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Evaluation of the efficacy of different metal chelates of DTPA in removing internally-deposited radionuclides

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The effectiveness of Co(II)-DTPA and Zn(II)-DTPA in preventing the retention of radiocerium and radioyttrium by the organs of the rat was studied and found to be insignificantly diminished as compared with Ca-DTPA. The therapeutic index of both chelates, on the other hand, is greater than that of Ca-DTPA because of their low toxicity. The possible therapeutic use of these chelates is discussed.

1. INTRODUCTION

The calcium-chelate of diethylenetriaminepentaacetic acid (DTPA) has proven to be a valuable antidote in poisoning with radioactive or stable metals (for detailed bibliography see Catsch 1964a). The toxicity of Ca-DTPA is markedly higher than that of Co(II)- and Zn(II)-DTPA (Catsch 1964b). This is in keeping with earlier observations with ethylenediaminetetraacetic acid (Steffensen 1957, Sullivan 1960). It may be assumed tentatively that the reduced toxicity of the more stable chelates, i.e. Co(II)- and Zn(II)-DTPA, is due to the fact that the chelated exogenous metals are exchanged, if at all, to a lesser extent against essential endogenous metal ions than it is the case with Ca-DTPA.

Provided that Co(II)- and Zn(II)-DTPA do not suffer a marked loss in the therapeutic efficacy, i.e. of their ability in mobilizing toxic metals from the body, their therapeutic index (defined as the ratio of toxic to therapeutic doses) should surpass that of Ca-DTPA. The resulting practical implications are obvious. The present study is concerned with the comparison of the DTPA-chelates referred to above in lowering the retention of radiocerium and radioyttrium by the organs of the rat.

2. MATERIALS AND METHODS

The animals used were male rats from the Heiligenberg-strain, 7 to 15 weeks old. Carrier-free ^{144}Ce(III) or ^{91}Y(III) was injected intravenously as chloride (3 to 5 μc per animal). The following chelates were used: (i) Na_3Ca-DTPA, (ii) Na_3Co-DTPA (excess of free DTPA $\leqslant 0{\cdot}5$ per cent), and (iii) Na_3Zn-DTPA (excess of free DTPA $\leqslant 0{\cdot}2$ per cent). Different dosages of these chelates were administered either intravenously and simultaneously with ^{144}Ce or intraperitoneally on the 2nd, 5th and 8th day following the injection of ^{144}Ce and ^{91}Y, respectively. In the former experiment, the animals were sacrificed 48 hours after the administration of ^{144}Ce, in the latter series—on the 12th day.

The activity of the ashed organs was assayed by means of an end-window counter. The radionuclide content of the skeleton was assumed to be 20 times that of a femur. In the experiments where Co(II)- and Zn(II)-DTPA labelled by ^{60}Co and ^{65}Zn, respectively, were used, the retention of the radionuclide was determined by *in vivo* whole-body counting; a plastic scintillator (NE 102; 7 in. diameter, 5 in. deep) was used.

3. Results

The maximal effect of the metal chelates is obtained with simultaneous administration. The results of this series are shown in figures 1 to 3, the ^{144}Ce-content of the organs being expressed as percentage of the average of 20 untreated animals. In the control group, liver, skeleton and kidneys retained

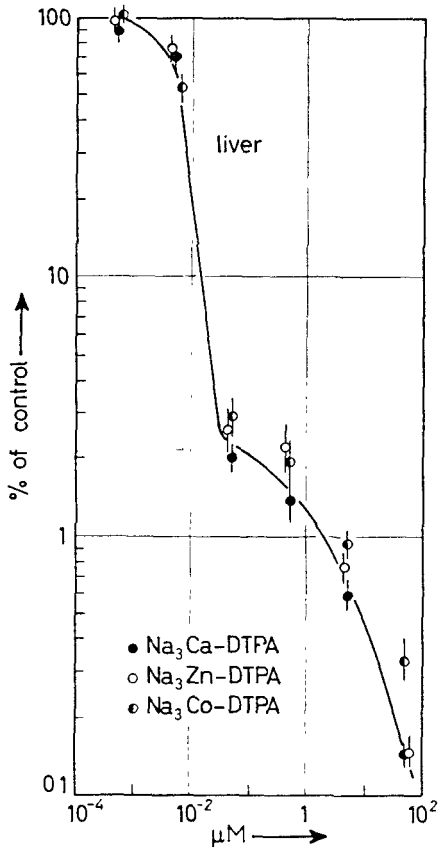

Figure 1. Retention of ^{144}Ce by the liver as influenced by different doses (μM per animal) of DTPA-chelates. DTPA was administered simultaneously with ^{144}Ce. Each point is the average of 6 to 10 rats ; the vertical bars indicate fiducial limits ($P = 0{\cdot}05$).

35·8, 33·7 and 2·32 per cent, respectively, of the administered ^{144}Ce-amount. In no case was a linear dependence of effectiveness upon dosage obtained. On the other hand, the shape of the dose-effect-curves is obviously identical for all three chelates. There is no significant difference in the efficacy of Ca- and Zn(II)-DTPA while the effectiveness of Co(II)-DTPA is apparently reduced as compared with Ca-DTPA.

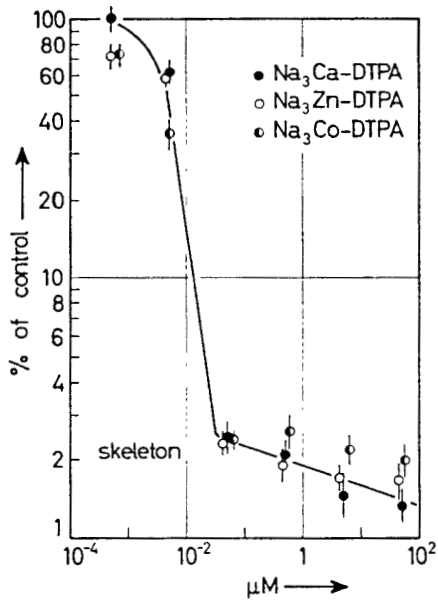

Figure 2. Retention of ^{144}Ce by the skeleton as influenced by different doses (µM per animal) of DTPA-chelates. DTPA was administered simultaneoulsy with ^{144}Ce. Each point is the average of 6 to 10 rats ; the vertical bars indicate fiducial limits ($P=0{\cdot}05$).

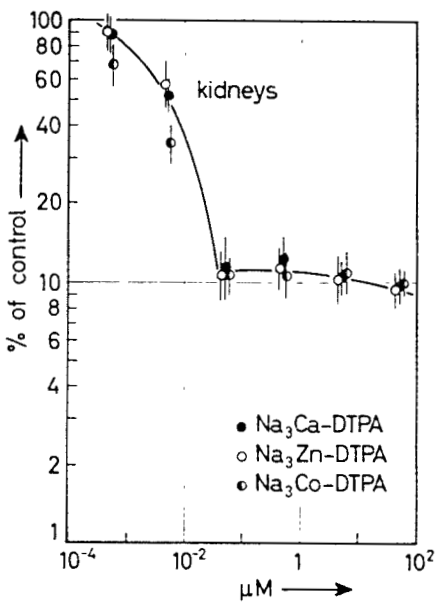

Figure 3. Retention of ^{144}Ce by the kidneys as influenced by different doses (µM per animal) of DTPA-chelates. DTPA was administered simultaneously with ^{144}Ce. Each point is the average of 6 to 10 rats ; the vertical bars indicate fiducial limits ($P=0{\cdot}05$).

In the experimental series where the chelates were given with a delay and the activity of the organs was assayed on the 12th day, the liver in the control group (23 rats) retained 15·0 per cent of the ^{144}Ce-dose, the kidneys—0·61 per cent. Although it is known that there is no elimination of ^{144}Ce from the bones, the skeletal content in the control group (18·5 per cent) was lower than in the preceding experiment. This is due to the different age of the animals in both experiments, a factor which exerts a significant influence on the skeletal retention (Schmautz 1964). In the ^{91}Y-control group (27 animals), liver, skeleton and kidneys retained 0·95, 64·8 and 1·32 per cent respectively.

In all cases, parallel dose-effect curves were obtained so that (after omission of the lowest dosages in the ^{144}Ce-series which deviate distinctly from a linear regression) the relative potency of Ca-DTPA could be calculated (cf. Finney 1952). The relative potency ρ indicates the factor by which the effectiveness of Ca-DTPA is higher than that of Co(II)- and Zn(II)-DTPA. Dividing the actual dosages of the last mentioned chelates by the ρ-values listed in the table, all experimental points can be satisfactorily fitted by the common dose-effect curves presented in figures 4 and 5.

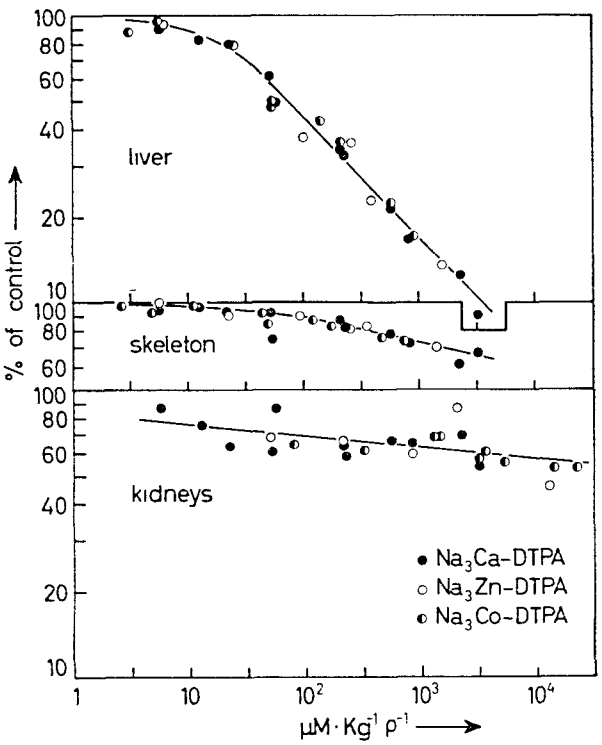

Figure 4. Retention of ^{144}Ce by the organs as influenced by different doses of DTPA-chelates. DTPA was administered on the 2nd, 5th and 8th day. The actual doses of Zn(II)- and Co(II)-DTPA are divided by the ρ-values listed in the table. Each point is the average of 10 rats.

The retention of ^{60}Co and ^{65}Zn after a single or repeated administration of labelled Co(II)- and Zn(II)-DTPA, respectively, is shown in figures 6 and 7. Using the data obtained in the single exposure study, the expected retention of

multiple doses was calculated for the 11th day. Whereas the predicted build-up of ^{60}Co (0·3 per cent) agrees satisfactorily with the actually observed 0·22 per cent, the retention of multiple ^{65}Zn-doses(2 per cent) is lower than the expected (5 per cent).

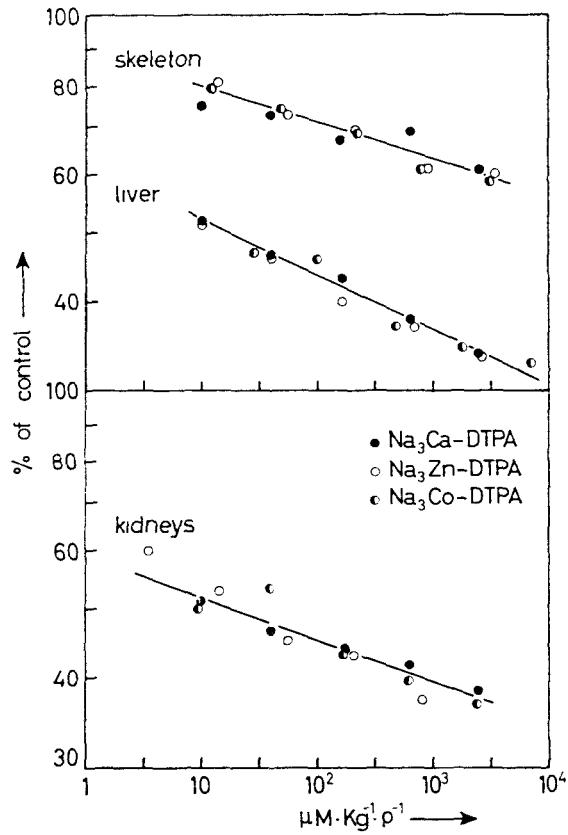

Figure 5. Retention of ^{91}Y by the organs as influenced by different doses of DTPA-chelates. DTPA was administered on the 2nd, 5th and 8th day. The actual doses of Zn(II)- and Co(II)-DTPA are divided by the ρ-values listed in the table. Each point is the average of 10 rats.

Radionuclide	Chelate	Liver	Skeleton	Kidneys
^{144}Ce	Zn(II)-DTPA	2·04 (1·40 – 2·96)	2·28 (1·20 – 4·37)	0·24 (0·03 – 2·81)
	Co(II)-DTPA	4·10 (2·98 – 5·00)	4·65 (2·41 – 8·98)	0·15 (0·02 – 1·59)
^{91}Y	Zn(II)-DTPA	1·20 (0·42 – 3·42)	0·87 (0·44 – 1·72)	3·45 (0·87 – 13·7)
	Co(II)-DTPA	0·44 (0·16 – 1·23)	1·00 (0·49 – 2·04)	1·10 (0·31 – 5·29)

Potency (ρ) of Ca-DTPA relative to Co(II)-DTPA and Zn(II)-DPTA. The figures in brackets indicate the fiducial limits ($P = 0·05$). For further explanation see text.

4. Discussion

Extending the semiquantitative treatment by Heller and Catsch (1959), the effectiveness of any metal-chelate ML in removing a carrier-free radiometal $^*M$ from the body, in a first approximation, should be proportionately related to:

$$\frac{K^{*M}_{ML} \cdot (L)_{\text{total}}}{\alpha + K^{\text{Ca}}_{\text{Ca}L} \cdot (\text{Ca}) + K^{M}_{ML} \cdot (M)}.$$

K are the stability constants of the corresponding 1 : 1-chelates, the brackets indicate concentrations, and the influence of pH is taken into account by the distribution coefficient α (Heller and Catsch 1959). It can be reasonably assumed that (Ca), (Co) and (Zn) in the mammalian organism are virtually invariable. The log-values of the stability constant of Co(II)- and Zn(II)-DTPA are 19·27 and 18·55, respectively, whereas $K^{\text{Ca}}_{\text{Ca}L}$ equals 10·89 (Anderegg, Nägeli, Müller and Schwarzenbach 1959). Since the effectiveness of all three chelates lies within the same order of magnitude, it may be concluded that $K^{\text{Co}}_{\text{Co}L} \cdot (\text{Co})$ as well as $K^{\text{Zn}}_{\text{Zn}L} \cdot (\text{Zn})$ do not differ significantly from $K^{\text{Ca}}_{\text{Ca}L} \cdot (\text{Ca})$. This implies necessarily that (Co) and (Zn) are negligibly small, even if $(\text{Co})_{\text{total}}$ and $(\text{Zn})_{\text{total}}$ are raised by the administration of the corresponding DTPA-chelate.

Depending on the organ taken as basis for the calculations, on the kind of radionuclide, and, finally, on the time of DTPA-treatment, the determination of the relative potency of Ca-DTPA yields inconsistent figures (see table and figures 1 to 3). The existent discrepancies cannot be easily explained, if they turn out to be real, and additional assumptions would be needed. From the practical view-point and taking into account the rather broad fiducial range of the relative potencies listed in the table, the apparent differences should by no means be overestimated, and it can be stated that there are, if at all, only minor differences in the efficacy of the three chelates.

For technical reasons, i.e. the limits set up by the solubility of the chelates, our earlier studies (Catsch 1964b) did not succeed in exact evaluation of the toxic dosages of Co(II)- and Zn(II)-DTPA. It could be stated only that in chronic toxicity tests Zn(II)-DTPA is at least ten times, and Co(II)-DTPA about four times less toxic than Ca-DTPA. If a ρ-value of 2 for Ca-DTPA/Zn(II)-DTPA (after averaging the figures of the table) is assumed, it follows that the therapeutic index of Zn(II)-DTPA is at least five times higher than that of Ca-DTPA. With Co(II)-DTPA, the increase of the therapeutic index is less pronounced.

So far, no data are available for the mobilization by Zn(II)- and Co(II)-DTPA of other practically important metal ions, such as Pb, Fe or ^{239}Pu. It is fairly unlikely, however, that relative potencies would be obtained fundamentally different from ^{144}Ce and ^{91}Y.

The non-linear relationship between dosage and effect which was demonstrated with simultaneous administration of the chelators (see figures 1 to 3) suggests a 'compartmentalization' of the given organ, i.e. the organ has to be conceived as composed of several compartments, each of which is characterized by different affinities toward the radiometal and, therefore, by differing response to the chelator (Catsch 1961, 1964a). Delayed administration of the chelator (see figures 4 and 5) is followed by a pronounced loss in effectiveness as well as

by a marked change of the shape of the dose-effect curves. Obviously, the bulk of the radiometal becomes transferred from the loosely bound state into the compartment(s) with a more stable binding. It is not at all unexpected that different radiometals, such as radiocerium and radioyttrium, behave in this

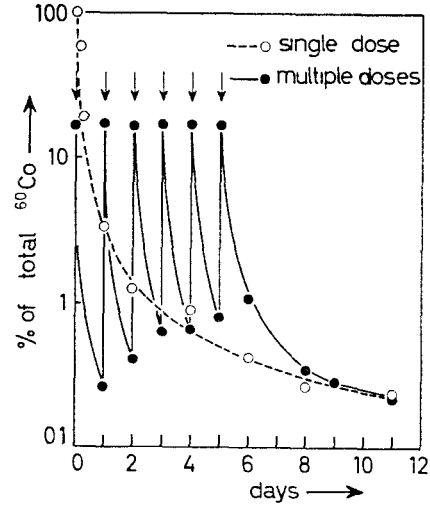

Figure 6. Whole-body retention of ^{60}Co administered intraperitoneally as DTPA-chelate. In the case of multiple doses, the percentage refers to the total dose. Each point is the average of 3 rats.

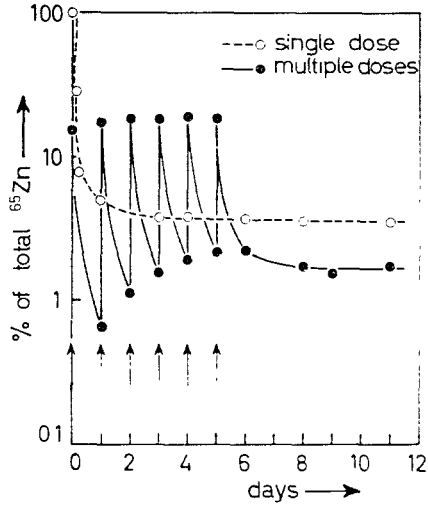

Figure 7. Whole-body retention of ^{65}Zn administered intraperitoneally as DTPA-chelate. In the case of multiple doses, the percentage refers to the total dose. Each point is the average of 3 rats.

respect quite individually. The relatively easy mobilization of ^{144}Ce from the liver is in sharp contrast to other tissues as well as to the response of ^{91}Y. Pertinent to the following considerations is the relatively small slope of the

dose-effect curves. This is in keeping with analogous findings of Catsch and Seidel (1963), Norwood (1962), Rosoff, Ritter, Sullivan, Hart and Spencer-Laszlo (1961), Schubert, Fried, Rosenthal and Lindenbaum (1961), Taylor and Sowby (1962).

The greater therapeutic index of Co(II)- and, in particular, of Zn(II)-DTPA makes a higher dosage than with Ca-DTPA feasible. However, if the small slope of the dose-effect curves is taken into account, the actual gain in therapeutic effectiveness will remain insignificant. Therefore, the sole, though essential, advantage offered by the less toxic chelates is the gain of an increased safety. Taking into account that the toxicity of Zn(II)-DTPA, for instance, is at least ten times, but the effectiveness merely two times lower than that of Ca-DTPA, the therapeutic index is increased by a factor of $> 10/2 = > 5$.

For the related ethylenediaminetetraacetic acid a dosage of 0·12 mM per kilogram and per day is generally recommended for humans (e.g. Foreman, Finnegan and Lushbaugh 1956, Seven 1960). Because of the two times higher toxicity of DTPA (Catsch 1964b), the dosage should be reduced to 0·06 mM Ca-DTPA per kilogram and per day. With Co(II)- and Zn(II)-DTPA (allowing for their possibly slightly reduced efficacy) a dosage of 0·15 mM per kilogram and per day may tentatively be considered as appropriate.

Before an unrestricted usage of both chelates in humans can be recommended, however, a crucial point remains to be settled. Although the repeated administration of relatively large doses of Zn(II)- and Co(II)-DTPA was not followed by lethality and nephrotic damage, there still remains the possibility that other and possibly relevant side-effects escaped observation. Referring to the results presented in figures 6 and 7, approximately 4 per cent of Zn and 0·7 per cent of Co (both administered as chelates) are retained by the body. If the assumption is made that these percentages do not depend on the size of the dose, each single dose would give rise to the splitting-off of approximately 0·3 mg Zn per kilogram and 0·05 mg Co per kilogram†. We are not aware of any pertinent investigations which prove unequivocally that the repeated (though restricted to a relatively short time-period) administration of doses of this order of magnitude leads to any harmful side effects. Nevertheless, experiments aiming at the elucidation of this question and giving direct evidence are urgently needed.

Acknowledgments

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On a étudié l'éfficacité du Co(II)-DTPA et du Zn(II)-DTPA à empêcher la rétention du radiocérium et du radioyttrium par les organes du rat. On l'a trouvée seulement très peu inférieure à celle du Ca-DTPA. L'index thérapeutique de ces deux chélates est, d'autre part, beaucoup plus élevé que celui du Ca-DTPA, en raison de leur faible toxicité. On va envisager les conséquences théoriques et pratiques de ces résultats.

† Recent investigations, as yet uncompleted, indicate that the apparent 'build-up' of ^{60}Co and ^{65}Zn observed in figures 6 and 7 is caused not by a genuine deposition, but mostly by an ordinary *exchange* between the administered and the corresponding endogenous metal ions.

Der Einfluß von Co(II)-DTPA und Zn(II)-DTPA auf die Verteilung von Radiocer und Radioyttrium im Organismus der Ratte wird untersucht. Die Retentionsverhinderung beider Chelate ist nur unwesentlich kleiner als die von Ca-DTPA. Dagegen ist der therapeutische Index beider Chelate weger ihrer geringen Toxicität wesentlich höher als bei Ca-DTPA. Die aus den Resultaten sich ergebenden theoretischen und praktischen Fragen werden diskutiert.

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