydrogenase deficient, a genetic trait known to contraindicate BAL chelation due to risk of hemolysis.[99]

## F. Prussian Blue

The possible need for extensive, long-term human use of Prussian Blue (PB) due to nuclear facility accidents and radioactive environmental pollution make data on chronic toxicity of PB important. During the clinical use of PB, it is confined to the gastrointestinal tract and any toxicity would be suspected to be local or due to interference with trace element kinetics. The chelating properties of PB depend on the synthesis and further preparation. PB occurs in various chemical forms with different physical properties, **15**.

$KFe(Fe(CN)_6)$
Potassiumferrichexacyanoferrate, colloidal soluble PB, SPB.

$Fe_4(Fe(CN)_6)_3$
Ferrichexacyanoferrate, insoluble PB, IPB

$NH_4Fe(Fe(CN)_6)$
Ammoniumferrichexacyanoferrate

**15**

The acute toxicity of PB is very low (Table 2). In his review on PB toxicity, Pearce[100] concluded from available experimental animal and human data that PB is without any important toxic effects, even in long-term use. A considerable number of long-term feeding studies in several species did not reveal an adverse effect of PB added to drinking water or feed. Only one study, which is a summary of several previous experiments not offering the original data,[101] found effects on weight and hematological and renal status in rats exposed to 5% PB in feed.

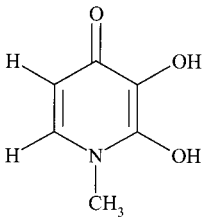
## G. DFOA

DFOA is poorly absorbed in the gastrointestinal tract and must therefore be administered by iv infusion or injection. Its distribution volume is extracellular, and the protein-binding in plasma is low, less than 10%. Its renal excretion is biphasic with the slow half-life being about 6 h. The acute toxicity is rather low (Table 2), and iv infusion is safe if care is taken not to administer the dose rapidly which can result in hypotension. However, a wide range of side effects have been noted during continued use in iron-overload patients including ophthalmic and auditory toxicity, bacterial and fungal infections, changes in blood histology, allergic and skin reactions, and pulmonary, renal, and neurological effects.[102]

## H. 1,2-Dimethyl-3-hydroxypyrid-4-one (L1)

The advent of L1 **16** has offered an alternative to DFOA in the treatment of transfusional Fe overload in thalassemia, due to its low price compared to DFOA, the possibility of continued treatment of patients not tolerating DFOA, and it can be admin-

istered orally. Also, in Al overload and acute Fe



16

intoxications, L1 is a promising new antidote. L1 is rapidly absorbed in the gastrointestinal tract and normally appears in serum a few minutes after oral administration. The main excretion route is via kidneys, with a half-life of 47−134 min.[103,104] The recovery from urine is close to 100%; the main species are free L1, the Fe and Cu complexes, and the glucuronide.

The acute toxicity of L1 is somewhat lower than that of DFOA (Table 2). The present clinical experience (up to 5 years treatment of iron overload patients) indicates a number of side effect, e.g., gastric discomfort, increase in antinuclear antibodies and rheumatoid factors, zinc depletion, transient agranulocytosis, or transient musculoskeletal and joint pain. Some patients suffering side effects have received continued treatment with lower, more frequent doses (reviewed by Kontoghiorghes[102]).

## *10. Treatment of Metal Intoxications*

### A. Aluminum

Aluminum is the most common metal in the Earth's crust. Aluminum in water normally occurs as solvated $Al^{3+}$ ions. As this complex is hydrophilic and has a very low ligand exchange rate, the reactivity and membrane passage of Al is minimal. As a consequence, the diffusional intestinal Al absorption is very low and Al and its salts normally have very low toxicity. This allows use of aluminum in kitchen utensils and in over-the-counter antacids. The intake of Al as antacids may occasionally amount to several grams per day, apparently without toxic effects. $Al^{3+}$ forms a citrate complex that is rapidly and much more efficiently absorbed in the intestines than solvated $Al^{3+}$, conceivably due to citrate-mediated increased paracellular transport.[119] This may lead to extensive, potentially toxic, systemic Al exposure if antacids are taken with juice or other citrate-containing beverages.

Care was not taken in the past to prevent systemic exposure of patients to Al during haemodialysis in chronic renal failure or during total parenteral nutrition. This led to development of serious neurodegenerative or bone disease in some patients.[120] The medical technologies have been accordingly changed, i.e., use of reverse-osmosis treated water, avoidance of Al-containing phosphate binders and antacids, and citrate-treated blood products for renal patients. Also, extensive care is taken in avoiding Al contamination of preterm infant nutrition formulas and total parenteral nutrition solutions.

Besides iatrogenic exposures, long-term extensive occupational exposure to Al may lead to development of neurobehavioral toxicity.[121,122] The possible involvement of Al in various neurodegenerative diseases such as Alzheimer's disease, amyotrophic lateral sclerosis, and Parkinson's dementia is a matter of debate, and some data indicate that such patients may benefit from chelation treatment increasing Al excretion.[120,122,123]

Even though improved medical treatment reduced the incidence of dialysis encelophaty, end-stage renal disease patients are still at risk for developing osteomalacia, adynamic bone disease, or microcytic anemia due to the compromised or absent renal excretion of Al from, e.g., the diet. The most common treatment of such Al overloaded patients is chelation with DFOA, which apparently enhances the excretion of vascular and well perfused extracellular Al pools. Animal experimental data indicate that DFOA chelation decreases bone and brain levels of Al.[124,125] Apparently, DFOA does not have access to brain deposits of Al,[126] and it is presently not known whether DFOA can interact directly with bone Al. Possibly, DFOA could mobilize these Al depots indirectly by reversing the net flux of Al to go from brain and bone, instead of to bone and brain.

The common therapy involves intravenous infusion of DFOA during the last period of dialysis, leading to a rapid increase in blood Al, which remains high until the subsequent course of hemodialysis, where a significant fraction of blood Al is removed. Thus, this treatment conceivably mobilizes tissue Al, mainly from bone.[127] Alternatively, extracorperal chelation with immobilized DFOA in a device attached to the dialysis apparatus may be applied.[128]

DFOA is not an ideal chelating agent because it can only be administered parenterally and due to the frequency of side effects. The hydroxypyridones can be administered orally and were initially developed as alternatives for iron chelation by DFOA.[102] These compounds have subsequently been studied as prospective Al chelators: Several hydroxypyridones efficiently enhanced the excretion of Al in animal studies,[125,129] however, their toxicity prevented clinical use. In limited clinical trial, oral administration of L1, the most promising of these compounds, efficiently enhanced Al excretion in renal dialysis patients.[130]

In conclusion, different forms of chronic Al exposure still cause severe human toxicity, and due to the toxicity of the most efficient Al chelators, an ideal chelation treatment is presently not available. Accordingly, studies to improve the possibility of Al chelation are needed in the future.

### B. Antimony

Antimony and its compounds are used in alloys, pigments, pesticides, and drugs for the tropical disease Schistosomiasis; the classical drug is "tartar emetic", antimony potassium tartrate which due to its extensive gastrointestinal toxicity is administered by iv infusion. In 1954, Friedheim et al.[131] introduced the antimony(II)−DMSA complex as antischistosomiasis drug. Human intoxications are very rare;

however, an oral acute poisoning in a child was treated successfully with DMPS[132] and two patients mistakenly given an extensive dose of tartar emetic iv instead of sucrose survived after DMSA chelation.[133] Lauwers et al.[134] treated four men orally intoxicated with a modest dose of tartar emetic with BAL; one of the patients with a history of heart disease died, while the three remaining survived. In animal experiments with various antimony compounds, DMSA was consistently more efficient than BAL in reducing mortality; in several experiments BAL was even without antidotal effect (reviewed by Ding and Liang[10]). A study of 16 chelating agents in mice showed that few had antidotal potency against ip potassium antimonyl tartrate administered at $>2\times LD_{50}$.[135] The most effective antidotes were DMSA, while BAL and $CaNa_2EDTA$ were not effective as the antidotes. As DMSA is less toxic than DMPS (Table 2), it is should be chosen as antidote in acute antimony intoxication.

## C. Arsenic

Arsenic compounds have been used medically for centuries, but most arsenic-containing drugs have now been phased out in almost all countries. Present industrial uses are, e.g., in dyes, alloys, semiconductors, and pesticides, the latter use causing frequent acute intoxications in the developing countries.

For most metals, only few case reports on chelation treatment of acute intoxications have been published. For As, a considerable amount of material is available, however, it does not offer a basis for future treatment recommendations, which still rest on results of animal experiments: The classical antidote in human poisonings with various arsenic compounds is BAL but DPA has also been used. Several research groups have, however, demonstrated that DMPS and DMSA are more efficient than BAL and DPA in experimental animals including mice, rats, and rabbits.[10,110,111,136−139] DPA was virtually without antidotal effect, and BAL was more than 1 order of magnitude less efficient than DMPS and DMSA. Also, DMPS and DMSA were superior to BAL in treatment of the systemic toxicity of percutaneously administered Lewisite.[140]

Schäfer et al.[141] demonstrated that organ As depots after injection of $AsO_3$ into mice could be mobilized by oral administration of DMPS or DMSA without increasing the brain deposition; however, oral administration of BAL extensively increased the brain deposition.

BAL and DPA are, however, still used in human intoxications. The major reason is probably that DMSA and DMPS are only slowly becoming generally available. In one mass poisoning in Argentina,[142] 718 individuals were poisoned by sodium arsenite added to meat by vandals. The authors mention that the supplies of BAL were very low, so only patients with high urinary levels of As were given BAL im, 10 or 16 doses of 2 mg/kg, when u-As was >0.75 mg/L (49 patients) or >5 mg/L (12 patients), respectively, administered over 10 days. None of the patients reported symptoms related to arsenic toxicity at 1 month and 2 years after the incidence.

In one suicidal case, a man ingested 2 g of $As_2O_3$. He was initially treated with gastric lavage and administration of charcoal. As his condition was rapidly deteriorating, he was transferred to another hospital, and 21 h after the ingestion of arsenic, chelation therapy was started. The patient survived after oral administration of $4 \times 300$ mg of DMSA per day for 3 days, even though he had clinical signs of polyneuropathy. The urinary As elimination was 27.03 mg during the 3 days of chelation. After cessation of DMSA chelation, u-As decreased extensively. Due to the very low toxicity of DMSA, it could have been administered for a much longer time period.[143]

A 22-month-old child ingested about 0.65 g of sodium arsenate. She was pale and lethargic when hospitalized with gastrointestinal symptoms and initially treated by gastric lavage and im BAL. The next day she was treated with DPA, then from day 8 she was given DMSA. For 12 days, the 24 h u-As declined from almost 5 mg/L to below 0.1 mg/L. The patient was discharged without symptoms.[144]

A 20-year-old male drug addict drank a herbicide solution containing about 80 g of monosodium methanearsenate to commit suicide. After extensive vomiting, he was admitted to intensive care in a state of shock with early liver and renal involvement. He was treated with DMSA, 30 mg/kg/day for 4 periods of 5 days over 30 days. In this period the s-As declined from more than 2.8 mg/L down to 6 $\mu$g/L and the u−As from almost 80 mg/L to 20 $\mu$g/L. While kidney function was normalized, elevated s-transaminases indicated liver damage, most likely due to a subsequently diagnosed chronic hepatitis.[145]

Due to food contamination with $As_2O_3$, 117 individuals with gastrointestinal symptoms typical of acute As intoxication were all treated with oral or parenteral DMSA for 6 weeks. Several patients developed neuritis, liver or cardiac toxicity symptoms. All patients recovered.[146]

A 23-year-old male ingested about 1 g of $As_2O_3$. Gastrointestinal symptoms did not start until after 7 h. During the next 5 h, he drank 5 L of water. As the major symptoms had subsided when he was admitted to the hospital 20 h later, the only treatment instituted was BAL chelation.[147]

Suicidal ingestion of about 10 g of sodium arsenate resulted in cardiovascular collapse, anuria, and hepatic damage. Immediately after hospital admission, the patient was treated with gastric lavage, oral charcoal, supportive measures, and hemodialysis. The next day hemodialysis was repeated and 250 mg of BAL was administered im. The hemodialytic As clearance was not affected by BAL chelation. The patient was discharged after 15 days.[148]

Three workers in a chemical factory developed mild symptoms of As intoxication after exposure to Vinyzene, 10,10′-oxydephenarsine. All three were discharged without symptoms after a few days BAL chelation.[149]

A 33-year-old woman ingested a dental paste containing about 1.8 g of $As_2O_3$. She rapidly developed gastroenteritis. The patient was treated with

hemodialysis and chelated with DMPS and recovered without complications.[150]

Two men (19 and 21 years) ingested approximately 1 and 4 g, respectively, of $As_2O_3$ in the belief that the white powder was a narcotic. On hospital admission, both had gastroenteritis and one had acute renal failure. Both were chelated with DMPS and recovered without renal or neurological symptoms.[151]

The case reports reviewed above describe several survivals, apparently without sequelae, despite ingestion of extensive doses of organic or inorganic arsenic compounds, several times the lethal doses. Due to the delay and low intensity of the chelation treatment in some of the cases, survival is likely to be due to supportive treatment.

## D. Beryllium

Beryllium is used in the electronic and the air and space industry. Its salts are strong immunotoxins. Only little is known about the possibility of chelation treatment of beryllium intoxication. One experimental study in rats indicates that chelation treatment with DMPS after repeated dosing with $Be(NO_3)_2$ enhanced the fecal excretion and decreased the taget organ concentration of Be. Further, histological and biochemical indicators of Be-induced tissue damage were decreased. DMSA was also efficient but less than DMPS.[152]

## E. Bismuth

Bismuth salts have been used in medicine for centuries. Before the advent of antibiotics, im injection of bismuth salts alone or in combination with organic arsenic compounds were used to treat syphilis. Previously, extensive doses (several grams of bismuth salts per day) were occasionally taken against various gastrointestinal diseases, resulting in about 1000 cases of encephalopathy, 70 fatal, during a 5-year period alone in France.[153] The presently important medical use of bismuth is treatment of ulcers due to *Helicobacter pylori* infections in the gastric mucosa with DeNol (tripotassium dicitratobismuthate, colloidal bismuth subcitrate, CBS). Even though the daily dosage has been significantly reduced due to the previous frequent intoxications, CBS may still cause systemic toxicity (renal failure, encephalopathy) due to continued use for extended time periods or accidental or deliberate overdose.[154] The intestinal absorption of CBS was calculated to be about 0.35% in rats, while only 0.08% absorption was found for basic bismuth salicylate.[155] Humans taking CBS had higher p-Bi than humans receiving basic bismuth salicylate.[156]

After a single parenteral administration of a lethal dose of $Bi(NO_3)_3H_2O$ to mice, DMSA and DMPS effectively protected against mortality.[157] Further, DMSA reduced the deposition of bismuth in liver and kidneys. In a study in rats loaded with a sublethal CBS dose by multiple injections, ip administration of BAL, DMPS, or DMSA mobilized Bi from various organs, BAL even from the brain, while DFOA, EDTA, and DPA were rather inefficient and EDTA even increased the brain deposition of Bi. Due to the high toxicity of BAL, this study indicates that DMPS would be the chelator of choice for treatment of human Bi intoxication.[158]

Only a few cases of chelation treatment of humans exposed to bismuth are reported. BAL, DPA, DMPS, and DMSA have been used. According to Molina et al.,[159] two patients with Bi encephalopathy recovered rapidly after BAL chelation. In other case studies, BAL was considered without beneficial effects.

A 68-year-old patient with chronic renal failure developed encephalopathy after he, by mistake, took twice the prescribed daily dose of DeNol liquid to treat gastrointestinal bleeding for two months. The patient was chelated with oral DMPS for 10 days, and during this period, the renal Bi clearance was increased about 10 times compared to the clearance before and after chelation. The patient completely recovered from the cerebral dysfunction.[160]

A 21-year-old man was hospitalized in an anuric state 48 h after taking 50−60 DeNol tablets corresponding to about 7 g of bismuth. He was initially treated im with BAL and activated charcoal and orally with poly(ethylene glycol) and subsequently dialyzed. Daily dialysis was continued for 6 days simultaneously with repeated iv infusions of DMPS for 4 days, followed by oral administration of DMPS for the next 14 days. Urine production resumed on day 8. While BAL treatment failed to mobilize bismuth to the dialysate despite a blood level of 590 $\mu$g/L, iv DMPS combined with dialysis led to extensive mobilization of Bi into the dialysate and rapidly reduced blood Bi to below 50 $\mu$g/L. The mobilization of Bi during dialysis and DMPS chelation amounted to almost 5 mg of Bi during the first 4 days.[161]

Taken together, the limited available data indicate that DMPS is an efficient antidote in even very severe acute Bi intoxication; however, Bi intoxication could be a type of intoxication, where BAL may have a role in the future.

## F. Cadmium

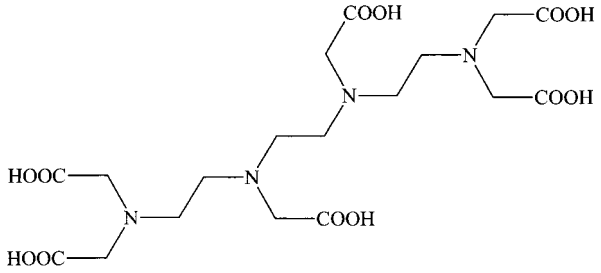
Cadmium is used in pigments, plastic stabilizers, electroplating, alloys, batteries, and the nuclear industry. Acute human intoxications with cadmium are due to suicidal oral intake of cadmium compounds, cadmium contamination of food items, or inhalation of cadmium fumes after accidental welding or flame cutting cadmium-coated items. While such poisonings have become rare, health effects due to chronic low-level cadmium exposure are still a problem in some industrial situations and areas with high environmental cadmium pollution,[162] creating a clinical need for chelation of aged cadmium deposits in the liver and kidney.

There is extensive literature on animal experiments, both to optimize treatment of acute cadmium intoxication and to develop chelating agents capable of mobilizing aged cadmium depots to prevent or alleviate chronic toxicity. The problem in cadmium chelation is the special toxicokinetics of cadmium, which, after systemic entrance, is rapidly localized intracellularly, mainly in the liver, then bound to metallothionein (MT), and subsequently slowly transferred as the MT complex via blood to be deposited

in proximal tubular cells in the kidney. This is the background for the toxicity of chronic cadmium exposure leading to urinary loss of filtrable proteins, calcium, and other small molecules, which may in turn lead to development of osteomalacia,[162] and also for the rapid decline in efficacy of treatment of acute cadmium intoxication with hydrophilic chelators demonstrated by Eybl et al.[163] and confirmed several years later by Cantilena and Claassen.[164]

Effects of a large number of chelating agents belonging to several chemical compound groups have been investigated. In the majority of acute toxicity studies in experimental animals, both cadmium and chelator were injected, reducing the relevance in relation to acute human intoxications. In brief, hydrophilic dithiol compounds and polyaminopolycarboxuylic acids most effectively reduced mortality in such injection studies and also enhanced excretion of cadmium, if the chelator was administered rapidly after cadmium.[10,105,165−167] Already in 1955, BAL was shown to potentiate the toxicity of Cd in rabbits by increasing cadmium-induced weight loss, anemia, and proteinuria.[22] Together, the cited studies demonstrate that DMSA has some effect but that DTPA is especially an efficient antidote during the special experimental condition of injecting both metal and chelator and that BAL is contraindicated in human Cd intoxication.

We developed an experimental model relevant for acute human oral metal intoxication.[73,168] Oral administration of chelating agents reduced mortality, histological tissue damage, and intestinal cadmium absorption after oral administration of a highly toxic cadmium dose.[53,54] Similar results were obtained by Basinger et al.[169] Also, parenteral administration of chelators reduced the toxicity of oral cadmium.[54] The combined data indicate that after oral administration of highly toxic doses of cadmium, oral administration of DMSA efficiently reduced toxicity and intestinal cadmium uptake while ip-administered DMSA had only a marginal antidotal effect. Also, DMPS was an efficient oral antidote. Oral administration of the disodium salt of DTPA enhanced survival and extensively reduced intestinal absorption of orally administered cadmium. However, ip injection of the monocalcium salt of DTPA very efficiently reduced oral cadmium toxicity. Also, triethylenetetraminehexaacetic acid (TTHA; **17**) was a highly efficient antidote toward oral cadmium toxicity.[54] On the basis

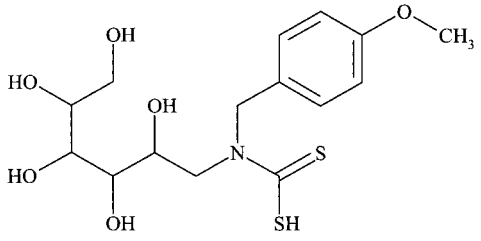



**17**

of these experimental data, the optimum chelation treatment of acute oral human cadmium intoxication using chelators accepted for clinical use would very likely be oral administration of DMSA combined with parenteral administration of Ca−DTPA.

The development of chelating agents capable of mobilizing aged cadmium depots has previously been reviewed by Jones[170] and Jones and Cherian.[171] Originally, this work was initiated by the demonstration that BAL[172,173] and diethylthiocarbamate[35] could promote the excretion of aged body depots of cadmium, presumably by a combination of intracellular action and mobilization of MT-bound cadmium. However, DDC enhanced the brain deposition of injected[36,37] or orally administered[47] cadmium and increased the acute toxicity of oral cadmium.[46] It is therefore clear that neither BAL nor DDC have a role as antidotes in acute cadmium intoxication.

On the basis of these initial cadmium mobilization studies, Jones and Singh and their research associates in several laboratories have developed chelating agents that during prolonged parenteral administration are capable of mobilizing a considerable fraction of aged cadmium depots from the liver and kidneys without increasing the brain deposition of cadmium or enhancing cadmium toxicity. Only a few of the many articles are cited below. Series of compounds have been synthesized and tested for mobilizing efficiency based on the molecular structures of diethyldithiocarbamate and BAL, respectively. Effective compounds are monoalkylesters or monoalkylamides of *meso*-2,3-dimercaptosuccinic acid[174−176] and amphipathic carbodithioates as, e.g., *N*-(4-methoxybenzyl)-D-glucamine carbodithioate[177] (**18**) and N-(4-methoxybenzyl)-4-0-(*β*-D-galactopyranosyl)-D-glucamine carbodithioate[178] (**19**). Of interest, monoisoamyl-

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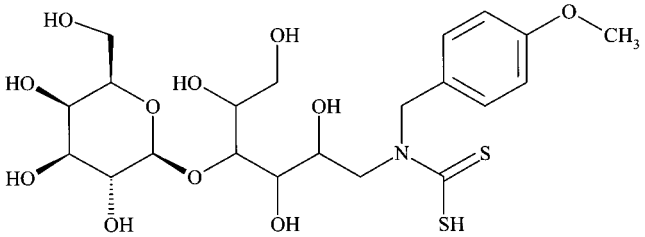


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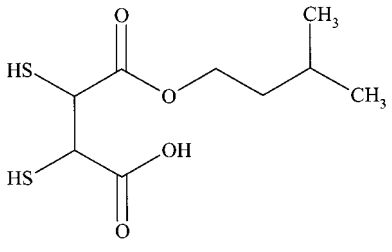
DMSA (**20**) was effective after oral administration.[174]

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Continued studies of mobilizing effects of carbodithioate derivatives on aged cadmium depots demonstrated that *N*-aryl-4-*O*-(β-D-galactopyranosyl)-D-glucamine-*N*-carbodithioates in general are very efficient, especially with the aryl groups benzyl[179] or 4-methylbenzyl.[180,181] The supposedly intracellular action of DMSA esters is conceivably mediated by active transport, as inhibitors of organic ion transporters reduced the mobilization of Cd by these compounds.[175,182]

A general problem with these compounds is their toxicity, presently precluding human application. However, even though these compounds presently have been used only in experimental animals, the most promising types could well lead to development of clinically useful chelating agents in the future. A major problem in chelation mobilization of cadmium in humans will be, however, that the persons eligible for chelation due to large hepatic and renal cadmium depots will most often suffer from only marginal renal damage (reduced proximal tubular reabsorption capacity leading to increased urinary calcium loss) which is not a serious condition by itself but could eventually lead to development of more severe renal disease and osteomalacia. The reversibility of this condition and thereby the potential usefulness of mobilization of aged cadmium depots by chelation is a matter of debate.[162] Accordingly, such chelation treatment must be very safe and without any side effects to be ethically acceptable. This would require prohibitively expensive toxicity testing, possible only under conditions comparable to those of the Orphan Drug Act.[183]

## G. Cobalt

Cobalt is mainly used in pigments and alloys. Previously, the use of cobalt sulfate in beer led to cobalt-induced cardiomyopathy. Acute intoxications are presently rare, but industrial exposures may lead to allergic dermatitis or asthma.

Intravenous or intraperitoneal administration of EDTA, DTPA, or DMSA rapidly after ip administration of cobalt chloride to mice reduced mortality as well as the levels of cobalt in various organs[184−186] while BAL enhanced the toxicity of cobalt.[186] In these studies, EDTA and DTPA were more efficient antidotes than DMSA. It is difficult to advance a chelating agent for acute human cobalt intoxication as long as animal experiments with a more relevant cobalt exposure route than the parenteral are not available and clinical experience is absent.

## H. Copper

The present major use of copper is in electrical equipment. Previously, the use of copper for kitchen utensils could led to extensive exposure. The major cause for chronic copper intoxication is Wilson's disease, originally treated by chelation with DPA; since 1973, TETA has become the drug of choice in the western world[7−9] (see chapter on Wilson's disease).

DPA, TETA, DMSA, and DMPS were efficient antidotes toward acute intoxication due to injection of copper salts into mice,[10,187] DMPS being the most efficient antidote. Further, these chelating agents reduced copper-induced hemolysis of human red blood cells in vitro.[188] Studies in several species have demonstrated increased urinary Cu excretion after administration of TETA or DPA to normal or cupper-loaded animals. Also, oral administration of DMSA increased the urinary copper excretion in rats injected with copper.[189]

In China, hundreds of patients with Wilson's disease have been treated with oral DMSA over the last 30 years, resulting in clinical improvement and increased urinary Cu excretion. Even in late stages of the disease, DMSA treatment was associated with improvement of clinical features and laboratory parameters.[190]

Only few cases of chelation treatment of acute human Cu intoxications have been reported: A 42-year-old male ingested about 250 g of crystalline $CuSO_4$ in a suicide attempt. He arrived at the hospital vomiting, fully alert. Chelation with im BAL was started immediately. After 10 h, vomiting subsided and activated charcoal and magnesium sulfate were given, and oral DPA chelation was initiated. After 3 days, blood levels of liver enzymes indicated acute liver damage. The patient recovered completely.[191]

An 86-year-old woman accidentally drank a solution of 3 g each of copper and zinc sulfate ("Eau de Dalibour", used in diluted form to treat skin ulcers). She was admitted vomiting, and soon after she delivered watery diarrhea. Endoscopy revealed an erythematous gastric mucosa with areas of bleeding. After gastric lavage and intravenous fluid to correct for hypovolumenia, chelation treatment was initiated with im BAL and DPA via nasogastric tube. The next day the patient became hypotensive. Fiber-optic bronchoscopy on day 3 revealed inflammation and ulceration in the bronchial tree. Due to deteriorating respiratory function, mechanical ventilation was instituted. During the following days, renal function deteriorated. From day 11, the patient started to recover. The authors could not conclude that chelation increased zinc and copper excretion, as the fecal excretion was not quantified.[192]

## I. Gold

Besides gold's use in jewelry, gold salts have medical importance in the treatment of rheumatoid arthritis. As the hitherto most used compound, gold thiomalate requires weekly im injections; a lipophilic preparation, auranofin, has been developed for oral use. Despite a high incidence of toxic side effects including dermatits, colitis, nephropathy, or hepatic damage, in some cases with a fatal outcome, gold treatment is still important in the management of rheumatoid arthritis.[193] In human cases of severe gold intoxication, chelation with BAL or DPA has traditionally been applied.[194] The previous high incidence of severe toxicity led to dosage reduction and careful monitoring of gold therapy on an individual patient basis. This has extensively reduced the incidence of side effects.

Experimental animal studies have demonstrated that DMSA and DMPS are efficient antidotes against

acute gold intoxication and efficiently reduce the toxicity of ip-administered sodium bis(thiosulfato)-gold(I)[194] or the nephrotoxicity of injected sodium thiomalate.[195] In these and other studies,[196−199] MSA, DMPS, and DPA significantly enhanced the excretion of gold after injection of gold compounds to rats or mice.

Unfortunately, neither of the cited studies compared the two chelators previously used in human gold intoxication, BAL and DPA, directly with DMSA and DMPS. The conclusion based on available data that DMPS and DMPA are more efficient antidotes and mobilizing agents than BAL and DPA in gold poisoning is therefore preliminary.

## J. Iron

Acute iron intoxication is among the most common childhood poisonings. According to the annual reports of the American Association of Poison Control Centers toxic exposure surveillance system, about 20 000 intoxications involving iron-containing medications in children are reported each year. Most of these are mild, e.g., due to ingestion of pediatric combined vitamin−mineral preparations and do not require chelation treatment; during 1983−1994, 6744 cases of DFOA treatment were reported, probably an extensive underestimation.[200] Even though development of more efficient methods of supportive care and the advent of chelation with DFOA has improved the possibility for treating severe iron poisoning, the frequency of such poisonings and the number of fatalities due to ingestion of concentrated iron supplements have increased.[201] The symptoms include gastrointestinal injury due to corrosive effects of iron salts, hypotention, metabolic acidosis, coagulopathies, and multiorgan failure. Treatment may include mechanical removal of remains of tablets, GI decontamination, careful management of body fluid volume, and chelation with DFOA. Clinical experience indicates that in acute iron intoxication, iv infusion of DFOA is the optimal and efficient route of chelation treatment.

Several clinical series of pediatric iron poisonings have been unable to demonstrate a beneficial effect of DFOA chelation treatment in mild iron intoxications. As adverse effects of DFOA treatment occur in some patients, objective criteria for chelation treatment or discontinuation of instituted chelation treatment would be useful. However, as the clinical and laboratory indicators of the severity of iron intoxication may be falsely negative, such criteria are difficult to establish. Acknowledging the risk for the patient associated with omission of chelation treatment, Mills and Curry[201] discussed the validity of these criteria and suggested that chelation treatment should be instituted based on one indication rather than on confirmed severe poisoning.

## K. Lead

Lead has widespread industrial uses in alloys, pigments, batteries, and many other applications. A major source of environmental lead pollution, organolead addition to automobile gasoline, has now been extensively reduced with a concomitant reduction in severe organolead poisonings due to gasoline sniffing and reduced general environmental lead pollution. However, because of lead's comparatively low melting point and high vapor pressure, industrial uses of inorganic lead may cause extensive local environmental pollution. Childhood lead intoxication has been and still is a serious problem in some groups of less affluent living in low-standard housing. The most important source of childhood lead exposure is lead paint in old houses, which has been estimated to involve more than 1 million children below 7 years of age alone in the United States, despite extensive efforts by the U.S. Centers of Disease Control to reduce childhood lead exposure.[202]

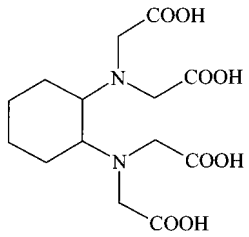
Epidemiological evidence indicates a risk of cognitive impairment in children at blood lead levels lower than 250 $\mu$g/L.[203−206] Accordingly, the U.S. CDC has revised the definition of childhood lead poisoning downward from 250 $\mu$g/L b-Pb to 100 $\mu$g/L.[202] The CDC lead update[207] indicates that 4.4% of children aged between 1 and 7 years are lead intoxicated according to this definition. Present recommendations by the U.S. Committee on Drugs[208] indicate a possible need for chelation treatment at b-Pb > 250 $\mu$g/L, at lower levels environmental intervention should be instituted. At b-Pb 250−450 $\mu$g/L, chelation should be instituted if aggressive environmental lead abatement does not lower b-Pb levels. At b-Pb > 450 $\mu$g/L, chelation treatment is indicated, preferably with DMSA, alternatively with EDTA. Besides these two chelators, BAL and DPA have been used as chelating agents in human lead intoxication.

As indicated by the brief summary above, environmental lead intoxication is the most common preventable childhood disease in the United States, and an important issue is whether the treatment should be abatement of lead pollution alone or abatement combined with chelation treatment. Accordingly, there is a need for solid knowledge about the optimal chelation treatment in subclinical lead intoxication and the beneficial effects to be expected. The majority of exposed children may be without clinical signs of lead intoxication discernible on an individual basis, yet they are at risk for developing irreversible neuropsycologic dysfunction. Present epidemiological evidence does not clearly prove whether chelation treatment with EDTA and abatement of lead pollution is more efficient in improving cognitive function[209] or reducing bone lead[210] than abatement alone. In 1991, DMSA was licensed in the United States for oral chelation treatment of children with b-Pb > 450 $\mu$g/L.[211] Several small studies and case studies indicate that DMSA effectively mobilizes soft tissue lead, in agreement with data from animal experiments. As DMSA has many advantages over BAL, DPA, and EDTA, a large, randomized trial of the efficacy of oral DMSA chelation treatment of young children with moderately elevated b-Pb would be very useful for deciding the future treatment of childhood lead poisoning. Such a study supported by the U.S. NIEHS and NIH has been initiated with enrollment of children between 1994 and 1996. Children eligible according to 200 < b-Pb < 440 $\mu$g/L and other

inclusion criteria were randomly allocated to placebo or DMSA treatment. The baseline data on this study have been published,[212] but we must await the final results for some time. In the meantime, the presently available data on effects of chelating agents in experimental lead intoxication and in acute and chronic human lead intoxication are reviewed below.

DMSA was more efficient than DPA in reducing tissue levels and enhancing the urinary excretion of lead in mice and rats, thus DMSA reduced the brain, bone, spleen, liver, and kidney levels of lead.[213,214] Also, DMSA reduced tissue and bone lead and enhanced urinary lead excretion and corrected lead-induced disturbances in porphyrin metabolism in rabbits.[215]

Injected DMSA and injected cyclohexanediaminetetraacetic acid (CDTA; **21**) were most efficient among 16 chelating agents tested as antidotes against acute parenteral lead intoxication in mice. In this

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study, injected DMPS, EDTA, and DPA did not protect against mortality, data for BAL are not given. DMPS was, however, more efficient than DMSA and several other chelators including EDTA in removing lead from brain and kidneys. EDTA had some effect on lead in bone and other organs, while DPA increased the brain lead level. In a limited chelation experiment, BAL reduced bone lead. EDTA and DPA were only marginally efficient after oral administration, while oral administration of DMSA significantly reduced liver, kidney, brain, and bone lead concentrations.[216]

When injected 15 min after injection of lead acetate, 9 tested chelators enhanced the 24 h urinary lead excretion, EDTA, DTPA, and CDTA, much more effectively than BAL, DMSA, and DMPS. DTPA, CDTA, and DMSA increased the 24 h fecal excretion of lead, while other chelators had marginal effect or even reduced the excretion.[217]

In acute parenteral lead intoxication in rats, injected EDTA and orally administered DMSA were equally efficient in increasing the erythrocyte $\delta$-aminolevulinic acid dehydrase activity and decreasing the urinary excretion of $\delta$-aminolevulinic acid toward normal levels. When administered together, these chelators were even more effective. In the same study, oral DMSA was more effective than injected EDTA in lowering blood, liver, kidney, and brain levels of lead, EDTA slightly increased the brain level. There was no difference in the urinary lead excretion. Again, combined chelation treatment was more effective in enhancing urinary lead excretion and reduction in organ lead levels, except in the brain.[28]

In a more recent dose−response experiment with the same dosing regime, similar results were obtained. The most important result in that study was an extensive and consistent reduction in brain and bone lead levels in groups treated with various combinations of both chelators or with one at a comparatively high dosage.[29]

In the studies reviewed above, chelation was initiated briefly after short-term lead administration. After prolonged exposure of rats to lead in drinking water, which is a more realistic dosage regimen in relation to occupational or environmental lead poisoning, repeated injections of DMSA resulted in extensive reductions in blood, brain, liver, and kidney lead levels; however, bone lead levels were not affected. Four months after cessation of DMSA chelation, blood and soft tissue lead levels had increased due to redistribution of bone lead.[25] In another experiment, parenteral EDTA chelation of rats previously exposed for several months to lead temporarily increased the brain level of lead, up to twice the prechelation level; a 5 day treatment did not decrease the brain lead level, even the blood, kidney, and bone lead levels were lower than in unchelated rats.[27] Intraperitoneal administration of 1−4 daily doses of DMPS to rats starting 4 days after 86 days of exposure to lead acetate in drinking water slightly reduced the brain lead concentration.[24]

Jones et al.[218] gave mice 10 or 20 ip injections of lead acetate over 12 or 26 days. Various subsequent parenteral chelation treatments over 4 or 8 days with EDTA, DTPA, or DMSA consistently reduced brain and kidney lead levels. Bone lead was mobilized by some treatments with EDTA or DMSA but less effectively than soft organ lead. The authors summarize available data on effects of DMSA and EDTA on brain lead levels and conclude that DMSA chelation reduces brain lead levels under all conditions hitherto examined.

Recently, Smith et al.[219] studied relationships between brain and blood lead levels during oral DMSA chelation treatment of rats exposed to lead in the drinking water for 30 or 40 days. Seven days of chelation reduced b-Pb much more than brain lead. Continued chelation for a total of 21 days did not reduce b-Pb further but did decrease the brain lead level compared to unchelated animals. This study indicates that the blood lead level, the hitherto most widely used parameter of the success of chelation treatment, does not predict an important outcome of the treatment, reduced brain levels of lead.

Together with the results of Flora et al.[28] and Tandon et al.,[29] these studies suggest that either oral DMSA alone or the combination oral DMSA and parenteral EDTA may be the most efficient chelation treatment presently available. Also, a widely used EDTA provocation test for diagnostic estimation of the lead body burden must be considered obsolete and dangerous for the patient, in agreement with a recommendation by the U.S. Committee on Drugs.[208] Also, the scientific basis and the validity of the results of the test have been seriously challenged.[220] The combined animal experimental data indicate, together with the human data reviewed below, that

DMSA preferentially mobilizes soft tissue lead and recently deposited bone lead depots whereas aged bone lead deposits are inaccessible to DMSA. As opposed to DMSA, EDTA is capable of mobilizing aged bone lead deposits; however, EDTA is less efficient than DMSA in mobilizing soft tissue lead.

An important question in management of lead intoxication is the possible enhancing or inhibiting effect of chelating agents on intestinal lead absorption. Using a single oral dosage of $^{203}Pb^{2+}$, whole-body $\gamma$-counting, and $\gamma$-counting of total urine collected in metabolism cages, Kapoor et al.[221] found that parenteral administration of EDTA, DMSA, DPA, or BAL immediately after administration of lead increased the intestinal lead uptake estimated as the 144 h postdosage whole-body retention plus the urinary lead output; however, the net retention of lead estimated as the whole-body retention was not affected by chelation, except for a slight increase in BAL-treated animals. Also, oral administration of the chelating agents immediately after the oral lead dosage did not increase the intestinal lead absorption. The whole-body retention was, however, extensively reduced in groups given EDTA or DMSA orally but not in groups given BAL or DPA orally.

Pappas et al.[222] studied effects of oral DMSA administration on urinary excretion of $\delta$-aminolevulinic acid and lead, blood zinc protoporphyrin levels, and organ levels of lead in rats exposed to lead in drinking water for 35 days, after cessation and during continuation of lead exposure. Even in rats still exposed to lead, DMSA reversed the hematological effects of lead, increased urinary lead excretion, and decreased blood, brain, kidney, liver, and bone lead levels.

Oral administration of DMSA to adult humans immediately after ingestion of 200 $\mu$g of the stable lead isotope $^{204}Pb$ decreased the fecal and increased the urinary output of $^{204}Pb$ and decreased the fraction of the dose recovered after the experimental period. In this situation, DMSA increased the intestinal lead absorption, at variance with the results of the animal experiments reviewed above. The study involved only four controls and four individuals in each of two DMSA dosage groups, and the differences were not statistically significant.[223] Until more unambiguous human data are available, caution is needed to ensure removal from lead exposure before DMSA chelation is instituted. After oral administration of cadmium or mercury to mice, oral administration of some chelating agents including DMSA and DMPS reduced intestinal metal uptake.[53−55]

In a behavioral study using a forced swim model in mice, chelation with DMSA for 7 weeks concomitantly with lead exposure in the drinking water exacerbated lead-induced behavioral anomaly.[224] A previous study had shown similar effects due to chelation of lead intoxication with EDTA,[225] but the enhancement of the neurotoxicity of lead could possibly be explained by a transiently increased brain deposition of lead induced by EDTA,[27] an explanation not at hand for the effect due to DMSA chelation. Both results, but especially that with DMSA, are disturbing: In the few studies and case reports on lead-exposed children or occupationally exposed adults, chelation reversed lead-induced behavioral anomalies and neuromotor impairment in some studies[98,226−229] but was without a definitive effect in others.[209] Also, in a further experimental study of lead-induced hyperactivity, DMSA chelation from 6 weeks after cessation of lead exposure alleviated lead-induced behavioral anomaly and reduced b-Pb in both sexes but more effectively in male than in female mice.[230] In a study of lead-induced behavioral hyperactivity during habituation, chelation with DMSA after cessation of lead exposure reduced lead neurotoxicity in the rat.[231]

A number of human studies of chelation treatment of chronic low-level lead poisoning are available. Most studies of effects of chelation focus on b-Pb lowering and correction of biochemical indicators of lead toxicity against the hematological system, while effects on neuropsychiatric symptoms are not evaluated quantitatively in a blinded fashion, except in few studies. Even though the present knowledge of effects of chelation on lead-induced neurotoxicity is based mainly on subjective evaluations of small groups or single cases, there is no doubt that chelation effectively alleviates the neurotoxicity of lead in severe poisonings of children or adults; the question is whether there is a beneficial effects of chelation on lead-induced neuropsychologic dysfunction in low-level lead intoxication of children. The TLC trial[212] will offer important data to answer this question.

A major problem in chelation treatment of chronic lead intoxication is the rebound of b-Pb upon cessation of chelation, which occurs after all the chelating agents used in lead intoxication. This rebound, which is conceivably predominantly due to mobilization of bone lead, may necessitate repeated chelation schedules.

Several small cohort studies and case studies of chelation treatment of lead-intoxicated humans have been published: Five lead-poisoned lead smelter workers were treated with increasing oral doses of DMSA (8.4−12.7 mg/kg up to 28.1−42.2 mg/kg at day 6). This treatment reduced the blood lead level by about 50%.[232]

Nine lead-poisoned workers were chelated with 30 mg/kg/day DMSA for 5 days, except two subjects started on lower doses, one because of a history of atopy and the other was treated for 15 days due a high initial blood lead level. The b-Pb levels were reduced by 35−81%, and the urinary lead excretion was on an average increased by 1 order of magnitude. Three weeks after the chelation treatment, clinical indices of lead poisoning had stabilized or improved in all patients.[233]

A chronic poisoning due to ingestion of lead-contaminated flour ground in a primitive flour mill was diagnosed by the $CaNa_2EDTA$ provocation test followed by oral DMSA challenge, resulting in an 11-fold increase in urinary lead excretion. A 5-day chelation treatment with DMSA corrected clinical lead poisoning symptoms, increased urinary lead excretion, and decreased b-Pb. Clinical signs of lead intoxication did not reappear during the several months of follow-up. This case suggests that oral

DMSA may offer a diagnostic provocation test in lead poisoning that is safer than the traditional EDTA test.[234]

A problem in chelation of lead intoxication with BAL or EDTA is management of lead-induced iron deficiency, as both BAL and EDTA form toxic iron complexes,[32,33] precluding iron therapy. Haust et al.[227] treated a severe occupational lead intoxication for several years with extensive oral doses of DMSA and simultaneously administered iron im, which increased the serum−ferritin to normal values without any toxic effect. Before DMSA chelation, therapy with BAL/$CaNa_2$EDTA and subsequently $CaNa_2$EDTA for 3 years to a total of 64.5 g had not improved the patient's clinical condition and his b-Pb was 900 $\mu$g/L at 4 years after cessation of lead exposure. He suffered from anorexia, colic, insomnia, and various neuropsychiatric symptoms.

At the time this case was reported, a total of 189 g of DMSA given during 6 courses in 141 days of chelation treatment over 2 years had mobilized about 375 mg of Pb. The urinary lead excretion was increased about 7 times, and the b-Pb was reduced to about 1/5 of the pretreatment values during the chelation courses but gradually rebounded to the pretreatment levels after each treatment. Also, subjective and clinical signs of lead intoxication were alleviated but gradually reappeared with the increased b-Pb after the chelation courses. As the lead body-burden of this patient was still immensely high, he is probably bound for repeated chelation for life.

A similar but less severe case was reported by Grandjean et al.[98] The b-Pb was initially reduced by a factor of about 10 by DMSA chelation but gradually increased after cessation of chelation. As the patient developed hypersensitivity to DMSA, chelation treatment was discontinued after three courses. The treatment apparently had a long-lasting effect both on b-Pb and on the patient's mental capacity.

Graziano et al.[220,235] treated groups of lead-exposed workers and children with DMSA chelation. In these studies, DMSA decreased b-Pb, increased lead diuresis, and corrected biochemical indices of lead toxicity. As the cumulative urinary lead excretion was extensively larger than the decrease in the blood lead store, DMSA chelation also mobilized lead from other tissues. The studies of Liebelt et al.[236] and Besunder et al.[237] confirm the efficacy of DMSA. Comparison of the intervention studies presently available of childhood low-level lead exposure cohorts, unchelated or chelated, indicate that lead abatement alone may reduce b-Pb and biochemical indicators of lead toxicity; the fall is, however, larger in groups also chelated by EDTA, BAL + EDTA, DPA, DMSA, or EDTA + DMSA; among these chelators, DMSA must be considered the most safe and efficient[235−242]

Taking into consideration the rather small differences in the lead mobilizing capacity of BAL, EDTA, and DMSA at relevant clinical dosage and that DPA is the least effective, the extensive differences in toxicity, and frequency of unpleasant side effects of these, DMSA being the least toxic and best tolerated, as well as the option of oral use of DMSA, this chelator is considered the first choice in low to moderate lead poisoning. Acute lead encephalopathy previously occurred frequently, most often due to oral intake of lead-based paint chips in infants, and led to very high mortality and chronic morbidity. For this intoxication, which is fortunately much more rare today, combined EDTA and BAL treatment has been advocated and extensively used originally based on Chisholm's[243] finding that the combination more effectively lowered b-Pb than either agent alone. The reason for the continued use of the BAL−EDTA combination is that the increasing effect of EDTA on brain lead levels should be counteracted by the soft tissue mobilizing effect of BAL. Today, this argument seems invalid, as DMSA, which does not increase brain lead, is available as an alternative to EDTA. Unfortunately, animal studies comparing the effect of BAL−EDTA combined chelation with DMSA chelation in severe experimental lead intoxication are, to my knowledge, not available. Such studies would show whether DMSA is the superior antidote in all kinds of lead intoxication.

## L. Manganese

Manganese is mainly used in alloys, dry batteries, and pigments. Acute exposure to manganese-containing dust may lead to chemical pneumonitis, chronic exposure to a Parkinson-like dementia. Experimental studies on antidotal and mobilizing effects of chelators in manganese intoxication are scarce. In a comparison of the effect of injection of various polyaminopolycarboxylic acids on the kinetics of injected $MnCl_2$ in the rat, all tested compounds efficiently increased the urinary Mn excretion and reduced the hepatic and renal Mn deposition, DTPA most efficiently but EDTA was also effective.[244] DMSA seemed to be without mobilizing effect on parenterally administered manganese in the rat.[245] Seven daily ip injections of DTPA or CDTA to rats starting immediately after a 1 h inhalation exposure to an aerosol of $MnCl_2$ mobilized extensive amounts of Mn to urine, while the excretion was almost nil in controls and rats exposed to DMPS. When the chelation treatment was postponed for 7 days, the mobilization by CDTA or DTPA was even more efficient.[246] In chronic human Mn intoxication, EDTA extensively increased urinary Mn excretion in several studies[247−249] while DMSA was without effect. In these studies, neither EDTA nor DMSA chelation had beneficial effects on the clinical symptoms.

## M. Mercury

The treatment of intoxications by mercury compounds is complicated by extensive differences between the toxicokinetics and critical target organs of inorganic salts, Hg(0) vapor, and various types of organomercury compounds. Due to the extensive use of elementary mercury in many industrial and scientific operations and in dentistry, exposure to mercury vapor has been a widespread occupational health problem. Also, the use of inorganic mercury in the chemical industry and organic mercury compounds in seed dressing and in the paper industry has resulted in human exposures and extensive environmental pollution.

Inorganic mercury salts are poorly absorbed, about 10% in the intestinal tract of the mouse[250] and deposited mainly in the kidney, which is a target organ for $Hg^{2+}$. The net retention of elemental mercury vapor is about 80% after low-level pulmonary exposure. At high acute dosage, mercury vapors induce extensive, often rapidly fatal, alveolar necrosis. In the blood, some Hg(0) is oxidized to $Hg^{2+}$ to be excreted in urine. Due to the lipophilicity of Hg(0), a fraction is deposited in the brain and other organs with subsequent oxidation. As the $Hg^{2+}$ ion does not pass cell membranes easily, this mechanism leads to capture of Hg in the central nervous system, which is the important target organ of Hg(0). Organic mercury compounds such as, e.g., $CH_3Hg^+$ are highly lipophilic and thus absorbed completely after oral exposure[251] to be deposited in the brain or pass the placenta barrier. Accordingly, neurotoxicity and fetotoxicity are important toxic effects of these compounds.

Human poisonings by various forms of mercury have been treated with the chelators BAL, DPA, NAPA, DMSA, and DMPS. The traditional treatment was BAL initially, followed by oral DPA. Because BAL increases the brain deposition of $CH_3Hg^+$,[21] this chelator is contraindicated in organic mercury intoxication. Due to the low toxicity and high efficacy of DMSA and DMPS, these compounds are the antidotes of choice in various forms of mercury poisoning. Even though methylmercury apparently does not form chelates, the bonds formed between the methylmercury ion and a deprotonated thiol group from various thiol compounds have a high degree of covalent character. Accordingly, thiol chelators influence the toxicokinetics of methylmercury by forming stable complexes.

Several studies explored antidotes against acute intoxication by inorganic or organic mercury. Thus, DMSA was an efficient antidote and mobilizing agent in acute systemic $HgCl_2$ or $CH_3HgCl$ intoxication in experimental animals, reducing Hg deposits in various organs and increasing the urinary excretion of Hg.[252,253] In the latter study, DMSA chelation increased the urinary Hg excretion more than 10 times and chelation treatment with DMSA in the drinking water reduced the whole-body, brain, liver, and kidney levels of injected $MeHg^+$ in mice by 50−70%. Also, in the studies of Aaseth and Friedheim,[26] oral administration of DMSA for 8 days after injection of $CH_3Hg^+$ reduced the brain Hg level by 75%. DMPS, DPA, and NAPA were less effective. The high efficacy of DMSA in acute $CH_3HgCl$ intoxication warranted investigation into the effect of this chelator on the teratogenicity of $CH_3HgCl$. Subcutaneous administration of DMSA immediately after oral administration of a teratogenic dose of $CH_3HgCl$ to mice on day 10 of gestation induced a dose-dependent reduction in the frequency of embryo lethality, skeletal anomalies, and cleft palate.[254] This study was followed by a comparison of the effects of BAL and DMPS on $CH_3HgCl$-induced maternal toxicity and embryotoxicity in mice. While BAL afforded little or no protection against maternal and fetal mortality or structural malformations from acute $CH_3HgCl$ poisoning, DMPS reduced maternal and fetal toxicity and teratogenicity in a dose-dependent fashion.[255]

In a comparison of the antidotal efficacy of DMSA, DMPS, BAL, DPA, and NAPA injected 20 min after injection of a $HgCl_2 > LD_{98}$, BAL was the least effective chelator.[256] In a subsequent study[257] using the same $HgCl_2$ dose and administration route, NAPA, DPA, and DMPS were more efficient in reducing mortality than DMSA and BAL.

In several studies in mice or rats injected with mercury salts, DMSA or DMPS effectively increased Hg elimination estimated from reduced body burden or kidney levels or increased urinary excretion of Hg, DMPS being somewhat more efficient than DMSA.[184,188,258,259] However, repeated injections of either chelator eventually mobilized similar amounts of renal mercury.[259] In a recent comparison of the protective effects of parenterally administered BAL and DMSA against Hg-induced nephrotoxicity after parenteral administration to the rat, BAL at the lowest dose decreased the urinary Hg excretion. At the highest dose applied, BAL exacerbated Hg-induced proteinuria, while DMSA afforded dose-related increased urinary Hg output and significantly decreased renal Hg deposition.[260]

These studies almost solely used parenteral administration of Hg and chelators. Two studies are, however, of special interest in relation to acute human Hg intoxication due to employment of relevant exposure routes: Buchet and Lauwerys[259] reported that DMSA could mobilize mercury accumulated in the kidney and to a lesser extent in the liver and could enhance the urinary mercury excretion in rats pretreated with different concentrations of mercury vapors. DMSA, however, was inefficient in removing mercury accumulated in the brain after exposure to mercury vapors.

Oral administration of $^{203}HgCl_2$ to mice induced dose-related mortality. After oral or parenteral administration of chelating agents 15 min after $HgCl_2$, DMSA and DMPS were superior to BAL and NAPA in reducing mortality and intestinal stasis induced by $HgCl_2$. Also, DMSA and DMPS reduced the whole-body retention and brain deposition of orally administered Hg more efficiently after oral than after parenteral administration and DMPS more efficiently than DMSA.[55]

The monoalkylesters of DMSA developed for mobilization of aged cadmium deposits have been evaluated as mobilizing agents for mercury. Parenteral administration of several such esters were more effective than DMSA and DMPS in reducing the whole-body retention and increasing the urinary Hg excretion in Hg-loaded mice, the DMSA monoisoamyl ester being most effective.[261] Also in rats, these monoalkyl esters of DMSA mobilized Hg more efficiently than DMSA, the iso derivatives most effectively.[262] Also at protracted time after $Hg^{2+}$ administration, the isoamyl ester was more effective than DMSA in decreasing whole-body and organ levels of Hg, except in the brain, where both chelators were equally effective.[263]

The combined data indicate that DMSA and DMPS are efficient antidotes in animal experimental acute

inorganic and organic mercury intoxication. While monoalkylesters of DMSA, in particular the isoamyl ester, are more efficient mobilizers of body stores of Hg than DMSA and DMPS, human use of this type of compounds must await development of less toxic compounds and extensive safety testing. Also, an increasing body of evidence indicates that DMSA and DMPS can be used safely in human poisonings by various mercury compounds, as indicated by the case and cohort studies reviewed below. Due to the possibility of DMSA or DMPS chelation for extended time periods via the oral route with a low rate of adverse side effects, these compounds are superior to BAL, DPA, and NAPA.

A number of case and cohort studies on chelation treatment of Hg intoxications are available: Four men exposed to mercury vapor during smelting of silver from dental amalgam in a private home were admitted to the hospital 24 h later with severe pulmonary incapacitation. Despite chelation with BAL, all four died 9−23 days after from severe pulmonary damage.[264] In a similar case of silver extraction from dental amalgam, four members of a family died from respiratory failure despite BAL chelation.[265] A gold prospector arrived at the hospital with fever, tachypnea, and headache. Despite chelation with BAL and DPA, the patient died from acute respiratory distress.[266] Two jewelers inhaled Hg vapors for about 30 min during melting a block of gold with an unknown content of Hg. They were admitted to the hospital 16 h later, short of breath with fatigue, nausea, and pain at various sites. Renal function was normal. Both were chelated with im BAL for 5 days, followed by oral DMSA for 5 days. Both blood and urine Hg concentrations rapidly fell from initially high values. The data suggest that the urinary Hg elimination was increased after the change from BAL to DMSA chelation while the blood Hg remained at the same levels.[267]

A man injected 40 mL of elemental mercury intravenously to commit suicide. After apparently remaining free of symptoms of Hg intoxication for 3 years, he arrived at the hospital with sweating, mild neuropsychiatric symptoms, intermittent pain, and mild peripheral neuropathy. X-ray examination disclosed multiple fine granules of elementary mercury all over the pulmonary circulation and a mercury deposit in the right ventricle of the heart. The patient was chelated with oral DMPS for 6 months without side effects. While chelation decreased the b-Hg only from about 100 to about 90 $\mu$g/L, the 24 h urinary Hg excretion was increased from a prechelation value of about 600 to over 2000 $\mu$g.[268]

Another case of intravenous self-administration of elementary mercury was treated with oral DMPS on a long-term basis. Despite extensive urinary Hg excretion, blood-Hg remained high and Hg deposits still remained in the body at the 5-year follow-up, where the patient had normal liver, lung, and kidney function but suffered tremor and lower extremity weakness.[269]

A young woman who developed acute renal failure after ingesting approximately 1 g of $HgCl_2$ for suicidal purposes was treated with plasma exchange, hemodialysis, and peritoneal dialysis and chelated with BAL. Anuria persisted for 14 days. At a 4 month follow-up, renal function was normal.[270]

Oral ingestion of a stool fixative containing 675 mg of $HgCl_2$ was rapidly treated with BAL chelation and extensive hydration, and the patient remained without systemic signs of mercury intoxication.[271]

A 38-year-old male intentionally drank 100 mL of a solution with an unknown concentration of $HgCl_2$. He was admitted to the hospital with consistent vomiting and bloody diarrhea. After treatment with gastric lavage, activated charcoal, and im BAL, his condition rapidly deteriorated with renal failure. About 10 h afterward, iv DMPS chelation and hemodialysis was started together with plasma expander due to hypovolumic chock and hemodialysis. Despite continuing extensive blood Hg levels (>2 mg/L), kidney function was regained after 10 days. Parenteral DMPS chelation was reduced from 1.5 to 0.75 g/day for 4 weeks, then oral DMSA, 0.9 g/day, was administered for another 3 weeks. The patient recovered completely.[272]

A 44-year-old man drank a solution containing about 5 g of the bactericide thiomersal (sodium 2-(ethylmercurio)-thiobezoic acid) in a suicide attempt. He was admitted to the hospital vomiting with nausea and hemorrhagic gastritis. After gastric lavage, he received a solution of DMPS via nasogastric tube. On day 1 he developed acute renal failure which persisted until day 40. During the next days he was chelated with alternating nasogastric tube and iv administrations of DMPS. From day 17−29, he received alternating doses of DMPS and DMSA; from day 33 until day 70, oral DMSA was given. During the early treatment he developed fever and delirium that increased to coma over a few days. Later he developed polyneuropathy and various cutaneous lesions. At 148 days, all neuropathy except sensory defects in two toes had completely resolved.[273]

$Hg_2Cl_2$-containing creams have been and are still used in some countries for dermal application, resulting in acute or chronic poisoning. A Russian investigation[274] described symptoms and treatment of 56 patients with such intoxication. Patients have with gastrointestinal, renal, hepatic, or dermal disorders. The highest b-Hg recorded was 800 $\mu$g/L. The patients were treated with DMPS, the most serious cases in combination with hemoperfusion. Another study[275] described 12 patients using a $Hg_2Cl_2$-containing facial cream for 2−10 years. All had high urinary Hg excretion rates. Upon chelation with DMPS, the urinary Hg output was further increased.

DMSA and DMPS have both been employed as diagnostic tools to estimate exposure to mercury vapor and body stores of Hg. In a study of three groups of workers, with present exposure to high mercury vapor levels, with reduced exposure and removed from exposure, the average increase in 24 h urinary Hg excretion due to one oral DMSA dose varied between 20 $\mu$g Hg in the group removed from exposure and 600 $\mu$g in the group with present high exposure, as compared to 4 $\mu$g in the unexposed control group. The ratio between average 24 h urinary Hg excretions after and before oral DMSA

chelation varied between 4 and 2.5, with the higher values in groups with the highest and most recent exposure to Hg vapor. The correlations between pre- and post-DMSA urinary Hg excretions were statistically significant in all groups, most strongly in the control and in the group removed from exposure, indicating that DMSA-induced excretion could indicate renal Hg deposits in individuals without recent exposure.[276] Both results indicate that DMSA efficiently mobilizes Hg present in more shallow depots and the extent of mobilization therefore could indicate the intensity of recent exposure.

In a study[277] of mercury vapor exposed industrial workers, dentists, controls with amalgam fillings, and controls without amalgam fillings, a single oral dose of DMPS significantly increased the urinary Hg excretion in all groups. The factorial increase in Hg excretion was much larger than in the study of Roels et al.[276] (a 3−12 times increase versus 2.5−4 times increase), despite the much lower dose (0.3 g of DMPS versus 2 g of DMSA). The correlations between urinary Hg excretions before and after DMPS administration and between plasma Hg before and urinary Hg excretion after DMPS were highly significant. Also, the excretions for 6 and 24 h after DMPS were strongly correlated, obviating the need for the long urine collection period. Even when applied to a group of 7 men at 3 years after cessation of long-term mercury vapor exposure, a DMPS mobilization test (300 mg single oral dose) increased the average 24 h urinary Hg excretion by a factor of 7.6.

This test was extended into treatment in a group of 10 mercury-exposed workers with basal urinary levels of 50 $\mu$g of Hg/g of creatinin or higher. The urinary Hg excretion was significantly increased for 5 days of DMPS chelation.[278]

The DMPS mobilization test has been used to evaluate the mercury exposure and systemic load of mercury in nonoccupationally exposed populations. Aposhian et al.[279] found a significant correlation between the DMPS-provoked urinary Hg excretion and the "amalgam score" (calculated from the number and diameters of dental amalgam fillings) in a group of volunteers. In two similar studies, DMPS increased the average urinary Hg excretion 6−7-fold[280] and 9-fold.[281] The excretion was larger in subjects with amalgam fillings both before and after DMPS provocation than in amalgam-free individuals in both studies, and a significant correlation with the "amalgam filling index" was demonstrated in the second study.

The DMPS mobilization test was used to study the urinary Hg excretion in dentists and dental technicians compared with non-Hg-exposed controls. As in the other studies, the post-DMPS urinary Hg excretion was extensively higher than and strongly related to the pre-DMPS value, highest in dentists. In this study, the amount of mobilized Hg was significantly inversely related to the score for several endpoints in neurobehavioral tests, indicating its value in predicting adverse exposure.[282]

The DMPS mobilization test has mainly been used to assess exposure to mercury vapor, but it is apparently a useful diagnostic tool in individuals exposed to other mercury compounds as well. When the test was applied to 11 workers making a skin lotion containing $Hg_2Cl_2$, 8 users of the skin lotion and 9 controls without apparent Hg exposure, the 6 h urinary Hg excretion was increased between 1 and 2 orders of magnitude in exposed individuals. The average 6 h Hg excretion was more than 5 and 1.4 mg in the workers and the users, respectively.[283] After a considerable time lag, 8 workers decided to participate in a second mobilization test and to receive chelation treatment with DMPS. This time, the avarage 6 h urinary Hg excretion after DMPS was almost 7 mg. The workers had normal kidney function. The workers received three courses of 400 mg of oral DMPS per day lasting 8, 7, and 6 days, respectively, resulting in an average mobilization of more than 24 mg of Hg including the mobilization test.[284]

A group of 53 men were exposed to elemental mercury vapor during repair work on Hg tubes in a chloralkali factory. Due to flame cutting of the pipes, boiling Hg was spread over the workers who also inhaled Hg vapor. Subsequently, several workers became ill. After several days, mercury poisoning was diagnosed based on elevated u-Hg levels. A total number of 26 men were hospitalized from day 19 or later after the exposure. Beginning 26 days after exposure, 14 days of chelation treatment with DMSA or NAPA was given. At 73 days after exposure, 12 patients with persistently elevated urinary Hg levels were chelated with NAPA or DMSA for 4 days. While DMSA increased the urinary Hg excretion 3.5−5-fold, NAPA increased the excretion only 2−2.5-fold.[285]

Animal experimental data, especially, but also limited human case and cohort data indicate that DMPS is the optimum chelation antidote in acute and chronic intoxications with inorganic Hg compounds, including elementary Hg vapor, while DMSA is more efficient than DMPS in poisonings with organic Hg compounds. To further verify this and offer a solid basis for possibly dismissing BAL in treatment of mercury intoxication, experimental animal studies quantifying and comparing the efficacy of DMPS and DMSA with that of BAL in both inorganic and organic mercury intoxication would be very useful.

## N. Nickel

Nickel is widely distributed in the occupational and general environment from alloys and nickel-plated items. While acute nickel poisoning is very rare, occupational exposure to insoluble crystalline nickel compounds is a recognized nasal and pulmonary carcinogenic hazard. Accordingly, the carcinogenicity of nickel governs occupational standards for airborne nickel, even though the human carcinogenicity of more soluble nickel species is still under debate. The soluble nickel compounds are strong skin allergens, the $Ni^{2+}$ ion acting as a hapten. The prevalence of nickel allergy in women is about 10% in western populations, placing nickel among the most important dermal allergens.[286] Oral nickel intake due to the use of nickel-containing kitchen utensils or intake of food items high in nickel may exacerbate dermatitis from skin contact with nickel. Accordingly, the

recommended treatment of severe nickel contact dermatitis is a diet low in nickel and avoidance of nickel-containing items in the household.

Few chelators have been tested clinically for alleviating effects on nickel contact dermatitis. Two principles have been employed: Systemic chelation with an oral chelator to reduce body stores of nickel, and use of skin creams with small molecular weight chelating agents or ion-exchange resins added to prevent uptake into the skin and block the antigenic activity of nickel. Several clinical studies have indicated healing of skin eruptions after an initial flare in some nickel allergic patients after oral intake of disulfiram or diethyldithiocarbamate. The mechanism is believed to involve increased excretions of body depots of nickel as indicated by increased serum and urinary nickel levels.[287] In most studies, the rate of improvement among patients was, however, not high and a fraction of treated patients developed hepatotoxicity.[288−292]

In an animal experiment, administration of DDC or disulfiram dramatically changed the kinetics of Ni orally administered Ni, as the intestinal uptake of orally administered nickel was increased by a factor of 10 and the brain deposition by a factor of about 700 in mice.[49] Accordingly, this chelation treatment of nickel allergic patients cannot be recommended.

Effects of creams and ointments containing various chelating agents on skin penetration of $Ni^{2+}$ in vitro are quite variable. On the other hand, creams containing EDTA,[293,294] DDC,[295] or dimethylglyoxim[295] reduced the response to patch testing in nickel allergic patients with nickel coins or solutions of nickel salts. The apparently most effective chelating agent for this kind of application is clioquinol.[294] Due to a range of different toxic effects, this chelator is, however, unsuitable for general use in prevention of Ni dermatitis.[286] A cream with a cation-exchange resin had some protective effect.[296] The combined experience indicates that even the skin penetration of Ni is not always extensively reduced; the antigenic potential of the nickel ion is reduced by chelation, as suggested already in 1954 by Kurtin and Orentreich.[297] For further discussion of this subject, see the review by Gawkrodger et al.[286]

Xie et al.[298−300] investigated various chelating agents as potential antidotes toward acute nickel intoxication. The general result of these studies was that intraperitioneal injection of DMSA consistently afforded some protection against the acute toxicity of injected $NiCl_2$ in mice estimated as pulmonary, testicular, renal, or hepatic lipid peroxidation, increased the urinary and fecal Ni excretion, and decreased the nickel levels in various organs. Similar results were obtained by Tandon et al.[301] in the rat. In all these studies, *N*-benzyl-D-glucaminedithioate was also effective in mobilizing nickel; however, this compound is an experimental antidote presently not available for human use.

## O. Platinum

The therapeutic use of the highly efficient antitumor drug *cis*-dichlorodiammineplatinum(II) (*cis*-Pt) is associated with various side effects, nephrotoxicity being dose-limiting.[302] The toxic mechanism is believed to involve GSH depletion and induction of oxidative stress, and animal experimental and clinical studies have demonstrated that various nucleophilic thiocompounds can protect against *cis*-Pt nephrotoxicity in protocols not affecting its antitumor activity.[303−305]

Among chelating agents, DDC[306] and derivatives of DDC[307−310] protected against nephrotoxicity, decreased renal and hepatic Pt levels, and increased especially the biliary excretion of Pt after *cis*-Pt administration, conceivably protecting the kidney by changing the excretion route from preferentially renal to biliary. In acute toxicity studies, sc DMSA reduced mortality after ip administration of 50 mg/kg of $H_2PtCl_6$ to mice.[10] Also, repeated ip injections of DMSA after iv administration of *cis*-Pt to rats reduced the renal and hepatic Pt levels and increased the urinary Pt excretion in rats.[311] However, even the Pt mobilizing effect of DMSA was comparable to that of the more efficient dithiocarbamate derivatives; DMSA was unable to alleviate Pt-induced renal damage but on the contrary increased urinary *N*-acetyl-*β*-D-glucosaminidase and creatinine excretion. The indicated renal damage was verified histologically. The effect of DMSA was conceivably due to the increased urinary Pt excretion. In the same study, BAL increased the organ levels of Pt; DFOA reduced the hepatic Pt level even more than DMSA but enhanced the *cis*-Pt toxicity.[311] Also, Planas-Boehne et al.[312] observed reduced renal Pt levels after ip administration of DMSA after iv administration of *cis*-Pt to rats.

## P. Organic Tin

Organic tin compounds are strong neurotoxins and induce thymus atrophy and bile duct damage. The metabolism involves subsequent dealkylation reactions; accordingly, tri- or tetralkyl tin exposure results in systemic exposure also to the mono- and dialkyl compounds. Although DMSA did not reduce dibutyltin-induced morality in mice, it reduced thymus and bile duct damage more efficiently than did BAL and was also an antidote in rats.[313,314]

Two women were poisoned by drinking red wine with trimethyltin added for homicidal purpose. One woman died after 1 week with multiorgan failure despite intravenous DMSA chelation. The other gradually recovered over several months from severe neuropsychiatric symptoms. She was chelated for several weeks with oral DMSA, apparently improving her clinical condition.[315]

## Q. Thallium

Thallium is used industrially in semiconductors, alloys, pigments, glass, imitation jewelry, and as catalysts. Formerly, thallium salts' widespread use as rodenticides led to many homicidal and suicidal human poisonings. Even anticoagulants and other biochemically acting poisons are commonly used as rodenticides today; thallium poisonings still occur. Although Tl-containing rodenticides were banned in several western countries in the 1970s, Tl salts are

still readily available around the world. In acute oral Tl poisoning, local and systemic toxicity rapidly evolve. The toxicity of $Tl^+$ is partly due to its physical similarity with the $K^+$ ion, i.e., substitution of $K^+$ in various biochemical processes, most notably in $Na^+/K^+$ ATPases. Further, $Tl^+$ has a high affinity for HS groups in proteins. The similarity to $K^+$ leads to high intestinal uptake after oral intake and to high reabsorption after glomerular filtration. Accordingly, supportive treatment in Tl intoxication is iv administration of $K^+$ to compete for access to biochemical sites and to increase renal clearance by competing for reabsorption.[316,317] Also, forced diuresis could increase Tl excretion.[318] After acute oral exposure, gastric lavage and laxative treatment have been used; however, the ion exchanger PB originally proposed by Heydaulf[319] is the most effective antidote for Tl studied so far. If PB is not available, activated charcoal can be employed as a less efficient alternative. As enterohepatic circulation is important in Tl kinetics, an efficient decorporating procedure also in chronic Tl intoxication is intestinal trapping of Tl by PB, which is also used for decorporation of cesium. PB occurs in various chemical forms with different physical properties that have been used clinically, see section 9.

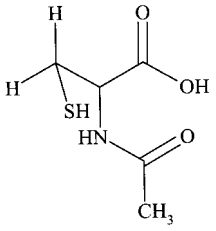
AFCF has been widely used in animal experimental and human detoxification of Tl and Ce. Among and within the main types of PB, the synthesis and further preparation may determine the Tl-binding capacity, particle size, i.e., specific surface area, and *K* content apparently being the main determining factors for Tl binding capacity.[320−322]

Leloux et al.[323] compared the different treatments available for Tl mobilization in acute oral Tl intoxication in the rat. The most efficient single treatments were increased urinary excretion forced by $K^+$ administration and enhanced fecal elimination after oral administration of PB. While forced diuresis by furoseimid administration was ineffective by itself, the total urinary and fecal excretion of Tl after combined oral PB and ip furoseimid administration was extensive. This interesting article advises a promising avenue for future research on the management of acute Tl intoxication that can be fruitful in studies of other intoxications as well.

Among clinically available antidotes, neither EDTA, DTPA, BAL, nor DPA were able to increase Tl excretion.[319,324−326] DPA caused redistribution of Tl to the brain of rats and increased Tl induced mortality.[327,328] DDC increased the urinary Tl excretion, however, as with most other metal cations, also increased the brain deposition of Tl, thereby augmenting the neurotoxicity.[41] Accordingly, these two antidotes are contraindicated and the others mentioned are certainly not recommended.

Recent and future studies in treatment of experimental Tl poisoning should relate to optimization of the chemical and physical form of PB and to combined treatment with PB and other compounds. Kravzov el al.[329] compared the efficiencies of a commercially available preparation of PB and freshly prepared colloidal PB in lowering organ and blood levels of Tl when administered orally 24 h after ip administration of Tl to mice. While both preparations of PB effectively lowered Tl levels in all investigated compartments, the freshly prepared PB was significantly more efficient. This study confirms the previous investigations cited above.

Combined treatment with oral PB and ip DPA for 3 days commencing 1 day after ip administration of thallium acetate to rats resulted in significantly less cerebellar damage estimated histologically than treatment with PB alone, which only marginally protected against cell toxicity. Administration of DPA alone enhanced the brain damage. The mortality in the groups tightly followed the amount of brain damage.[330] Meggs et al.[331] compared the efficiency of *N*-acetylcystein (NAC; **22**) and PB alone or in combination in acute Tl intoxication in mice. NAC was




**22**

marginally effective in reducing mortality after a sc dose of thallium acetate corresponding to $LD_{90}$, while PB and the combination of PB and NAC were only slightly more effective. At a dose $>LD_{100}$, the only treatment offering some protection was PB alone.

## R. Zinc

Zinc is used for numerous industrial products including pigments, alloys, and for anticorrosion treatment. Welding on zinc-plated items may cause acute pulmonary symptoms ("zinc fever") due to inhalation of zinc oxide. Zinc salts used in soldering flux solutions are highly corrosive. Various skin creams and ointments contain levels of zinc oxide that are toxic upon oral intake.

In a comparison of the mobilizing effects of several polyaminopolycarboxylic acids in zinc-loaded rats, DTPA and TTHA most efficiently decreased organ levels and increased urinary and fecal zinc excretion.[332]

In a comparison of several chelating agents as antidotes against mortality of acute parenteral zinc intoxication in mice, DMSA efficiently reduced acute mortality.[333] In a similar study by Llobet et al.,[334] EDTA, DTPA, and CDTA were the most effective antidotes, together with DMSA, while DMPS and DPA afforded some protection at a higher ratio between chelator and zinc dose. DPA was rather inefficient both in preventing mortality[334] and in enhancing zinc excretion in acute zinc intoxication.[335,336] It is well established that EDTA and even more efficiently DTPA mobilize Zn in humans (reviewed by Catsch and Harmuth-Hoehne[72]). Knowledge on the efficacy of BAL in acute zinc intoxication is almost absent.

A limited number of cases of chelation treatment of acute zinc intoxication have been published: A 16-month-old boy ingested about one tablespoon of a

soldering flux liquid containing $ZnCl_2$. The child was admitted vomiting in a lethargic state with progressing respiratory symptoms. Supportive care included iv fluids, iv penicillin, and occasional humidified oxygen. Repeated endoscopy over the days indicated progressing esophageal and gastric ulcerations, mucosal sloughing, and coagulative necrosis. Decreasing hemoglobin values, presumably due to gastric bleeding, necessitated tranfusion of packed erythrocytes. Kidney function appeared normal and transient hepatobiliary and pancreatic dysfunction improved rapidly. Due to declining mental status and increasing hypertension, chelation therapy with im BAL and iv EDTA was initiated on day 4. Extensive esophageal and gastric scarring remained, and the boy was discharged on total parenteral nutrition. Several weeks later, an operation was necessary to reestablish gastric−duodenal passage.[337]

A 20-year-old woman ingested a metal scouring solution containing 385 g/L of $ZnCl_2$ in a suicide attempt. Endoscopy revealed only moderate esophageal damage but extensive ulcerative and necrotic lesions in the gastric mucosa. Fiber-optics bronchoscopy revealed no damage to the trachea or the bronchial tree. Chelation treatment with im BAL was started 6 h postingestion and increased the urinary Zn clearance. Supportive care included mechanical ventilation and iv fluid administration. The patient was discharged on day 18 after a complete recovery.[338]

In the Cu−Zn intoxication reviewed in the section on Cu[192] it is difficult to assess whether Cu or Zn was responsible for the major toxic insult. The two chelators administered are both grossly ineffective in Zn intoxication, and it is fair to state that only the Cu intoxication was treated by the chelation schedule applied.

## 11. Conclusions

During the last 15 years, several important developments have occurred in the possibility and clinical practice of chelation treatment of acute and chronic metal poisoning. During this period, DMSA and DMPS have gained more general acceptance among clinicians, undoubtedly improving the management of many human metal intoxications.

The available literature on clinical treatment of cases of acute metal intoxications indicate, however, that the older, more toxic, and less efficacious chelators, especially BAL, are still used in surprisingly many cases. This is odd as DMPS and DMSA are available as cheaper, more stable preparations for oral or parenteral use, as compared to the presently available unstable preparations of BAL suited for im use only.

The development in experimental animals of chelating agents efficiently mobilizing aged intracellular cadmium deposits offers possible future further development of less toxic compounds that may also be clinically used with lead and mercury, a development already successfully initiated experimentally for several other metals than Cd.

The development of a safe DMPS mobilization test for inorganic mercury may hopefully lead to development of further diagnostic tools that can be administered more safely than the traditional EDTA test for lead exposure.

Still, further knowledge is needed in several basic research areas within the field of in vivo chelation of metals and call for studies on, e.g., (1) Molecular mechanisms of action of clinically important chelators, (2) Intracellular and extracellular chelation in relation to mobilization of aged metal deposits and the possible redistribution of toxic metal to sensitive organs as, e.g., the brain, (3) Effects of chelators on metal biokinetics during continued exposure to the metal, especially possible enhancement or reduction of intestinal metal uptake, (4) Combined chelation treatment with lipophilic and hydrophilic chelators, which presently has a minimal clinical role, (5) Minimization of the mobilization of essential trace elements during long-term chelation, (6) Fetotoxic and teratogenic effects of chelators, (7) Development of orally administrable chelators, (8) Development of less toxic chelators for chronic chelation treatment of diseases due to metal storage.

Especially with the two last points, continued development of orally administrable chelating agents for efficient, nontoxic mobilization on a home-patient basis over extended time periods (even life-long chelation) of aged deposits of toxic metal such as Al, Cd, Fe, Hg, and Cu will probably be a main future research issue. Also, even such work is without high scientific merit, extensive animal experimental comparison of the efficacies of classical chelators, especially BAL with those of DMSA and DMPS in acute intoxications using relevant exposure routes, i.e., oral administration of relevant species of the metals, as well as inhalation of Hg vapor, is a prerequisite for outphasing the old chelators in uses where a more effective alternative is now available. Thus, if BAL were suggested as a new drug today, it would most likely not be approved for clinical use. Due to the advent of the more efficient and safe drugs DMSA and DMPS, we have now reached the state where BAL most likely should be made unavailable as a drug.

## 12. Abbreviations

| | |
|---|---|
| AFCF | ammoniumferric hexacyanoferrate complex |
| BAL | 2,3-dimercaptopropanol |
| b-Pb | whole-blood concentration of lead |
| CBS | colloidal Bismuth Subcitrate, Tripotassium dicitratobismthate |
| CDTA | cyclohexanediaminetetraacetic acid |
| DDC | diethyldithiocarbamate |
| DFOA | desferrioxamine |
| DMSA | dimercaptosuccinic acid |
| DMPS | dimercaptopropanesulfonic acid |
| DPA | D-penicillamine |
| DTPA | diethylenetriaminepentaacetic acid |
| E | efficiency (of a chelator for mobilizing a metal) |
| EDTA | ethylenediaminetetraacetic acid |
| IDA | iminodiacetic acid |
| ip | intrapritoneal |
| iv | intravenous |
| L1 | 1,2-dimethyl-3-hydroxypyrid-4-one |
| LD | lethal dose (subscript indicates percent mortality) |

| | |
|---|---|
| ME | (metal) mobilizing effectiveness of a chelator (see MEF and MEQ) |
| MEF | factorially increased (metal) output |
| MEQ | factorially decreased (metal) retention |
| MT | metallothioneine |
| NAPA | *N*-acetyl-D-penicillamine |
| NTA | nitrilotriacetic acid |
| PB | Prussian Blue |
| PBS | colloidal soluble potassiumferric hexacyanoferrate complex |
| PBI | insoluble ferric hexacyanoferrate complex |
| RE | relative efficiency of two chelators, ratio between effects at same dose |
| RP | relative potency of two chelators, ratio between equally effective doses |
| s-As | serum concentration of arsenic |
| sc | subcutaneous |
| TE | therapeutic effectiveness (see TEF, RP, and RE) |
| TEF | factorially increased LD ($LD_{50}$ or $LD_{99}$) |
| TETA | triethylenetetramine |
| TTD | tetratethythiuramdisulfide |
| TTHA | triethylenetetraminehexaacetic acid |
| u-As | urinary concentration of arsenic |

## 13. Reference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