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RADIOACTIVE METAL MOBILI ZATION

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THE BIOLOGICAL EFFECTS of carrier-free radionuclides absorbed by the living organism are, with very few exceptions, a consequence of the ionizing radiation emitted. Accordingly, the therapeutic aim in cases of poisoning with metals will differ according to whether the metals are or are not radioactive. If they are not, the conversion of the metal into an insoluble and thus pharmacologically inactive form or a change in its distribution pattern can sometimes be taken as a therapeutically satisfactory result; if on the other hand the metals absorbed are radioactive then the sole aim of the therapeutic measures taken must be their removal from the organism. More precisely, the aim is to reduce the radiometal concentration primarily in the critical organ, that is to say the organ in which injury due to deposited radioactivity would be most serious for the organism. We may say here that as far as most radiometals of practical interest or potential hazards are concerned, the critical organ is the skeleton.

When the problem of radioactive poisoning and its treatment first aroused attention in view of the increasing production and use of radioactive substances, no known complexing agent, or none at any rate that had been tested biologically, had the qualities required to make a substance effective: namely, a sufficiently broad therapeutic range, inert behavior in metabolism, and, above all, a high affinity for the radiometals to be removed from the organism. Dimercaptopropanol (BAL), which had proved relatively effective in animal experiments and also in clinical tests in cases of poisoning with various stable heavy metals, failed generally against radiometals of practical interest (49, 50, 61), with the one exception of polonium-210 (43, 44). The first important advance was made with ethylenediaminetetraacetic acid (EDTA), which for a considerable time remained the only chelating agent which had been at all extensively tested on animals and also showed favorable and encouraging clinical results (28, 62, 64). The earlier work on EDTA has in recent years been thoroughly surveyed (2, 6, 17, 64, 67, 71), and there is no need to go over its results in detail here. However, it will be useful to outline briefly the potentialities, drawbacks and limitations of the EDTA treatment.

The activity spectrum of EDTA is rather restricted by the fact that, even under optimum conditions (of dosage and time of administration), it is ineffective or only partially effective against a number of radiometals of practical importance. It has more than once been established that EDTA cannot prevent the accumulation of radium, radiostrontium or radiobarium in the skeleton. Our own experience (14) shows that it actually causes a 10–20 per cent increase in deposition, a fact which is consistent with Spencer's observation (77) on the inhibition of radiostrontium excretion in the urine in man after treatment with EDTA. EDTA also proved totally ineffective with ruthenium-106 (78). The increased excretion of radioactive light lanthanides, such as cerium-144, is entirely attributable to a reduced deposition in the soft tissues, especially the liver; the fixation of cerium in the skeleton is either unaffected by EDTA or slightly increased (9, 10). Radioyttrium and plutonium respond fairly well to EDTA. But here we come upon the second disadvantage of EDTA: the marked dependence of its effectiveness on the time of administration. If EDTA is administered under conditions of delayed treatment, that is at a time when the major part of the radiometal has already been deposited in the tissues, the amount of radionuclide which can be mobilized is often small and without practical significance in relation to the remaining body burden. This is also true when the EDTA treatment is extended over prolonged periods, which in any case would seem to be a questionable procedure in view of the nephrotoxic action of EDTA.

These facts governed the direction and aim of subsequent investigations: first, the analysis of factors generally relevant for biological effectiveness, and, secondly, the search for other substances more effective than EDTA and/or having a broader therapeutic range. Of course these two problems are closely connected, since the prerequisite of a rational and systematic search for new pharmacologically effective compounds is a sound knowledge of their mode of action. It was early recognized (42, 67, 71) that the effectiveness of a chelating agent is decisively affected by competition between the metal ion to be removed and natural body cations for the chelating agent. Provided the concentration of chelating agent in the organism is high, competition by trace metals present only in low concentrations can be ignored and further considerations are restricted to Ca ions and in some cases hydrogen ions. Without going more deeply into the theory (42), this means that the effectiveness of a chelating agent Z in regard to the

mobilization of a radiometal M should be related in a first approximation to a quantity designated E:

$$E = \frac{K^{M}_{MZ} \cdot Z_{total}}{\alpha + K^{Ca}_{CaZ}(Ca)}$$

This holds good on the simplifying assumption that the radiometal is free from carrier, or at least has high specific activity, that the chelates of Z with biological trace metals can be ignored, and that at physiological pH only simple 1:1 Ca or M chelates are formed. The figure α designates the proportion of the free anion Z^{-i} in the total of all uncomplexed protonated species of the acid H_iZ and is calculated from the acidity constants and the given pH:

$$\alpha = 1 + K^{H}_{HZ}(H) + K^{H}_{HZ} K^{H}_{H_2Z}(H)^2 + K^{H}_{HZ} K^{H}_{H_2Z} K^{H}_{H_3Z}(H)^3 + \cdots .$$

If information is available on the stability of the hydrogen and hydroxo complexes, the numerator in E should be multiplied by the distribution function γ to obtain the total of all complexed M species; as a rule only slight and negligible variations of the E values are obtained as a result. Since at pH 7 for EDTA and its related polyamino acids α is considerably smaller than K^{Ca}_{CaZ}, the competition of hydrogen ions needs to be considered only at considerably lower pH values. Thus, for example, in acid urine a rather more marked breakdown of the M chelates and—if the metal ions are reabsorbed in the tubules—a loss of effectiveness are to be expected. Moreover, extremely low pH values are found in the secreting cells of the stomach. Dudley (20) attributed to a similar instability of the chelates the high concentrations of radioyttrium which he observed in the stomach wall after administration of the metal in chelated form.

If the effectiveness of a chelating agent with various radiometals is considered in relation to the K^{M}_{MZ}/K^{Ca}_{CaZ} values, a satisfactory correlation is generally not obtained. Figure 1 shows the effectiveness of EDTA expressed as the radiometal content of the organ after EDTA treatment as a percentage of the content of the organs of untreated control animals, for a number of carrier-free radionuclides under identical test conditions of dosage and time of administration of the EDTA. The lack of a clear relationship between the two variables was to be expected, because one relevant factor had hitherto been ignored, namely the competition between the therapeutic chelating agent and endogenous high-molecular chelating compounds (proteins, nucleic acids, etc.), ion-exchanging structures and, in some cases, hydroxyl or other anions for the radiometal. It is at present difficult to give even a semiquantitative treatment of these relationships because the appropriate acidity and stability constants are not yet known, nor is there sufficient information about the type of chelates involved. If a chelating agent is to have any effect on the biological behavior of a metal ion, then the pM defined by E

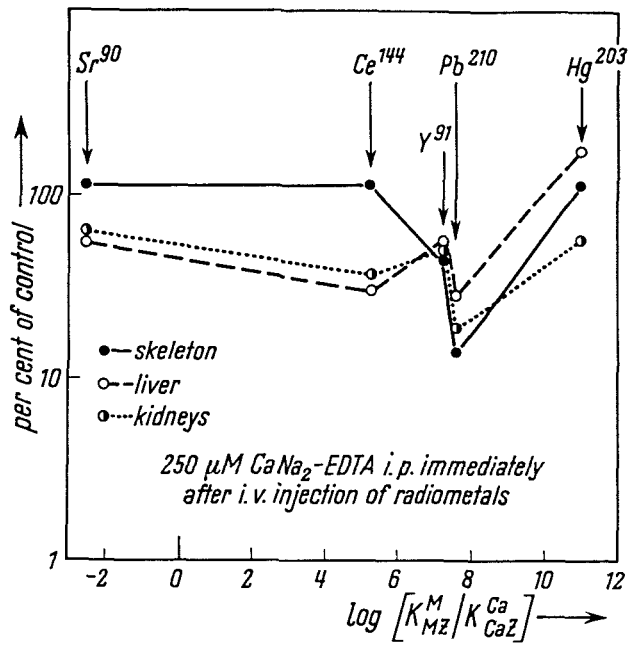
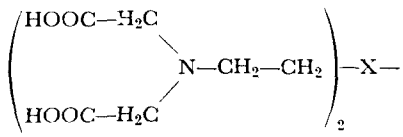


FIG. 1. Effect of EDTA under near optimal conditions on the retention of carrier-free radionuclides in the organs of the rat (Catsch *et al.* (10, 13, 14) and unpublished results).

must at least be as great as or greater than the pM determined solely by the biological milieu in the absence of Z, and the greater the difference between these two pM values the higher the effectiveness should be.

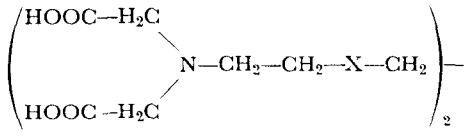
Since the concentration of competing endogenous ions and chelating agents and exchange capacity of the ion exchangers is nearly invariant and since the toxicity of the chelating agent makes it impossible to increase the dosage at will, the therapeutic effectiveness can—at this stage in the discussion—be improved only by varying the quotient K^{M}_{MZ}/K^{Ca}_{CaZ}, in other words, by the use of chelating agents which are significantly different in this respect from EDTA. There is no need to deal in detail here with the numerous chelating agents that have been examined during the past few years and proved inferior to EDTA. In order to emphasize again the significance of the *relative* chelate stability defined by K^{M}_{MZ}/K^{Ca}_{CaZ}, we may briefly mention only 1,2-diaminocyclohexanetetraacetic acid (CDTA), which shows substantially higher stability constants than EDTA for most metal ions, but only slightly different relative stabilities. Its biological effectiveness, which has so far been tested for plutonium (3, 73), radiocerium (10) and radioyttrium (13), is thus in all respects comparable to that of EDTA and not superior to it. On the other hand, a particular group of polyamino acids were likely to be more effective than EDTA, and, as will be shown later, this hypothesis was confirmed experimentally. These are substances with an alkylene bridge longer than that of EDTA and interrupted by one or more atoms other than carbon.



X = O: 2:2′-bis[di(carboxymethyl)amino]diethyl ether (BADE, BAETA)

= S: 2:2′-bis[di(carboxymethyl)amino]diethyl sulphide (BADS)

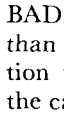
= N: diethylenetriaminepentaacetic acid (DTPA)



X = O: 1:2-bis[di(carboxymethyl)aminoethoxy]ethane (BAE)

= S: 1:2-bis[di(carboxymethyl)aminoethylthio]ethane (BASE)

= N: triethylenetetraaminehexaacetic acid (TTHA)



The reduction of chelate stability due to lengthening of the alkylene bridge and enlargement of the chelate ring is offset by the hetero-atoms (ether oxygen, sulfur or substituted nitrogen) usable by the metal as ligand; most of the metals so far examined have shown K^{M}_{MZ}/K^{Ca}_{CaZ} values several log-units greater then EDTA (1, 5, 16, 21, 41).

The first substance of this group examined was BADE, which showed a stronger mobilization effect than EDTA for radiocerium, also in regard to its deposition in the skeleton (9, 10). This also proved to be the case with plutonium (33), but not with radioyttrium (13) or thorium (34). A substance with a considerably greater effectiveness and, in particular, a broader activity spectrum than the polyamino acids containing ether oxygen is diethylenetriaminepentaacetic acid (DTPA). Table 1 shows some representative results obtained from experiments with rats, which show clearly the considerable differences in effectiveness between EDTA and DTPA, even under conditions of delayed treatment. It should be added that the favorable results obtained with plutonium have been fully confirmed by other workers (25, 76), and that a greater effectiveness of DTPA as compared with EDTA is to be seen in the effect on iron (23, 24), chromium, cobalt, zinc (24), manganese (35) and uranium (8) toxicity.

The dependence of chelating agent effectiveness on the quantity E should present a straight line in the log-log plot, provided a certain threshold value for E has been exceeded, that is, the affinity of the radiometal for endogenous chelating agents is less than its affinity for a chelating agent used therapeutically. From the

TABLE 1. *Effect of $CaNa_3$-DTPA and $CaNa_2$-EDTA on Retention of Different Radioactive Metals in Rats*

Radionuclide	Chelate	Time of Injection After Radionuclide	% of Administered Dose			Ref
			Liver	Skeleton	Excret.	
Y^{91}	control	—	3.4	56		(13)
	EDTA	2 minutes	1.7	25		
	DTPA	2 minutes	0.6	18		
	control	—	1.1	59		
	EDTA	7th, 9th, 11th, 13th day	0.7	48		
	DTPA	7th, 9th, 11th, 13th day	0.4	38		
Ce^{144}	control	—	42	30		(10)
	EDTA	2 minutes	13	32		
	DTPA	2 minutes	0.4	2.3		
	control	—	36	25		(11)
	EDTA	5th, 7th, 9th day	32	26		
	DTPA	5th, 7th, 9th day	2	16		
Th^{234}	control	—		60	1.5†	(34)
	EDTA	from 2nd to 5th day		60	5.1	
	DTPA	from 2nd to 5th day		43	26	
Pu^{239}	control	—	9.1*	6.6*		(75)
	EDTA	1 hour	4.9	4.6		
	DTPA	1 hour	2.7	0.9		
	control	—	7.9	64	2.4†	(33)
	EDTA	6th, 12th, 21st, 22nd, 25th, 26th day	11	46	6.7	
	DTPA	6th, 12th, 21st, 22nd, 25th, 26th day	1.6	40	20	
Am^{241}	control	—		40	40	(76)
	DTPA	6 hours		10	90	
	DTPA	7th day			80	

* Per cent/gm of dry tissue. † Urine.

definition of E it follows that this straight line represents the dependence of effectiveness both on the dosage of *one* particular chelating agent and on the relative chelate stabilities of a number of chelating agents. Thus the effectivities of two chelating agents having similar K values should overlap and, if adequate dosages are chosen, should behave identically. Figure 2 summarizes the corresponding data for radiocerium; four different polyamino acids (EDTA, DTPA, CDTA and HEDTA) were examined, six different dosages for DTPA being used. As regards skeletal effectiveness, it is possible to represent all experimental points within the limits of experimental error satisfactorily as a straight line, provided that E is greater than about 10^5. It should be stressed that with lower values for E the chelating agents induce a slight but statistically significant increase in radiometal deposition. The reason for this might be that the radiometal chelates are able to diffuse into the extracapillary space and thus bring enhanced amounts of radiometal into contact with the competing ion-exchanging or -adsorbing structures of the bone. Assuming, moreover, that the competition of the bone tissue is more important than that of the high molecular chelating

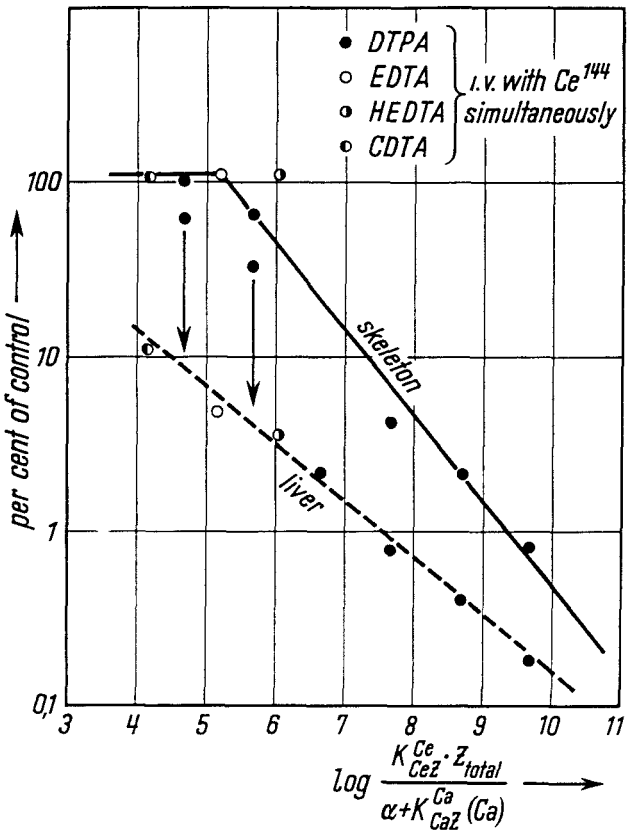


FIG. 2. Effect of several chelating agents and respective various doses of DTPA on the retention of Ce^{144} in the organs of the rat. Abbreviations: DTPA-diethylenetriaminepentaacetic acid, EDTA-ethylenediaminetetraacetic acid, HEDTA-hydroxyethylethylenediaminetriacetic acid, CDTA-1,2-diaminocyclohexanetetraacetic acid (Catsch and Lê (10), Catsch, unpublished results).

agents of the blood plasma, there must be a tendency towards increased deposition in the skeleton. As to effectiveness in the liver, the two lowest DTPA dosages of 10^{-8} and 10^{-9} mole/animal show a definite deviation from linear regression. The fact that extremely low dosages of chelating agent are less effective than expected is not surprising in view of the fact that an important prerequisite for the validity of the dependence of effectiveness on E, namely a considerable excess of chelating agent over the concentration of radiometal and of biological trace metals, is lacking in this case. It might be thought that appropriate extrapolation of these curves would throw light on the E values necessary to obtain still greater mobilization. However, a certain caution seems indicated here in view of the fact that the postulated dependence of effectiveness on E leaves a number of relevant factors out of account and so implies a certain degree of over-simplification. Our starting point hitherto has been that the behavior of the chelating agents within the organism is completely inert, that is, they are neither decomposed nor deposited but are within a short time excreted completely and unchanged. This is indeed true for polyamino acids like EDTA and DTPA, as Foreman (24, 30, 32) has shown, but does not apply necessarily or unconditionally to other chelating agents of different chemical structures.

The extent to which the efficiency pattern depends on the metabolic behavior of the chelating agent is strikingly shown by the condensed phosphates, regardless of whether we think of these (like Graham's salt) as chelating agents or, with Thilo (79), as soluble ion exchangers. As can be seen from Table 2, the polyphosphates when administered early have a fairly strong effect on the behavior of various radiometals, deposition in the bone tissue being reduced as compared with EDTA, while the effectiveness in the parenchymatous organs is considerably less or indeed deposition may be actually greater (10, 13, 73). Tests with polyphosphates labeled with P^{32} have shown (38) that they are present in the blood as nondiffusible and nonultrafilterable colloids and are deposited in the organs of the reticuloendothelial system and the kidneys. This also explains the increased deposition of radiometals in these organs and the unexpectedly high skeletal effectiveness. The fact that the condensed phosphates do increase the excretion of radiometals from the organism is explained by the hydrolytic breakdown of the polyphosphates to lower molecular poly- and metaphosphates, which can be excreted and have complexing properties (10, 73). Since the condensed phosphates have only a narrow therapeutic range and their effect in delayed treatment is negligible (10), they are of little practical importance.

An unexpected result was also found when the higher homologue of DTPA, ten-dentate triethylenetetraminehexaacetic acid (TTHA) was examined, (Table 3). TTHA if administered early inhibits deposition of radiocerium and radioyttrium in the skeleton much more than DTPA. On extrapolation of the appropriate curve in Figure 2 this would correspond to an E value of the order of 10^{10} or 10^{11}. As regards effectiveness in the liver, a reduction of the radiocerium content to less than 0.1 per cent of the control would be expected, whereas, what we have in fact is a reduction merely to 0.25 per cent. As TTHA has a number of ligand sites greater than the coordination number of the radiometals concerned, we must assume the formation to some extent of bimetallic chelates and, in certain circumstances, of polynuclear aggregates, which could lead to

TABLE 2. *Effect of 25 mg Sodium Polyphosphate (Graham's Salt) on Retention of Different Radioactive Metals in Rats**

Radionuclide	% of Control, Fiducial Limits ($P = 0.05$)			Ref
	Liver	Kidneys	Skeleton	
Y^{91}	132 (103–168)	450 (198–980)	51 (44–59)	(13)
Ce^{144}	23 (20–26)	250 (182–345)	48 (41–55)	(10)
Pu^{239}	82 (57–107)	189 (122–256)	34 (25–43)	(73)

* Injection ip 2 minutes after Y^{91} and Ce^{144}, 10 minutes after Pu^{239}.

TABLE 3. *Effect of DTPA and TTHA on Distribution of Ce^{144} and Y^{91} in the Organs of the Rat**

Radionuclide	Treatment	% of Control, Fiducial Limits ($P = 0.05$)	
		Liver	Skeleton
Ce^{144}	DTPA after 2 minutes ip	1.02 (0.78–1.30)	10.1 (8.8–11.6)
	TTHA after 2 minutes ip	1.50 (1.31–1.73)	5.20 (4.45–6.12)
	DTPA simultaneously iv	0.11 (0.08–0.14)	1.28 (0.92–1.79)
	TTHA simultaneously iv	0.23 (0.17–0.31)	0.45 (0.33–0.62)
Y^{91}	DTPA after 2 minutes ip	36 (27–49)	5.70 (5.00–6.50)
	TTHA after 2 minutes ip	42 (31–60)	3.27 (3.21–4.37)

* Dosages: 250 μM $CaNa_3$-DTPA ip, 100 μM $CaNa_3$-DTPA iv, 250 μM $CaNa_4$-TTHA ip; 100 μM Ca_2Na_4-TTHA iv (Catsch & Schindewolf-Jordan, unpublished results).

behavior similar to that of the colloidal condensed phosphates. A deviation in the behavior of TTHA was also established in the course of our investigation (8) into the influence of various chelating agents on the acute toxicity of uranyl nitrate: when a single dose of the chelating agent is administered early, the LD_{50} of uranium is increased from 6.7 mg/kg to 12.4 mg/kg by EDTA and to 16.2 mg/kg by DTPA. On the other hand TTHA, the toxicity of which is normally no different from that of EDTA or DTPA, was 100 per cent lethal to uranium injected animals, and, actually, within one hour of administration. We attributed this potentiation of TTHA toxicity by uranium to the formation of insoluble polynuclear uranium chelates in the blood. With regard to the figures given in Table 3, which show a clear increase of effectiveness as against DTPA, we feel that this substance calls for further investigations, which should also include plutonium.

To deal with the question of the dependence of effectiveness on the quantity of E, any deviations from linear behavior can be explained on different principles from those discussed earlier. There would seem to be some grounds for assuming that a given organ does not behave as a homogeneous unit in the absorption of radiometals, but rather as one composed of several compartments differing in affinity for radiometals and so, also, in the ability to mobilize the radiometal by means of chelating agents. Figure 3 shows schematically how the assumption that the organ consists of two compartments with different affinities for a radiometal and thus, also, different threshold dosages for E results in a nonlinear relation between effectiveness and E for the organ as a whole.

Let us now turn to radiostrontium as a special case. Here DTPA proved just as ineffective as EDTA (14, 51); comparable experiments on radiobarium and radium have still to be made, but it can be presumed with fair probability that DTPA will also fail with these. The

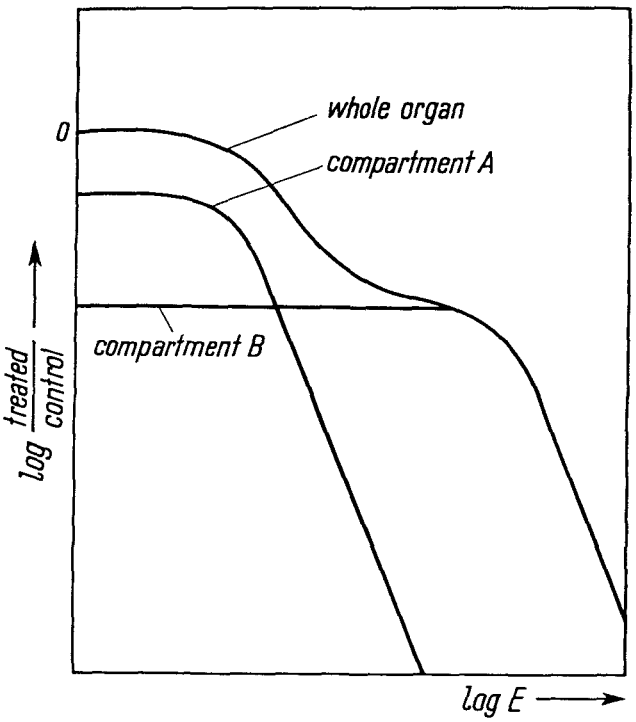


FIG. 3. Hypothetical dependence of the effectiveness of a chelating agent on the value E (see p. 207) under the assumption that the organ is subdivided in two distinct compartments characterized by a different affinity towards the radiometal.

total failure, or at best only slight effectiveness, of all chelating agents so far tested with radiostrontium is not fortuitous (as Schubert (68) has recently shown again in detail). Rather it is an expression of the general rule that chelate stability constants are inversely proportional to the ion radius of the alkaline earth atoms, in other words, that the competition of the Ca ions for the chelating agent will be notably stronger than in the case of other radiometals. It must be granted that the difference between the Ca and Sr stability constants of EDTA and DTPA, of some 2 log units, is exceptionally large, and that it is not difficult to find chelating agents with E values only a little below 1. But the effectiveness of these is still disappointingly low, the reduction of radiostrontium deposition in the skeleton being 10–20 per cent on early administration. Individual chelating agents which have been shown to have some, if only slight, effectiveness are sodium citrate (7, 14, 45–47, 70), tricarballylic acid (45–47), sodium thiosulfate (15), pyrocatecholdisulfonic acid (14, 51), low- and high-molecular meta- and polyphosphates (7, 14), BADS (14) and BADE (14, 53). Since rhodizonic acid is a selective precipitating agent for strontium, Lindenbaum and Fried (55) examined its effect on the distribution of carrier-free radiostrontium but found it relatively slight. This, moreover, was not confirmed by other investigators, admittedly working under somewhat different experimental conditions (51, 57, 81). So far the greatest reduction in radiostrontium deposition in the skeleton

TABLE 4. *Effect of BADE (BAETA) and BADS on Skeletal Retention of Sr^{85} in Rats (Catsch, unpublished results)*

Treatment	% of Control Fiducial Limits ($P = 0.05$)
250 μM Na_2-BADS simultaneously	82 (77–86)
250 μM $SrNa_2$-BADS simultaneously	66 (63–70)
500 μM $SrNa_2$-BADS simultaneously	61 (56–67)
100 μM Na_2-BADE simultaneously	89 (80–99)
100 μM $SrNa_2$-BADE simultaneously	71 (64–79)
30 μM $SrNa_2$-BADE simultaneously	86 (82–91)
250 μM $SrNa_2$-BADE simultaneously	66 (62–71)
500 μM $SrNa_2$-BADE simultaneously	52 (49–55)
500 μM $SrNa_2$-BADE after 2 hours	84 (76–92)
500 μM $SrNa_2$-BADE after 24 hours	93 (85–102)

(to 35 per cent of the control) has been obtained with Diamond Fast Blue (51); but since this dyestuff in effective doses has proved strongly toxic and sometimes lethal it has not any practical importance.

We cannot confirm Schubert's assumption (70) that the removal of radiostrontium by means of zirconium citrate is governed exclusively by the citrate component since we have shown (14) that zirconium citrate is definitely more effective (with a reduction to 65 per cent) than sodium citrate (80 per cent). According to Kroll (53) BADE shows a greater effectivity when used in the form of the Sr chelate, that is to say, the effect of the chelating agent is combined with that of an isotopic dilution of the radiostrontium. Table 4 shows that we were able to confirm that the effectiveness of Sr-BADE was greater than that of Na_2-BADE, though still inadequate, especially if it was administered several hours after radiostrontium. Naturally a low mobilization effect is better than none at all; but the results obtained so far are so insignificant (compared with those obtained by DTPA with other radiometals) that in my estimation there is at present no occasion to revise the generally pessimistic assessment of the therapeutic possibilities for radiostrontium poisoning; especially as the chances of finding a chelating agent with considerably higher relative stability or, indeed, with a selective affinity for strontium are extremely slight. There is the additional fact that the effective substances mentioned above prove ineffective if administered after a delay. Their ineffectiveness in delayed treatment is attributable mainly to the fact that the radiostrontium which is initially reversibly bound on the surface of the apatite crystals is relatively soon removed from the equilibrium as a result of the onset of recrystallization and is converted into a form which is no longer accessible to the chelating agent (60).

Since heavy metals of the sixth period prefer sulfur to oxygen as ligand atom, it seemed useful to include suitable substances in our tests. In conformity with the only slightly differing $K^{Pb}_{PbZ}/K^{Ca}_{CaZ}$ values of EDTA and DTPA both these chelating agents showed no significant variations of effectiveness either on early or delayed administration; Fried *et al.* (37) obtained the same result in exploratory toxicity experiments. Polyamino acids, which contain ether or thioether linkages, also showed no increase of effectiveness over EDTA. It should of course be mentioned here that sulfur in the form of thioether is in principle a much weaker coordination partner than in the form of mercaptide or sulfite. The only polyamino acid which gave us a definitely stronger mobilization of radiolead than EDTA was cystaminetetraacetic acid (CyTA). It is not yet clear whether CyTA is effective in itself or only after splitting of the disulfide bond, that is, in the form of the resulting mercaptoethyliminodiacetic acid. This latter assumption would seem to be supported by the toxicity of CyTA, which is about ten times higher than that of other polyamino acids and appears, primarily, in damage to the kidneys. The stability constants for the Pb-CyTA chelate have not yet been established, though that for mercaptoethyliminodiacetic acid is known (4). Assuming a complete splitting of the CyTA in the organism, and considering the effectiveness in relation to the E values for various dosages of mercaptoethyliminodiacetic acid, EDTA and DTPA, we get (as shown in Fig. 4) a satisfactory linearity within the limits of experimental error in the case of the liver and kidneys. As to the skeleton, CyTA proves considerably less effective than its behavior in the other organs might have led us to expect, the slope of the straight line obtained for various dosages of CyTA being apparently identical with the slope representing the effectiveness of various dosages of EDTA and DTPA. To explain this discrepancy we may assume as a working hypothesis that the splitting of the disulfide bond of CyTA occurs preferentially in the liver and kidneys, or that the mercaptoethyliminodiacetic acid has a specially high affinity for these organs. Results so far do not suggest that CyTA is of practical importance since its effectiveness in nontoxic dosages is of the same order as that of EDTA and DTPA.

The first results of experiments still in progress with carrier-free mercury-203 are shown in Figure 5. The only organs whose mercury content is affected by polyamino acids, and that only to a slight extent, are the kidneys. The graph connecting effectivity and the E values shows, in this case, only a negligible slope and a marked scattering of the points. One has the impression that it is the longer-chain polyamino acids that are considerably less effective than would have been expected from their E values. The reasons for this exceptional behavior are not yet clear; it is possible that the substances in question behave differently from the other polyamino acids in metabolism. The polyamines which we also examined were completely ineffective in spite of very much higher E values, which is most probably due to their metabolic breakdown. As with radiolead, CyTA proved the most effective, the mercury content of the kidneys being reduced, under identical test conditions, to about 25 per cent of the control, although a much slighter effect might have been expected from the E value of 10^8 (on the assumption that the mercapto-

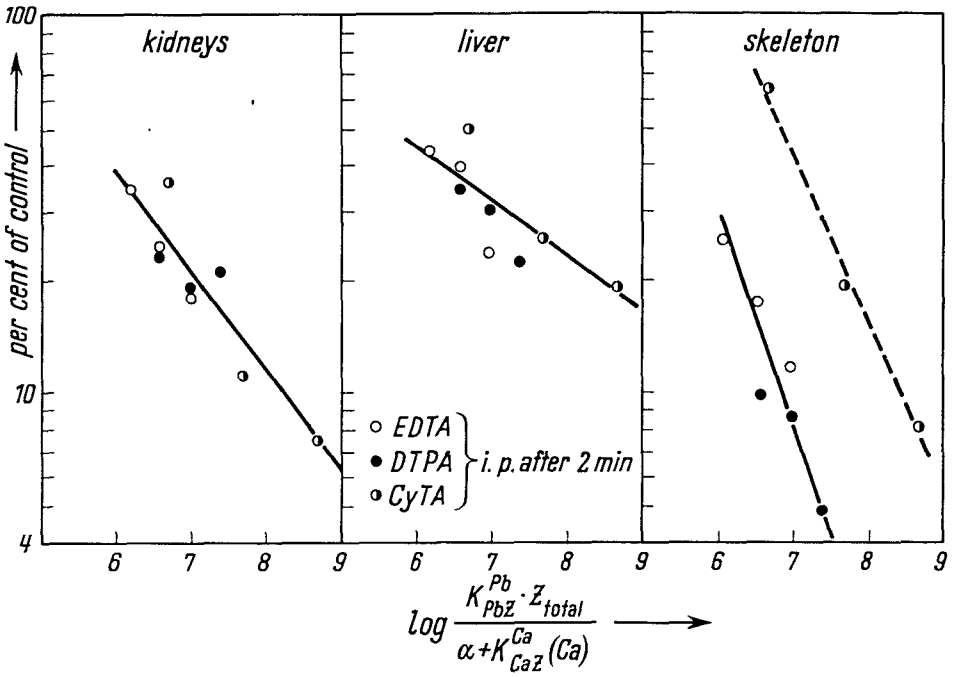


FIG. 4. Effect of various doses of several chelating agents on the retention of Pb^{210} in the organs of the rat. It is assumed that CyTA (cystaminetetraacetic acid) is completely split up into 2-mercaptoethyliminodiacetic acid (Catsch, unpublished results).

ethyliminodiacetic acid is the effective substance). It is interesting that other chelating agents with fewer ligand sites, such as iminodiacetic acid and ethylenediamine-N,N'-diacetic acid were effective to a relatively high degree. As Figure 6 shows, the effectiveness of CyTA when administered after a delay increased, in contrast to what happened with other chelating agents and other radiometals. It was stated at the outset that both toxicity and excretion of polonium-210 are favorably affected by BAL (43, 44) or its water-soluble sulfonic acid derivatives (22). No comparison has yet been made between the sulfhydryl compounds and DTPA or other polyamino acids.

The high effectiveness of DTPA, which is evident even at relatively low dosages, makes it possible to analyze its action in more detail than is possible with EDTA. Table 1 shows that the greater effectiveness of DTPA (as against EDTA) is maintained when it is administered after delay, but that the dependence of effectiveness on time is again unmistakable. To explain this generally valid circumstance a factor which has been ignored hitherto must now be taken into account, namely the rate at which the chelates are excreted from the organism. The high effectiveness observed on administration of the chelating agent immediately or within a short time is partly due to the fact that the freely diffusible metal chelate is excreted rapidly by the kidneys and thus is to a large extent withdrawn also from the competition of the endogenous chelating agents and ion exchangers. If a chelating agent is removed comparatively slowly from the blood, the partition of the metal chelate between excretion and intercellular space is shifted in favor of the latter, and in the light of the ideas discussed above we should expect a reduction of effectiveness. That this assumption is correct has been

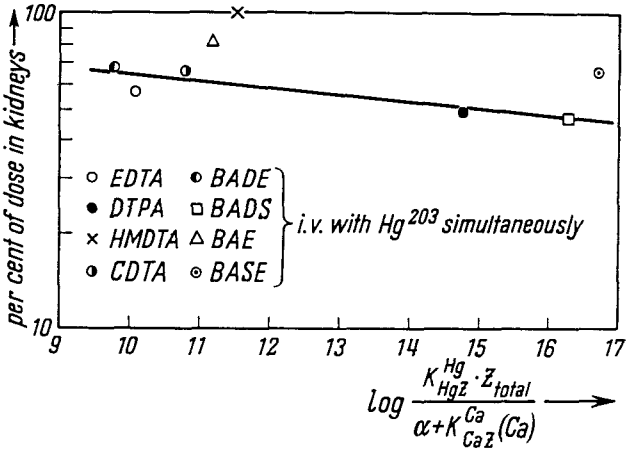


FIG. 5. Effect of several chelating agents on the retention of Hg^{203} in the kidneys of the rat. HMDTA-hexamethylenediaminetetraacetic acid; for further abbreviations see p. 208 and legend of Fig. 2 (Catsch and Nigrović, unpublished results).

impressively shown in a test carried out by Semenov *et al.* (72): Carrier-free radioyttrium was injected with an excess of EDTA into nephrectomized rats and the radioyttrium content of the organs was measured after 24 hours. The distribution differs hardly at all from that in the control animals which were given only radioyttrium. It should be mentioned that the blood concentration of the EDTA labeled with C^{14} remains constant over the same period of time in the case of the nephrectomized animals (32). This shows that as soon as the excretion factor is removed the stability of the Y-EDTA chelate is no longer sufficient to compete successfully with the tissues.

If a chelating agent is administered when the bulk of

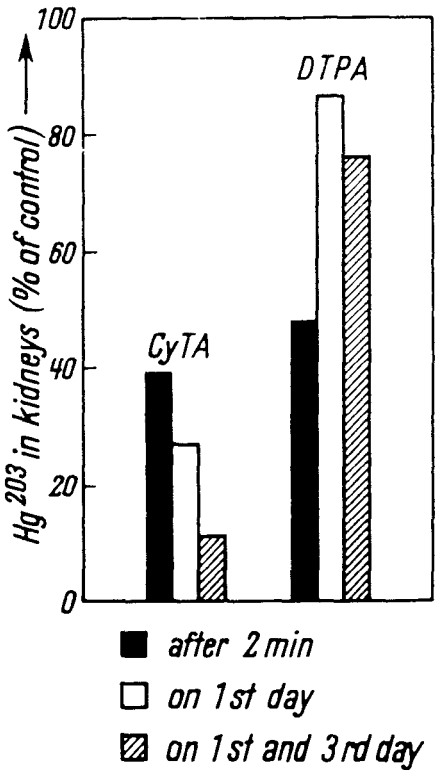


FIG. 6. Effectiveness of DTPA and CyTA (50 μM per rat and dose) in removing Hg^{203} from the kidneys of the rat (Catsch and Nigrović, unpublished results).

the radiometal is already deposited in the tissues and organs, its effectiveness depends in the first place on the relationship defined above between the various complexing affinities and also on the chelate excretion rate, which, however, here has the opposite effect to that discussed above. Mobilization of the radiometal deposited in the tissues is in principle possible only through chelation of the free metal ions and the consequent disturbance of the equlibrium with the cellular chelating agents or ion exchangers. If the rates at which the endogenous chelates decompose and/or the metal ions flow into the extracellular space are slow in relation to the rate of excretion of the chelating agent, then the effectiveness of the latter must decrease fairly rapidly. Provided a chelating agent is effective at all, that is, it exceeds the threshold value for E for the radiometal concerned, its effectiveness should be inversely proportional to the excretion of the chelate. The significance of the solubilizing rate may be exemplified by the findings of Schubert and Fried (69): the dissolution of an olated thorium hydroxide by means of DTPA *in vitro* is extremely slow in spite of the very high stability of the Th-DTPA chelate. The same thing occurs *in vivo*; if animals are injected with colloidal thorium hydroxide, excretion is only slightly intensified by repeated doses of DTPA, whereas if monomeric thorium citrate is injected the effectiveness of the DTPA becomes very marked indeed. The reduced effectiveness of chelating agents with certain isotopically diluted radiometals [e.g. radioyttrium (72)] is also attributable to the slow solubilization of the colloidal metal aggregates.

The excretion of radiometals from the various organs as a rule represents a multiexponential function in which the individual terms formally can be assigned to particular compartments which presumably possess different affinities for combination with the radiometal. Here it is of course a prerequisite that individual compartments are not interchangeable or that the appropriate rates of interchange are low in relation to the excretion rate. Since the mobilizing capacity of a chelating agent depends essentially on the stability of the endogenous radiometal complexes, it follows that the effectiveness is inversely proportional to the so-called biological half-time, and should decrease in the event of a multiexponential excretion function, indicating the progressive increase in the significance of the "slow" components with the passage of time. These suppositions were confirmed by tests with radiocerium, whose excretion from the liver during the first two months after administration can be expressed as the sum of two exponential terms with half-time values of 5 and 15 days, respectively, in rats. The "fast" component is predominant, that is, during the first four weeks, the effectiveness of single dosages of DTPA remains roughly constant, giving a fall in radiocerium content to about $\frac{1}{3}$, regardless of the time of administration (Table 5). It decreases only when the "slow" component is predominant. This is also the case when the readily mobilizable fraction of the radiocerium, corresponding to the "fast" component, has been removed by a previous dosage of DTPA, so that the effects of several successive DTPA dosages are no longer fully additive (Catsch, unpublished results). For the reasons already discussed in connection with radiostrontium, the progressive decrease in effectiveness in the skeleton is especially strongly marked. Examination of the distribution over the various sections of the femur has shown that radiocerium deposited in the metaphysis is mobilized by DTPA to a greater extent than from the epiphysis and diaphysis (Table 6). It can also be seen that here the progressive loss of effectiveness is more strongly marked, as would be expected given the exceptionally intensive growth processes taking place in the metaphysis. These results were obtained with young rats, and it may be asked whether the dependence of effectiveness on time would be as great if the animals were fully grown. Another factor which is still not clear is the effectiveness of chelating agents where the administration of radiometals has been maintained over a prolonged period, the result of which, as compared with a single dosage, is to give a much more uniform distribution of the radiometals in the skeleton.

The differences in the extent to which the radiometal in the liver and skeleton can be depleted by DTPA were shown by Fried *et al.* [quoted in (65)] for plutonium also: daily administration of DTPA over two weeks reduced the plutonium content of the liver to 3 per cent of the control, while that of the skeleton fell only to 50 per

TABLE 5. *Dependence of the $CaNa_3$-DTPA Effectiveness in Removing Ce^{144} from the Organs of Rats on the Time of its Administration**

Day of Administration	% of Control, Fiducial Limits ($P = 0.05$)		
	Liver	Kidneys	Skeleton
1st	21 (19–24)	54 (43–68)	72 (64–80)
3th	28 (23–33)	86 (63–117)	72 (62–82)
25th	24 (14–39)	84 (64–111)	90 (83–98)
7th, 9th, 11th, 13th	10 (7.6–13)	55 (43–68)	59 (52–68)
41st, 43rd, 45th, 47th	28 (18–43)	98 (80–123)	101 (90–112)

* Single ip dose: 250 μM (Catsch, unpublished results, and Catsch & Lê (11)).

TABLE 6. *Effect of $CaNa_3$-DTPA on Retention of Ce^{144} in Different Parts of the Rat Femur**

Time of Injection After Ce^{144}	% of Control, Fiducial Limits ($P = 0.05$)		
	Epiphysis	Metaphysis	Midshaft
2 minutes	14 (11–17)	9 (7–11)	12 (10–14)
5th day	87 (70–108)	64 (50–82)	82 (70–97)
5th, 7th, 9th days	71 (58–86)	46 (40–53)	72 (55–87)
25th day	90 (80–100)	85 (63–115)	90 (70–115)

* Single ip dose: 250 μM (Catsch & Schindewolf-Jordan, unpublished results).

cent. Tests by Foreman (25), in which administration of DTPA was begun one month after administration of plutonium and was continued over various periods of time, also suggest that the effectiveness of DTPA was pretty well exhausted after a four-week treatment. As to its effectiveness in man, we have so far only the observations of Norwood; in some cases, where the incorporation of plutonium took place several years previously, DTPA gave a considerably more (about 10 times) intensified excretion as against EDTA.

Absorption of DTPA from the gut is low (as with EDTA) and represents about 4 per cent of the total dosage (24). Provided the dosage is sufficient the fraction absorbed should be enough to show a clear effect. The effectiveness of DTPA administered orally has been experimentally shown for radiocerium (12) and in a particularly impressive manner by Foreman [quoted in (65)] for americium-241.

In contrast to the previously accepted view that the excretion of radiometals mobilized by polyamino acids is effected only by the kidneys, it has been shown that DTPA causes a marked increase in radiometal excretion in the feces, especially in the case of those which, like radiocerium, are deposited to a large extent in the liver (Fig. 7). After a single dosage of DTPA, for example, on the fourth day after administration some 11 per cent of radiocerium (above the control excretion rate) was excreted in the urine and 20 per cent in the feces. Because the fecal excretion rate of radiocerium is naturally high, the factor by which the pretreatment excretion level is increased by DTPA is considerably higher in urine. The

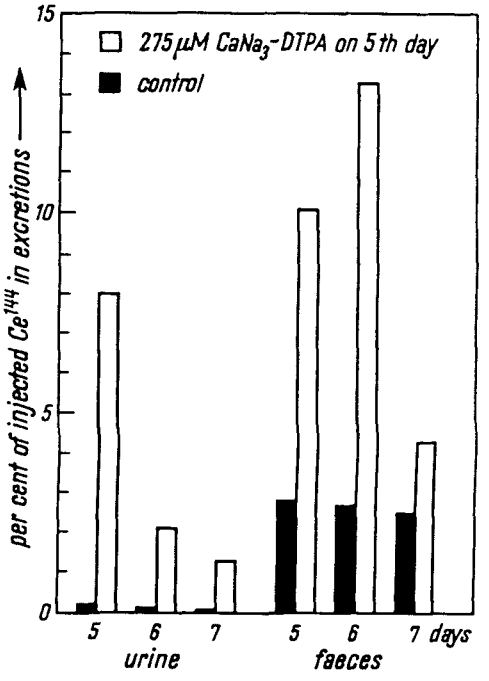


FIG. 7. Effect of a single DTPA dose on the excretion of Ce^{144} from the rat (Catsch and Lê (11)).

proportion of radiocerium mobilized by DTPA and excreted in the urine and feces is about 1:2, which is surprising in view of the fact that the corresponding proportion of excreted C^{14}-labeled DTPA is about 16:1 (24).

DTPA gave unexpected results in other respects as well. Earlier work (18, 72) had shown that EDTA is still effective when administered three hours *before* the administration of radioyttrium. In regard to the clear effectiveness of relatively small dosages of DTPA (cf. Fig. 2) a prophylactic effectiveness might be expected at even longer intervals of time and has in fact been established experimentally (12): 250 μM DTPA per rat, injected 12 hours before administration of radiocerium, reduced deposition of the latter in the skeleton to about 50 per cent and in the liver to 3 per cent of the control values. In this case the very pronounced liver effectiveness is surprising. According to Foreman (24), after 12 hours some 0.03–0.05 per cent of the total Ca-DTPA dosage is still present in the blood. This would in our experiment be equivalent to a dosage of about 0.1 μM, from which, however, on the basis of the results discussed above (cf. Fig. 2), one would have expected the liver content to fall to not less then 10–20 per cent of the control value.

From the earlier work on EDTA it was assumed that the enhanced excretion, in keeping with the rapid and complete excretion of EDTA, was limited to a fairly short period, about the first 24 hours. Fried *et al.* (33) however showed that plutonium clearly continued to be excreted in the urine in more than normal quantities several days after administration of DTPA. Our own tests with radiocerium had given the same results (Fig.

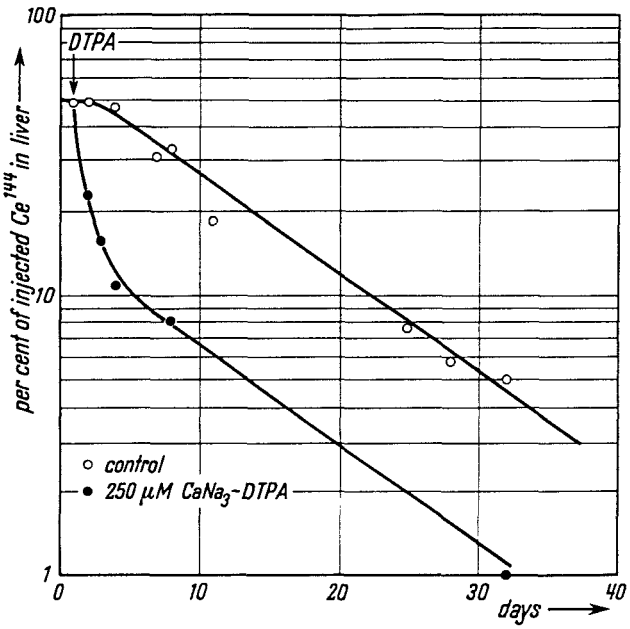


FIG. 8. Effect of a single DTPA dose given on the 1st day on the Ce^{144} content in the liver of the rat (Catsch, unpublished results).

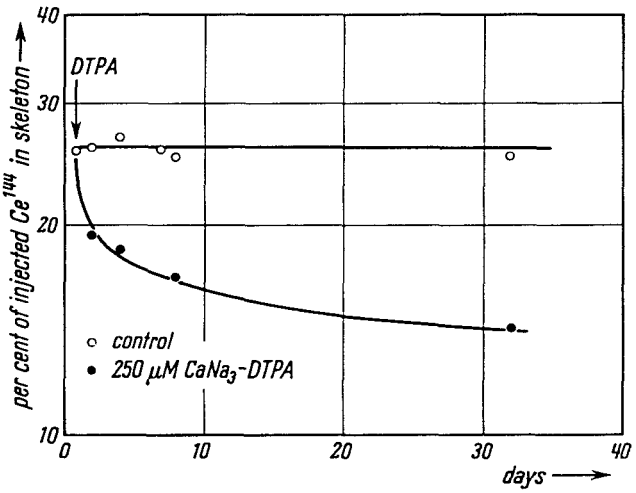


FIG. 9. Effect of a single DTPA dose given on the 1st day on the Ce^{144} content in the skeleton of the rat (Catsch, unpublished results).

8, 9): after a single dose of DTPA on the first day the liver shows a progressive decline in radiocerium content which continues over at least five days, and only on the eighth day does the effectiveness of DTPA appear to be exhausted, as thenceforward the curve runs parallel to the excretion curve of the untreated controls. In the skeleton the decrease is definite even after the eighth day. Here, of course, it is questionable whether this is due solely to the DTPA, and we suggest the following explanation: we know that a certain fraction of the radiocerium initially deposited in the liver returns to the blood stream and is absorbed into the skeleton. The constancy of the radiocerium content in the skeleton is thus only apparent, because the mobilization of radiocerium from the bones is to a large extent compensated by this redistribution, and cannot be observed until this factor has been removed, that is, after the radiocerium content of the liver has been reduced by DTPA.

The somewhat unexpected results discussed above—the partition of radiometal excretion between urine and feces, the surprisingly prolonged or high effectiveness after a single or prophylactic dosage of DTPA—seem to call for some revision of the accepted views on the behavior of polyamino acids in the organism. Since the Ca chelates of polyamino acids are water-soluble and usually anionic, it seemed from the first improbable that they would be able to penetrate cell membranes and enter the intracellular space to any great extent. This assumption was supported by the work of Foreman (24) on the behavior of Ca chelates labeled with C^{14}. Our own observations on the other hand could well be explained by the assumption that a fraction of the Ca-DTPA chelate, minor indeed but still effective, tends to concentrate in the intracellular space, at any rate, of the liver.

This tentative hypothesis is also favored by other observations that are not in a strict sense proof positive and only indirect: in the first place, the DTPA concentration in the liver 24 hours after administration is clearly higher than that in the blood (24). Studies by Lindenbaum and Schubert (56) on tissues from animals given plutonium show that the ultrafilterability of plutonium present in the tissues is increased following a single DTPA dosage and that this persists for at least a week. The information given does not of course show how the organ homogenates examined were obtained and prepared, so that the increased diffusibility of the plutonium could naturally be due also to traces of DTPA in the extracellular space. Our own work on radiocerium, in which the liver was thoroughly and very carefully perfused *in situ* before being homogenized, gave the same result: 5–12 per cent of the total amount of radiocerium contained in the liver homogenate remained freely dialyzable during the first five days after administration of the DTPA, whereas in the untreated control animals this fraction was practically nil.

We may also mention the results of work still in progress, which deals with the effect of DTPA on the intracellular distribution pattern of radiocerium. A state of equilibrium, that is a constant distribution of radiocerium over the various subcellular fractions of the liver homogenate, is reached only some days after the injection of radiocerium; during the first few days the protein-free fraction of the cytoplasm shows a fairly high and slowly decreasing radiocerium concentration, while that of the cytoplasm proteins and of the cellular organelles increases not only relatively but absolutely over the same period. It can thus be assumed that the supernatant obtained after precipitation of the cytoplasm proteins represents the quickly reacting cell fraction, and that the transfer of the radiocerium into the other subcellular fractions and/or its complexing by the

appropriate constituents take place relatively slowly. On the assumption that the Ca-DTPA chelate is present only in the extracellular space, it would be natural to expect the administration of DTPA to be followed by a reduction of radiocerium content of the protein-free cytoplasm fraction, at the very least equivalent to that in the slower reacting fractions. However, Figure 10 shows that in fact the opposite is the case: the radiocerium concentration of the cytoplasmic supernatant is clearly relatively higher during the first few days after administration of DTPA as compared with the other fractions, and shortly after administration is absolutely higher. This observation, together with the evidence obtained by electrophoresis for the presence of Ce^{141}-DTPA in the cytoplasm, favors our assumption that a small fraction of the DTPA is present within the cell for a fairly long time. At present we are trying with the help of autoradiographic investigations to show direct evidence of the presence of DTPA labeled with C^{14} in the cell. It seemed to me necessary to discuss more closely these observations, which at first sight seemed of only theoretical interest, because (as will be shown later) it afforded starting points of practical importance for further development.

The polyamino acids are as a rule administered in the form of their Ca chelates, which are only slightly toxic. As the free sodium salts give rise to some demineralization of the skeleton and as sufficient clinical experience is available to show the tolerance for Na_2-EDTA when slowly administered intravenously, the question arises whether use can be made of this to improve the effectiveness of the chelate. Cohn *et al.* (18) thought that the relatively effective mobilization of radioyttrium from the skeleton observed by them could be attributed to the alternating administration of $CaNa_2$- and Na_2-EDTA; there are no controls in this case, so that this conclusion does not seem justified as yet. Semenov and Tregubenko (72) have recently dealt in detail with the same question and were able by means of repeated doses of Na_2-EDTA to reduce the radioyttrium content of the skeleton to 64 per cent of the control, whereas $CaNa_2$-EDTA reduced it only to 80 per cent. However, both forms of EDTA proved equally effective as far as the parenchymatous organs were concerned, which is what would be expected. This observation is not however amenable to generalization and it was impossible to reproduce it with other radiometals such as cerium and plutonium.

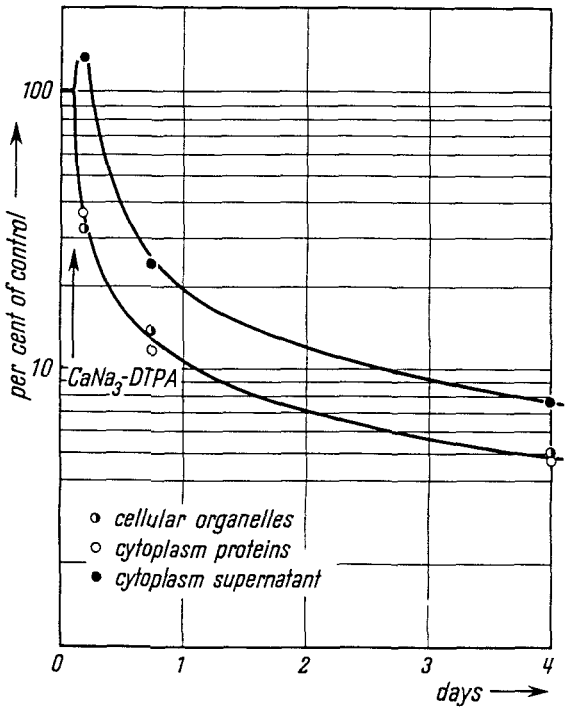
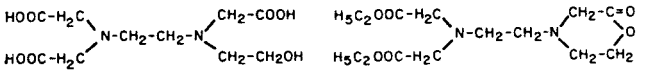


FIG. 10. Effect of a single DTPA dose given after 2.5 hours on the Ce^{141} content in the subcellular fractions of the liver homogenate (Catsch and Immel-Teller, unpublished results).

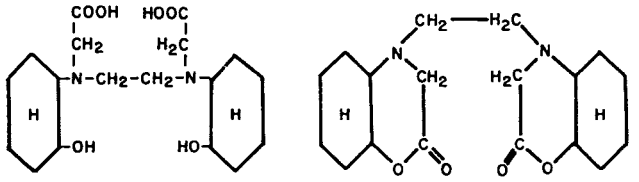
Attempts to increase the effectivity of chelating agents by simultaneous administration of diuretics, the cholagogue dehydrocholic acid or parathormone (11, 14, 39, 72), which intensify the bone resorption processes, have proved negative or unconvincing. A clear increase in the effectiveness of EDTA, however, was obtained in the mobilization of lead from the bones by combining it with vitamin A (59), which in massive dosages is known to enhance bone resorption.

A fundamentally different starting point for improving the therapeutic effectiveness of chelating agents is to be found in the observations already discussed in detail concerning the distribution of chelating agents in the extra- and intra-cellular space. In our view there are certain indications which suggest that the high effectiveness of DTPA is to a certain extent due to its penetration into the intracellular space, particularly in the liver; and it was therefore natural to expect that if this process were intensified there would be a corresponding increase in effectiveness. This possibility had already been discussed by Fried *et al.* (36) and even verified experimentally: by administering fluoroacetic acid, these authors were able to reduce the toxicity of nonradioactive lead by a factor of about 1.15. The mode of action of fluoroacetic acid is supposed to be that, as a metabolic inhibitor, it interferes with the Krebs cycle and leads to an intracellular accumulation of citrate, which is known to have a fairly high affinity for lead ions. The stability of the citrate chelates with the radiometals of practical interest to us is however too low to suggest that this principle could be satisfactorily applied in this case.

Another approach that seemed more promising was to use chelating agents with a high intrinsic permeance, for example esterified polyamino acids. By esterifying the carboxyl groups, the ligand groups necessary for chelation were, it is true, blocked, but it was reasonable to expect that the esters would be hydrolyzed at the intracellular pH, perhaps with the assistance of esterases, and that the effective ligand groupings would be liberated.



N′-(2-hydroxyethyl)ethylenediamine-N,N,N′-triacetic acid (HEDTA) and derivative.



N,N′-bis (2-hydroxycyclohexyl)ethylenediamine-N,N′-diacetic acid (DOC).

Among compounds which we have so far examined is the ethyl ester of HEDTA, which exists partly in a lactone form, and thus is a so-called inner ester. The compound is water soluble at pH 7 and is readily administered intravenously. The corresponding free polyamino acid, HEDTA, is a chelating agent comparable with EDTA 9, 13) in biological effectiveness. Also water soluble at pH 7 is the second compound examined by us, DOC, which occurs as a dilactone. As a control DOC was administered in the form of its $CaNa_2$ chelate which was prepared by hydrolyzing the lactone at pH 10 and 50°C and then adding an equimolar amount of Ca. Table 7, which summarizes the exploratory tests, shows that both esters 24 hours after intravenous injection of radioyttrium or radiocerium effect a clear mobilization of the radiometals from the liver, while the corresponding Ca chelates have no significant effect. An approximately equivalent effect was observed when the ethyl ester of HEDTA was administered orally. That the ester has no effectiveness in the skeleton is consistent with the basic suppositions about its mode of action. That no clear effect was established in the kidneys does not quite justify the conclusion that the esters are selectively taken up by the liver cells and/or are hydrolyzed by them. The results mentioned earlier had shown that radiocerium deposited in the kidneys is fairly quickly converted into a form that is difficult to mobilize (see Table 5). In the light of the observation that fecal excretion is intensified by the DTPA—present, as assumed, in the intracellular space—this was also to be expected in the case of esterified polyamino acids and was established experimentally (Fig. 11). The reduction of the radiometal content of the liver by some 30 per cent by the esters is in these circumstances rather low and not comparable with the effectiveness of DTPA, which in all probability is connected with the inadequate relative stability of the HEDTA and DOC chelates. These observations are, therefore, at present of practical importance only to the extent that they indicate a fundamentally new approach to the therapeutic use of chelating agents. Final conclusions can naturally only be drawn when we know something about the esters of more powerful chelating agents, such as DTPA.

Hitherto we have been concerned solely with the removal of radiometals already deposited in the organs or—under optimum conditions—still in the blood stream at the time when the chelating agent was administered. This leaves out of account a point of practical importance, namely the way in which contamination occurred. The experience of the past few years (28, 29, 54, 64) has shown that the inhalation of radioactive material and contamination of wounds are in practice by far the commonest ways. If the radiometals entering the body in this way show only a slight absorption rate and if in the case of contaminated wounds the radioactive deposit cannot be removed by surgical methods, an attempt must be made to intensify absorption with simultaneous excretion of the radiometal in order to prevent excessive local radiation. There can be no doubt that even in this special case chelating agents are the most promising means. Appropriate experimental investigations into this problem (19, 31, 40, 63) have so far been limited

TABLE 7. *Effect of HEDTA and DOC, Administered after 24 Hours as Calcium Sodium Chelate (Ch) or Respective Ester Lactone (E), on Distribution of Ce^{144} and Y^{91} in the Organs of the Rat**

Radionuclide	Treatment	% of Control, Fiducial Limits ($P = 0.05$)		
		Liver	Kidneys	Skeleton
Ce^{144}	HEDTA-Ch iv	98 (93–103)	90 (84–97)	99 (93–105)
	HEDTA-E iv	76 (73–79)	94 (88–101)	102 (97–107)
	HEDTA-E po	79 (73–86)	112 (96–130)	106 (96–117)
	DOC-Ch ip	87 (75–100)	103 (72–147)	98 (84–115)
	DOC-E ip	68 (59–79)	91 (63–130)	93 (79–109)
Y^{91}	HEDTA-Ch iv	94 (80–111)	98 (77–129)	90 (85–96)
	HEDTA-E iv	67 (56–79)	114 (87–150)	96 (91–101)

* 100 μM HEDTA, 250 μM DOC (Catsch).

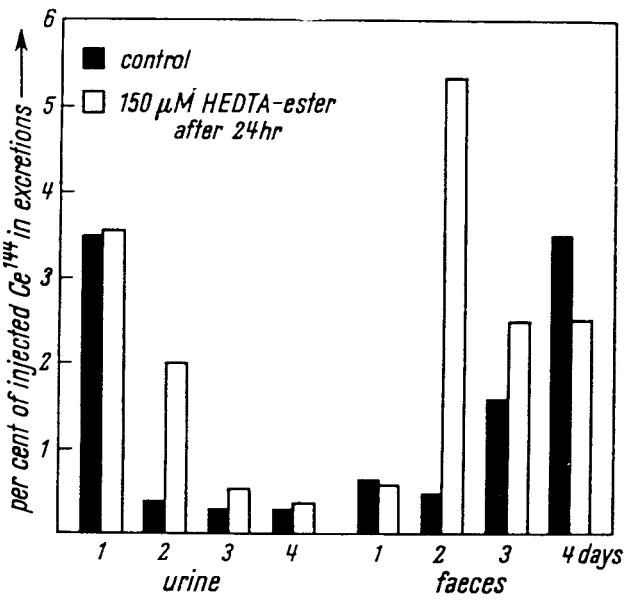


FIG 11. Effect of a single HEDTA-ester dose on the excretion of Ce^{144} from the rat (Catsch, unpublished results)

and carried out only with EDTA. They show, as does also analogous experience with man (64), that it is indeed possible in principle to intensify absorption in this way, but that the decontamination which is the final objective remains disappointingly slight, so that, first of all, it is not easy to decide whether the inherently low effectiveness of EDTA is to be blamed exclusively for the poor results obtained. The examination of DTPA which is *a priori* more effective seems therefore to be one of the most urgent tasks at the moment. In the special case of radiometals deposited in the respiratory tract, it would also be useful to test the effectiveness of a chelating agent administered in aerosol form. Exploratory tests by Semenov *et al.* (72) with this type of application have shown that EDTA is remarkably effective with radioyttrium. Both parenteral and oral administration of chelating agents seem to be contraindicated where radiometals are present in the digestive tract, since in some circumstances they could increase parenteral absorption of radiometals, and they may affect the effectivity of other materials administered for the purpose of inhibiting absorption (e.g., ion exchangers).

In conclusion we may deal briefly with the question of DTPA toxicity. As far as the decalcifying effects of Na_3-DTPA and the toxicity of single doses of $CaNa_3$-DTPA are concerned, exploratory tests (12, 23) suggest that the significant dosages are of the same order as those of EDTA. From the point of view of practical therapeutic use, the experiments with animals (27, 80) and the corresponding clinical trials (64, 74) have shown the exceptional importance of the nephrotoxic effects of *repeated* doses of chelating agents. Such effects are the limiting factor as regards the administration of chelating agents over as short a time as possible, which is desirable in itself. The question whether the nephrotoxic effect of DTPA differs quantitatively from that of EDTA still remains open. Since the exact cause of the kidney damage is still not clear (74), in our view, no *a priori* assertions can be made. Comparative toxicological tests with DTPA and EDTA and the determination of their therapeutic range are therefore desirable. Not until we have these results will we be justified in recommending the clinical use of DTPA without any restrictions.

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