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Excretion of ^{65}Zn-DTPA in the rat

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The doubly labelled ^{65}Zn–DTPA.^{14}C was injected into rats and the urine assayed for Zn, ^{65}Zn, and ^{14}C. A simple quantitative method is proposed which allows the metabolic fate of ^{65}Zn to be derived. It has been shown that the retention of ^{65}Zn is mainly due to isotopic exchange whereas the Zn-balance remains virtually unchanged. The exchangeable endogenous Zn-pool was estimated to be 5·5 μmoles . kg^{-1}, i.e. 1 per cent of the total body Zn. The implication of these findings for the therapeutic usage of Zn–DTPA is stressed.

1. INTRODUCTION

It has been shown by Catsch, Lê and Chambault (1964) that the administration of Zn–DTPA (diethylenetriaminepentaacetic acid), labelled by ^{65}Zn, gives rise to the retention of a certain fraction of ^{65}Zn by the rat. This may be attributed either to a genuine deposition of Zn in tissue and/or to isotopic exchange. The fact that a higher amount of previously administered ^{65}Zn can be removed from the body by Zn–DTPA than by Ca–DTPA (Catsch and Lê 1965) indicates that isotopic exchange does actually occur, although the simultaneous splitting-off and deposition of Zn cannot be excluded.

In view of the therapeutic use of DTPA in cases of poisoning with toxic or radioactive metal ions, this question is of some practical importance as the therapeutic index (defined as the ratio of toxic to therapeutic doses) of Zn–DTPA proved to be essentially higher than that of Ca–DTPA (Catsch 1963, 1964a, Catsch *et al.* 1964, Catsch and von Wedelstaedt 1965). The question whether and to what extent a genuine retention of Zn is involved, is crucial because of possible long-term side-effects of Zn.

The present study, using the doubly labelled chelate ^{65}Zn–DTPA . ^{14}C, is based on the following conception:

The parenterally-administered chelate solution can be characterized by the ratios $S_1 = {}^{65}\mathrm{Zn}/\mathrm{Zn}$ and $S_2 = \mathrm{Zn}/{}^{14}\mathrm{C}$. The corresponding ratios in the urine are designated by U_1 and U_2. Supposing that there is no retention of Zn and only isotopic exchange between chelated ^{65}Zn and endogenous stable Zn takes place, it is obvious that $U_1 < S_1$ and $U_2 = S_2$. The exchangeable fraction (Zn_{ex}) of the total endogenous Zn-pool can be derived from:

$$Zn_{ex} = Zn_{tot}(S_1/U_1 - 1), \qquad (1)$$

where the total dose of administered Zn is designated by Zn_{tot}, and Zn_{ex} and Zn_{tot} have the same unit of measurement.

If, on the other hand, there is retention of Zn and no isotopic exchange, we obtain $U_1 = S_1$ and $U_2 < S_2$. The retained fraction (Zn_{ret}) is given by:

$$Zn_{ret} = Zn_{tot}(1 - U_2/S_2). \quad (2)$$

When both processes occur, the retained fraction of ^{65}Zn equals

$$^{65}Zn_{ret} = 1 - U_1U_2/S_1S_2. \quad (3)$$

Of course, we are aware that other methods are possible, for instance, the determination of stable Zn and ^{65}Zn in the whole body and in the excretions. It is obvious, however, that such an experimental design is extremely laborious and particularly susceptible to artefacts due to the contamination of urine by Zn of spilled food, drinking water and faeces.

2. Methods and materials

Adult female rats of the inbred Heiligenberg-strain were used. Before the injection of DTPA into the caudal vein, the urethra was tied off with a single ligature. Four hours after the injection, the urine was sampled by puncturing the bladder with a syringe equipped with a platinum needle. Before (but not during) the experiment the animals had free access to drinking water and standard pellets ($20\,\mu g$ Zn . g^{-1}). Preliminary experiments (see § 3) had shown that the bladder contains, 4 hours following the intravenous injection of 0·5 ml. physiological saline, $1{\cdot}12 \pm 0{\cdot}003$ ml. of urine and that there is no marked back-pressure.

The following chemicals were used: (1) $Na_3[Zn\text{–}DTPA].4\frac{1}{2}\,H_2O$ (by courtesy of J. R. Geigy AG, Basle); the excess of free ligand was $\leqslant 0{\cdot}2$ per cent. (2) DTPA . ^{14}C as acid (by courtesy of Geigy Research Laboratories, Ardsley, N.Y.). The label is at the carboxylate groupings. The specific activity was $7{\cdot}14\,\mu Ci$. mg^{-1}. (3) Metallic zinc (analytical grade) dissolved in conc. HCl with a Zn-content $\leqslant 5 \times 10^{-7}$ per cent. (4) Carrier free $^{65}ZnCl_2$ in 1 N HCl (The Radiochemical Centre, Amersham).

The final solution was adjusted by NaOH to pH ~7. The injected volume (0·5 ml. per animal) contained $10\,\mu$moles DTPA, $9\,\mu$moles Zn, $14\,\mu Ci\ ^{14}C$ and $10\,\mu Ci\ ^{65}Zn$. The 10 per cent excess of free ligand has been chosen purposely since even a slight excess of unchelated Zn^{2+} would invalidate the experimental results. For technical reasons (small volume and radioactivity of the solution) the adjustment of complete equivalance was not attempted.

The total amount of urine was immediately measured and aliquots were assayed for radioactivity. An amount of 0·02 ml. was applied on a filter-paper strip (width 4 cm, Schleicher & Schüll, No. 2043b). The β-activity of the spots (diam. ~ 17 mm) was measured by scanning the paper strips at a constant speed (120 mm . h^{-1}) between a dual windowless methane flow detector ($\sim 4\pi$ geometry). The aperture of the counters was a rectangular slit (1×36 mm). The total activity of the spots was determined by a built-in integrator of peak intensity. The γ-activity of ^{65}Zn was determined by measuring the punched spots by a NaJ(Tl)-crystal of the well type. In order to obtain the activity due to ^{14}C only, a certain fraction (corresponding to ^{65}Zn) was subtracted from the total activity. This correction value was derived from measuring samples of pure ^{65}Zn under identical conditions by both counting equipments. The urine of each animal was assayed in triplicate. The standard error of the radiometric measurements as estimated by variance analysis of the data was 7·5 per cent and 1·1 per cent for ^{14}C and ^{65}Zn, respectively.

The urine was dry-ashed in platinum crucibles and analysed for Zn according to the method of Vallee and Gibson (1948). The standard error of the Zn-determination was found to be 3·5 per cent.

In order to avoid any contamination by external Zn, only highly purified reagents were used. All glassware and syringes were made Zn-free by treatment with disodium-Versenate and dithizone.

3. Results and discussion

In order to determine the urinary excretion of Zn in untreated animals under our experimental conditions, 20 rats with an average body weight of 217 g were injected with 0·5 ml. physiological saline. The average Zn-content of the 4 hour-urine was $1{\cdot}27 \pm 0{\cdot}16\,\mu g$ ($1{\cdot}13\,\mu g\,.\,ml.^{-1}$ urine). This compares well with the excretion of about $4\,\mu g$ during 18 hours (Millar, Fischer, Mawson and Elcoate 1954). The above value was subtracted from the actual Zn-amount of the urine of DTPA-treated animals.

As can be seen from the table, about 75 per cent of DTPA.^{14}C was excreted within the first 4 hours. This is in keeping with pertinent data of Foreman (1960) and Stevens, Rosoff, Weiner and Spencer (1962).

Body weight (g)	224 ± 7
Urine:	
Volume (ml.)	1·10 ± 0·12
^{65}Zn (imp . sec^{-1} . $ml.^{-1} \times 10^4$)	1·447 ± 0·217
Zn (μg . $ml.^{-1}$)	535 ± 87
^{14}C (imp . $ml.^{-1} \times 10^6$)	2·218 ± 0·292
^{14}C (percentage of dose)	73·3 ± 5·9
S_1	30·62
U_1	26·94 ± 0·40
S_2 (10^{-4})	2·124
U_2 (10^{-4})	2·282 ± 0·116
$U_1 . U_2$ (10^{-3})	6·140 ± 0·305
Zn_{ex} (μmol)	1·23 (0·91; 1·57)†
Zn_{ret} (μmol)	−0·67 (0·40; −1·74)†
$^{65}Zn_{ret}$ (percentage of dose)	5·6 (15·8)‡

† Fiducial limits for $P = 0{\cdot}05$.
‡ Upper fiducial limit for $P = 0{\cdot}05$.
Averages ± S.E. (13 rats). For abbreviations see §1.

Since U_2/S_2 is larger than unity equation (2) yields a negative figure. That implies that there is no retention but rather a mobilization of endogeneous Zn. The removed fraction amounts to 0·7 and maximum $1{\cdot}7\,\mu$moles. Keeping in mind that the injected solution contained an excess of $\sim 1\,\mu$mole free ligand, this result is quite likely. Furthermore, it should not be forgotten that the octadenate ligand can bind more than one Zn^{2+} (coordination number 4) and, thus, form bimetallic chelate species of an appreciable stability ($K^{Zn}_{Zn_2L} \sim 10^{4{\cdot}4}$, according to Anderegg, Nägeli, Müller and Schwarzenbach 1959). The free capacity of the

1 : 1 Zn–DTPA is also evident from the fact that the toxicity of the bimetallic Zn_2–TTHA (triethylenetetraaminehexa-acetic acid) is distinctly lower than that of Zn–DTPA (von Wedelstaedt and Catsch 1965). Due to the relatively broad fiducial range of the estimate for Zn_{ret}, the assumption of a mobilizing effect does not reach statistical significance, and even the opposite case, i.e. the retention of a maximum of 0·4 μmoles, cannot be excluded.

On the other hand, the difference between S_1 and U_1 is highly significant. The exchangeable endogenous Zn-pool, estimated according to equation (1), equals 1·23 μmoles. Referring to the data of Gilbert and Taylor (1956), the total Zn-content of a rat with a body-weight of 224 g amounts to approximately 100 μmoles, that of the soft tissues to about 65 μmoles. Our estimate is distinctly lower, but somewhat higher than the Zn-content of blood ($\sim$0·8 μmoles). Thus, we may deduce that the isotopic exchange is not restricted to the Zn of blood only.

The figure for the retention of ^{65}Zn, obtained by equation (3), is 6 per cent of the administered dose and is in fairly good agreement with data obtained by direct whole-body measurements (Catsch and Lê, in press).

The results of the present investigation have two main implications:

(i) They throw serious doubt on the possibility of interpeting the results of experiments dealing with the metabolic fate of ^{65}Zn-chelates (e.g. Foreman 1960, Stand, Rosoff, Williams and Spencer 1962, Rosoff, Methfessel and Spencer 1965, Spencer, Rosoff, Feldstein, Cohn and Gusmano 1965) in terms of a genuine retention exclusively. The same also applies, of course, to other radionuclides, such as ^{60}Co and ^{59}Fe.

(ii) Following the intraperitoneal administration of Zn–DTPA with a 10 per cent excess of free ligand there is, if at all, only a marginal retention of Zn by the rat, amounting to a maximum of 1·8 μmoles . kg^{-1}. The parenteral $LD_{50\ per\ cent}$ of Zn in small rodents is about 250–700 μmoles . kg^{-1} (Caujolle, Chang, Mamy, Moulas and Suong 1964, Franz 1962). In humans an amount of approximately 800 μmoles Zn is retained from a normal diet (McCance and Widdowson 1942, Scoular 1939, Stern, Nadler and Earle 1941). An intravenous dose of 8 μmoles Zn . kg^{-1} (as gluconate) was found to be well tolerated (Vallee, Fluharty and Gibson 1949). Finally it may be mentioned that the DTPA-dosage used in the present study (45 μmoles . kg^{-1}) is in the same order of magnitude as the one recommended for therapeutical purposes (Catsch 1964 b), i.e. 60 μmoles . kg^{-1} . d^{-1}. Taking into account all these facts the retention of 1·8 μmoles Zn . kg^{-1} (the worst estimate) can certainly be considered as safe. Consequently, a possible argument against the therapeutic use of Zn–DTPA in humans is invalidated. The question whether a higher amount of Zn will be retained without an excess of free DTPA remains open. One should emphasize, however, that an amount of 10 per cent free ligand in the dose-range used does not lead to toxic side-effects (Catsch 1964 b).

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On a injecté à des rats du ^{65}Zn–DTPA.^{14}C doublement marqué et déterminé la teneur en Zn, ^{65}Zn et ^{14}C des urines. Une méthode quantitative simple est proposée pour suivre le destin métabolique du ^{65}Zn dans l'organisme. On a pu montrer que la rétention du ^{65}Zn est principalement dûe à un échange isotopique, tandis que le bilan en Zn reste

pratiquement inchangé. La quantité de Zn endogène échangeable a pu être estimée à 5,5 μmoles . kg^{-1}, soit 1 pour cent de la teneur totale en Zinc de l'organisme. On insiste aussi sur la signification de ces constatations pour l'application thérapeutique du Zn–DTPA.

Doppelt markiertes ^{65}Zn–DTPA.^{14}C wurde Ratten injiziert und der Gehalt des Urins an Zn, ^{65}Zn und ^{14}C bestimmt. Es wird eine einfache quantitative Methode vorgeschlagen, die gestattet, das Verhalten von ^{65}Zn im Organismus zu erfassen. Es konnte gezeigt werden, daß die Retention von ^{65}Zn im wesentlichen durch isotopischen Austausch bedingt wird, während die Zn-Bilanz praktisch unverändert bleibt. Die austauschbare endogene Zn-Fraktion konnte zu 5,5 μmol . kg^{-1}, d.h. rund 1 Prozent des gesamten Körper-Zn, geschätzt werden. Es wird auf die sich aus diesen Befunden ergebenden Konsequenzen für die therapeutische Verwendung von Zn–DTPA hingewiesen.

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