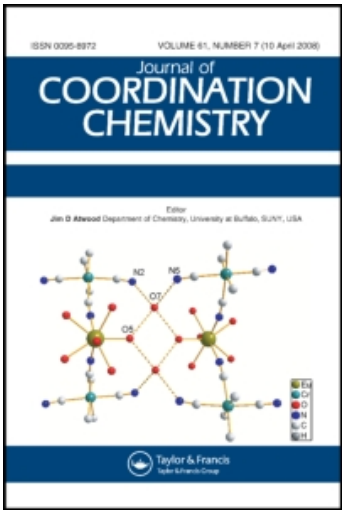

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THE DESIGN OF THERAPEUTIC CHELATING AGENTS

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The factors involved in the design of therapeutic chelating agents are outlined on the basis of the theoretical analyses of ligand design and experimental data obtained in animal studies. The starting point in such design must always be those factors which assure that a sufficiently high stability constant be achieved, and here the analyses presented by Martell and his co-workers furnish a general approach. If the removal of intracellular metal deposits is to be achieved, additional factors need to be considered to incorporate variables which govern the interaction of the chelating agent with the membrane systems of those organs within which the toxic metal is concentrated. For these, the QSAR (quantitative structure activity relationship) procedure of Hansch furnishes a useful guide. This allows the development of direct structure-efficacy correlations (DSEC) involving molecular parameters in addition to those which are directly involved in the determination of the stability constant. In several cases data are available which indicate how the relative efficacy of two chelating agents with essentially identical stability constant expectations is dependent upon structural features which govern the relative ease with which such molecules can gain access to intracellular deposits. The combination of these approaches allows the joint use of *in vitro* and *in vivo* data to design improved therapeutic chelating agents with an increased probability of success when tested *in vivo*.

Keywords: Therapeutic chelating agents, chelating agent design, design, therapeutic chelating agents, membrane permeability, chelating agents

INTRODUCTION

The design of chelating agents for the express purpose of removing toxic metals from the mammalian body is a field of growing importance as the perception of a decline in the quality of the human environment becomes more widespread and as the evidence for this decline becomes more extensive and convincing. Recent studies have emphasized the developmental damage in children which results from life in an environment in which high levels of contamination from lead are present,[1] the dangers which arise from the uncontrolled discharge of dusts from nuclear power plants[2] and the problems which arise when the kidney levels of a toxic metal such as cadmium reach high levels.[3] All of these are problems whose incidence and health effects can, in principle, be significantly reduced by treatment with appropriate agents which can enhance the excretion of the toxic metal.[4–6] Related problems include those which involve hereditary disorders which result either directly from the accumulation of toxic metals (such as that of copper in Wilson's disease[7]), or indirectly as a result of therapeutic measures. Examples of this latter situation are the thalassemias, whose treatment involves repeated blood transfusions throughout life, with a concurrent accumulation of iron.[8] Improved clinical treatments for each of these problems requires the design of one or more chelating agents which can induce a significant increase in the excretion of a specific toxic metal. It is this design process which will be examined here.

DESIGN FACTORS

The design of a chelating agent for use in the mobilization of a toxic metal requires, first of all, that the overall process be possible in the sense that one is not trying to violate the second law of thermodynamics. This, in turn, requires that the reaction of the chelating agent with the toxic metal will, in fact proceed to give a new complex, which one hopes is less toxic than the parent metal ion. Such a reaction will be possible if the chelating agent has a stability constant for the toxic metal which is sufficiently high, and it is in the considerations which enter in this part of the design that the studies of Martell[9-18] are of key importance. Once this difficult problem is solved, then the tailoring of the structures to achieve satisfactory interactions with the biological systems of importance can begin.

Achieving Desired Stability Constants

The extensive theoretical and experimental studies of Martell and others have examined and presented solutions for most of the problems involved in the relationship between chelate structure and stability constants for metal ions in a very general manner[18] and have also presented detailed examples for specific ions, of which the most thoroughly examined has been iron(III).[8] The iron(III) mobilization problem is of special interest because of its relationship to the treatment of iron-overload problems resulting from the long term, regular administration of blood transfusions.[8] The solutions are directly applicable to the design of new, more effective chelating agents for the removal of toxic metals from bound sites on serum proteins and other extracellular sites. While these studies have centred on iron(III), it must be emphasized that the analyses presented are quite general and apply to most other toxic ions.

An example of the type of information which has been developed and its potential application is the relationship between chelate structural features and donor atom basicity, which is, in turn, one of the major determinants of stability constants. One finds that the transformation of a primary amine to a secondary amine leads to an increase in basicity, so such a change should lead to an increase in stability constant, provided that there is not a simultaneous increase in steric strain to accommodate the added groups. In the case of simple amines such increases in basicity are achieved at the expense of an over-riding increase in steric interference to metal ion coordination with a net reduction in stability constants for many systems.[18] However, one can see that the transformation of triethylenetetramine into *N''*,*N'''*dimethyltriethylenetetramine would lead to a significant increase in basicity and an increase in the stability constant for the copper(II) complex. A chelating agent in which the methyl groups are in positions such as these, in which they should not interfere with the formation of a ring of donor atoms around the copper ion, should form more stable copper complexes than the unsubstituted parent compound. Such a compound should be superior to triethylenetetramine itself (which is a compound currently used clinically) in the mobilization of copper from hepatic deposits in individuals who accumulate copper as a result of Wilson's disease. The additional methyl groups might also assist in the transport of the substituted compound across the cellular membrane by making the structure somewhat more lipophilic.

Various studies have clearly delineated those factors which determine the thermodynamic possibility of forming a metal complex of interest and thus allow the separation of feasible candidates from those which cannot possibly succeed.[18] One

may well ask how this impressive mass of theory and data can be used to develop chelating agents capable of mobilizing toxic metals from intracellular sites. This point can be most readily addressed by a consideration of the factors which must be critical in the determination of the success of a compound for such purposes. Here we can define the effectiveness E, following Schubert[19] and Catsch[20] as

$$E = [ML]/[M]$$

where [ML] is the concentration of chelated toxic metal, M, and [M] is the concentration of uncomplexed toxic metal. In order to relate this directly to *in vivo* experiments the assumption is generally made that we are dealing with a homogeneous solution such as the serum. We can also use the measure of the chelating agent induced reduction of the concentration of the toxic metal in an organ introduced by Catsch,[20] the effectivity quotient, here designated Q_E,

Effectivity Quotient =

$$Q_E = [\text{M in tissue in presence of L}]/[\text{M in tissue in absence of L}]$$

(where the bracketed terms refer to the amount of residual M in a tissue (M_P), such as the liver, after treatment with the chelating agent L, and in the absence of chelate treatment with L (M_A), respectively) or, more briefly as follows.

$$Q_E = M_P/M_A$$

A very low value of the effectivity quotient is desired as this indicates that a large fraction of the toxic metal has been removed by chelate treatment. The problem of chelate design is then reduced to the determination of the variables which determine E and the manner in which they are affected by manipulations in the structure of the chelating agent. From basic principles we know that one of the major variables which determines the effectiveness, E, is the stability constant, so we can write the following.

$$E = E_1(K_{stab}).E_x(x_1, x_2, x_3, \ldots..), \text{ where } K_{stab} = [ML]/[M][L]$$

Here $E_1(K_{stab})$ represents the explicit dependence of E on K_{stab}. The situation involving the approximation

$$E = E_1(K_{stab})$$

which ignores the effects of the other variables, x_1, x_2, x_3., *etc.* has been examined by both Schubert and Catsch and one can write for the explicit relationship for chelating agents where there is a competition between serum calcium and the metal whose mobilization is desired

$$E_1(K_{stab}) = K_{ML}[L] = K_{ML}[L]_{total}/\{\alpha_L + 10^{-3}K_{CaL}\}$$

where α_L is defined as

$$\alpha_L = 1 + K_{HL}[H] + K_{HL}K_{H2L}[H]^2 + K_{HL}K_{H2L}K_{H3L}[H]^3 + \ldots$$

The factor α_L allows the calculation of the effective or conditional stability constant,

K_{ML}^{eff}, at a given $[H^+]$, which is dependent upon the pH, from the value for K_{ML}, which is not, *via* the relationship

$$K_{ML}^{eff} = K_{ML}/\alpha_L$$

where α_L is the value of α_L at the pH under consideration, usually 7.4, the pH of mammalian serum. From this it follows that a large difference between the values of K_{ML} and K_{CaL} is desirable with $K_{ML} \gg K_{CaL}$. More exactly what is desired is a large difference between the effective stability constants for the toxic metal and the calcium ion, which is the principal metal ion subject to complexation in the serum. For situations in which there is a competition between the toxic metal and several essential metals this treatment can be expanded to give relationships in which these are taken into consideration.

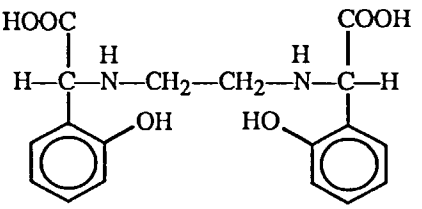
The relationship between E, the effectiveness, and Q_E, the effectivity quotient, can be approximated for the case where one assumes that the system is homogeneous, at equilibrium, that all of the complexed ion is excreted and that the only competition for the binding sites is the Ca^{2+} ion which is present. Under these conditions the following applies.

$$E = [ML]/[M] = \{M_A - M_P\}/M_P = (M_A/M_P) - 1;$$
$$\text{since } Q_E = M_P/M_A \text{ and } E = 1/Q_E - 1,\ Q_E = 1/(E + 1)$$

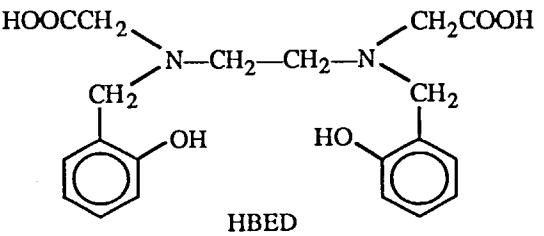
The larger E is, the smaller Q_E is, which is expected as a large E goes with a large stability constant. This treatment does not explicitly contain the limiting value of the stability constant for the initiation of metal mobilization, *i.e.* that value below which chelating agents cannot compete with the naturally occurring ligands which bind the toxic metal ion *in vivo*, though such a limit can be introduced for each specific metal of interest. The form of such expressions in situations where the metal ion undergoes hydrolysis or other complicating side reactions has been described in the literature.[4]

The goal of chelating agent design is seen as the development of a chelating agent for which the value of E is maximized and Q_E is minimized. The principles involved in this can be seen clearly in the studies of Martell and his co-workers.[9-18] The case of iron(III) is of special interest in this respect.

The exceptional iron-binding properties of the chelating agent *N,N'*-ethylenebis-[2-(*o*-hydroxyphenyl)]-glycine, EHPG, shown in Figure 1, were first described in detailed terms by Martell and his co-workers in 1957.[9] This compound is unusual in that it possesses a phenolic hydroxy group capable of binding to iron as well as amine nitrogen and carboxylate oxygen atoms typical of EDTA derivatives. The result is a greatly enhanced ability to bind iron(III), providing a stable, deep red, iron(III) complex over the pH range of 3–11. The exceptional stability of this iron(III) complex led to the design and ultimate synthesis of another chelating agent which utilized similar features in an improved structure in which the steric arrangements for the binding of iron(III) were expected and found to be better than those found in EHPG. This improved structure is found in *N,N''*-Di(2-hydroxybenzyl)ethylenediamine-*N,N'*-diacetic acid, HBED,[10] shown in Figure 1. While the log of the stability constant for EHPG with iron(III) is 33.91, that of HBED for iron(III) is 39.68.[10] HBED and EHPG were both subsequently examined as agents for the mobilization of iron(III) and found to have considerable activity, with HBED being the more promising for oral use in the clinic.[21]



EHPG



HBED

FIGURE 1 The structures of EHPG and HBED.

Studies of this sort on chelating agents for iron(III) of other structural types have been presented by Raymond,[22-24] Crumbliss,[25,26] Pitt,[27] Kontoghiorghes[28,29] and Hider[30,31] among others, and can furnish a very useful guide to similar studies with other toxic metals. These studies and the papers of Martell cited earlier, are of special importance as they include discussions of almost all of the numerous factors which have an influence on the values of the stability constants for metal complexes.

Other Variables: Direct Structure–Efficacy Correlations (DSEC)

The notion that the molecular requirements for effective therapeutic chelating agents are completely different from those of any other drug type is not supported by experimental evidence. For any type of drug one has general requirements which govern its toxicity, organ distribution, metabolism, pharmacokinetics, plus a specialized set of requirements which govern the "receptor"-type interactions by which it exerts its specific effects. Chelating agents for the mobilization of *intracellular* toxic metals must certainly form stable complexes with the metal ion of interest, but they must also meet a large number of other requirements *if they are to reach those intracellular sites at which the toxic metal is stored and form a readily excreted complex with this metal.* It is to an examination of these variables that we now turn.

The variables that govern the interaction of an organic compound, such as a therapeutic chelating agent, with a biological system in which the major sites of activity are within a cellular membrane of some sort, include those which govern the ability of the molecule to pass through this membrane. We can examine each of these factors for which evidence is available in turn.

Molecular Polarity, $\Sigma\pi$. The mot useful parameter for the estimation of this is the π parameter of Hansch[32] which is a number which provides an estimate of the contribution of a group in the molecule to the hydrophobic/hydrophilic balance of that molecule. For chelating agents it is convenient to examine $\Sigma\pi$, the sum of the π constants of the various groups in the molecule. For the chelating agents examined in

this manner to date[30,31,33-36] measures of efficacy show a maximum which occurs at some intermediate value of $\Sigma\pi$. For the removal of *renal* deposits of cadmium by dithiocarbamates derived from benzyl glucamine, the optimum efficacy is found to occur at a value of $\Sigma\pi$ close to -3.0.[35] The explicit relationship found for this is[35]

$$\{Cd_{treated}/Cd_{control}\} = 0.880 + 0.30(\Sigma\pi) + 0.054(\Sigma\pi)^2$$

where $Cd_{treated}$ is the cadmium concentration (μg/g) in the organ after treatment with the chelating agent and $Cd_{control}$ is the cadmium concentration (μg/g) in the same organ of the untreated control animals. Analogous relationships have been reported for related parameters involved in the chelate mobilization of iron[31] which utilize the partition coefficient of the chelating agent, a parameter which is closely related to $\Sigma\pi$.[32] The term $\Sigma\pi$ has the advantage over the partition coefficient in that it can be directly calculated for amphiphilic (soap-like) molecules for which the direct determination of the partition coefficient is attended by some difficulties as such molecules tend to concentrate at the interface and form foams.

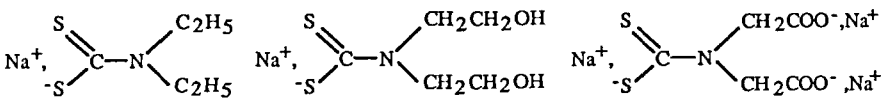
Molecular Weight, M. Another variable which affects the efficacy of a therapeutic chelating agent is the molecular weight of the molecule (M) or some closely related variable such as molecular volume. In the case where some toxic metal is to be removed from the liver *via* biliary excretion subsequent to complexation, this is a dependency which is well established by many pharmacological studies.[37] In the case where cadmium is to be removed from the liver by certain dithiocarbamate derivatives, such a dependence on molecular weight is also found.[35,36] In the case of cadmium mobilization by benzyl glucamine dithiocarbamates the specific relationship found for the reduction of hepatic cadmium is[35]

$$\{Cd_{treated}/Cd_{control}\} = 2.73 - 5.27 \times 10^{-3} M$$

The origin of this molecular weight dependence lies in two factors: the rate at which the molecule diffuses through the cytoplasm of the cell[38,39] and the way in which the energy of transport across the cellular membrane is dependent on the radius of the charged or polar molecular species being transported.[40] Note that Q_E for a given chelating agent is strongly dependent upon the organ under consideration.

Ionic Charge, Z. A factor which is important and cannot usually be directly incorporated into the $\Sigma\pi$ value because of a lack of available parameters, is the net charge on the chelating agent, Z. The two layers of the cellular membrane are made up of phospholipids and these bear oriented negative charges which give a membrane potential of about 90 mV. The passage of another negative species through this membrane is a process which requires a significant amount of energy because of electrostatic repulsions.[39,40] For this reason we find that the ability of chelating agents to gain access to intracellular deposits of metals generally drops off precipitously as the charge on the chelating agent increases. A clear example of this is seen in a comparison of the three compounds diethyldithiocarbamate (DDTC), di(hydroxyethyl)dithiocarbamate (NaY) and di(carboxymethyl)dithiocarbamate (NaX), (Figure 2) as agents for the induction of the excretion of cadmium from its aged, intracellular deposits.[41,42] In this case the stability constants for cadmium complexation are expected to be similar with the possible chelate rings of the compound containing the carboxylic acid group giving it some advantage. *In vivo*, however, the compound containing the carboxylic acid groups is almost completely ineffective because of its apparent inability to gain access to intracellular sites at which such cadmium is

stored. Another example is seen with diethylenetriaminepentaacetate (DTPA). About 90% of an injected dose of DTPA is excreted in the urine within 4 hours and only 0.12% is excreted in the bile during the first 24 hours.[43] This compound is typically only able to mobilize minute amounts of cadmium from intracellular sites, though the stability constant of its cadmium complex is larger than that of the dithiocarbamates which are frequently effective in the mobilization of such cadmium deposits.[44-47] The only exceptions to this difficult passage of ions across most cellular membranes appear to be molecules for which specific transport systems are incorporated into the membrane.



DDTC NaY NaX

FIGURE 2 The structures of three dithiocarbamates differing in ionic charge.

Attempts have been made to obviate this problem *via* the use of the esters of polyaminocarboxylates. Such compounds are much more effective in the removal of plutonium from intracellular deposits[48] but the compounds themselves are generally significantly more toxic than the parent compounds.[20] More recently, the esters of HBED have been shown to be of interest in accelerating the excretion of iron.[27,49]

Other Variables

When more detailed models of the mobilization of toxic metals from intracellular sites are considered, some of the additional variables become apparent.[50-52] One such group of variables are the rate constants for the various processes involving transport and ligand substitution of the chelating agent. These ligand substitution processes can, in fact, become the rate determining steps.[53] Another set of variables involves other molecular parameters such as chirality (R/S) and chain branching or connectivity (χ), both of which can exert significant effects.[54] In general, we can thus express the functional dependence of E in somewhat more detail as

$$E = E(K_{stab}, \Sigma\pi, M, Z, R/S, \chi, \ldots\ldots)$$

but the explicit equations describing these dependencies are not yet known for the mobilization of toxic metals from intracellular sites. Other variables include pharmacokinetic properties and toxicity. Little has been done on manipulations of the pharmacokinetic properties of therapeutic chelating agents, though the decrease in the hydrophilicity of a chelating agent generally reduces the rates of some of its reactions. The toxicity of a chelating agent and that of its metal complexes can generally be decreased *via* the addition of groups analogous to those which are placed on organic molecules during normal biological detoxification processes, *e.g.* groups analogous to glucuronide, sulfonate, *etc.*

The variables that are used here to describe the dependence of efficacy on structures are not continuous and certainly do not constitute an orthogonal set. The route to more effective compounds can be approached quite simply by the use of structural changes which allow us to move in a direction in which ($\Delta E/\Delta x_i$) has a

positive slope. Since the variables are quite directly related to structural properties of the chelating agent, these can be manipulated in a straightforward manner, especially in those cases where a sufficient amount of quantitative data is available. The formulation of an optimum structure is not yet possible on the basis of such relationships as have been developed to date between E and the various x_i quantities which determine its value. This must ultimately be done if the direct structure activity correlations (DSEC) developed for therapeutic chelating agents are to be used in the most effective manner. It must be emphasized that E (and not any single one of the independent variables) is to be maximized as well as the chemical structures allow. Thus attempts to maximize any single one of the independent variables (such as the stability constant) to the neglect of the others will not lead to a maximum value for E. The evidence for this can be seen clearly in the experimental data on copper mobilization.[53]

TABLE I

Relative renal and hepatic cadmium levels following chelating agent treatment; control (untreated) values = 1.00.

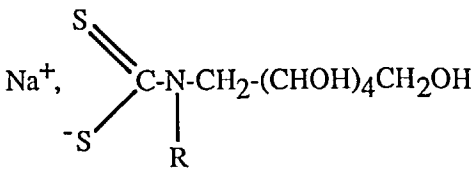
Compound	Species	Chelate Dosage*	Renal Cd	Hepatic Cd	References
NaNMGDTC	mouse	4.4 mmol/kg × 9	0.46	0.97	55
BGDTC	rat	0.40 mmol/kg × 7	0.52	0.28	44
BGDTC	mouse	0.40 mmol/kg × 7	0.99	0.96	44
BGDTC	mouse	1.00 mmol/kg × 5	0.57	0.74	45
MeOBGDTC	mouse	1.00 mmol/kg × 5	0.19	0.39	45
MeOBGDTC	mouse	1.00 mmol/kg × 2	0.36	0.38	36
MeOBGD	mouse	1.00 mmol/kg × 2	0.59	0.23	36
MeOBGDTC	mouse	0.40 mmol/kg × 5	0.68	0.75	59
BLDTC	mouse	0.40 mmol/kg × 5	0.39	0.38	59
MeBLDTC	mouse	0.40 mmol/kg × 5	0.39	0.20	59

* The chelate dosage shown is given as the individual dose administered times the number of such doses administered; there is a separation of 24 h between each chelate administration.

Abbreviations used: NaNMGDTC, sodium *N*-methyl-*D*-glucamine dithiocarbamate; BGDTC, sodium *N*-benzyl-*D*-glucamine dithiocarbamate; MeOBGDTC, sodium *N*-(4-methoxybenzyl)-*D*-glucamine dithiocarbamate; MeOBGD, sodium *N*-(4-methoxybenzyl)-*D*-gluco-L-talooctamine dithiocarbamate; BLDTC, sodium *N*-Benzyl-4-*O*-(b-*D*-galactopyranosyl)-*D*-glucamine-*N*-Carbodithioate; MeBLDTC, sodium *N*-(4-methylbenzyl)-4-*O*-(b-*D*-galactopyranosyl)-*D*-glucamine-*N*-carbodithioate.

Enhanced Efficacy Via *Manipulation of Pharmacologically Important Variables.*

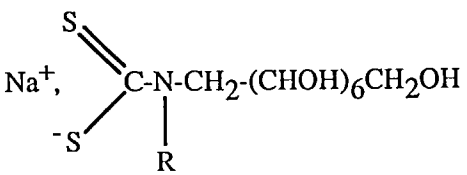
The achievement of enhanced efficacy *via* the manipulation of the variables which are of critical importance in the determination of the pharmacological behaviour of chelating agents, but having little or no influence on the stability constant, can be seen in the development of compounds of increasing efficacy for the mobilization of cadmium from its aged, intracellular deposits in the kidneys and liver of rodents (mice and rats). The data on these compounds are shown in Table I and their structures are shown in Figure 3. The starting point was the compound sodium *N*-methyl-*D*-glucamine dithiocarbamate (NaNMGDTC), which was effective and relatively non-toxic,[55] but had to be administered at a high dosage to remove an appreciable fraction of the intracellular cadmium in the kidney and the liver. The



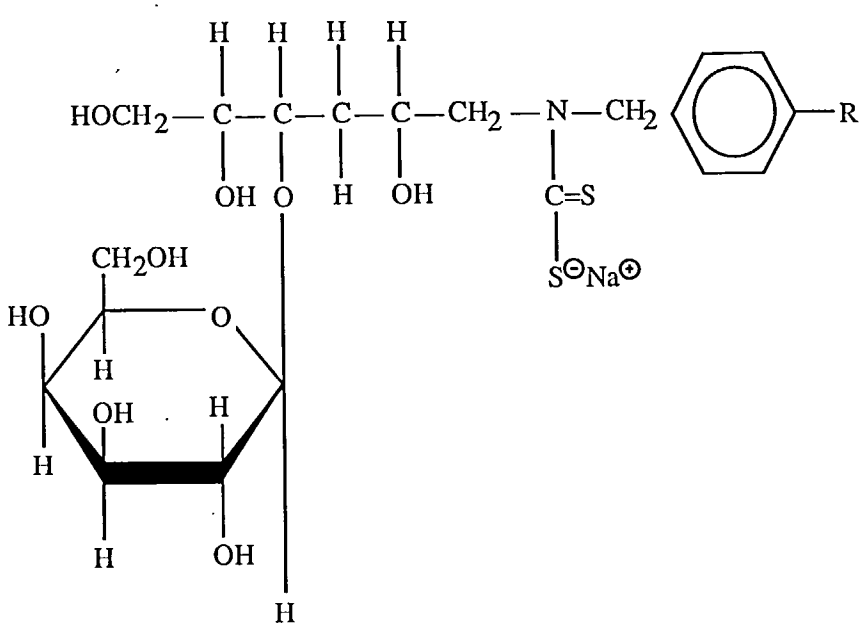
NaNMGDTC: R = CH_3-

BGDTC: R = C_6H_5- CH_2-

MeOBDTC: R = 4-CH_3-O-C_6H_4-CH_2-



MeOBGD: R = 4-CH_3-$OC_6H_4CH_2$-



BLDTC: R = H

MeBLDTC: R = CH_3

FIGURE 3 Structures of dithiocarbamates of increasing efficacy for cadmium mobilization from intracellular sites.

next step involved the preparation of sodium *N*-benzyl-*D*-glucamine dithiocarbamate (BGDTC), a compound of significantly higher molecular weight, which was effective in removing cadmium from organs at lower dosages.[44] Then an improved compound, obtained by the use of the Topliss scheme, was found in sodium *N*-(4-methoxybenzyl)-*D*-glucamine dithiocarbamate (MeOBGDTC),[45] which had a more nearly optimum value of $\Sigma\pi$ (about -2.08). Following a detailed study of many compounds of this type, direct structure activity correlations of the type shown earlier were developed[35] and these served as a guide for subsequent work. The key features in these correlations, in addition to those described above, was a slight dependence of the efficacy for renal cadmium mobilization on the molecular weight and a slight dependence of the efficacy for hepatic cadmium mobilization on $\Sigma\pi$. This, in turn, suggested the advantages of a compound of higher molecular weight, since both renal and hepatic cadmium mobilization were expected to improve as the molecular weight of the compounds increased, provided that the value of $\Sigma\pi$ was not too far from the optimum one. Follow up led to synthesis of the more effective compound sodium *N*-(4-methoxybenzyl)-*D*-gluco-*L*-talooctamine dithiocarbamate (MeOBGD),[36] which had a higher molecular weight than the compounds previously prepared and studied. A significant improvement over this compound was obtained, in turn, by using lactose, rather than glucose, as the reducing sugar, to obtain sodium *N*-Benzyl-4-*O*-(b-*D*-galactopyranosyl(-*D*-glucamine-*N*-carbodithioate) (BLDTC).[58] This compound had a much higher molecular weight than any of the previously prepared compounds, but did not have an optimum value of $\Sigma\pi$. The preparation of the analogous compound from 4-methylbenzylamine provided a compound with a value of $\Sigma\pi$ closer to the optimum value, MeBLDTC, which, once again, was found to be still more effective than BLDTC.[59] In this series of compounds, the steps leading to compounds of greater efficacy involved changes in $\Sigma\pi$ and M to obtain more nearly optimum values of these parameters. It is apparent that one of the major changes as we pass through this series is the significant change in molecular weights, going from just over 300 for the first entry to approximately 550 for the final one. All of these compounds possess an amphiphilic structure. The process was facilitated by the fact that both renal and hepatic cadmium deposits were more readily removed by compounds of greater molecular weight, provided that the value of $\Sigma\pi$ was not too far from the optimum one for renal efficacy. The numerical data on these compounds, summarized in Table I, indicate how these compounds compare with each other. In examining this data it is necessary to note that for a given chelating agent it is significantly easier to mobilize intracellular cadmium from rats than from mice. Also, as one proceeds down the table the dose of chelating agent administered decreases very significantly. Additional data demonstrating these trends is available in the literature cited in Table I. None of these compounds caused any observable histopathological damage when they removed cadmium from intracellular deposits in the kidneys and liver.

Chelating Agent Toxicity and Dose–Response Curves

A factor which is rarely discussed during the process of chelating agent design, but which is of great practical significance is the chelating agent toxicity and the manner in which it affects the usable portion of the dose–response curve for toxic metal mobilization. The effectiveness of a chelating agent in removing a toxic metal from a living mammal increases with increasing dose of the chelating agent.[36] For a toxic chelating agent, however, high doses cannot be used *in vivo*, because of their potential

lethality. The problem was first explicitly recognized in the 1940's by the prominent British physiologist Danielli, who noted that a major disadvantage in the use of BAL (2,3-dimercapto-1-propanol) was its considerable toxicity.[56] Danielli, exploiting the fact that one of the natural biochemical processes by which mammals detoxify compounds is *via* glucuronidation, demonstrated that BAL-glucoside was a significantly less toxic and potentially more effective chelating agent than BAL itself.[56] This type of structural modification has been exploited recently in the development of very effective compounds for the mobilization of cadmium from its aged renal and hepatic deposits.[35,36,45]

CHELATING AGENT SPECIFICITY AND SELECTIVITY

In biological systems, especially whole animals such as small mammals (mice and rats), selectivity for the toxic metal ion of interest often turns out not to be as acute a problem as is sometimes suggested.[57] There are at least two reasons for this. The first is that for many toxic metals such as cadmium or mercury, typical stability constants for the chelating agent for the toxic metal ion are almost always much larger than that for the corresponding essential metal ion in the group (in this particular case, zinc), because the toxic metal ions are the heavier metal ions. The essential metal ions are, with the sole exception of molybdenum, derived from the elements in the first transition series or the elements of comparable position in the periodic table, such as zinc, while the typical toxic metal ions are generally derived from those of the second or third rows in the corresponding positions of the periodic table. The second, and possibly more important factor is that the toxic metals are generally present in the environment in amounts which are far smaller than the essential metal ions. As a result they are not normal constituents of the diet of the typical animal or person except under unusual circumstances. The removal of essential metal ions from an animal is **rapidly** followed by an increased uptake of that essential element from the diet while the toxic metal is generally not present in this diet in comparable amounts and is not replaced in a similar fashion from the diet. Thus problems from the enhanced excretion of essential metal ions due to a lack of chelating agent selectivity are rarely found in animal experiments or human clinical experience unless no effort at all is made to replace the essential metal ions. Typical data of this sort can be seen in experiments in which dithiocarbamates were used to remove cadmium from mice.[44] In these experiments no significant decreases in the zinc, copper or manganese contents of the liver, kidney or brain were reported following treatment with dithiocarbamates which reduced cadmium levels in the liver to approximately 75% of the initial values. Of perhaps greater relevance is the fact that of the chelating agents currently used in the clinic, only deferoxamine exhibits a practical selectivity for the metal ion which it is used to mobilize, iron, and even here this compound has also been used to mobilize aluminium. This is why deferoxamine is suitable for the long term therapy required in the treatment of β-thalassemia. Thus EDTA, BAL, *D*-penicillamine, *meso*-2,3-dimercaptosuccinic acid (DMSA), 2,3-dimercaptopropanesulfonate (DMPS), triethylenetetramine (TREN), and diethylenetriaminepentaacetate (DTPA), are chelating agents which each complex a variety of metal ions including several of the essential metals ions. Thus practically all form quite stable complexes with zinc, manganese and copper, and several cause a significant temporary increase in the urinary excretion of one or more of these elements. However, only extended continuous daily administration or high doses of these compounds result in damage due to an excessive loss of an essential metal.

A MORE GENERAL RELATIONSHIP

A summary of many of the factors which may be of importance in the design of a chelating agent for the removal of a toxic metal ion from its *in vivo* deposits is presented in Table II. The design of therapeutic chelating agents would be much facilitated by the development of explicit general relationships which incorporate more of the variables upon which their *in vivo* efficacy depends. For example, the effects of changes carried out to enhance stability constants often have a deleterious effect on the ability of the chelating agent to reach the biological sites at which the toxic metal is stored or, when a macrocycle is involved, on the rate with which the chelating agent reacts with the toxic metal. For these more general relationships to be elaborated the functional relationship between metal mobilization efficacy and the variables which determine it must first be determined experimentally. This is a task for the future which should well repay the efforts which it will require.

TABLE II
Some factors of importance in the design of therapeutic chelating agents.

Effective stability constant of metal complex formed
Net charge on chelating agent at physiological pH
Partition coefficient/$\Sigma\pi$/amphiphilic nature
Molecular weight
Rate of reaction with *in situ* toxic metal deposits
Rate of metabolism
Toxicity of chelating agent
Chirality of chelating agent
Chain branching in chelating agent
Water solubility (for injection)
Chemical stabilty
In vivo distribution of unexcreted metal complex
Route of excretion of metal complex

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