The Role of Rate Determining Steps in the Decorporation of Toxic Metal Ions

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Abstract

An examination of a variety of experimental data shows that several types of rate determining steps can drastically limit the ability of a chelating agent to react with a toxic metal ion *in vivo*. Such limitations are important for many metal–chelate formation reactions with very favorable overall thermodynamic changes. Two of the most important of these are the slow ligand exchange reactions and slow rates of movement of ionic chelating agents across cellular membranes to achieve contact with intracellular deposits of toxic metal ions. They have drastic effects on the overall stoichiometry of the decorporation process. A simple model is developed which allows rates of mobilization to be estimated. This model is also applied to the problem of overly rapid metal mobilization which frequently leads to adverse effects. The nature of synergistic effects in chelate therapy is also examined and it is seen that many of these can be explained on the basis of the catalysis of ligand exchange processes or on the basis of the different *in vivo* distribution of lipophilic and hydrophilic chelating agents.

Introduction

The search for new therapeutic chelating agents for toxic metal ions is usually based, in large part, on an evaluation of the stability constants of the complexes formed by the chelating agents with that toxic metal ion [1–6]. This method has the enormous advantage that it allows the rapid and reliable selection of those chelating agents whose reactions with the toxic metal are most favored thermodynamically. In essence, it tells us which of the proposed processes under study are not forbidden by the laws of thermodynamics. This allows us to eliminate a large number of conceivable but thermodynamically forbidden processes. Unfortunately, other factors may operate to render a compound less useful *in vivo* than would be expected on the basis of such a model [7, 8]. As a result, the efficiency of a chelating agent may be limited by kinetic processes of metabolism (transport, chemical modification) or exchange of endogenous and exogenous ligands. Many of these other factors are related to the rates of various processes involved in the overall detoxification procedure. Previously the rate at which the chelating agent is removed from the serum by excretion has been shown to affect the conclusions drawn from a purely thermodynamic model [9]. The purpose of the present study is to continue the examination of the influence of rate factors on the predictions of the thermodynamic model. The principal benefit to be derived from such a study is a better appreciation of the ways in which the structures, properties and modes of administration of chelating agents must be modified in order to make them more useful *in vivo*. Here we will examine several related aspects of this overall problem: (I) mobilization processes in which the rate determining step is probably the rate of exchange of the metal ion from the natural set of *in vivo* binding sites to the therapeutic chelating agent, (II) the stoichiometry of commonly utilized clinical decorporation procedures, (III) a model of the decorporation process which allows us to optimize metal mobilization via manipulation of the dosing schedule, (IV) the disadvantage of overly rapid mobilization and (V) the nature of synergistic effects in the overall detoxification or mobilization processes. An examination of these factors allows the development of more rational schemes for the optimum mobilization of toxic metals from those sites to which they are bound *in vivo*.

I. Metal Exchange Processes as Rate Determining Steps

In vitro, the divalent metal ions (e.g. Mn^{2+}, Cu^{2+}, Pb^{2+}, Cd^{2+}, etc.) are classed as kinetically labile (equilibration with a new set of ligands within the time of mixing) but *in vivo* where we are dealing with

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multidentate ligand exchange and with low concentrations, this assumption of lability, especially for the transition metal ions may not be valid [41, see however 92].

An examination of the literature reveals several cases where the transfer of the metals from their *in vivo* binding sites on proteins to the therapeutic chelating agents is a slow process and may be rate determining in the overall process. These include those reactions involved in the removal of iron from transferrin [10–12] where the rates of such reactions tend to be slow in comparison to the rate of excretion or metabolism of the chelating agent added to mobilize the iron. Thus, the half-time for the clearance of desferrioxamine (desferal) from the plasma is only 5–10 min [13]. This has a very disadvantageous effect on the iron decorporation process since a 100:1 mole ratio of desferrioxamine to transferrin is only able to remove 5% of the iron after 30 min [14, 15]. This undoubtedly partially explains the low efficacy sometimes found with desferrioxamine. This efficacy, however, improves very significantly in the presence of ascorbic acid and under conditions of high iron overload, as described in the next section. Analogous reactions of aluminum are much more rapid [94].

Copper in serum is present primarily bound to ceruloplasmin [16]. This protein contains over 90% of the copper in human plasma and binds copper in at least three types of sites [16]. When ceruloplasmin is treated with some multidentate chelating agents, little effect is found on its oxidase activity [17, 18], which is, however, inhibited by certain monodentate ligands such as N_3^- and CN^-. The copper binding sites are apparently not readily accessible and one expects a slow rate of reaction between such chelating agents and the copper bound in ceruloplasmin. This is, in fact, found with certain macrocyclic chelators [7] and the removal of copper from ceruloplasmin does not occur at all in the presence of phenanthroline [19]. That serum copper which is not bound to ceruloplasmin is present in serum albumin [20]. Under physiological conditions the rate of copper removal from serum albumin is expected to be very slow [20].

Another element for which some data are available is cadmium. This element rapidly attains intracellular spaces and induces the synthesis of metallothionein to which it is ultimately and rapidly bound. This cadmium metallothionein (CdMT) releases cadmium to chelating agents such as EDTA [21, 95] and dithiocarbamates [22]. These are also rather slow reactions in comparison with the rate at which the chelating agents are metabolized or excreted [9] whereas metal interchange reactions involving metallothionein may be rapid [92, 93]. The pseudo first order rate constants for these reactions are shown in Table I.

TABLE I. Pseudo First-order Rate Constants for Reaction of CdMT with Chelating Agents

Chelating agent	T (°C)	k_1 (s^{-1})	Reference
EDTA	25	3.7×10^{-6}	21
Sodium diethyldithiocarbamate	25	5.9×10^{-5}	22
Sodium *N*-methyl-D-glucamine dithiocarbamate	25	9.6×10^{-5}	22

These rate constants are such that a period of several hours is required for the removal of appreciable amounts of Cd from CdMT. The serum half lives of these chelating agents are all less than 30 min. While little cadmium remains in the serum 24 h after administration, what does remain is bound to serum albumin and α_2-macroglobulin [23, 24]. This type of complexed cadmium also reacts quite slowly with most chelating agents [25]. With 0.19 μmol EDTA/ml, 52% of such serum Cd is mobilized after 2½ h [25].

In most cases where rate studies have been carried out it is found that the rate of removal of a toxic metal from its binding sites (extra- or intracellular) is fairly slow. For a large number of systems of considerable interest, rate data of this sort are simply not available. Indirect evidence on these rates can be obtained from information on the stoichiometry of metal decorporation.

II. Stoichiometry of Metal Decorporation

The limiting effects of the rates of various processes on the overall decorporation process for toxic metals, in humans and other mammals, can be seen quite clearly in a comparison of the mole ratios of metal excreted to chelating agent administered. Before examining the data it is well to realize that, for therapeutic chelating agents as with other drugs, the types of processes which form the basis of action fall into three main phases; the pharmaceutical phase (the processes involved in the release of the active agent), the pharmacokinetic phase (the absorption, distribution, metabolism and excretion of the drug) and the pharmacodynamic phase (the molecular interaction of the active agent with its specific site of action) [26]. However, in the case of therapeutic chelating agents, the sequence of these processes is not necessarily the same as for drugs with specific, localized receptors. An examination of the data on the mole ratios of metal excreted to chelating agent administered is quite revealing as it shows the importance of factors not often given adequate consideration. Data of this sort on iron excretion are collected

TABLE II. Iron Excretion Induced by Chelating Agents (all humans have medically induced iron overload)

Species	Chelating agent		Iron excretion (mmol)	mol Fe/mol chelator	Reference
	name	(mmol)			
Human	Desferrioxamine (750 mg + 750 mg ascorbic acid i.v.) (m.w. of methanesulfonyl = 656.81)	1.142	0.895	0.783	97
Human	Desferrioxamine 16000 mg	24.36	3.22	0.132	97
Human	Desferrioxamine 750 mg	1.142	0.179	0.156	28, 97
Rats (hyper-transfused)	2,3 dihydroxybenzoic acid	0.65	0.0143	0.022	98
Human	2,3 dihydroxybenzoic acid	1.905	0.080	0.042	98
Human	Desferrioxamine	1.525	0.18–75	0.118–0.492	99
Human	Desferrioxamine	1.200	0.03–0.27	0.025–0.23	100
Human	Desferrioxamine	0.761	0.322	0.423	101
Human	Desferrioxamine	6.10	1.79	0.293	102
Mice (iron overload)	1,2-dimethyl-3-hydroxypyrid-4-one (m.w. = 139)	1.439	0.076	0.053	103
Mice (iron overload)	Desferrioxamine	0.304	0.041	0.135	103
Human	1,2-dimethyl-3-hydroxypyridin-4-one	86.33 (p.o.)	0.329	0.0038	104
Human	Desferrioxamine + ascorbate	11.41 s.c.	0.390	0.0342	104
Human	1,2-dimethyl-3-hydroxypyridin-4-one (p.o.)	61.15 p.o.	0.376	0.006	104

TABLE III. Lead Excretion Induced by Chelating Agents

Species	Chelating agent		Lead Excretion (mmol) (urine)	mol Pb/mol chelator	Reference
	name	(mmol)			
Human (with chronic Pb intox)	$Na_2CaEDTA$ (i.v.)	2.44	0.015–0.174	0.004–0.072	105
Human (chronic Pb intox)	DMSA (oral)	46	0.0648	0.0014	106
Human (chronic Pb intox)	EDTA (i.v.)	12.2	0.112	0.009	106
Rats	DMPS	~0.030	~0.00011	0.0037	107
Rats	BAL	~0.030	~0.0017	0.0057	107
Rats	DMPS	0.20	0.000058	0.00029	108
Human	EDTA	2.44	0.015	0.006	109
		2.44	0.021	0.008	109
Rats	EDTA	0.268	0.00044	0.0016	110
	BAL	0.268	0.00071	0.0026	110

in Table II. The excretion of iron after the administration of desferrioxamine, described in several entries in this table is roughly related to iron overload [27]. Iron overload arises most commonly in individuals who receive blood transfusions continuously, such as those with thallasemia. The mole ratio of iron excreted to desferrioxamine administered increases considerably when ascorbic acid is also administered. With individuals who are extremely overloaded this ratio can rise to values close to unity [27].

Data on the stoichiometric relationships between lead mobilized and chelating agents administered are presented in Table III. In these cases the degrees of exposure differ as do the organ distributions of the lead.

The third element for which data of this sort are presented is copper. The excretion of this metal is of special interest in connection with the clinical control of Wilson's Disease, a hereditary disorder in which copper accumulates in various organs until lethal levels are reached. Walshe has developed the use of

TABLE IV. Copper Excretion Induced by Chelating Agents

Species	Chelating agent		Copper excretion (mmol)	mol Cu/mol chelator	Reference
	name	(mmol)			
Human (Wilson's Disease)	D-Penicillamine (m.w. = 149.2)	4.02	0.020	0.005	111
Human (Wilson's Disease)	D-Penicillamine	6.70	0.0141	0.002	112
Human (Wilson's Disease)	Triethylenetetramine dihydrochloride (m.w. = 219.16)	4.56	0.0321	0.007	113
Human (Wilson's Disease)	D-penicillamine (i.v.)	1.51	0.0163	0.011	114
Human (Wilson's Disease)	BAL (m.w. = 124.21) (i.m.)	1.61	0.0142	0.009	114
Human (Wilson's Disease)	Triethylenetetramine dihydrochloride	6.05	0.0150	0.0026	115
Human (Wilson's Disease)	D-Penicillamine	3.35	0.0169	0.005	115
Rat (unloaded, normal)	D-Penicillamine	0.670	0.00037	0.0006	116
Rat (unloaded, normal)	Triethylenetetramine dihydrochloride	0.455	0.00031	0.0007	116

D-penicillamine for the treatment of this disorder and has also shown how other compounds, such as triethylenetetramine, can be used with individuals who show adverse reactions when given D-penicillamine. The natural accumulation of copper in this disorder leads to individuals who have accumulated varying amounts of copper, depending upon the seriousness of their disorder and the length of time it has gone untreated. Some data on the mole ratios of copper excreted to chelating agent administered are collected in Table IV. The rate and extent of copper removal from Wilson's Disease patients upon treatment with D-penicillamine is very much dependent on the status of the patient. In early stages of treatment extent and rate of copper removal by D-penicillamine can be very dramatic.

A common factor in some of these chelate treatment regimes is that the ratio of metal removed to chelate administered decreases as the amount of chelate administered increases. This is shown very clearly in the data on daily urinary iron excretion at various levels of desferrioxamine administration [28]. As might be suspected, the *in vivo* data on the reactions between chelating agents and very toxic metals are limited by the fact that high levels of a toxic metal cannot be attained in such experiments because of their lethality and usual clinical practice is to give as high a dosage of antagonist as feasible.

Another aspect of the mobilization of a toxic metal is that in a series of closely spaced chelate administrations, the first treatment generally induces a greater mobilization of the toxic metal than subsequent ones. This is due to the fact that the majority of therapeutic chelating agents are restricted to the extracellular compartment and the initial supply of chelating agent will react with a larger pool of serum bound toxic metal than subsequent injections. As the toxic metal 'reequilibrates' (actually a kind of steady state) among intra- and extracellular metal, this occurs rather slowly. Therefore the mole ratio of metal mobilized to chelating agent administered decreases for subsequent doses of chelating agent. For any particular toxic metal examined, it is found that the overall rate of toxic metal excretion is reduced by several factors. These rate reducing steps, in the order in which they occur are (a) limited absorption of the chelating agent (of especial importance in oral administration of the chelating agent), (b) pharmacokinetics of processes in which the chelating agent is excreted or metabolized or transported across cell membranes, (c) a slow rate of transfer of toxic metal from the *in vivo* binding sites to the administered chelating agent, (d) release of toxic metal ion from the metal chelate complex (after it has been formed) to tissues through which it passes during metabolism of the metal–chelate complex (e.g. to the liver or kidney).

III. Metal Mobilization Model

It is very helpful to develop a model of the metal mobilization process, and for this a purely kinetic model possesses several advantages. The basic assumption made in the model developed here is that the mobilization of the metal ion involves the reaction of the chelating agent, Ch, with the protein-bound metal in the serum, MP, to give as products the chelated form of the toxic metal ion, MCh, and the metal-free serum protein:

$$\mathrm{Ch} + \mathrm{MP} \xrightarrow{k_1} \mathrm{MCh} + \mathrm{P} \qquad (1)$$

The second reaction which must be considered involves the removal of the chelating agent by such processes as excretion and destruction by metabolic processes:

$$\mathrm{Ch} \xrightarrow{k_2} \text{inactive or excreted products} \qquad (2)$$

That metal which is transformed into low molecular weight chelated forms is then assumed to be rapidly filtered out at the glomeruli and to pass into the urine.

The rate equations for these processes are postulated to be

$$\mathrm{d[MP]}/\mathrm{d}t = -k_1[\mathrm{Ch}][\mathrm{MP}] \qquad (3)$$

and

$$\mathrm{d[Ch]}/\mathrm{d}t = -k_1[\mathrm{Ch}][\mathrm{MP}] - k_2[\mathrm{Ch}] \qquad (4)$$

The removal rate of the metal in this simple model is taken to be the rate of formation of MCh by reaction (1),

$$\mathrm{d[MCh]}/\mathrm{d}t = k_1[\mathrm{Ch}][\mathrm{MP}] \qquad (5)$$

It is first assumed that the chelating agent is administered rapidly by intravenous injection (over a time period short in comparison with the time scales of reactions (1) and (2)). Without further restrictions and approximations it is not possible to solve these equations in closed (analytical) form. It is easy to develop numerical solutions, however, and this has been done. This type of modeling process can readily be adapted to more complex rate mechanisms, such as those found for the reaction of the nickel bovine serum albumin complex and histidine [29]. We have generated numerical solutions for eqns. (3) and (4) for various assumptions about the values of k_1 and k_2; these are shown in Figs. 1–4 as functions of time. In all cases the initial concentrations of MP and Ch are taken as 1 (in arbitrary units), and the time interval is 3, again in arbitrary units. In Fig. 1 it is assumed that $k_1 = 1$ and $k_2 = 0$, that is, the chelating agent is neither metabolized nor excreted. About

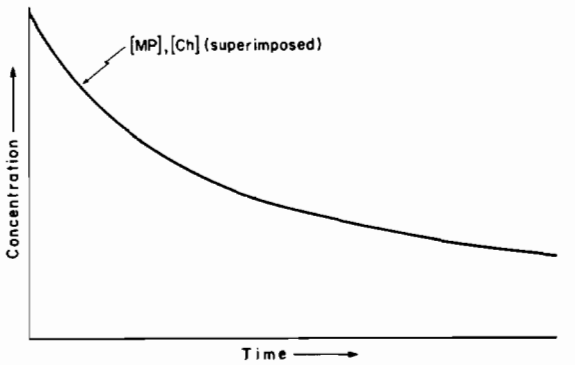

Fig. 1. Clearance of metal by chelate. $k_1 = 1$, $k_2 = 0$, initial [MP] = 1, initial [Ch] = 1, run duration = 3.

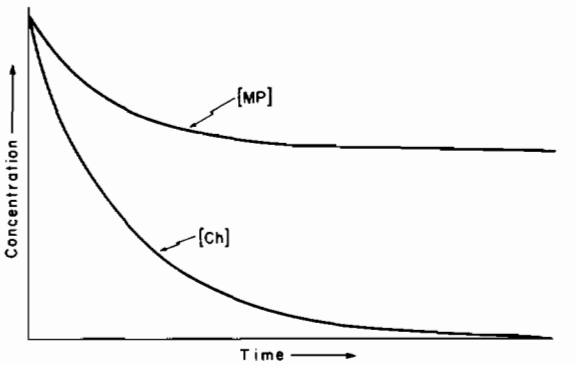

Fig. 2. Clearance of metal by chelate. $k_1 = 1$, $k_2 = 1$, initial [MP] = 1, initial [Ch] = 1, run duration = 3.

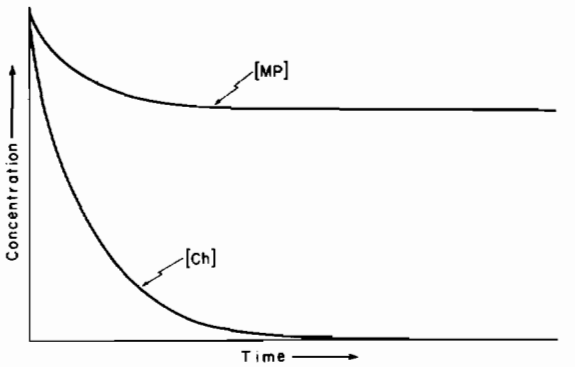

Fig. 3. Clearance of metal by chelate. $k_1 = 1$, $k_2 = 2$, initial [MP] = 1, initial [Ch] = 1, run duration = 3.

76% of the metal present initially has been removed. In Fig. 2, it is assumed that $k_1 = 1$ and $k_2 = 1$; under these conditions about 44% of the metal has been removed. In Fig. 3, $k_1 = 1$ and $k_2 = 2$; in this run only about 30% of the metal has been removed by the simulated treatment. In Fig. 4 the concentration of chelating agent is plotted for the case where $k_1 = 0$ and $k_2 = 1$; this curve corresponds to the first order removal of chelating agent by excretion and metabolism, without reaction with metal.

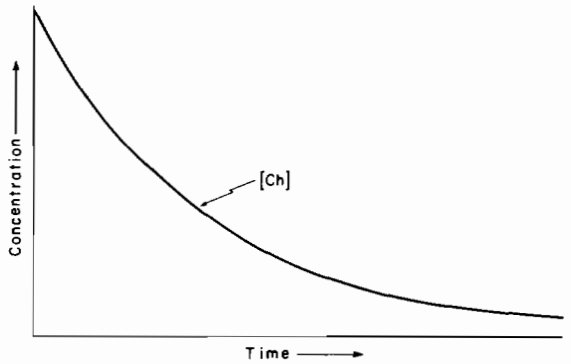

Fig. 4. Clearance of metal by chelate; metabolism and excretion of chelate in the absence of reaction with metal. $k_1 = 0$, $k_2 = 1$, run duration = 3.

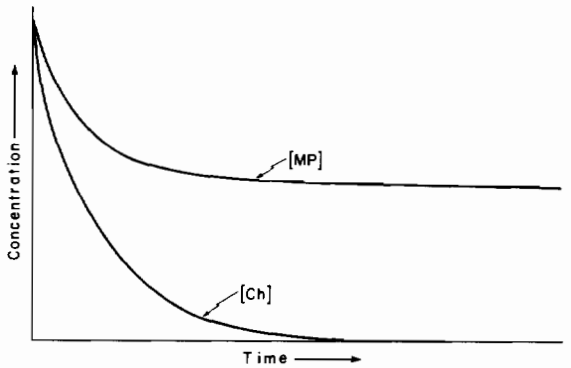

Fig. 5. Clearance of metal by chelate. $k_1 = 1$, $k_2 = 2$, initial [MP] = 1, initial [Ch] = 2, run duration = 3.

These runs demonstrate very clearly what one would expect intuitively – that the mobilization of the metal ion is very significantly decreased by the metabolism and excretion of the chelating agent. If the time scale for chelate removal by metabolism and excretion is comparable to or shorter than the time scale for its reaction with the metal–protein complex, the chelate will be used very inefficiently.

Figures 3, 5 and 6 demonstrate that one can partially overcome this problem (within the limitations of the toxicity of the chelating agent) by increasing the dose of chelate. In all these runs $k_1 = 1$, $k_2 = 2$, the initial concentration of MP is 1, and the duration of the run is 3. In Fig. 3 the initial chelate concentration is 1, and 30% of the metal is removed. In Fig. 5 the initial chelate concentration is 2, and 53% of the metal is removed. In Fig. 6 the initial chelated concentration is 3, and 68.8% of the metal is removed.

There are not a large number of systems for which data of the sort needed to use models such as this are directly available. There are some cases, however, where pharmacokinetic data on the chelating agents are available and where some kinetic data on the rate

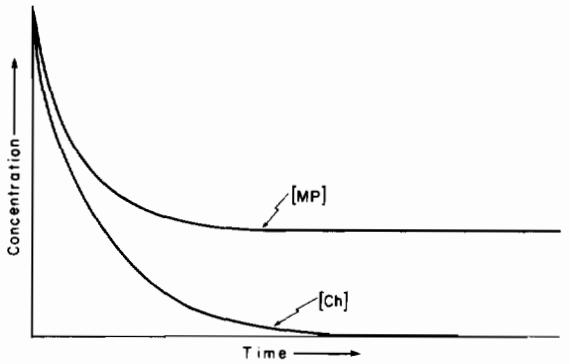

Fig. 6. Clearance of metal by chelate. $k_1 = 1$, $k_2 = 2$, initial [MP] = 1, initial [Ch] = 3, run duration = 3.

of reaction of the chelating agnet with a key metal binding protein are available.

The numerical methods used with this model are very easily extended to more complex representations of the chelation therapy. We discuss one briefly. The proposed model is as follows.

$$\text{Tissue-bound metal (MT)} \xrightarrow{k_1} \text{serum-bound metal (MS)} \quad (6)$$

$$\text{Serum-bound metal (MS)} \xrightarrow{k_2} \text{tissue-bound metal (MT)} \quad (7)$$

$$\text{MS} + \text{Ch} \xrightarrow{k_3} \text{rapidly excreted species} \quad (8)$$

$$\text{Ch} \xrightarrow{k_4} \text{excreted or metabolized} \quad (9)$$

The differential equations describing this model are

$$d[\text{MT}]/dt = -k_1[\text{MT}] + k_2 \frac{\nu_s}{\nu_t}[\text{MS}] \quad (10)$$

$$d[\text{MS}]/dt = +k_1 \frac{\nu_t}{\nu_s}[\text{MT}] - k_2[\text{MS}] - k_3[\text{MS}][\text{Ch}] \quad (11)$$

$$d[\text{Ch}]/dt = -k_3[\text{MS}][\text{Ch}] - k_4[\text{Ch}] + s(t)/\nu_s \quad (12)$$

Here $s(t)$ is the rate at which chelating agent is being administered, ν_s is the effective volume of the serum phase, and ν_t is the effective volume of the tissue phase.

Figures 7 and 8 show some representative simulations with this model. The parameters are given in the captions in the figures. The serum chelate concentration increases rapidly at first, then more slowly; it actually approaches a limiting value determined by

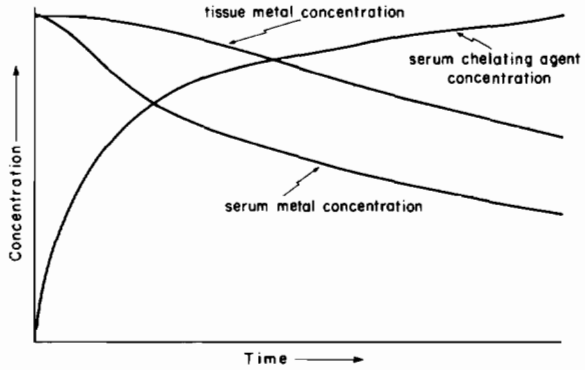

Fig. 7. Clearance of metal by chelate, 2-compartment model. Volume of tissue = 1000 ml, volume of serum = 1000 ml, k_1 (tissue → serum) = 0.25, k_2 (serum → tissue) = 1, k_3 (serum + chelate) = 1, k_4 (chelate metabolism and excretion) = 1, chelate dosage rate = 1000, duration of run = 6.4.

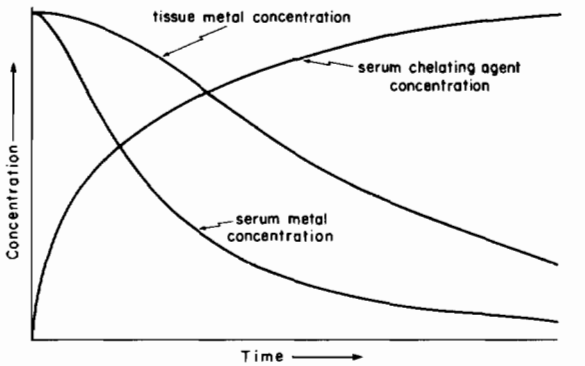

Fig. 8. Clearance of metal by chelate, 2-compartment model. Parameters as in Fig. 7 except that k_2 = 0.25 and the duration of the run is 10.

the rate of administration and the rate of metabolism and excretion. The serum metal concentration decreases rapidly at first, followed by a slower drop which is controlled by the rate at which metal moves from tissue to serum. As expected, the tissue metal concentration is depleted more slowly than the serum metal concentration, but controls the rate of decrease of the serum metal concentration over longer times.

IV. Overly Rapid Metal Mobilization

The disadvantage of overly rapid mobilization of toxic metal ions has been established in the cases of several such species. For iron-overloaded patients, the combined use of desferrioxamine and ascorbic acid can lead to a very impressive enhancement of urinary iron excretion which, when carried out intensively, is accompanied by cardiac arrest in an unacceptably high percentage of the patients [30]. In the case of lead intoxication, intensive decorporation regimes lead to renal failure in perhaps as many as 5% of the individuals [31, 32]. The cause of this may be the inherent renal toxicity of EDTA itself [33] which arises, at least in part, from its ability to significantly increase the urinary excretion of essential metal ions such as Zn^{2+}, Mn^{2+}, Ca^{2+}, Cu^{2+}, etc. [34], or to the inherent toxicity of the unacceptably large amounts of the Pb^{2+}–EDTA complex as it is transported through the kidney. The standard de-leading regime based on $Na_2CaEDTA$ is quite safe [35]. For cadmium intoxication, the chelate mobilization of significant amounts of cadmium by certain chelating agents to the kidney can result in significant renal damage [36]. With some other situations, such as copper overload which occurs in Wilson's Disease, there do not appear to be any clinical cases which indicate that overly rapid excretion of copper has led to problems. This may be due to the fact that copper complexes of either Cu(II) or Cu(I) are generally much less toxic than Cu^{2+}.

By using the first of the models presented previously, one can design a therapeutic regime to maximize mobilization below the limit at which the adverse effects occur. This is developed as follows. We set x = [MP] and y = [Ch], giving rate equations

$$dx/dt = -k_1xy \tag{13}$$

$$dy/dt = -k_1xy - k_2y + s(t) \tag{14}$$

$s(t)$ = dosage rate per unit volume of patient

If we choose $s(t)$ so as to maintain a constant [Ch] = $y = y_0$, then $dy/dt = 0$, and

$$dx/dt = -k_1y_0x \tag{15}$$

so

$$x = x_0 \exp(-k_1y_0t) \tag{16}$$

yielding an exponential removal rate of the metal, which is perhaps somewhat slow. Nevertheless, let us determine the dosage rate which will yield this rate of metal chelation. Solve eqn. (14) for $s(t)$, given the requirement that $dy/dt = 0$ and that $y(t) = y_0$; this gives

$$s(t) = y_0[k_1x(t) + k_2] \tag{17}$$

Substituting eqn. (16) into this result gives

$$s(t) = y_0[k_1x_0 \exp(-k_1y_0t) + k_2] \tag{18}$$

The rate of clearance of metal in this model is given by

$$|dx|/|dt| = k_1y_0x_0 \exp(-k_1y_0t) \tag{19}$$

Let D be the maximum rate of clearance which can be tolerated without injuring the patient. We note that the maximum rate of clearance, according to eqn. (19), occurs at $t = 0$, from which it is seen that

the criterion which must be satisfied to avoid patient injury is

$$y_0 < \frac{D}{k_1 x_0} \tag{20}$$

Thus the dosage rate must be less than

$$s_{\max}(t) = \frac{D}{k_1 x_0}\left[k_1 x_0 \exp\left(-\frac{k_1 D}{k_1 x_0} t\right) + k_2\right]$$

$$= D\left[\exp\left(-\frac{Dt}{x_0}\right) + \frac{k_2}{k_1 x_0}\right] \tag{21}$$

A dosage regime for which $s(t) < s_{\max}(t)$ for all t should then be safe for the patient. The dosage regime specified by eqn. (21) clears metal from the patient exponentially, with a half-life of 0.693 x_0/D.

We next explore the feasibility of decorporating metal from the patient more rapidly than exponentially while avoiding injury. Again we use our first model, given by eqns. (13) and (14). Let x_0 = initial metal concentration, y_0 = initial chelate concentration, and D = the desired metal removal rate, $|dx/dt|$. Then the model eqns. (13) and (14) become

$$-D = -k_1 xy \tag{22}$$

and

$$dy/dt = -D - k_2 y + s(t) \tag{23}$$

From eqn. (22) we see that the initial dosage of chelate should be such as to make the initial blood level of chelate equal to $y_0 = D/k_1 x_0$. Also, if the removal rate of the metal is to be a constant, D, then

$$x(t) = x_0 - Dt, \quad t < x_0/D \tag{24}$$

So from eqn. (22) we get

$$-D = -k_1(x_0 - Dt)y \tag{25}$$

from which

$$y(t) = \frac{D}{k_1(x_0 - Dt)} \tag{26}$$

gives us the desired chelate blood level function during the course of the treatment.

We must next determine the dosage function $s(t)$ which yields this chelate blood level function. We solve eqn. (14) for $s(t)$, obtaining

$$s(t) = dy/dt + k_1 xy + k_2 y \tag{27}$$

which on use of eqn. (22) gives

$$s(t) = dy/dt + D + k_2 y \tag{28}$$

We obtain an expression for dy/dt by differentiating eqn. (26) to get

$$dy/dt = \frac{D^2}{k_1(x_0 - Dt)^2} \tag{29}$$

Substitution of this result in eqn. (27) then yields for the dosage function

$$s(t) = \frac{D^2}{k_1(x_0 - Dt)^2} + D + \frac{k_2 D}{K_1(x_0 - Dt)} \tag{30}$$

To obtain maximum rate of clearance of metal from the patient, then, one gives an initial dose of chelate to yield an initial blood level of $y_0 = D/k_1 x_0$, and then doses at a rate $s(t)$ for $0 < t < t_{\text{final}}$, where t_{final} must be less than x_0/D. In actual fact, the toxicity of the chelate itself may force one to stop increasing the dose rate at a lower concentration, but this dosage function could be used up to that point to obtain the maximum rate of safe clearance.

Two courses of therapy using this scheme were modeled by computer, and the results are shown graphically in Figs. 9 and 10. In the simulation plotted in Fig. 9, the patient was dosed according to eqn. (30) for the first 80% of the treatment period. He was then dosed at the level reached at this time for the remaining 20% of the treatment period (4 days). Then treatment was stopped, and the chelate level is seen to fall off rapidly at the end of the run. Note the extremely linear decrease in metal concentration, as well as the very constant rate of metal removal, both indicating the ability of $s(t)$ as given by eqn. (30) to maintain a constant rate of metal removal.

In the simulation shown in Fig. 10, the patient is dosed at an accelerating rate according to eqn. (3) for the first 65% of the treatment period; the dosage is then maintained at the level reached at the end of

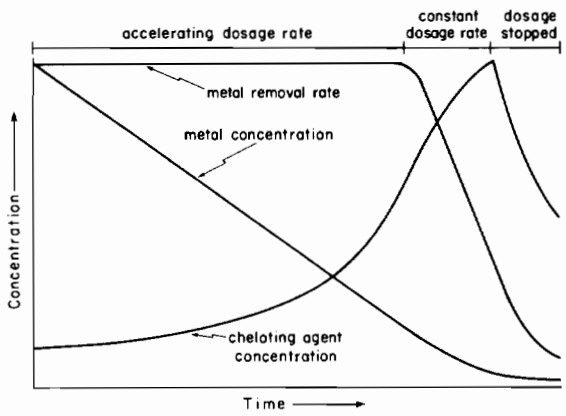

Fig. 9. Clearance of metal by chelate, simple model, dosage rate function $s(t)$ given by eqn. (30). $k_1 = 1$, $k_2 = 1$, initial [MP] = 1, desired removal rate = 0.25, run duration = 4. An accelerating dose rate was constant for the remainder of the run. The calculated maximum rate of metal removal was 0.2510.

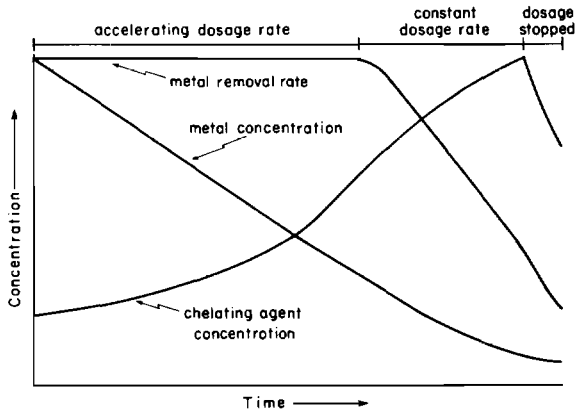

Fig. 10. Clearance of metal by chelate, simple model, $s(t)$ given by eqn. (30). Parameters as in Fig. 9, except that an accelerating dose rate was used for the first 65% of the run; the dose rate was constant for the remainder of the run. The calculated maximum rate of metal removal was 0.2505.

this time for the remaining 35% of the treatment period (4 days), at which point dosage ceases. Again we see that the chosen dosage function does an excellent job of yielding a constant rate of metal clearance from the patient.

V. Synergistic Effects and Catalytic Processes

Notwithstanding the occasional problems that may arise from the overly rapid mobilization of a toxic metal *in vivo*, it is generally advantageous to be able to accelerate the metal mobilization rate and to get a more favorable ratio of mobilized metal to chelator. A careful examination of the factors involved when toxic metal mobilization is accelerated is of considerable use in providing insights needed to extend such phenomena to other toxic metal systems. While the use of two different chelating agents to form a mixed complex of greater stability has been discussed in some detail by Schubert [37, 38] and others [39, 40, 96], the actual data presented in support of such a claim is largely unconvincing [42–48]. Claims regarding the formation and enhanced stability of mixed complexes involving multidentate ligands are questionable.

The earliest experimental use of mixtures of chelating agents was prior to 1955 [50] in Hamilton's laboratory in Berkeley, but the earliest comprehensive presentation of the successful enhancement of metal ion decorporation by such mixtures is found in the work of Volf [51], who used mixtures of DTPA and desferrioxamine (DF), and mixtures of each of these with citrate. These three mixtures were all about equally effective and led to residual levels of plutonium at the injection site and in the skeleton, liver and kidneys which were significantly lower than the levels obtained with either DTPA or DF. Although citrate stimulates the excretion of plutonium, citrate itself is metabolized with sufficient rapidity that its participation in mixed complexes is not too probable [52]. The description of its action on the effect of other chelating agents as synergistic [4, 44] is more accurate. As we shall see from the evidence presented below, very low molecular weight chelating agents can be excellent catalysts for the transfer of a metal ion from a very large chelating agent, such as a protein or enzyme, to a smaller one. Table V contains a listing of a number of cases in which synergistic effects have been reported in studies on the decorporation of metals. In addition to cases where there appears to be a catalysis of the metal transfer, some examples involve combinations of hydrophilic and lipophilic chelating agents.

For some of these systems, the underlying chemical processes have been studied in some detail. The catalytic transfer of iron from transferrin to desferrioxamine has been examined using as a catalyst pyrophosphate [73, 78], aerobactin [74], adenosine

TABLE V. Synergistic Combinations in Metal Detoxification

Metal	Compounds used	Reference
Fe	Desferrioxamine plus DTPA	51
Fe	Desferrioxamine plus citrate	51
Fe	DTPA + dipicolinate	51
Fe	DTPA + citrate	51
Hg	D-penicillamine plus sodium maleate	53
Cd	BAL + DTPA	54
Fe	Desferrioxamine plus ascorbate	55, 56, 57
Fe	Pyridoxal isonicotinoyl hydrazone + 2,3-dihydroxybenzoic acid or catechol	58
Fe	Desferrioxamine + DTPA	59, 60
Fe	Desferrioxamine plus citrate or NTA	61
Pu	DTPA + ascorbic acid	62
Pu	DTPA + citrate + nicotine-hydroxamic acid	63
Cd	Cysteine plus ascorbate	64
Zn, Pb	Cysteine plus ascorbate	65
Cd	ZnDTPA + DMSA	91
Pb	Cysteine plus ascorbate	66
Pb	EDTA plus ascorbate	67
Pb	EDTA + thiamine, folic acid or pyridoxine	68
Pb	DMSA or HEDTA + vitamin B complex	69
Pb	EDTA + Zn	70
Fe	Desferrioxamine + ACATA (2-(β-aminoethoxy)cyclohexylamine tetraacetate)	71
CH_3HgCl	Dimercaptosuccinate plus dimercaptopropane-sulfonate	72
Hg	Spironolactone plus dimercaptopropane-sulfonate	117

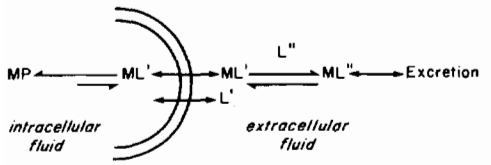

Fig. 11. Model of a synergistic process facilitating metal decorporation. L′ is a low molecular weight species which can pass into the cell and displace M from MP to give ML′. L″ is a multidentate chelating agent which cannot pass into the cell.

diphosphate or triphosphate, guanosine triphosphate or diphosphoglycerate [75, 76] and aminoalkylphosphonic acids [77]. The underlying rate aspects of the metal transfer processes involving iron interactions with various hydroxamic acids have been very elegantly developed by Crumbliss and his collaborators [79–82]. As Raymond and his co-workers have shown, the rate of reaction of desferrioxamine with transferrin bound iron is quite slow [15] and this is responsible for the frequently very modest efficacy of this compound in the clinic. For other systems which involve two very different types of chelating agents, such as the lipophilic BAL plus the hydrophilic DTPA [54], the lipophilic compound may well serve to transfer intracellular cadmium to the extracellular hydrophilic DTPA.

Another simple scheme for use in the selection of ligand species which may act synergistically is shown in Fig. 11. Here MP represents metal bound to some protein or other metal-binding species in the intracellular fluid (or even possible within an organelle such as the nucleus), L′ is a small molecular weight ligand which can pass through the cellular membrane and facilitate the movement of the metal out through the membrane. However, in the extracellular fluid there may well be strong competition for the metal, so we must add L″, a ligand which binds the metal more strongly than L′ (e.g. L″ may be multidentate ($\geqslant$4) and bear a high negative charge) to facilitate metal decorporation. Also, with a highly multidentate ligand the rate of dissociation of ML″ would be slower, which would also be advantageous. With certain toxic metal ions (e.g. Cd^{2+}, Hg^{2+}, Pb^{2+}, etc.), L″ would have the same advantages if it were of lower denticity but contained one or more thiol groups (e.g. BAL, cysteine, D-penicillamine as listed in Table V). The combination of BAL and DTPA used so successfully by Cherian [54] would appear to be a system of this sort. The process here has many obvious similarities to the mechanism of homeostastis proposed by Williams [41].

There thus appear to be at least two methods whose use has clearly resulted in significant increases in the rate of mobilization and excretion of toxic metals. The first of these uses a small chelating agent to catalyze the transfer of a metal between two larger ones, while the second uses a combination of a lipophilic and a hydrophilic chelating agent. These processes appear to be of some considerable generality and it seems reasonable to expect that they can and will be extended to additional toxic metal ions, as well as undergoing considerable refinement for the toxic metal ions listed in the table. The use of a non-toxic metal ion such as Zn^{2+}, to displace a toxic one such as Pb^{2+} [70] has been applied to other systems, though not in combination with a chelating agent. Needless to say, there are examples in this table for which the mechanism does not appear to be obvious. The development of useful synergistic processes to accelerate the mobilization and excretion of toxic metals is, in at least some ways, equivalent to the development of a more powerful and selective chelating agent for that toxic metal.

VI. Conclusions

While it is apparent that measurements of the stability constants of the complexes of toxic metal ions and potential therapeutic chelating agents is an essential first step in the characterization of such agents, it, in itself, is not a sufficient criterion to guarantee success *in vivo*. Such parameters allow us to select those processes which do not violate the laws of thermodynamics. The trend to search for metal chelates which are more thermodynamically stable however, may also be a simultaneous move towards metal chelates which are formed more slowly [7] or towards species with an extremely limited ability to penetrate cellular membranes and remove intracellular deposits of toxic metals. There would seem to be good reason to believe that a systematic exploitation of species which can catalyze the transfer of toxic metals from their usual *in vivo* binding sites to therapeutic chelating agents might well prove to be useful in attaining significant improvement in the decorporation of certain metal ions. However, it is also necessary to note that there may well exist maximum rates at which toxic metal complexes may be transported through the kidneys without causing very serious damage. A relationship of this renal damage to the stability constants of the chelating agents used seems possible (*i.e.* complexes stable enough to be filtered but not stable enough to pass through the nephron without reacting), but the ability of some chelating agents to affect essential metal ions involved in the maintenance of proper kidney function may also play a role. Thus the superiority of *meso*-2,3-dimercaptosuccinic acid over the polyamino-carboxylic acids as antagonists for lead intoxication [83] may well be due to the rather feeble complexes which it forms with Mg^{2+}, Ca^{2+}, and Mn^{2+} in contrast to the much more stable analogous complexes of the polyaminocarboxylates.

Desiderata for Therapeutic Chelating Agents

One can compile a list of desiderate for therapeutic chelating agents which expands upon those given previously [2, 60–66]. This summarizes the full range of behavior and properties which we expect for an ideal compound of this type:

(1) Possess a low inherent toxicity.

(2) Rapidly form very stable complexes with the metal ion which are less toxic than the metal ion itself.

(3) Are soluble in water and chemically stable.

(4) Undergo a minimum of metabolic change *in vivo*.

(5) The complexes which they form with toxic metals are rapidly excreted in the urine or feces.

(6) Capable of oral administration.

(7) Have minimal interactions with essential biological molecules and structures.

(8) Are capable of penetrating into intracellular deposits of toxic metals.

(9) Possess some degree of selectivity for the toxic metal ion.

(10) Are slowly removed from the organism until *after* they form complexes with toxic metal ions.

(11) React rapidly, *in vivo* with toxic metal ions complexed to serum albumin, enzymes, cellular membranes and other serum or intercellular constituents. Such reactions may be direct or require the assistance of some other species acting synergistically.

Of these desiderata, 4, 5, 6, 7, 8, 10 and 11 are dependent directly upon rate processes. It is quite conceivable that the development of methods for manipulating some of these rates may prove very advantageous in formulating improved methods of chelate therapy for the clinic.

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