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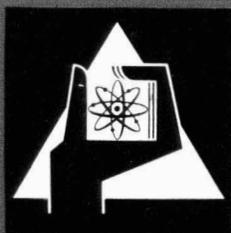
Principles and Trends in Therapeutic Removal of Internally Deposited
Radionuclides

Retention in the Skeleton of Radiostrontium as influenced by Tetracycline

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PRINCIPLES AND TRENDS IN THERAPEUTIC REMOVAL OF INTERNALLY DEPOSITED RADIONUCLIDES

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(Presented by A. CATSCH)

Abstract—The general principles governing the therapeutical removal of radiometals by chelating agents are outlined. Experimental data are presented demonstrating the dependence of chelate effectiveness on the number and the nature of ligand atoms as well as on the molecular configuration of the ligand. Implications derived from experimental data and from the multi-compartmental nature of a body organ for working out an optimal dosage schedule are discussed.

BEFORE we enter into the discussion of problems and experimental data concerning the therapeutic removal of internally deposited transuranic elements, I would like briefly to outline the principles governing this field and to show the direction in which it is developing. Although the corroborative experimental findings that I will quote do not deal directly with plutonium, the conclusions which can be drawn from them, particularly if lanthanides are involved, should be, to a certain extent, applicable and valid for the transuranic elements. The circumstance that all papers which will be presented in this session are confined to the action of chelating compounds is not fortuitous. Rather, it is the consequence of the important progress made quite recently in this direction, whereas all other approaches remain without any essential results. The experimental research on chelating agents at present pursues two main goals: first, the development of highly effective chelating compounds; and second, the elaboration of an optimal therapeutic regime for a given chelator which will guarantee a maximal radiometal mobilization and simultaneously avoid harmful side effects from the toxic action of the chelator.

The factors relevant to the therapeutical effectiveness of chelating agents have been reviewed comprehensively⁽¹⁻³⁾ and there is no need to repeat again the underlying ideas. It may be useful only to recall that the effectiveness of a chelator is related, among other factors, to the ratio of the chelate stability constant for the

radiometal to the stability constant for calcium, an ion which markedly impairs the chelate effectiveness by competition. Generally speaking, this ratio can be influenced by varying the number of the electron-donor atoms, the nature of the donor groups and, finally, the molecular configuration of the ligand.

In regard to the number of ligand atoms, a pronounced polydentate nature of the ligand can give rise to a marked increase in chelate stability. As a rule, this increase will be more pronounced the higher the coordination number of the metal ion involved. In keeping with this argument, we find for higher-dentate polyaminopolycarboxylic acids a marked superiority in mobilizing radiocerium, radioyttrium,^(4,5) and thorium,⁽⁶⁾ i.e., metals for which a maximal coordination number of 8 is likely^(7,8) (Fig. 1). The stability constants for calcium, possessing 6 coordination sites only, are almost identical in the case of EDTA (ethylenediaminetetraacetic acid), DTPA (diethylenetriaminepentaacetic acid), and (very probably) TTHA (triethylenetetraaminehexaacetic acid). Somewhat unexpected is the observation that TTHA affects the deposition of radiocerium and radioyttrium in the liver to a lesser degree than DTPA. The reasons for this discrepancy are not yet fully understood. Because the higher homologue of DTPA, 12-dentate TPHA (tetraethylenepentamineheptaacetic acid), shows a relatively poor mobilization ability, we may conclude that the "polydentate principle" cannot be applied

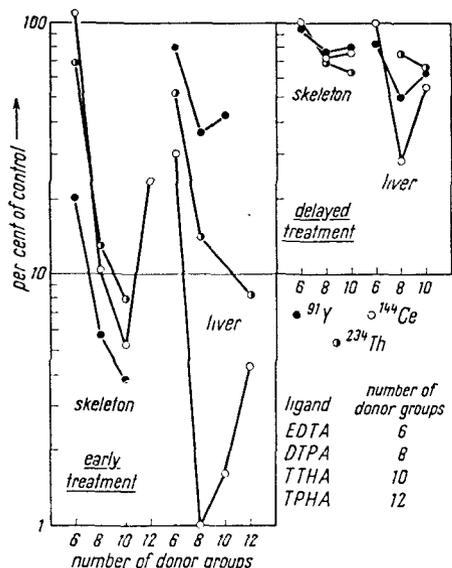


FIG. 1.⁽⁴⁻⁶⁾ Effect of EDTA, DTPA, TTHA, and TPHA on the retention of radiometals by organs of the rat. The calcium chelates (250 μ M/rat) were administered a few minutes post-injection (early treatment) or on the third day (delayed treatment).

indiscriminately. In the case of TPHA, the formation of less stable bimetallic chelate species appears to be likely and to be responsible for the loss in effectiveness. As for plutonium, a superiority of TTHA over DTPA should be anticipated.

Apart from the number of donor groups, the

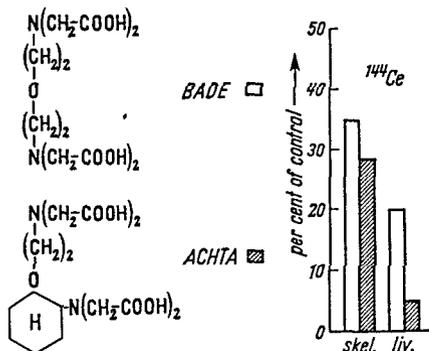


FIG. 2.⁽⁵⁾ Effect of BADE and ACHTA (2-(β -aminoethoxy)cyclohexylaminetetraacetic acid) on the retention of Ce^{144} by organs of the rat. The calcium chelates (250 μ M/rat) were administered a few minutes post-injection.

molecular configuration of the ligand may have a marked effect on its coordination tendencies. This might be either by influencing the basicity of the donor atoms or by impairing (or making easier) the metal-ligand orientation. However, our attempts^(5,6) to obtain such specifically effective compounds for the mobilization of radiocerium or thorium have been unsuccessful so far. A derivative of 2:2'-bis[di(carboxymethyl)-amino]diethyl ether (BADE), possessing a cyclohexyl ring instead of an ethylene group, gives rise to a higher excretion of radiocerium than BADE (Fig. 2). The similar substitution, however, in the DTPA molecule was not followed by a comparable increase in effectiveness. Furthermore, TTHA promotes a higher mobilization of radiocerium and thorium than a

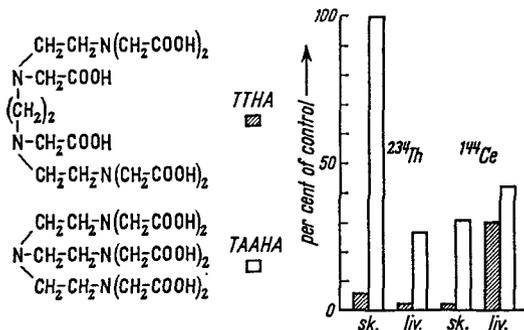


FIG. 3.^(5,6) Effect of TTHA and TAAHA (tri-(2-aminoethyl)aminehexaacetic acid) on the retention of Th^{234} and Ce^{144} by organs of the rat. The calcium chelates (250 μ M/rat) were administered a few minutes post-injection.

related compound, although both agents have the same number and kind of coordination sites (Fig. 3).

Several heavy metals, such as lead, mercury, or polonium, prefer sulphur or nitrogen as a coordination partner over oxygen while calcium behaves in the opposite fashion. In conformity with the higher stability constant ratios, we find that particular sulphur-containing polyamino acids (e.g., 2-mercaptoethyliminodiacetic acid and cystaminetetraacetic acid) reduce the radiolead content of the kidneys and other organs to a much larger extent than do ordinary polyamino acids or structurally different SH-compounds with fewer donor groups.⁽⁹⁾ As expected for the rare earths, the sulphur compounds do not show

any advantage;^(10,11) this should obtain for plutonium also, considering the similar coordination behaviour of lanthanides and actinides.

The effectiveness of the chelating agents is greatly reduced with delayed treatment (Fig. 1), and the difference between DTPA and TTHA disappears. The same observations were reported quite recently for DTPA and BADE.⁽¹²⁻¹⁴⁾ Although DTPA is superior to BADE if administered early, the amount of plutonium, radiocerium, and radioyttrium that can be removed by both compounds with delayed treatment is almost identical. This may occur because as time passes, a major fraction of the radiometal deposited in the tissues is withdrawn from equilibrium and becomes unavailable to the chelators. The remaining and only loosely bound fraction could then be mobilized even by relatively weak chelators. Possible differences in the metabolic behaviour of different chelating agents, caused by structural dissimilarities or differences of the charges of the chelate ions, may also be taken into account. The practical implication is obvious. More experimental investigation is needed with as many different compounds as possible.

The main reasons for the more or less pronounced dependence of effectiveness on the time of chelate administration are the progressive transfer of the radiometal into biological compartments that bind it more tightly and/or the formation of so-called inert or sluggish chelates with endogeneous constituents. As has been pointed out by SCHWARZENBACH,⁽¹⁵⁾ these two parameters (i.e., the chelate stability constant and the constant of the velocity by which the association and dissociation of a chelate proceeds) are not necessarily correlated.

To corroborate the possible importance of sluggish exchange reactions by an experimental result, I would like to mention an observation we have made in the course of our experiments with radioruthenium now under way.⁽¹⁶⁾ To obtain the maximum mobilization effect, we injected radioruthenium simultaneously with different chelating agents (Table 1). Among the compounds so far tested (polyamines, mercaptanes, and polyaminopolycarboxylic acids), the highest reduction of deposition was observed for radioruthenium chelated by 1:2-bis[2-di(carboxymethyl) aminoethylthio] ethane

Table 1. Retention of Ru¹⁰⁶ by organs of the rat after an intravenous injection of different Ru¹⁰⁶ chelates

Chelate	Per cent of control				
	Blood	Muscles	Liver	Kidneys	Skeleton
Trien*	16	13	51	44	23
Penten*	94	119	93	94	101
DMPS*	44	32	36	158	36
DMPA*	14	14	28	270	61
EDTA	104	110	81	81	96
DTPA	10	19	13	19	27
BAEE*	89	111	86	89	103
BATE	2.0	1.8	4.7	9.5	64

* trien = triethylenetetraamine; penten = tetrakis(β -aminoethyl)-ethylenediamine; DMPS = 2,3-dimercaptopropanolsulphonic acid-(1); DMPA = 2,3-dimercaptopropionic acid; BAEE = 1:2-bis[di(carboxymethyl)aminoethoxy]ethane.

(BATE), a compound that proved rather ineffective for all other radiometals previously tested. The effectiveness of BATE and of all other chelators quoted in Table 1 was almost completely abolished if they were administered shortly—even 1 min—after the injection of radioruthenium. This negative result is the more unexpected as radioruthenium is cleared from the blood rather slowly. By means of electrophoretic studies (Fig. 4), we have found that the stability of the Ru-BATE chelate is decisively higher than the binding of ruthenium by the serum proteins. When radioruthenium was added to a solution of BATE in serum, the fraction bound by the albumin and globulins remained negligible and the bulk of the activity migrated together with BATE. If, however, BATE was added to a solution of radioruthenium in serum, the binding ratio reversed, even if the solution was incubated for several days. These findings led us to the conclusion that the ruthenium-protein complex represents a typical inert chelate and that the formation of predominantly covalent bindings is probably the responsible factor.

In this context, may I mention also a similar observation concerning the mobilization by DTPA of radiocerium from an intramuscular depot (Table 2). In the case of carrier-free radiocerium, its absorption from the injection site and excretion from the body can be largely

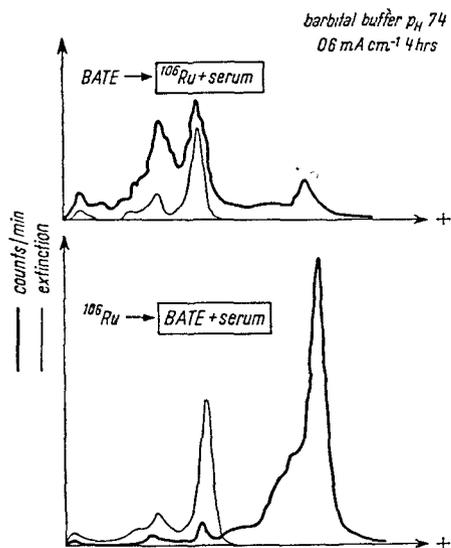


FIG. 4.⁽¹⁶⁾ Electrophoretic behaviour of Ru^{106} in serum containing BATE. Separation on cellulose acetate foils.

enhanced by relatively few doses of DTPA, whereas both the absorption and the effectiveness of DTPA are significantly reduced with isotopically diluted radiocerium. It can thus be assumed that in the latter case a precipitate forms that is extremely slowly solubilized by the chelator.

Let us now turn to questions concerning the effectiveness of multiple chelate doses and the elaboration of an optimal dosage schedule. There are reasons to believe that the individual terms of a multiexponential function, representing the retention of a given radiometal by an organ, can be assigned to particular organ compartments that presumably possess different

affinities toward the radiometal. Because the mobilizing capacity of a chelator depends essentially on the stability of the endogenous radiometal complexes, it follows that its effectiveness is inversely proportional to the biological half-time and should decrease in the event of a multiexponential excretion function, indicating the progressive increase of the "slow" component(s). For further consideration, the assumptions made about the arrangement of the compartments are essential. If we deal with so-called parallel compartments (Fig. 5a), all of them standing in direct exchange with the extracapillary space, the radiometal in the "fast" compartment can be removed fairly quickly by a chelator. Further administration only gives a relatively slight effect, due to the more stable binding of the radiometal by the "slow" compartment (Fig. 5b). A second possibility is the

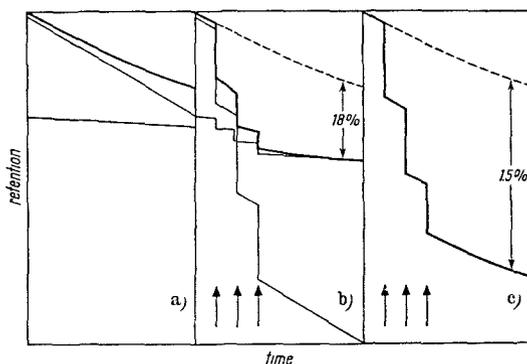


FIG. 5. Retention (log scale) of a radiometal by an organ composed of two parallel compartments (a); hypothetical effect of multiple chelate doses in the case of compartments arranged in parallel (b) and in a series (c).

Table 2. Effect of daily intraperitoneal doses of $\text{CaNa}_3\text{-DTPA}$ ($250 \mu\text{M}$ on 3rd–7th day) on the retention of intramuscularly injected Ce^{144} by rats (means and standard errors)

Tissue	Per cent of dose			
	Ce^{144} (17)		Ce^{144} plus 1 mg Ce	
	Control	DTPA	Control	DTPA
Injected leg	17.5 ± 1.2	8.2 ± 0.3	83.4 ± 3.2	73.7 ± 4.8
Opposite leg	2.6 ± 0.1	1.4 ± 0.04	< 0.1	< 0.1
Liver	16.4 ± 0.5	1.5 ± 0.07	0.32 ± 0.04	0.14 ± 0.02

serial arrangement of the compartments—only one compartment communicating directly with the extracapillary space. If the compartments are arranged in order of the increasing affinity toward the radiometal and the transfer of the radiometal within the series proceeds rather slowly, a quite different effectiveness pattern results and the net amount of excreted radiometal will be markedly higher (Fig. 5c). As to the retention of radiocerium by the liver, our experimental data⁽¹⁸⁾ favour the assumption of at least two compartments arranged in parallel (the effects of several successive DTPA doses were not fully additive). Analyzing the data of SCHUBERT *et al.*⁽¹⁹⁾ with plutonium (Fig. 6), we

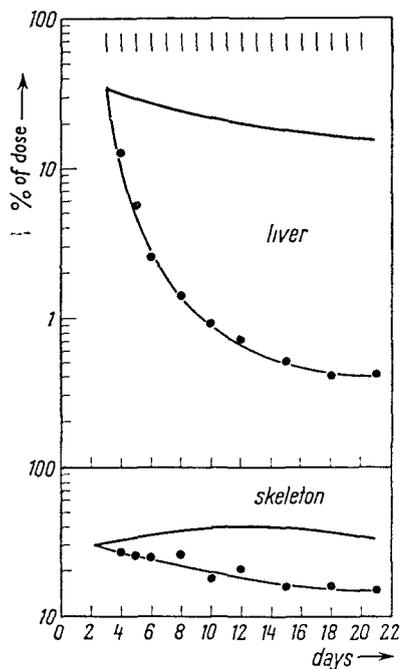


FIG. 6.⁽¹⁹⁾ Removal of monomeric Pu^{239} by multiple DTPA doses (500 mg/kg) from organs of the mouse.

come to the same conclusion. DTPA was given here in daily doses for 18 days and its effectiveness was relatively quickly exhausted. It is likely that the same net effect might have been obtained by a smaller number of doses. Furthermore, one might be in doubt about the appropriateness of the dosage schedule used (i.e., the daily administration of DTPA). The action of

a highly effective compound such as DTPA is sustained over several days.^(12,13,18-22) Consequently, we must consider the possibility that successive and too narrowly spaced doses are less effective than if each dose is given only after the complete cessation of the action of the preceding dose (Fig. 7). To elucidate this point, we carried out the following experiment. Rats injected

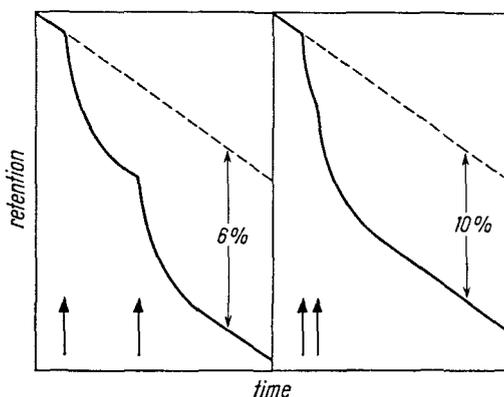


FIG. 7. Hypothetical retention (log scale) of a radio metal as affected by two chelate doses. The second dose is given after different time intervals.

with radiocerium were given three doses of DTPA on the third, fourth and fifth day in the first group, and on the third, sixth and tenth day in the second. The foregoing assumption can be verified by referring to Table 3. Multiple chelate doses given within a short time lower the radiocerium content of the liver to only 21 per cent of the control value; the less narrowly spaced dosage gives a reduction to 16 per cent. This difference is statistically significant. On the other hand, the response of skeleton and kidneys to both dose schedules is almost identical. This is in keeping with our earlier statement⁽¹⁸⁾ that the sustained action of DTPA is confined to the mobilization of radiocerium from the liver. We may therefore deduce from these findings that a protracted dose schedule is preferred—at least in dealing with liver-seeking radiometals—because it gives a higher mobilization and ought to be less harmful.

In conclusion, I might deal briefly with the effect of esterified polyaminoacids. Some time ago we reported^(3,9,23) that the ester-lactones enhance the mobilization of radiocerium,

Table 3. Retention of Ce¹⁴⁴ by organs of the rat as affected by multiple CaNa₃-DTPA doses (250 μM/rat) given on the third, fourth and fifth day (experiment A) or on the third, sixth and tenth day (experiment B). All animals were sacrificed 14 days after injection of Ce¹⁴⁴.

Ten animals per group.

(Means and standard errors)

Organ	Per cent of dose			P (A vs. B)
	Control	Experiment A	Experiment B	
Liver	12.4 ± 0.88	2.65 ± 0.16	1.94 ± 0.21	0.02-0.01
Kidneys	0.50 ± 0.020	0.36 ± 0.019	0.35 ± 0.027	0.8 -0.7
Skeleton	24.8 ± 0.65	16.5 ± 0.46	17.6 ± 0.48	0.2 -0.1

radioyttrium, and lead from the parenchymatous organs to a higher degree than the ordinary calcium chelates of the underlying polyamino acids. It was thought that the higher effect of the ester is brought about by its ability to permeate cellular membranes. Since then we have tested a new compound, the ester-lactone of BADE, and are able to confirm our earlier findings. The effect of this compound, however, remains low as compared to DTPA. Final conclusions about the value of this approach can be drawn only when the ester of DTPA becomes available. Nevertheless, considering the relatively high effectiveness of BADE in removing plutonium,⁽¹²⁾ a screening test on the effect of the BADE-ester in this case might be justified.

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Retention in the Skeleton of Radiostrontium as influenced by Tetracycline

SEVERAL recent investigations have directed attention to the influence of tetracycline on the metabolic behaviour of radiostrontium. The results, however, are ambiguous. In contrast to Nakatsuka *et al.*¹, who were not able to observe any significant effect, it was claimed by Ogawa *et al.*² that tetracycline administered immediately after radiostrontium injection prevents its skeletal deposition and enhances its urinary excretion. This effect was thought to be due to the chelating properties of the compound. Positive results were also obtained by Richards *et al.*³. Repeated daily doses of tetracycline—given 4 days prior until 24 days after the administration of radiostrontium—did not affect the initial deposition of radiostrontium, but rather accelerated its elimination from the bone. The treatment schedule used by the foregoing authors, however, does not permit deciding whether the pre-treatment, or post-treatment, or both, were responsible for the effect.

In order to elucidate this question, albino rats and mice, injected with tracer amounts of carrier-free ⁸⁵SrNO₃, were treated with tetracycline hydrochloride (by courtesy of Farbenfabriken Bayer AG, Leverkusen) in different ways. The dosage of tetracycline was the same as that used by Richards *et al.*; it proved, however, to be toxic, and several animals died in the course of the experiment. Both femurs were ashed at 600° C and their activity assayed by use of a sodium iodide (TI)-scintillation crystal. The strontium-85 content of the whole skeleton was assumed to equal the ten-fold activity of both femurs.

The results (Table 1) show that neither post-treatment nor the simultaneous administration of tetracycline gives rise to a reduced skeletal retention of strontium-85. On the contrary, one experimental series appears to indicate that the elimination of strontium-85 from the bone is inhibited. A significantly reduced deposition was achieved by tetracycline only if given prior to injection of strontium-85.

Table 1. STRONTIUM-85 CONTENT OF THE SKELETON (MEAN AVERAGE ± S.E.)

Treatment (mg tetracycline/kg)	No. of rats (R) or mice (M)	Average body-wt. (g)	Day of death	⁸⁵ Sr-dose (per cent)	P
Control	6 R	179	2	53.6 ± 1.05	
53 Simultaneously	6 R	188	2	50.9 ± 1.34	0.14
Control	6 R	164	3	46.2 ± 1.06	
150 4 days prior	5 R	140	3	34.7 ± 3.12	0.006
Control	10 R	169	13	32.7 ± 1.09	
100 10 days after *	11 R	162	13	31.1 ± 1.02	0.3
Control	7 M	29	12	30.4 ± 1.42	
150 5 days prior	6 M	28	12	21.5 ± 1.53	0.002
150 10 days after *	4 M	29	12	35.4 ± 1.88	0.06

* Treatment was started 24 h after injection of strontium-85.

These findings make it unlikely that the effectiveness of tetracycline can be attributed to the chelation of radiostrontium. On the other hand, taking into account the pronounced affinity of tetracycline to bone tissue⁴⁻⁷ (which is thought to be due to the formation of calcium chelates *in situ*⁷), we may tentatively assume that this reaction leads to an impairment of the mechanism(s) responsible for the retention of radiostrontium by bone. As to the mode of action, final conclusions can be drawn only if more detailed investigations on the effectiveness of different tetracyclines, dose dependence, time and duration of treatment, are made available. Such experiments are now under way and will be published elsewhere.

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