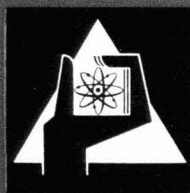# KERNFORSCHUNGSZENTRUM

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Rare Earths and Ruthenium: Metabolism and Removal
from the Mammalian Body

A. Catsch und D. Seidel

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RARE EARTHS AND RUTHENIUM: METABOLISM AND REMOVAL FROM THE MAMMALIAN BODY

A. CATSCH AND D. SEIDL
INSTITUT FÜR STRAHLENBIOLOGIE
KERNFORSCHUNGSZENTRUM, KARLSRUHE
FEDERAL REPUBLIC OF GERMANY

Abstract — Résumé — Аннотация — Resumen

RARE EARTHS AND RUTHENIUM: METABOLISM AND REMOVAL FROM THE MAMMALIAN BODY. The effectiveness of a chelating agent in removing an internally deposited radiometal is essentially determined by: (i) its affinity toward the radiometal as related to the competition of endogenous cations and ligands; (ii) the velocity by which the exchange reaction between therapeutic and endogenous ligands proceeds.

The limitation of the therapeutic effect due to the last mentioned factor is exemplified by experimental data with radioruthenium. Although the chelation of ruthenium by several compounds, particularly the 2:2'-bis [di(carboxymethyl) aminoethylthio] ethane, exceeds markedly the binding affinity of proteins, the mobilized fraction is small because of the extremely sluggish dissociation of the ruthenium-protein complexes.

On the other hand, a very high mobilization of radiocerium is guaranteed by its fast co-ordination reactions, provided that suitable chelators are chosen. Structural factors determining the relative chelate stability are discussed. Ligands with a pronounced polydentate structure, e. g. diethylenetriaminepentaacetic acid and triethylenetetraaminehexaacetic acid, proved to be most effective. Experimental data are presented allowing one to work out the optimal dosage schedule which is the parenteral administration of only a few medium doses, separated by several treatment-free days.

TERRES RARES ET RUTHENIUM: METABOLISME ET ELIMINATION CHEZ LES MAMMIFÈRES. L'efficacité d'un agent de chélation dans l'élimination d'un radiométal déposé à l'intérieur de l'organisme est déterminée essentiellement a) par son affinité pour le radiométal par rapport à celle des cations endogènes et des coordinats; b) par la vitesse à laquelle s'opère la réaction d'échange entre coordinats thérapeutiques et endogènes.

La limitation de l'effet thérapeutique due au deuxième facteur est illustrée par des données expérimentales sur le radioruthénium. Bien que la chélation de Ru par plusieurs composés, notamment le 2:2'-bis [di(carboxyméthyl) aminoéthylthio]-éthane excède notablement l'affinité des protéines, la fraction mobilisée est faible en raison de la dissociation extrêmement faible des complexes Ru-protéines.

D'un autre côté, une mobilisation très élevée du radiocérium est assurée par ses réactions rapides de coordination, à condition de choisir des agents de chélation appropriés. Les auteurs étudient les facteurs structuraux qui déterminent la stabilité relative du produit de chélation. Les coordinats caractérisés par une structure prononcée de polydentate, tels que l'acide diéthylènetriamine penta-acétique et l'acide triéthylènetétraamine hexa-acétique, se sont révélés les plus efficaces. Le mémoire contient des données expérimentales qui permettent d'élaborer le plan de dosage optimum, qui consiste dans l'administration parentérale d'un petit nombre de doses moyennes, avec des intervalles de quelques jours pendant lesquels aucun traitement n'est appliqué.

РЕДКИЕ ЗЕМЛИ И РУТЕНИЙ: МЕТАБОЛИЗМ И УДАЛЕНИЕ ИЗ ОРГАНИЗМА МЛЕКОПИТАЮЩЕГО. Эффективность внутрикомплексных соединений при удалении отложившихся в организме радиоактивных элементов определяется а) прочностью химической связи с радиоактивным металлом в/отношении к конкурирующему средству эндогенных катионов и адденд и б) скоростью, с которой протекает реакция обмена между терапевтическим и эндогенными аддендами.

Ограниченность терапевтического действия благодаря наличию фактора б) демонстрируется с помощью экспериментальных данных с радиорутением. Хотя комплексирующая способность рутения с некоторыми комплексообразователями, в особенности с 2:2 бис [ди(карбоксметил)аминоэтилтио] эта-

ном, значительно превосходит прочность связи протеинов с рутением, "растворимая" фракция мала ввиду исключительно медленной диссоциации комплексов Ru-протеин.

В то же время очень большая растворимость радиоцерия обеспечивается быстротой комплексообразования при условии выбора соответствующих внутрикомплексных соединений. Обсуждаются структурные факторы, определяющие относительную стойкость комплексов. Адденды с известной полидентичной структурой, например, диэтиленовая триаминопентауксусная кислота и триэтиленовая тетрааминогексауксусная кислота являются наиболее эффективными. Приводятся экспериментальные данные, позволяющие выработать оптимальный порядок дозировки, который представляет собой парентеральное введение всего нескольких средних доз через определенные промежутки времени.

TIERRAS RARAS Y RUTENIO: METABOLISMO Y ELIMINACIÓN DEL CUERPO DE LOS MAMÍFEROS. La eficacia con que un agente de quelación elimina un metal radiactivo depositado internamente depende, ante todo, de a) su afinidad por el metal, en relación con la competencia entre los cationes y los ligandos endógenos, b) la velocidad con que se produce la reacción de intercambio entre los ligandos terapéuticos y endógenos.

Ejemplo del límite que impone al efecto terapéutico este último factor son los datos obtenidos experimentalmente con radiorutenio. Aunque el poder de quelación de diversos compuestos, particularmente el 2, 2'-bis [di(carboximetil) aminoetiltio] etano, por el Ru supera considerablemente a la afinidad de las proteínas por dicho metal, la fracción movilizada es pequeña porque los complejos Ru-proteína se disocian muy lentamente.

En cambio, la movilización de radiocerio es muy elevada cuando se eligen agentes de quelación adecuados porque sus reacciones de coordinación se verifican con rapidez. La memoria discute los factores estructurales que determinan la estabilidad relativa de los quelatos. Se encontró que los ligandos que poseen una estructura muy ramificada, por ejemplo, los ácidos dietilentriaminopentaacético y trietilentraaminohexaacético son los más eficaces. Los autores presentan resultados experimentales que permiten determinar la posología óptima, que consiste en administrar parenteralmente algunas dosis medias, con intervalos de varios días.

The use of chelating agents has been proved to be the most promising approach to the problem that mainly concerns this meeting: radioactive metal mobilization. The progress achieved during the last decade in the application of chelating agents is well known [1] and there is no need to emphasize their effectiveness once again. On the other hand, however, it should not be overlooked that there are essential drawbacks and limitations to the therapeutical applicability of chelating compounds. This is particularly true of the often questionable results with delayed treatment.

Primarily, there are three factors which determine the effectiveness of a given chelator:

(i) The velocity of the reactions involving the exchange of the radiometal between endogenous ligands (or other biological acceptors) and the therapeutical chelating agent. If this exchange is sluggish and the velocity is slow, in comparison with the velocity at which the chelate is excreted from the organism, the fraction of the radiometal mobilized from the tissues will be negligible.
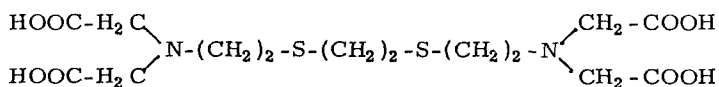
(ii) The affinity of the chelator to the radiometal should be as high as possible in order to achieve the maximum effect. In determining the effectiveness, the competition of endogenous cations (calcium and hydrogen mainly) and of endogenous ligands or ion-exchangers competing with the chelator for the radiometal must be taken into account.

(iii) It frequently turns out to be necessary to administer the chelating compound repeatedly because a single dose does not give a satisfactory mobilization effect. In this case, we may face the limit set up by the toxic side effects of the chelator.

This paper is an attempt to corroborate these statements by experimental findings and also to present some preliminary data bearing on the last-mentioned point; i.e. the working out of a treatment schedule which guarantees a maximum therapeutic effect and simultaneously avoids harmful side-effects.

The practical significance and potential hazard of Ru^{106} is emphasized by numerous investigations concerned with its metabolic behaviour and radiotoxicity [2 - 7] . It is somewhat surprising, therefore, that the possibilities of its removal from the body have not been the subject of experimental studies so far. This may be the result of the rather involved chemistry of ruthenium, as well as of the unavailability of quantitative data dealing with the co-ordination tendencies of ruthenium [8] .

In order to find effective compounds, it was necessary to include into the screening tests as many structurally different compounds as possible: Several polyaminopolycarboxylic acids, polyamines, sulfhydryl-containing compounds, condensed phosphates, as well as agents used in classical analytical chemistry (such as rubeanic acid, phenantroline, dipyridyl) were tested. In order to get the maximum effect, the compounds were injected intravenously and simultaneously with Ru^{106} chloride or nitrosyl nitrate. The detailed results will be discussed in a later publication [9] . Here, it may suffice to mention that the most pronounced reduction of Ru^{106} deposition in the tissues was achieved by 1:2-Bis-[2-di(carboxymethyl)amino-ethylthio] ethane (BATE):

$$\begin{matrix} HOOC-H_2C \\ \\ HOOC-H_2C \end{matrix} > N-(CH_2)_2-S-(CH_2)_2-S-(CH_2)_2-N < \begin{matrix} CH_2-COOH \\ \\ CH_2-COOH \end{matrix}$$

The superiority of BATE over diethylene-triamine-pentaacetic acid (DTPA) and over the analogous compound possessing ether oxygen instead of sulphur as heteroatoms emphasizes the participation of the sulphur atoms in chelating ruthenium.

The influence of BATE on the metabolic behaviour of Ru^{106} may be characterized by Figs. 1 - 5. Whereas $Ru^{106}Cl_3$ (dissolved either in water or in blood serum) is retained by the blood over a relatively long period, the Ru-BATE chelate is cleared from the blood rather quickly (Fig. 1). Fig. 2 compares the retention of the different ruthenium forms by the liver; the approximately tenfold lower deposition of the ruthenium chelate was also obtained with the other organs and tissues. The chloride, as well as the chelate, are excreted mainly in the urine (Fig. 3). Noteworthy is the reduced effectiveness of BATE in preventing the deposition of Ru^{106} administered as nitrosyl nitrate (Fig. 4).

In view of the exceedingly slow blood clearance of Ru^{106} , a fairly high effectiveness for the separate and delayed administration of BATE could be anticipated. It was, therefore, unexpected and highly surprising to note that BATE, even when injected immediately after Ru^{106}, proved to be almost without any effect (Table I). Neither the faecal nor the urinary excretion rates were significantly affected by a six-times-repeated administration of the chelator (Fig. 5) and it may be pointed out that identical negative results were also obtained with other chelating compounds [9] .
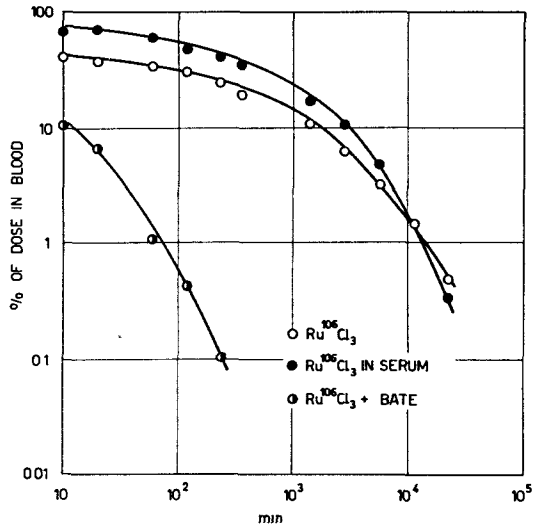
Fig. 1

Clearance of $Ru^{106}Cl_3$ (dissolved in water, serum, or in 10^{-1} molar Na_2-BATE, and injected i. v.) from the blood
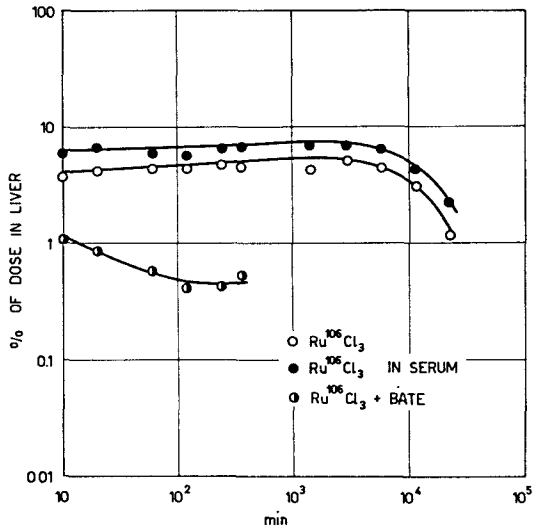
Each point is the mean average of 5 rats

Fig. 2
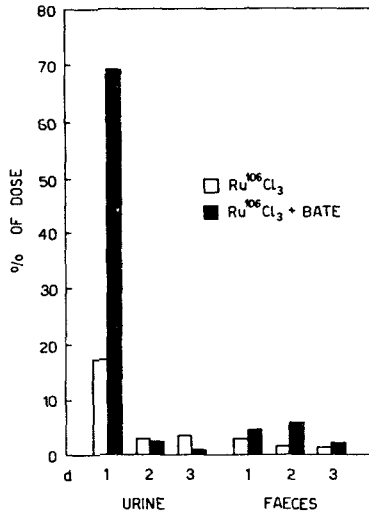
Retention of $Ru^{106}Cl_3$ by the liver

See See Fig. 1

Fig. 3

Excretion of $Ru^{106}Cl_3$ (dissolved in water or 10^{-1} molar Na_2-BATE, and injected i. v.)
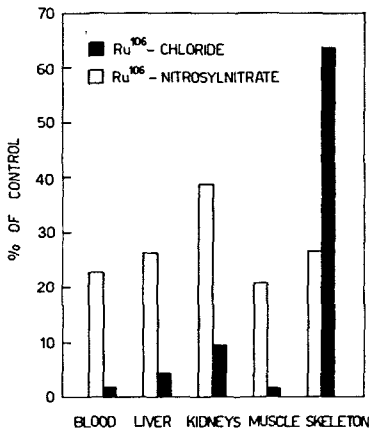Each point is the mean average of 6 rats

Fig. 4

Effect of 50 µM Na_2-BATE (simultaneous i. v. injection) on the
distribution of Ru^{106} - chloride and Ru^{106} - nitrosyl nitrate
5 rats per group

We are able to corroborate this obvious discrepancy by in vitro studies.
It was shown by electrophoretic techniques that the slow clearance of Ru^{106}
from the blood is due to its binding by all protein fractions of the plasma.
It must be assumed that the stability of the protein complexes is lower than
that of the Ru-BATE chelate; this is exemplified by the fact that Ru^{106} , if
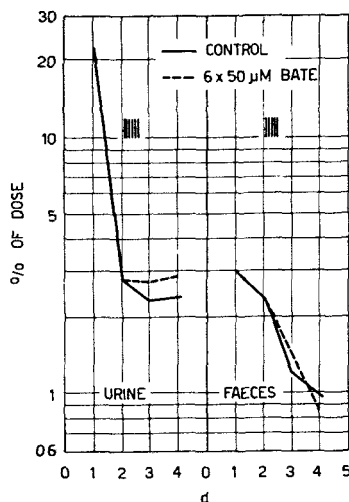added to a BATE solution in serum, migrates almost entirely as the chelate,

Fig. 5

Effects of Na_2-BATE on the excretion of i. v. injected $Ru^{106}Cl_3$
3 rats per group.

whereas the activity of the protein fractions remains negligible. However, if BATE is added to serum already containing Ru^{106}, the fraction with higher activity remains bound by the proteins, Even if the serum is kept for several days before the electrophoretic separation, the amount of Ru^{106} removed by BATE from the proteins increases only slightly. Consequently, we have to conclude that the ruthenium-protein complex represents an "inert co-ordination" compound which is characterized by an extremely slow dissociation. By analogy with the co-ordination chemistry of metal ions of the platinum group, it is likely that the formation of predominantly covalent bindings is the responsible factor.

It is easy to understand why the chelators are almost ineffective when they are given after the administration of Ru^{106}, that is after the formation of ruthenium association compounds with the serum and tissue proteins. Likewise, it explains the reduced chelate effectiveness in mobilizing Ru^{106} - nitrosyl nitrate, in which case a preformed ruthenium complex is involved.

In contrast with ruthenium, the exchange reactions of cerium are relatively fast. It is possible, as will be seen later, to remove significant amounts of radiocerium from the body by suitable chelating agents, even if the treatment is started with marked delay. We have pointed out at the outset that a necessary prerequisite for a high effectiveness of a chelator is its relative chelate stability, i.e. its stability relative to the competition of endogenous cations, especially calcium. The cerium(III) ion has a co-ordination requirement of eight, whereas calcium (II) possesses a co-ordination number of only six. Consequently, the stability constant of a cerium chelate is influenced by the number of electron-donor atoms of the ligand to a higher extent than that of the calcium chelates. Likewise, the biological effectiveness of chelators increases with increasing number of ligand atoms (Fig. 6). These data show, however, that the "polydentate principle" cannot

TABLE I

EFFECT OF BATE ON RETENTION OF $^{106}RuCl_3$ BY THE ORGANS OF THE RAT

Mean average ± S.E.

No. 1 and 2: 5 animals per group, sacrificed on day 2.

No. 3: 10 animals per group, sacrificed on day 8.

BATE was administered in 6 fractions (every 30 min).

| No.	μM BATE	time of injection	% of Ru^{106} dose				
			blood	muscles	liver	kidney	skeleton
1	-	-	7.10 ± 0.44	16.3 ± 2.08	5.38 ± 0.10	3.57 ± 0.08	6.03 ± 0.13
	50	simultaneously i.v.	0.058 ± 0.005	0.13 ± 0.03	0.35 ± 0.02	0.34 ± 0.02	3.82 ± 0.07
2	-	-	7.99 ± 0.46	18.6 ± 1.51	7.15 ± 0.29	4.24 ± 0.13	6.62 ± 0.17
	100	1 min i.p.	6.50 ± 0.79	12.6 ± 1.43	6.50 ± 0.41	3.77 ± 0.21	6.15 ± 0.18
3	-	-	1.50 ± 0.15	14.6 ± 0.62	3.46 ± 0.22	3.44 ± 0.06	3.77 ± 0.19
	300	10 - 160 min i.p.	1.06 ± 0.05	13.3 ± 0.87	2.87 ± 0.13	2.87 ± 0.05	3.94 ± 0.07
	300	72 - 75 h i.p.	1.46 ± 0.07	13.6 ± 1.00	3.30 ± 0.09	3.40 ± 0.06	3.80 ± 0.19
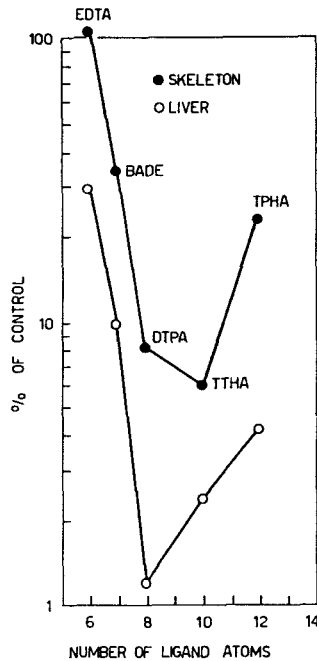
Fig. 6

Effect of several polyaminopolycarboxylic acids (injected i. p. after 2 min)
on the retention of i. v. injected $Ce^{144}Cl_3$ by the organs of the rat [15]

be applied unlimitedly. The ten-dentate triethylene-tetraamine-hexaacetic
acid (TTHA), it is true, gives rise to a higher reduction of the skeletal
deposition of Ce^{144} than the eight-dentate DTPA, not to speak of the six-
dentate ethylene-diamine-tetraacetic acid (EDTA). The retention of Ce^{144}
by the liver, however, is affected by TTHA to a lesser degree. The next
higher homologue, the twelve-dentate tetraethylene-pentaamine-heptaacetic
acid (TPHA), proves to be inferior to DTPA with regard to all organs. It
seems likely that this loss in effectiveness is caused by the formation of
less stable bimetallic and possibly polymerizing chelate species. It may
be mentioned in this context that the order of effectiveness of the different
chelating agents quoted in Fig. 6 also holds for other metal ions with higher
co-ordination requirements, such as yttrium, thorium, plutonium, and
americium [1] .

Let us now turn to the dependence of chelate effectiveness on several
essential parameters. Since the enteral absorption of DTPA and related
compounds is relatively low, a parenteral route of application is usually
preferred. However, considering the relatively high effectiveness of even
small doses of DTPA [1] , as well as the practical advantage offered by
oral administration, the possibility of using this route should not be neglected
entirely. As a matter of fact, numerous investigations [10 - 12] have demon-
strated unequivocally a quite satisfactory mobilization of different radio-
metals by oral DTPA. In addition, one gains the impression from some of

these studies that the effectiveness of oral DTPA might be higher than expected from the experimentally determined value for the absorption from the intestinal tract. Properly considered, a higher effect might be expected if it is tentatively assumed that orally administered chelator absorbed from the gut reaches higher concentrations in the liver than it would following intravenous injection. In order to elucidate this point, we compared the effectiveness of a dose of 50 μM DTPA applied intraperitoneally with that of 1000 μM administered orally. The chosen ratio of doses is based on the fact that the enteral absorption of DTPA is approximately 5% [13] . As can be seen from Table II, both application routes give an almost identical effect; the slight apparent superiority of oral DTPA does not reach statistical significance.

TABLE II

EFFECT OF ORAL CHELATE DOSES ON THE RETENTION OF
$Ce^{144}Cl_3$ BY THE ORGANS OF THE RAT

Mean average ± S.E.

No.1: 7 animals per group, sacrificed on day 6. Chelates were administered on the 3rd day after the intravenous injection of Ce^{144} .

No.2: 10 animals per group, sacrificed on day 4. Chelates were administered 24 h after the intravenous injection of Ce^{144}.

No.	Treatment	% of Ce^{144} dose		
		Liver	Kidneys	Skeleton
1	Control	35.5 ± 1.39	0.93 ± 0.05	23.5 ± 0.69
	1 mM DTPA p.o.	21.1 ± 1.84	0.73 ± 0.06	17.3 ± 0.91
	0.05 mM DTPA i.p.	23.3 ± 1.39	0.82 ± 0.05	19.7 ± 0.70
2	Control	32.5 ± 0.76	1.61 ± 0.11	26.2 ± 0.76
	0.25 mM Ca-DTPA p.o.	21.8 ± 0.69	1.44 ± 0.14	22.7 ± 0.91
	" Ca-TTHA "	25.2 ± 1.07	1.38 ± 0.09	21.9 ± 0.50
	" Ca_2-TTHA "	23.8 ± 2.58	1.19 ± 0.04	20.9 ± 0.51

Recently it was claimed by BALLOU [10] that the superiority of TTHA over DTPA in removing internally deposited plutonium becomes still more pronounced if the chelators are given by the oral route. In a similar experiment with Ce^{144} , however, we were not able to confirm this observation. As can be seen from the data presented in Table II, a higher amount of Ce^{144} is removed by DTPA. This holds for the 1:1-Ca-TTHA chelate as well as for a molar ratio of 2 Ca : 1 TTHA. Both chelate species differ in the number of their negative charges which in turn could be reflected by different enteral absorption rates.

As to the final conclusions about the feasibility of oral treatment, it should not be overlooked that fairly high doses of DTPA (such as those used in our experiments) result in severe diarrhoea and certain mortality.

It was mentioned previously that the effectiveness of a given chelator depends essentially on the stability of the binding of the radiometal by endogenous constituents. Since these stabilities are reflected by the "biological half-time", it follows that the extent of radiometal mobilization by a chelator should be inversely proportional to the biological half-time and in the event of a multi-exponential retention function, the mobilization should decrease progressively with time.

Initially about 40 to 50% of Ce^{144} is retained by the liver, the larger fraction being excreted fairly quickly with a half-time of a few days [14] . A clear diminution of the excretion rate becomes manifest only after a lapse of several weeks. In keeping with this behaviour, the effectiveness of a single DTPA dose remains constant over a period of 3 - 4 weeks and the relative amount of Ce^{144} removed decreases only when the departure from exponential linearity, referred to above, becomes manifest. The higher effectiveness noted with very early administration of DTPA is because shortly after injection the distribution of Ce^{144} has not yet reached equilibrium and an essential and easily removed fraction is still in the extracellular space. In the case of the kidneys (retaining approximately 2% of the Ce^{144} dose) a higher fraction, comprising about 50%, is bound rather tightly and retained with a half-time of at least 200 d. Correspondingly, the dependence of DTPA effectiveness on the time of its administration is much more pronounced than in the liver. The same is true for the skeleton; this is in keeping with the fact that the amount of Ce^{144} retained by the bones (20 - 30%) remains constant over a longer period of time.

If these time dependence data are considered, one might expect that with prolonged treatment with or without an increase of chelate dose, a point will be reached where the organs or organ compartments containing the easily removable radiometal fractions are depleted and that a further increase of the total chelate dosage would not give rise to a correspondingly higher mobilization effect. In order to test and verify these assumptions, rats were injected with Ce^{144} and received three doses of DTPA which varied by a factor of 100; the treatment was started either on the 7th or on the 31st day. As can be seen from Table III and Fig. 7 the liver shows only a slight departure from linearity at the highest dose, indicating that the postulated "saturation" level of effectiveness is not yet reached. As to the removal of Ce^{144} from the skeleton, there is clear linear regression of effectiveness with dose for early treatment, whereas in the delayed treatment series the effect remains constant over a relatively wide range of dosage. Thus, we gain the impression that at this stage already 80% of the skeletal Ce^{144} is withdrawn from equilibrium and no longer accessible to the chelating agent. A similar indication is given by the fact that the superiority of TTHA over DTPA observed with early treatment (Fig. 6) is completely nullified when both compounds are given after some delay [15] .

A somewhat unexpected behaviour is to be seen with regard to the kidneys (Fig. 7 and Table III), namely, the decrease of effectiveness of DTPA at higher dose levels. The same situation was also encountered in another experimental series (No. 3 in Table III and Fig. 8), in which the size of a single dose of DTPA (amounting to 200 μM per animal) was kept constant, whereas the number of doses were varied from 1 to 6. DTPA was given

TABLE III

EFFECT OF DIFFERENT DTPA TREATMENT SCHEDULES ON THE
RETENTION OF INTRAVENOUS INJECTED $Ce^{144}Cl_3$ BY THE ORGANS
OF THE RAT

Mean average ± S.E.
No. 1: 16 animals per group, sacrificed on day 19.
No. 2: 8 animals per group, sacrificed on day 42.
No. 3: 6 animals per group, sacrificed on day 42. Na_3Ca-DTPA was administered in all series intraperitoneally.

No	µM DTPA per dose	on day	% of Ce¹⁴⁴ dose		
			liver	kidneys	skeleton
1	0	-	8.11 ± 0.61	0.47 ± 0.024	21.6 ± 0.46
	5		6.04 ± 0.42	0.39 ± 0.018	19.6 ± 0.40
	16	7, 11, 15	4.52 ± 0.19	0.34 ± 0.017	18.6 ± 0.41
	50		3.25 ± 0.22	0.29 ± 0.014	17.3 ± 0.37
	160		1.82 ± 0.11	0.30 ± 0.016	16.4 ± 0.34
	500		1.75 ± 0.20	0.37 ± 0.016	14.4 ± 0.39
2	0	-	1.70 ± 0.19	0.30 ± 0.015	21.9 ± 0.87
	5		1.63 ± 0.28	0.31 ± 0.024	19.6 ± 0.47
	16	31, 35, 39	1.54 ± 0.26	0.27 ± 0.021	19.4 ± 0.54
	50		1.56 ± 0.17	0.24 ± 0.021	17.7 ± 0.49
	160		0.87 ± 0.046	0.23 ± 0.015	17.7 ± 0.79
	500		0.70 ± 0.064	0.24 ± 0.026	17.4 ± 0.29
3	0	-	see experiment No. 2		
		20	1.46 ± 0.12	0.17 ± 0.009	19.3 ± 0.75
		20, 24	1.11 ± 0.070	0.19 ± 0.018	19.3 ± 0.99
	200	20, 24, 28	0.80 ± 0.034	0.21 ± 0.017	17.0 ± 0.24
		20, 24, 28, 31	0.83 ± 0.058	0.16 ± 0.008	15.7 ± 0.51
		20, 24, 28, 31, 35	0.57 ± 0.079	0.24 ± 0.028	17.7 ± 0.59
		20, 24, 28, 31, 35, 38	0.45 ± 0.058	0.22 ± 0.029	16.9 ± 0.82

on every 4th day and the animals of all experimental groups were sacrificed on the 42nd day after administration of Ce^{144} . In spite of the marked scattering of the experimental points, there is a clear and significant decrease of kidney effectiveness with increasing number of DTPA doses. Furthermore, it should be emphasized that in the case of high total doses the variability within any one group is significantly higher than for low dose levels or other organs. If the well-known nephrotoxicity of the polyamino-polycarboxylic acids is taken into account, a plausible tentative assumption might be that the loss in effectiveness noted at high dose levels is caused by an impairment of the renal excretion functions. As for the dose-dependence of the effectiveness in liver and skeleton, the results summarized in Table III and Fig. 8 agree in essentials with the previous experiment presented in Fig. 7.

    In all the above experiments, the interval between individual DTPA doses was three days. In order to elucidate the question whether and to what
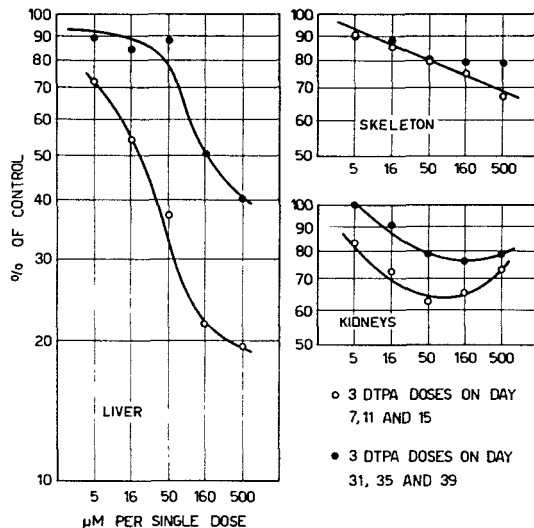
Fig. 7

Dependence of DTPA effectiveness on dose

Experimental series No. 1 and 2, Table III; calculated after logarithmical transformation

extent the effectiveness of the chelator is affected by the length of the treatment-free interval, the rats were given three doses of 200 μM.DTPA, either daily or on every 4th day and the treatment was started on the 3rd or on the 40th day after injection of C^{144}. As can be seen from the data of Fig. 9, the effectiveness of both treatment schedules was identical with regard to kidneys and skeleton. In the case of the liver, however, the narrowly spaced multiple doses proved to be less effective; the differences are statistically significant. This finding agrees with and is explained by the observation of several investigators [14, 16 - 19] that the action of highly effective chelators such as DTPA is sustained over several days and is mainly confined to the mobilization of radiometals from the liver. It may be deduced that a protracted administration of a given chelator affords a better "utilization" of the effect of each single dose, at least if we are dealing with "liver-seeking" radionuclides.

In addition, a protracted treatment schedule might prove to be favourable from another point of view, namely, in avoiding toxic and harmful side effects of the chelator. Our present knowledge of the toxicity of chelating agents belonging to the group of synthetic polyamino-polycarboxylic acids may briefly be outlined as follows. It has been learned with experimental animals, as well as by clinical experience, that repeated administration of sufficiently high chelate doses can give rise to severe (although in principle reversible) damage to renal functions and morphology, i. e. nephrotic changes [13, 20] . Whereas in the case of EDTA, a compound which in the last decade has found wide experimental and clinical use, the order of magnitude of the toxic dosage may be regarded as adequately established, the quantitative aspects of DTPA toxicity are still rather vague. The acute toxicity of EDTA
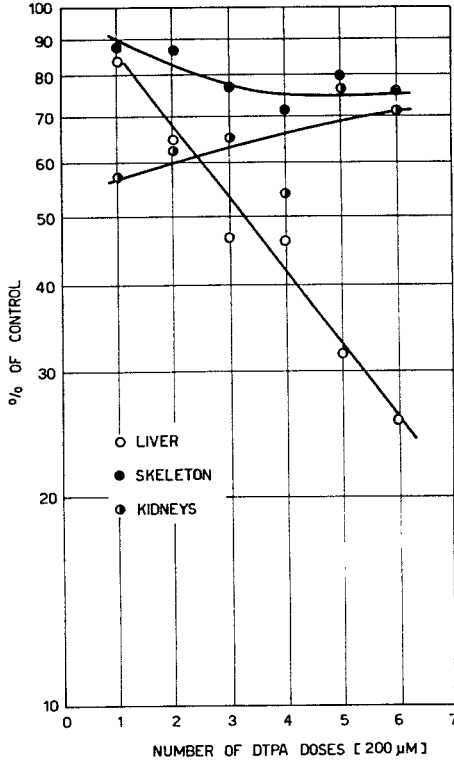
Fig. 8

Dependence of DTPA effectiveness on number of doses
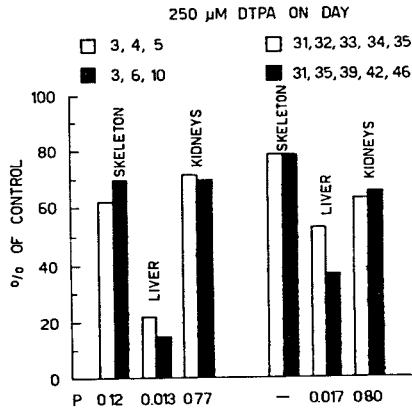Experimental series No. 3, Table III; calculated after logarithmical transformation

Fig. 9

Removal of i. v. injected $Ce^{144}Cl_3$ by DTPA from the organs
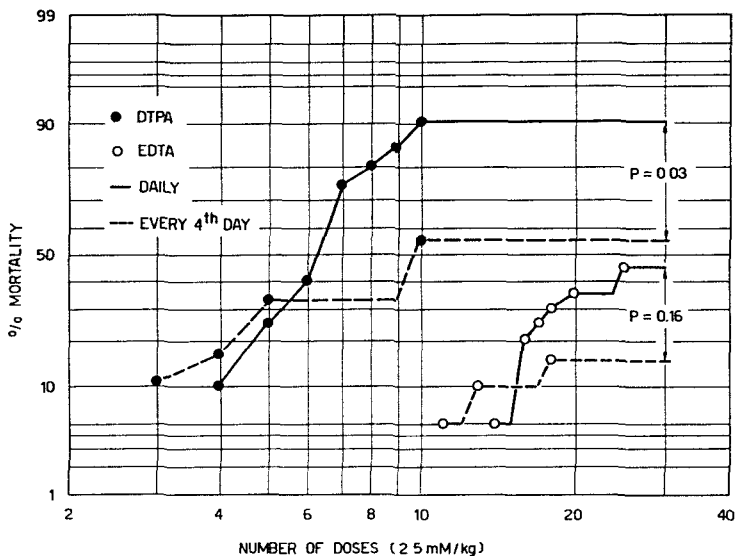10 rats per group

Fig. 10

Toxicity of Na₂Ca-EDTA and Na₃Ca-DTPA (i. p. injection) in rats
20 animals per group

and DTPA administered as a single dose is almost identical [11] , but the same is not necessarily true of the toxicity and long-term effects of multiple chelate doses. We have recently begun a detailed study aimed at elucidating this question, but the experiments are still far from completion at the present and only preliminary data are available. Fig. 10 shows time-mortality curves for rats which received a total of 30 chelate doses intraperitoneally, each of them amounting to 2.5 mM Na₂Ca-EDTA, or Na₃Ca-DTPA per kg. The injections were given either daily or on every 4th day. The comparison of the two daily-treatment series shows that, on a molar base, DTPA is approximately 3 to 5 times more toxic than EDTA. In this context it may be mentioned that FOREMAN et al. [21] observed nephrotic lesions with relatively low doses of DTPA (ineffective in the case of EDTA). The second important result of this experiment (Fig. 10) is that the protracted treatment schedule proves to be less harmful. Because of the limited number of animals, however, statistical significance is reached only for the DTPA groups. The question of why the time-effect curves appear to be truncated, even when the time co-ordinate is logarithmically transformed, is still unanswered. In other words, it is not yet fully understood why a certain proportion of animals appear to be completely unaffected by relatively high total dosage

In summarizing our experimental data and considerations, we may draw the following conclusions:

(i) Ru¹⁰⁶ is chelated by different compounds, in particular by BATE, rather efficiently. Nevertheless, the practical value of all chelating agents is minimal, since exchange reactions of ruthenium between exogenous and endogenous ligands proceed too slowly by orders of magnitude to guarantee a sizable mobilization effect.

(ii) A marked removal of internally deposited Ce^{144} can be achieved by appropriate chelating agents, even when treatment is started with delay. At the present stage of our knowledge, DTPA and TTHA should be the compounds chosen for therapeutical purposes.

(iii) Experimental data are presented on the toxicity of DTPA and the dependence of its effectiveness on dose, time and mode of administration. They permit recommendation of a treatment schedule which guarantees a maximum therapeutical effect and simultaneously avoids harmful side-effects of the chelator. According to this schedule the treatment should be started as early as possible, the number of individual doses should be relatively limited and the size of the individual doses should lie in the moderate range, since an unlimited increase of the total dosage is not followed by a correspondingly higher effect. In contrast to the prevailing practice of daily or even twice-daily administrations of the chelating agents, a more effective and less harmful schedule is to be preferred, the essence of which is that each individual chelate dose is followed by several treatment-free days.

(iv) Although these conclusions and recommendations are in detail only valid for radiocerium and other rare earths, preliminary data on other radiometals (such as plutonium or americium) indicate that, at least in principle and with only minor corrections, they hold for other radiometals as well.

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