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COMMENTARY

NEW DEVELOPMENTS IN METAL ANTIDOTAL PROPERTIES OF CHELATING AGENTS*

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For several years chelating agents (ligands) have occupied a well established place in the therapeutic arsenal for the management of heavy metal intoxications as well as of incorporation of radioactive metals [reviewed in Refs. 1, 2]. It should not be overlooked, however, that a critical analysis of experimental studies as well as of clinical experiences leaves much to be desired with respect to the actual attainable results. The amount of toxic metal that can be mobilized by chelating agents is often too small to be satisfactory from the therapeutic point of view. This, in turn, calls for long-term therapy which may create difficulties because of untoward side-reactions. In this paper we shall stress some possibilities for achieving the optimum chelation therapy which have either been under scrutiny during recent years for their applicability or which we consider as promising starting points for future studies.

The ability of any chelating agent to mobilize a toxic metal as well as the toxicity of the chelator depends on two factors: the so-called conditional stability constant and the pharmacokinetics of the chelating agent, mainly its physiological distribution space and its excretion rate from the body. The term 'conditional constant' must be introduced in order to allow for the competing side reactions of the therapeutic ligand L with endogenous hydrogen and metal ions. As has been explained in more detail elsewhere [1], the conditional constant for a metal M to be decorporated can be defined in a first approximation as

$$K'_M = \frac{K^M_{ML}[L]_{tot}}{\alpha_L + K^{Ca}_{CaL}[Ca^{2+}]} \quad (1)$$

K^M_{ML} and K^{Ca}_{CaL} are the stoichiometric stability constants of the simple 1:1-chelates ML and CaL, respectively, α_L takes into account the competition of protons at a given pH value, while $[L]_{tot}$ depicts the molarity of the chelating agent in the distribution space. The concentration of $[Ca^{2+}]$ in blood plasma is 10^{-3} M and is assumed to be virtually constant because of homeostatic control. In the formulation of equation (1) the competition of Ca^{2+} only has been taken into account because, compared with calcium, other metal ions either form chelates which are considerably less stable or they are present in relatively low concentrations. Equation (1) only holds if simple 1:1-chelates are formed, while the conditional constant of an ML_2-chelate is defined as

$$K''_M = \frac{\beta_2[L]^2_{tot}}{(\alpha_L + K^{Ca}_{CaL}[Ca^{2+}])^2} \quad (2)$$

where

$$\beta_2 \equiv K^M_{ML} \cdot K^L_{ML_2} = \frac{[ML_2]}{[M][L]^2}$$

It goes without saying that the conditional constants as defined by equations (1) and (2) represent an oversimplification insofar as the competition of endogenous ligands for the metal to be decorporated has not been considered. It is quite clear that no mobilization can occur if the stability of the compounds formed between the metal ion and any endogenous acceptor groups surpasses the affinity of the chelating agent defined by K'_M or K''_M. The interaction of chelating agents with essential trace metals, while of no consequence for their mobilizing efficacy, can become of critical importance for their toxic action. This statement does not imply that the toxicity of all chelating agents depends exclusively on the binding of endogenous metals.

The importance of the pharmacokinetics is easily understood [3] if one keeps in mind that the concentration of the chelating agent in the physiological distribution space is contained in the definition of the conditional constant and, furthermore, that the metal mobilization has to be considered as a ligand exchange. If the rate at which the exchange reaction takes place is smaller than the rate of excretion of the chelating agent from the body, the mobilization effect will remain poor in spite of high K'_M values. There are several reasons why exchange reactions may be slow. Firstly, there is some experimental evidence that metals may form co-ordination compounds with endogenous ligands of high molecular weight which behave as so-called inert complexes which, by definition, are characterized by sluggish exchange reactions. Secondly, we have to consider those situations where the chelating agent has a physiological distribution volume which differs from that of the metal; in this case, mobilization can only take place in such a way that initially the chelating agent

* Abbreviations used in the text: EDTA, ethylenediaminetetraacetate; DTPA, diethylenetriaminepentaacetate; DFOA, desferrioxamine B; PA, D-penicillamine; EAP, ethylenebisaminoisopropylphosphonate; DMPS, 2,3-dimercaptopropane-1-sulfonate; trien, triethylenetetraamine.

Table 1. Conditional stability constants log K'_M for some selected metal ions and chelating agents

Metal ion	EDTA	DTPA	DFOA	PA	EAP	trien
Be^{2+}	−2·3	−1·1			>5·6	
Fe^{3+}	13·4	15·6	20·7			13·5
Cu^{2+}	7·1	9·6	4·2	8·9 (10·1)	11·0	12·4
Zn^{2+}	4·6	6·5	1·2	2·4	4·0	3·7
Ce^{3+}	4·3	8·5				
Hg^{2+}	10·1	14·8		9·9 (11·9)		16·9
Pb^{2+}	6·3	7·2		4·8 (5·7)		2·0

The values in brackets represent log K''_M. For the calculations pH = 7·4, $[L]_{tot} = 10^{-4}$ M and $[Ca^{2+}] = 10^{-3}$ M have been assumed. The stoichiometric stability constants and the acidity dissociation constants have been taken from the compilation of Sillén and Martell [4], and in the case of EAP from Djatlowa *et al.* [5]. See text for abbreviations.

binds only that assumedly small fraction of the metal which shares the same distribution space. The binding of additional amounts becomes possible through the disturbed equilibrium and the resultant transfer of dissociated metal ions into the distribution space available to the chelator. It is quite conceivable that the transfer of a metal ion through a compartment system characterized by complex biological structures is not necessarily a rapid process. Finally, if mobilization occurs via the formation of an intermediate ternary complex, i.e. it is dependent on the direct reaction between chelating agent and endogenously bound metal, a difference in distribution space between chelating agent and metal will be absolutely prohibitive.

The above rather general statements will be exemplified by the behaviour of EDTA and its more effective congener DTPA (diethylenetriaminepentaacetate). Both compounds are known to form chelates with a large number of metal ions which are characterized by very high stoichiometric as well as conditional stability constants (see Table 1); the only exceptions are found in the special cases of Sr and Be. With both these metal ions the conditional constant is less than unity and, correspondingly, both chelators are ineffective. Even in those cases where K'_M is considerably larger than unity, it may not be sufficiently large to overcome the affinity of the metal towards endogenous binding sites and the effect remains unsatisfactory. This as well as the fact that even small differences in the conditional constant may at times result in a drastic increase in effectiveness, will be exemplified by the response of Ce(III). Even under optimal conditions, i.e. with simultaneous administration and high dosage, EDTA is not able to prevent the retention of Ce by the bones. In contrast, DTPA with a conditional constant which is higher by a factor of 10^4 (see Table 1) will reduce the skeletal deposition of Ce to less than 1/10 of the control. Analogous conditions are encountered with the actinides. The mobilizing efficacy of DTPA with delayed treatment, i.e. at a time when the bulk of the metal ions has left the extracellular fluid and has been taken up by the tissues, is, as a rule, considerably diminished. The underlying reason must mainly be looked for in the pharmacokinetics of DTPA. Ca-DTPA has multiple negative charges (for prevention of calciprivic effects it must be administered as Ca-chelate) and, therefore, is not able to permeate cell membranes and is predominantly distributed throughout the extracellular space from which it is relatively rapidly excreted through the kidneys. Its plasma clearance has a half-time of 20–30 min. The possible limitation of efficacy by these pharmacokinetical features has been argued before.

In discussing the possibilities of optimizing chelation therapy the search for and use of chelating agents possessing higher conditional constants than DTPA may serve as a starting point. We have chosen DTPA as a reference compound because it exhibits relatively high conditional constants, excepting the few explicitly mentioned cases, and is frequently (but wrongly) considered as a universal metal antidote. If our following reasoning centers primarily on the conditional constant as the very criterion, this is only partially justified. Undoubtedly this argument has proved successful from the heuristical point of view, as for instance for the use of DTPA instead of EDTA in the case of lanthanides and actinides. On the other hand, it cannot be overlooked that the theoretically required positive correlation between the conditional constant and the mobilizing effectiveness is frequently missing. There are several reasons which can be held responsible. The oversimplified formulation in Equa-

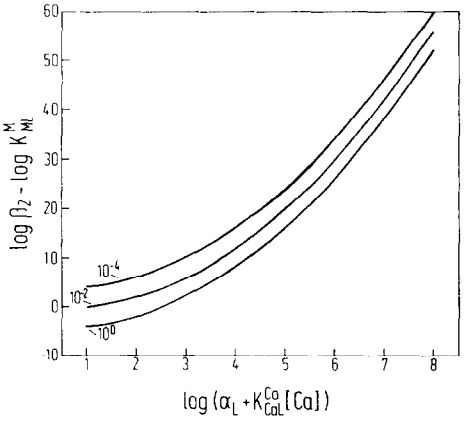

Fig. 1. Dependence of the quotient β_2/K^M_{ML} on the denominator in equation (1) for various values of $[L]_{tot}$ which are shown next to the curves. For the given quotients the condition $K'_M = K''_M$ is fulfilled.

tion (1), for instance, does not take into account the formation of ML_n-chelates and other chelate species as well as peculiarities in the physiological behaviour of the chelating agent and/or of the metal chelate and, finally, the possibility of the metabolic degradation or transformation of the chelating agent.

Pertinent compilations [4] reveal that in the case of ligands with few electron-donating atoms the stoichiometric *brutto* stability constant β_2 can occasionally be larger than the stoichiometric constant K^M_{ML} for a high-dentate compound. This can lead to the idea that it may be feasible to obtain an increased decorporating effectiveness by using suitable chelating agents with high β_2-values. The quadratic terms in equation (2), however, stipulate in most cases that the difference $K^M_{ML} - K'_M$ will exceed $\beta_2 - K''_M$. Figure 1 shows the dependence of the quotient β_2/K^M_{ML} on the denominator in equation (1) for various values of $[L]_{tot}$ under the condition that K'_M should equal K''_M. It can be seen immediately that only with extremely high β_2-values or high dosage of the chelating agents (which would be unrealistic for any practical application) could a substantial removal be expected. At the same time this explains why in the past all investigations based on high β_2-values of particular chelatings agents yielded virtually negative results.

An increase in the number of ligand atoms of the polyaminopolycarboxylic acids, as for instance the transition from the octadentate DTPA to the decadentate triethylenetetraaminehexaacetate or to even higher-dentate homologues, does not evoke substantially increased conditional constants nor an improved mobilizing efficacy. In contrast, the substitution of the acetate group by the bifunctional phosphonate group seems, in certain cases, to offer advantages and makes it desirable to continue investigative efforts along this line. In the case of EDTA, for instance, the substitution of two acetate groups by methylphosphonate results in a definitely increased removal of Ce(III) and Pu(IV); in the former case it is paralleled by a correspondingly increased conditional constant. If, however, all acetate groups of EDTA are substituted by methylphosphonate, a compound is obtained which is less effective than EDTA. Consequently, it might be promising to synthetize and test for biological effectiveness a derivative of DTPA in which 2 or 3 acetate groups are substituted by methylphosphonate. Alkyleneamines with phosphonate groups are of particular interest because they form, in contrast to the polyaminopolycarboxylic acids, exceptionally stable Be-chelates [5]. This is particularly true for ethylenebisaminoisopropylphosphonate (EAP) which is listed in Table 1. A positive influence of EAP on the excretion and toxicity of Be has already been demonstrated in animal experiments [6]. Furthermore, it is noteworthy that the conditional constant of Cu(II)-EAP is approximately 100 times as high as that of D-penicillamine (PA) which is generally chosen as the chelator to deplete the Cu-stores in Wilson's disease. A comparison of the efficacy of these two compounds, therefore, should be worthwhile.

The importance of the nature of the ligand groups is particularly evident in the case of desferrioxamine B (DFOA) which possesses 3 hydroxamic acid groups. It can be seen from Table 1 that the conditional constant of its Fe(III)-chelate is so exceptionally high and surpasses by far the constants for other metal ions that DFOA represents a virtually selective chelator for Fe(III). Consequently, it is not surprising that DFOA plays a unique role in the treatment of iron-storage diseases. Unexpectedly, DFOA exhibits also a very high efficacy in removing Pu(IV) which clearly surpasses that of DTPA [7]. This is only true provided that Pu is still bound to apotransferrin. The reason why a delayed treatment with DFOA is reduced in efficacy and DFOA becomes clearly inferior to DTPA, could probably be related to the fact that DFOA is subject to a rather extensive metabolic degradation. It should be promising to extend the investigations on Pu to other ligands with hydroxamic acid groups. Finally, in view of the synergistic action between DFOA and DTPA, which will be discussed in a different context, special attention should be given to those ligands containing both hydroxamic as well as carboxylic acid groups.

The so-called B-metals, which form cations with 18 electrons in the binding shell ($s^2p^6d^{10}$), are known to exhibit an affinity towards S or N. Although EDTA and DTPA form chelates of considerable stability with metals of practical importance such as Hg(II) and Pb(II) (Table 1), one would expect chelating agents with S and/or N as ligand atoms to have a higher biological efficacy. Numerous studies on Hg confirm this assumption, but, at the same time, raise a number of questions. In spite of the fact that the conditional constant of Hg(II)-DTPA is higher than that of Hg(II)-PA, the latter is distinctly more effective biologically. The discrepancy cannot be explained by the formation of Hg-PA_2, since its conditional constant is, at least within the usual dose range, considerably lower than that of Hg-DTPA. A valid explanation could be based on the fact that PA, in contrast to DTPA, is able to permeate to some extent into the intracellular space. Furthermore, DTPA retards the excretion of Hg from various organs which could be due to the formation of ternary complexes with endogenous binding sites. This reaction, in turn, could delay the kinetically controlled dissociation of Hg. A higher efficacy than for DTPA has been also demonstrated for other thiols including 2,3-dimercaptopropanol (BAL), its water-soluble derivative 2,3-dimercaptopropanesulfonate (DMPS) and mercaptoethyliminodiacetate. In the case of Pb the situation is different insofar as the efficacy appears to be higher, compared with DTPA, only with those compounds which possess carboxylate groups in addition to sulfhydryl groups; for Pb this is, obviously, an optimal combination of ligand groups, for instance in the case of mercaptoethyliminodiacetate.

Considering the facts and arguments discussed above, it appears to be very promising to extend the studies dealing with Hg and Pb to include as many other S-containing ligands as possible. In doing so, the toxicity of the ligands deserves special attention because it is well known that thiols show pronounced differences in this respect. Thus, the therapeutic index of mercaptoethyliminodiacetate is so low that it cannot be considered at all for clinical use. Although it is known that the number of sulfhydryl groups, the molecular configuration and the physiological distribution space are the main determinants for the tox-

icity of thiols, a basic understanding of the significance of all these factors is still missing at present.

Hg and Cu(II) favor N over O as electron-donor; consequently, a closer examination of such ligands, as for instance the polyamines, would suggest itself for the future. In particular, the use of triethylenetetraamine (trien) for the decorporation of Cu or Hg may be promising because of the considerably high conditional stability constants (Table 1). So far, in one patient with Wilson's disease trien proved to be at least equivalent to PA with respect to Cu-excretion [8]. The high efficacy of PA in removing Cu is somewhat unexpected since the conditional constant of Cu(II)-PA as well as of Cu(II)-PA_2 does not differ markedly from that of DTPA (Table 1) and DTPA is distinctly inferior to PA. In an attempt to explain this apparent discrepancy, Peisach and Blumberg [9] argue that only PA is able to participate in an oxidation–reduction reaction whereby Cu(II) is reduced to Cu(I) and the ligand sulfur oxidized to the radical Ṡ. The conditional constant of Cu(I)-PA is 10^3 times higher than that of Cu(II)-PA.

Through the synthesis of macrocyclic polyethers the co-ordination chemistry has received new and interesting impulses [for references see 10, 11]. Such ligands, containing closed cavities, may form with several metal ions inclusion compounds of the so-called cryptate type. In contrast to the acyclic ligands, which have been discussed so far, the cyclic compounds possess an entirely new parameter relevant for the stability, namely the ratio of the cation diameter to the cavity diameter. Close agreement between both diameters can result in a relatively high selectivity towards certain metal ions. The selectivity for B-metals could be further increased through a partial or total substitution of the ether-oxygen of cyclic polyethers by S or N, respectively. No binding of lanthanides and actinides to cyclic polyethers has been observed, obviously because of the high solvation energy of the tri- and tetravalent cations. Further improvement towards higher selectivity as well as stability can be obtained with bicyclic ligands [10]. As far as their biological application is concerned, cyclic ligands could also prove useful because of their favourable membrane permeability properties. At the present, the final evaluation of the usefulness of cyclic ligands for decorporation purposes seems premature, because the few results available are by no means systematic. The stoichiometric stability constant of the Sr-complex of 4,7,13,16,21,24-hexaoxa-1,10-diazabicyclo-(8,8,8)-hexacosane equals 10^8. In calculating the conditional constant, $K^K_{KL}[K^+]$ must be considered as an additional additive term in the denominator of equation (1), so that K'_{Sr} becomes $10^{1.6}$ if one assumes $[L]_{tot}$ to be 10^{-4}. This corresponds to an exceptionally high efficacy *in vivo* [12] which has also been confirmed for Ra [13]. The conditional constant for Pb is $10^{5.6}$, thus being lower than in the case of DTPA. The finding that in animal experiments the bicyclic compound was able to reduce the body burden of Pb by only 30% [13] could, at first sight, be interpreted as showing a good correlation between conditional constant and efficacy. This conclusion, however, may be premature as the experimental design does not allow any evaluation of whether the bicyclic ligand, if used with delayed treatment for the mobilization of intracellular Pb, is also inferior to DTPA. In view of the aforementioned affinity of Hg and Cu towards S- and N-ligands, it should indeed be worthwhile to examine the efficacy of respective cyclic ligands. To exemplify this point: the stoichiometric constant of the Cu(II)-complex of the acyclic *N,N'*-di(2-aminoethyl)propylenediamine equals $10^{23.9}$ and is increased to 10^{28} for the corresponding cyclic compound 5,7,7,12,14,14-hexamethyl-1,4,8,11,-tetraazacyclotetradecane [14].

At first sight, the combined administration of two chelating agents in order to obtain an increased effectiveness seems to be reasonable only if the two ligands differ with respect to their pharmacokinetics and, accordingly, their efficacy pattern. Otherwise the overall effect of the combination of two ligands with widely differing conditional constants should be determined by the chelating agent with the higher constant. If the constants are comparable, the combined administration should not improve the effectiveness any more than could be obtained by a correspondingly increased dose of a single chelator. Schubert [15], however, pointed out that in the presence of two ligands *A* and *B* the formation of a mixed ligand complex *AMB* is, on a statistical basis, always more favoured than the formation of MA_2 and MB_2, respectively. Furthermore, the mixed ligand complex may be more stable than would be expected from solely statistical effects. This argument is valid primarily for ligands with few electron-donating atoms, and for these it could be demonstrated that the increase in stability of the mixed complex exceeds the theoretically expected value [16]. The differences between the expected and observed values, however, are relatively small (0·3 to 0·7 log-units), and the same criticism must be applied as discussed in an earlier context (Fig. 1). In addition, Schubert pointed out that with high-dentate ligands the probability of the formation of mixed ligand complexes is low. At best, it could be expected to occur with ligands having different ligand groups. Despite these limitations, the administration of combinations of chelating agents proved to be useful. The absorption of Pu(IV) from an intramuscular deposit can be substantially enhanced if DTPA is injected into the contaminated tissue. The efficacy of DTPA can be drastically potentiated if citrate or DFOA is administered simultaneously [17]. Likewise with intraperitoneal administration of DTPA and DFOA the combination proved to be more effective in lowering the systemic Pu-burden than each of the two chelating agents on its own [7]. It is suggested that the enhanced effectiveness is due to the formation of mixed ligand complexes, as postulated by Schubert [15], since the favourable effect can only be observed before the bulk of Pu leaves the blood, and at this point the different pharmacokinetics of the two compounds could not have any influence. This inference is supported by analogous studies on Am(III) which showed that DFOA can likewise potentiate the efficacy of DTPA while it was virtually without effect if administered alone [18]. These preliminary positive results are encouraging for the continuation of studies along the same line. At the same time, they open up another possibility. Assuming that the formation of mixed ligand complexes is favoured under the condition that

the participating ligands contain different ligand groups, one may conjecture, that one high-dentate ligand with different kinds of ligand groupings and an appropriate steric configuration should be even more effective, in accordance with the so-called chelate effect of Schwarzenbach [19], than the combination principle. This may explain the previously mentioned enhanced efficacy of ethylenediamine-*N*,*N*'-diacetate-*N*,*N*'-dimethylphosphonate compared with EDTA and ethylenediamine-*N*,*N*,*N*',*N*'-tetramethylphosphonate, respectively.

In the above sections emphasis was put predominantly on the conditional stability constant. In the introduction as well as during the discussion of the cyclic ligands we have pointed out that the ability of any chelating agent to enter the intracellular space represents an equally relevant factor. The question of whether the lipophilic cyclic ligands will bring about a real improvement remains to be proved by future studies. In the case of DTPA its Ca-chelate, because of its negative charges and hydrophilic nature, is predominantly distributed in the extracellular space, and the attempt to improve its cellular permeability by esterification of the acetate groups seemed promising [20]. Provided that the ester bonds are hydrolysed within the cell in order to set free the groupings essential for chelation, an increased removal of intracellularly deposited toxic metals was to be expected. Respective positive results with Ce [20] and Pu [21] did indeed confirm the validity of the basic assumption but, at the same time, revealed an exceptionally high toxicity of the esterified DTPA [21] which, most likely, is due to an interaction with intracellular essential metal ions. Thus, no further argument is needed to exclude the DTPA-ester from any practical use.

A different approach is the so-called encapsulation of the chelating agent within lysomotropic lipid spherules which might be phagocytized by the cells [22]. In fact, it has been shown that the administration of encapsulated ^{14}C-labelled EDTA leads to a markedly higher and long lasting retention of the chelating agent by liver and spleen. The use of liposomes labelled with ^{3}H-amyloglucosidase or ^{3}H-cholesterol provides autoradiographic evidence that the liposomes are distributed rather homogenously over the hepatic cells [23]. As to the action of encapsulated DTPA, the first preliminary studies revealed a somewhat higher removal of Pu from the liver and of inorganic Hg from the kidneys as well as a promotion of faecal excretion of colloidal Au [24, 25]. Although these results are quite promising, a final evaluation of the practical applicability of this principle requires the clarification of the question of whether, as in the case of esterified DTPA, enhanced toxicity of the encapsulated DTPA may be a limiting factor.

The final aim for the optimation of chelation therapy concerns the increase of the therapeutic index which, in turn, presupposes the knowledge of the mechanism(s) of toxic side-effects. As to Ca-DTPA and related compounds, there is ample direct and indirect evidence [1, 2, 26] that the toxicity demonstrated in animal experiments is due to an interaction with essential trace metals, mainly with Zn and Mn, although it would still be premature to propose a consistent and completely verified picture of the pathogenesis of DTPA-toxicity. We want to emphasize that the most important finding which supports the hypothesis of an interaction with trace metals, is the fact that numerous toxic manifestations after administration of high doses of Ca-DTPA do not appear with equimolar doses of Zn-DTPA [1, 2]. To avoid any misconceptions, it should be stressed that in the human no untoward reactions of Ca-DTPA, at a dosage which is considered as safe, have been observed and that the toxic doses in animal experiments are at least 100 times higher than those recommended for clinical use. Thus, the question of the increase of the therapeutic index seems to be of purely academic interest. That this is not the case, however, becomes evident from the following argument. The unsatisfactory efficacy of DTPA with delayed treatment depends, as we stressed previously, at least partially on the very short residence time of the chelate in the organism. Thus, its action may be enhanced by maintaining a high concentration of DTPA in the blood over an extended time period. In practice it could be accomplished by an infusion over a long time or by a highly fractionated administration regimen. Recent studies, however, have shown that a highly protracted treatment schedule leads to a drastically enhanced toxicity of Ca-DTPA: when it is administered to rats daily on 5 consecutive days, the cumulative LD_{50} is 11·5 m-moles/kg. If, however, the treatment schedule is changed so that the daily dose is given in 5 fractions every 2 hr, the cumulative LD_{50} drops to 5·2 m-moles/kg [27]. In dogs the potentiation of toxicity following a highly fractionated treatment regiment was even more impressive [28]. The reason for the pronounced dependency of the toxicity on the treatment schedule may be due to the fact that a long-lasting high concentration of the chelator in the blood will prevent the replenishment of essential metal stores. This conjecture is supported, firstly, by the finding that a dose of Ca-DTPA when given in 5 fractions intensifies the urinary excretion of Zn 1·8 times more than an equimolar single dose [27]. Secondly, Zn-DTPA does not show any dependence on the timing of the dosage and no mortality in the above mentioned fractionated regimen occurred with a cumulative dose of 25 m-moles/kg [27]. Thus, it seems obvious to recommend Zn-DTPA instead of Ca-DTPA for practical use. Immediately the question arises as to the therapeutic efficacy of Zn-DTPA. The denominator in equation (1) in this case needs to be extended by an additive term $K_{ZnL}^{Zn}[Zn^{2+}]$. The value of Zn^{2+}, not exactly known, must be quite small; on the other hand, K_{ZnL}^{Zn} is about 10^7 times larger than K_{CaL}^{Ca}, and, consequently, the newly introduced term may become by no means negligible. This assumption is corroborated by experimental results. Investigations in which equimolar doses of Ca-DTPA or Zn-DTPA were administered shortly after Ce, Y [29], Am [30] and Pu [7] showed a distinctly reduced effectiveness of Zn-DTPA. The relative potency of Ca-DTPA, defined as the ratio of equally effective doses, ranged from 2 to 45 in the different experiments depending on the kind of the metal as well as the organ. It is remarkable that the differences in the efficacy of both chelates with delayed treatment become smaller and finally

disappear, so that in the cases of repeated doses Zn-DTPA becomes virtually equivalent to Ca-DTPA [31]. This phenomenon which, at first sight, seems somewhat unexpected, can be explained by the fact that the slope of the dose–effect curves is inversely related to the time interval between incorporation of the metal and administration of the chelating agent [30]. Although the relative potency of Ca-DTPA must be considered as a constant and as time-independent, it will be seemingly less manifest in the case of a flat dose–effect curve than it would be in cases of pronounced dose-dependency. In other words, the difference in efficacy, though still existent, will be too small to be assessed experimentally. Even in acute situations the loss in efficacy of Zn-DTPA is not aggravating as it can be compensated for by a higher and still safe dose. Furthermore, the use of Zn-DTPA permits a treatment schedule aiming at the maintainance of a higher chelate concentration over a longer period and it goes without saying that this would prevent the build-up of a systemic body burden, e.g. following contamination of wounds with or inhalation of Pu, more effectively than a single dose of Ca-DTPA.

For B-metals 2,3-dimercaptopropanol (BAL) could still be considered as the optimal antidote, provided the evaluation of efficacy is solely based upon the amount of excreted metal. There is considerable reluctance to its clinical use, however, because of the low therapeutic index and the relatively high incidence of untoward reactions. Since the penetration of BAL into the intracellular space is of importance for its toxicity, it seemed reasonable to substitute the propanol group by propanesulfonate resulting into a nonlipophilic and, thus, less toxic compound [32]. In fact, 2,3-dimercaptopropane-1-sulfonate with an acute LD_{50} of 5·0 m-moles/kg is considerably better tolerated than BAL with a LD_{50} as low as 0·8 m-moles/kg. The first results with this compound have been promising, but an exact comparison of its efficacy with that of BAL and other thiols is still missing.

Finally, a somewhat restraining comment is necessary. Toxic metal ions, within a rather short time, may give rise to irreversible damage. Consequently, the intensified excretion of the metal induced by a chelating agent is not necessarily paralleled by a genuine therapeutic effect. At the same time, this constitutes a challenge with regard to the methodology of experimental studies which during recent years were mainly concentrated on the excretion of the metal while the evaluation of the eventual therapeutic benefit has been almost neglected.

In summary, information discussed has revealed a certain, though not spectacular progress. We assume, hopefully, that the pursuit of the possibilities pointed out by us will lead to further improvements and will give new impulses to chelation therapy which was, at least until recently, threatened by stagnation. This, however, presupposes a close cooperation between medical scientists and chemists, i.e. the willingness of chemists to suggest and/or to synthetize pertinent compounds.

* Only selected pertinent references are listed. References to the other experimental data mentioned, are cited in the comprehensive bibliographies [1, 2].

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