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The Effect of Kinetic Factors on the Thermodynamic Evaluations of Therapeutic Chelating Agents

MARK M. JONES*

Department of Chemistry and Center in Molecular Toxicology, Vanderbilt University, Nashville, Tenn. 37235, U.S.A.

and PETER M. MAY

School of Mathematical and Physical Sciences, Murdoch University, Murdoch, W. A. 6150, Australia

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Abstract

The effect of certain kinetic factors on thermodynamic evaluations of several therapeutic chelating agents is examined. This allows the *in vivo* behavior of these drugs to be understood in more detail. The main kinetic factors examined are: (a) the pharmacokinetics of the chelating agents *in vivo*; (b) the kinetics by which the toxic metal ions are distributed between the serum and other organs; and (c) the rates of the reactions between the therapeutic chelating agent and the complexes formed by the toxic metal ion with serum constituents. A new parameter is proposed for predicting the efficacy of metal binding in plasma. This takes into account changes in the chelating agent concentration with time. Predictions of the relative efficacy of the therapeutic agents based solely on thermodynamic calculations are shown to provide a useful differential indicator for spontaneous detoxification processes, whereas kinetic factors become more important in delayed treatments or when the chelating agents exhibit similar thermodynamic behavior.

Introduction

The use of stability constants to select promising therapeutic chelating agents for toxic metal ions was first explored by Schubert [1]. His ideas were refined and put to a series of direct *in vivo* tests by Catsch and his co-workers [2, 3]. In these studies the ability of the chelating agents to bind toxic metal ions in biological systems was quantified in terms of an 'effective stability constant', a parameter which attempted to take into account the most important competitive chemical interactions occurring in plasma (particularly with respect to the proton and some other metal ions).

However, these early calculations suffered from a lack of generality: it was especially difficult to extend the treatment as the number of interactions considered was increased. Thus, simplifying assumptions concerning the number and type of complexes formed in plasma were often made but were not always justified.

Many of these difficulties were overcome by the development of computer simulation methods which could incorporate, in a relatively easy way, multiple equilibria among serum constituents. These computer-based approaches stem from the initial models developed by Perrin [4–6] and have now matured to the stage where many thousands of possible equilibria can be examined simultaneously. The calculations have greatly facilitated the study of the relative efficacy of therapeutic agents and they have been widely used in the search for new antagonists of toxic metals [4–15]. The results are most conveniently expressed in terms of a quantity called the Plasma Mobilizing Index (*PMI*), defined [11] as follows:

$$PMI = \frac{\text{(total concentration of low-molecular-weight metal complex species in the presence of drug)}}{\text{(total concentration of low-molecular-weight metal complex species in normal plasma)}}$$

Formulated in this way, *PMI* provides a good measure of the relative thermodynamic ability of any chelating agent to compete for a given metal ion in blood plasma, provided only that the metal ion concentration in the low-molecular-weight fraction remains small compared with that bound to plasma proteins such as albumin and transferrin. The *PMI* factor is particularly useful because it is independent of the exact extent of metal–protein binding [11].

However, like all purely thermodynamic parameters, the *PMI* concept is unable to allow for changes over time of the sort which often characterize experiments with chelating agents *in vivo*. The present study was undertaken to examine how some of the

*Author to whom correspondence should be addressed.

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more important kinetic factors may affect the conclusions which can be drawn from *PMI* calculations.

Theory

Three kinetic factors seem most likely to affect the conclusions which can be drawn from *PMI* calculations. These are: (a) the rate at which the chelating agent disappears from the plasma; (b) the rate at which the metal ion and its complexes are cleared from the serum; and (c) the rate at which the metal ion complexes of serum constituents, especially the transport proteins like albumin and transferrin, react with the administered chelating agents in plasma.

Chelating agents may disappear from the plasma because they are excreted, because they pass into the tissues, because they are metabolized or because of a combination of all three of these. Some agents such as ethylenediaminetetraacetic acid (EDTA), diethylenetriaminepentaacetic acid (DTPA) and sodium 1,2-dihydroxybenzene-3,5-disulfonate (Tiron) undergo very little metabolic change and do not pass, in any significant amount, across cell membranes [16]. Others, such as sodium diethyldithiocarbamate (DDC), undergo all these processes. Clearly, the effect of this type of process is to reduce the plasma concentration of the agent with time and hence to lower its effectiveness relative to other compounds with identical PMI values which do not disappear as quickly.

Many heavy metal ions entering the plasma tend to be removed rather rapidly and hence become separated from the direct sphere of action of the chelating agent. This is well illustrated by cadmium ion, the rapid passage of which to intracellular sites has been extensively studied by Klaassen and his co-workers [17–19]. However, it is pertinent to note that, at first, this does not alter the relative effectiveness of various agents since, in the plasma, all are equally disadvantaged. Of course, with time, the ability of different agents to promote metal ion excretion may well be reversed, depending on how well each agent is capable of following the metal ion to its major or most labile site of deposition.

It is also necessary to consider the rates at which chelating agents remove target metal ions bound to plasma constituents, such complexes invariably being the predominant form of the metal ion in this body fluid. The entire success of chelation therapy often depends on this rate of complexation: if it is rapid in comparison with the rates at which the agent and/or the metal ions disappear from plasma, the probability of successful decorportation is considerably enhanced. For example, it is well recognized that the slow rate of reaction of desferrioxamine B (DFOA) with iron bound to plasma transferrin severely limits the ability of this compound to mobilize iron [20]. The same kind of restriction applies to the sequestration of copper by certain macrocyclic amines [21]. It follows that when the target metal ion is held in a plasma-protein complex of significant kinetic stability, the rate at which the metal ion can be transferred from the protein to the chelating agent may seriously limit the utility of the PMI curves as normally calculated.

The lability of metal ions in aqueous environments has been studied in much detail. The relative rates of water exchange for the aqua ions [22] is in the order $Hg(II) > Cu(II) > Cd(II) > Zn(II) > Fe(II) > Ni(II) > Cr(III) > Be(II) > Fe(III)$. However, substitution processes slow down as the denticity of the displaced chelating agent increases [23]. Also, when a metal ion has to be removed from a site within a large plasma-protein molecule into a closed chelate ring, the rate may become very slow [21].

In comparing *PMI* values of various chelating agents, however, there are good reasons why the rate at which the metal ion is transferred between the protein and the chelating agent is not usually a problem for the computer simulation models. To make any difference, the chelating agents being compared must react with the protein complex at different (rather than just slower) rates; such variations tend to be rather less important than the overall reduction in the ability of all agents to sequester the particular (inertly bound) metal ion. Moreover, mediation by naturally occurring low-molecular-weight ligands such as citrate is probably an important factor in most such transfer processes [24] and, when this occurs, it will tend to even out differences in the rates of acquisition by various chelating agents.

Based on these concepts, it is reasonable to draw two conclusions. The first is that predictions regarding the ability of chelating agents to promote excretion of target metal ions based on *PMI* values are strictly only appropriate shortly after the metal enters the plasma and before there has been time for a significant amount to be deposited into the tissues. Secondly, it is necessary to take account of the relative concentration profiles of each agent considered in the plasma with respect to time.

To fulfill these requirements, we now suggest the calculation of a parameter called the 'Cumulative Plasma Mobilizing Index', *CPMI*. This may be defined as follows:

$$(CPMI)t = \int_0^t (PMI)\,\mathrm{d}t$$

where *PMI* is integrated as a function of the changing plasma concentration of the chelating agent under consideration. An arbitrary choice of time, t, is necessary but ideally should correspond to the period in which the target metal ion remains available

in plasma, *i.e.* before it has largely disappeared into the tissues or been excreted. In the examples presented below t has been set at 60 min.

It is proposed that this parameter, *CPMI*, will provide a better predictive measure of the relative ability of chelating agents to promote urinary excretions of toxic metal ion administered in animal experiments when pharmacokinetic differences between the chelating agents are important.

Results

Calculations of *PMI* functions for Cd(II) with a variety of chelating agents have been published previously [12]. Based on these results, the way in which *PMI* values vary with time have been determined for several chelating agents for which pharmacokinetic data are available regarding the gross rate of disappearance of the compound from the plasma. While it would be desirable to have all the pharmacokinetic data on the same species under identical conditions, this is presently not possible. We have accordingly used such limited data as are available and assumed that the general trends are similar.

The pharmacokinetics of DDC have been studied in some detail in dogs [25]. There are two main routes by which it is removed from the plasma. Some (27% in total) is S-methylated with a first-order rate constant of 5.69×10^{-2} min^{-1} (*i.e.* $t_{1/2} = 12.2$ min) while the rest is eliminated by other routes with a first-order rate constant of 1.48×10^{-1} min^{-1} (*i.e.* $t_{1/2} = 4.68$ min). The variation in concentration with time is thus given by the two-term expression

$$C = C_0 e^{-0.0569t} + C_0 e^{-0.148t}$$

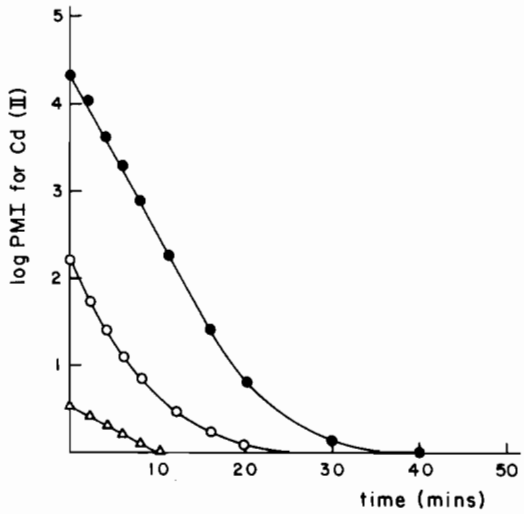

Fig. 1. Log *PMI vs. t* for the cadmium(II)–sodium diethyldithiocarbamate system (• curve is for $C_0 = 10^{-3}$ mol dm^{-3}, ○ is for $C_0 = 10^{-4}$ mol dm^{-3} and △ is for $C_0 = 10^{-5}$ mol dm^{-3}).

The effect of such a decrease on *PMI* values for DDC with Cd(II), starting with various initial serum concentrations, can be evaluated and is shown in Fig. 1. The *PMI* values are seen to fall off quite rapidly, reaching zero within about 40 min. A rapid decrease in effectiveness in removing serum cadmium with time is typical of this compound.

Another chelating agent, which behaves somewhat differently, is sodium 2,3-dimercaptopropane 1-sulfonate (Unithiol). From the data of Klimova [26], the half-life in plasma can be estimated to be about 20 min (*i.e.*, $k_1 = 3.46 \times 10^{-2}$ min^{-1}). Unlike DDC, this compound does not pass rapidly through cell

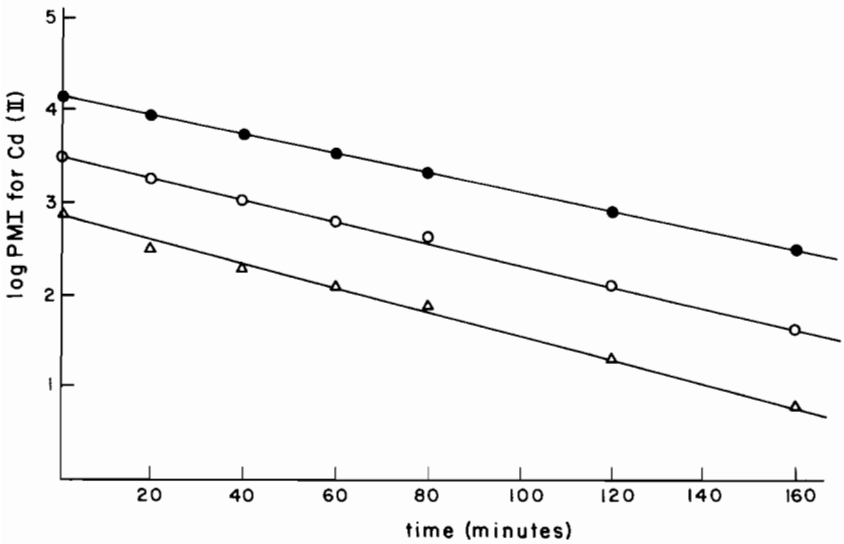

Fig. 2. Log *PMI vs. t* for the cadmium(II)–sodium 2,3-dimercaptopropane 1-sulfonate system (• curve is for $C_0 = 10^{-3}$ mol dm^{-3}, ○ is for $C_0 = 10^{-4}$ mol dm^{-3} and △ is for $C_0 = 10^{-5}$ mol dm^{-3}).

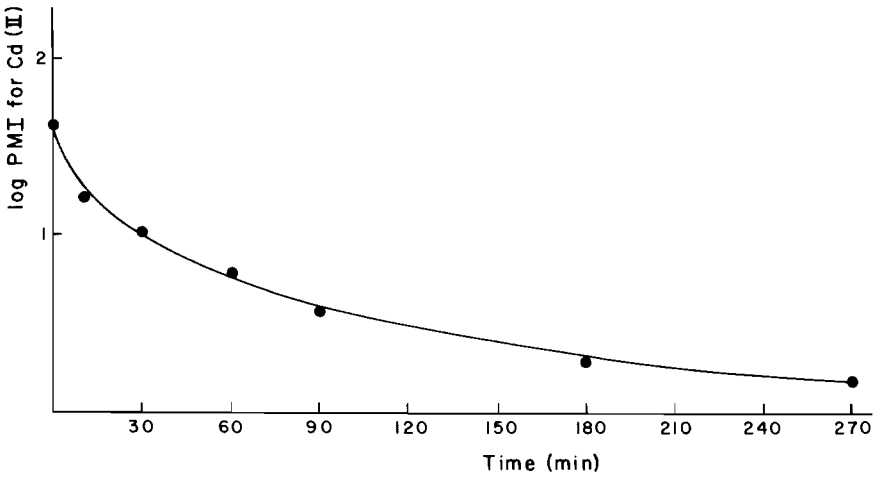

Fig. 3. Log *PMI vs. t* for the cadmium(II)–D-penicillamine system (• curve is for $C_0 = 10^{-3}$ mol dm^{-3}, ○ is for $C_0 = 10^{-4}$ mol dm^{-3} and △ is for $C_0 = 10^{-5}$ mol dm^{-3}).

membranes and is mainly eliminated through the kidneys. Although subject to oxidation *in vivo*, the half-lives of such redox processes are perhaps 2–3 h, sufficiently slower than the rate of renal clearance to be neglected. As can be seen in Fig. 2, the *PMI* values drop quite slowly with time. From an initial value of about 1.6×10^4, they fall to 5.6×10^3 by the end of 40 min and are still well over 10^3 after 100 min.

D-Penicillamine (PEN) is a chelating agent whose metabolism and pharmacokinetics have been well studied in both rats [27] and man [28, 29]. The compound binds very firmly to plasma proteins including albumin, α-globulins and caeruloplasmin via disulfide linkages. Furthermore, the mixed cysteine–penicillamine disulfide and penicillamine disulfide are both formed in the plasma. Radioactively labeled penicillamine has been shown to clear much more slowly from tissues containing collagen and elastin than it does from the liver and kidney [27–29]. Gross plasma levels of PEN in man following intravenous administration have been measured by Wiesner *et al.* [29], who reported a rapid distribution phase ($t_{1/2} < 10$ min) after which there was a period with a significantly slower elimination rate ($t_{1/2} = 63$ min). Using the actual data from the original study [30], one can estimate the variation of PEN levels in plasma with time. The corresponding changes of *PMI* are plotted in Fig. 3. It is clear that PEN has a long period over which it acts, although its *PMI* values for Cd(II) are never very large in comparison with most other chelating agents considered.

EDTA and DTPA behave rather similarly to one another. ^{14}C-Labeled compounds are rapidly cleared from mice with a half-life of 17 min and there is very little metabolic conversion to other products [31]. Neglecting the form in which the compounds are administered (*i.e.* for example, whether as the Ca(II)

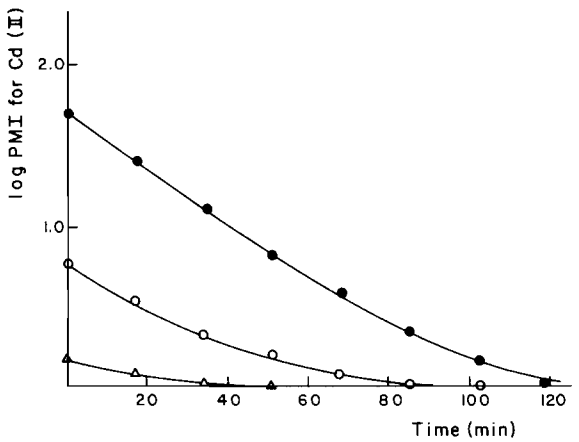

Fig. 4. Log *PMI vs. t* for the cadmium(II)–EDTA system (• curve is for $C_0 = 10^{-3}$ mol dm^{-3}, ○ is for $C_0 = 10^{-4}$ mol dm^{-3} and △ is for $C_0 = 10^{-5}$ mol dm^{-3}).

or Zn(II) salt), one can estimate the effect of this clearance rate on the *PMI* values of EDTA and DTPA on Cd(II) with time as shown in Figs. 4 and 5. Note that this assumes an essentially instantaneous equilibration of the Ca(II) and Zn(II) in plasma with tissue stores of these metal ions – something which is only realistic if the chelating agent is in fact administered with enough of the metal to which it is predominantly bound at equilibrium (*i.e.* as CaEDTA and as ZnDTPA). The effect of administering these agents in the form of other salts, e.g. CaDTPA, has been discussed elsewhere [32].

The half-life of dimercaptosuccinic acid (DMSA) in male juvenile monkeys (*Macaca mulatta*) has been reported to be approximately 45 min [36] and this value has been used in our calculations. The half-life is somewhat longer in rabbits [37] but about this same value (43 min) in dogs [38]. The calculated

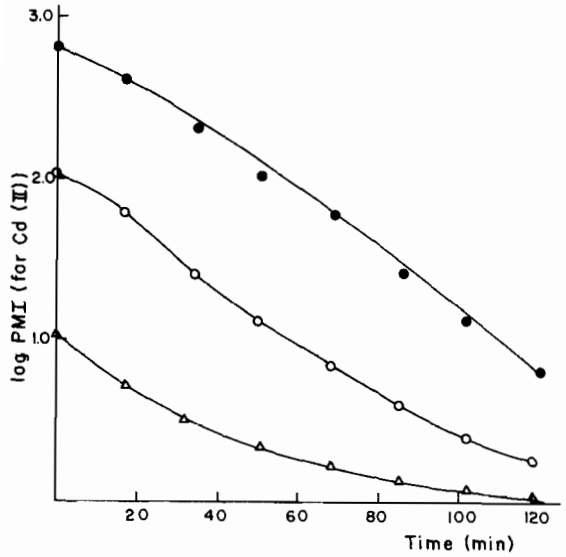

Fig. 5. Log *PMI vs. t* for the cadmium(II)–DTPA system (• curve is for $C_0 = 10^{-3}$ mol dm^{-3}, ○ is for $C_0 = 10^{-4}$ mol dm^{-3} and △ is for $C_0 = 10^{-5}$ mol dm^{-3}).

variations of *PMI* with time, using a half-life of 45 min, are shown in Fig. 6.

Graphical integrations of the *PMI* curves shown in Figs. 1 to 6 have been performed and the *CPMI* values for each agent calculated over a period of the first 60 min. The results are shown in Table I. Compared with the order of the chelating agents based on *PMI* values alone [12], a considerable reduction in efficacy of both DDC and PEN is exhibited.

TABLE I. *CPMI* Values (h) for Cd(II) as a Function of Initial Ligand Concentration in Plasma (integration is from $t = 0$ to $t = 60$ min)

	Initial plasma concentration (mol dm^{-3})		
Ligand	10^{-3}	10^{-4}	10^{-5}
DMSA	4.62	3.77	2.70
Unithiol	3.76	3.06	2.42
DTPA	2.45	1.55	0.52
DDC	1.21	0.37	0.06
EDTA	1.16	0.38	0.05
PEN	1.07[a]	[b]	[b]

[a]Initial peak plasma concentration = 0.673×10^{-3} mol dm^{-3}. [b]Experimental data do not readily lend themselves to calculation of CPMI for these concentrations.

Discussion

It is a fairly obvious, but nevertheless important, conclusion of this study that all the kinetic factors considered tend to reduce the efficacy of the chelating agents compared with that which might be expected on the basis of *PMI* considerations alone. In large measure, this explains why the differences observed between viable therapeutic regimens are often only small, whereas the thermodynamic affinities in blood plasma (and hence the theoretical capacity of the chelating agent to enlarge the target metal ion's low-molecular-weight fraction) generally need to be portrayed on a log scale. It is also clear that the relative order of efficacy, calculated in terms of *PMI*, may be drastically altered by disadvantageous rate processes, particularly those in which the chelating agent is rapidly excreted, metabolized, or transported into the tissues. Conversely, it can be expected that *PMI* curves will correspond most closely to the relative efficacy of injected chelating agents under conditions such as occur in the early stages of acute intravenous intoxication with water soluble metal salts.

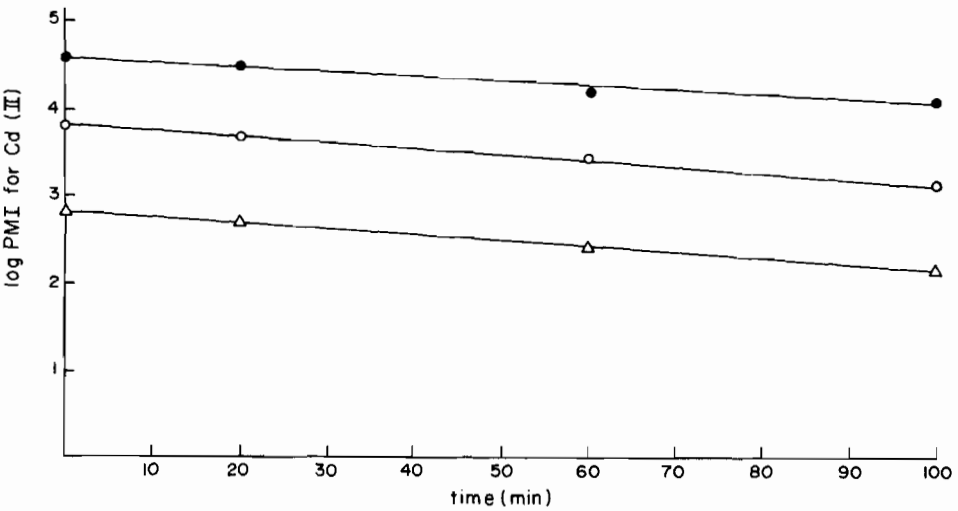

Fig. 6. Log *PMI vs. t* for the cadmium(II)–DMSA system (• curve is for $C_0 = 10^{-3}$ mol dm^{-3}, ○ is for $C_0 = 10^{-4}$ mol dm^{-3} and △ is for $C_0 = 10^{-5}$ mol dm^{-3}).

PMI values quantify the metal binding potential of chelating agents in plasma at given concentrations. This may well be related to (but is not the same as) the ability of the chelating agent to cause metal ions to be excreted, either in the urine or the feces.

In this regard, as in the case of improvements made to the thermodynamic data on which the calculations are based [15], the results of the computer model are all too frequently wrongly perceived and, hence, misused. When the predicted order of binding in plasma matches the observed order of chelating agents promoting excretion of a target metal ion, it can be concluded that thermodynamic factors probably dominate the critical stages of the *in vivo* process. When there is no such agreement, the only justifiable conclusion is that some 'kinetic factors' have become significant: there is no test of the model since there is currently no direct way to observe the degree of binding in plasma directly.

The same rationale applies to the calculation of the Cumulative Plasma Mobilizing Index proposed in this paper. When the *CPMI* order corresponds to observed biological effects and the *PMI* order does not, it is reasonable to think that the relative changes in plasma concentration of the chelating agent will play a decisive role in determining the outcome of the particular chelation therapy. The tabulated *CPMI* values in Table I reveal a striking superiority for DMSA. This is due to both its high *PMI* values and its longer serum half-life, with the latter factor being responsible for the fact that the *CPMI* values for DMSA are larger than those of Unithiol. A direct test for the validity of the *CPMI* concept, using previously published data, is unfortunately not yet possible. This is because such data were obtained in experiments which invariably utilized different routes of injection for the cadmium and the chelating agent, even when the cadmium was administered intravenously [18, 39].

Due to a lack of data, it is also not yet possible to incorporate other factors influencing the behavior of chelating agents *in vivo* into the computer models in a general way. It should be most desirable to take account of the relative rates at which chelating agents and their complexes can pass through biological membranes. Together with data on the relative labilities of metal deposits in various tissues, this would extend the time scale of the simulations, greatly enhancing the information which can be obtained from them. The ultimate objective must be to amalgamate all the available knowledge in a single model encompassing both the thermodynamic equilibria of chelating agents *in vivo* and their rate-limiting processes, such as described in the kinetic simulation by Jones [33]. Only then will we truly understand why the agents behave as they do and what can be done to modify their behavior in any way which may be required.

In the meantime, current models have to be used despite their limitations. *PMI* calculations have a good record in picking out chelating agents with promise [34]. Most importantly, they provide a screen for testing large numbers of proposed compounds, revealing which are thermodynamically feasible antagonists for particular toxic metal ions. As such they represent a *sine qua non* of therapeutic chelating agent efficacy. However, of the thermodynamically feasible antidotes, only those with favorable kinetic profiles are potentially useful and, of these, only a few meeting further criteria (such as low inherent toxicity) are actually capable of clinical application. Even then, obstacles having nothing to do with their physiological properties may prevent new chelating agents from being listed in the Pharmacoepia [35].

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