THE EFFECT OF ZIRCONIUM AND SODIUM CITRATE ON THE DISTRIBUTION AND EXCRETION OF SIMULTANEOUSLY INJECTED THORIUM AND RADIOSTRONTIUM

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(Received for publication, June 29, 1949)

The potential health hazards of radioelements taken into the body are well known (1, 2). The present study is part of a program designed to test the effect of zirconium and other salts on the metabolism of long lived radioelements. It has been shown that the early administration of zirconium salts to experimental animals previously injected with plutonium (Pu$^{239}$) and radioyttrium (Y$^{93}$) resulted in a marked increase in the urinary excretion of these radioelements and a considerable reduction of their concentration in bone (3, 4).

Thorium (Th$^{232}$) and radiostrontium (Sr$^{89,90}$) are of particular interest because of the marked differences in the nature of their deposition in bone (2). Strontium is closely related metabolically to calcium and the other alkaline earths (2) and is deposited throughout the mineral structure of bone, while many elements, including thorium, plutonium, zirconium, and the rare earths, are deposited primarily in the non-mineralized areas and possibly on the superficial surfaces of the mineralized structure (1, 2). Conditions that affect the metabolism of Ca and Sr do not appear to disturb that of Pu or Y (1, 2). It appeared unlikely, therefore, that zirconium administration would affect the metabolism of Sr.

Until recently there has been little information available on the quantitative distribution and excretion of soluble thorium salts following parenteral administration (2, 6). It has been stated (2) that tracer studies and bone radioautographs showed "no significant differences between the metabolic characteristics of thorium and plutonium." Therefore, it seemed reasonable to expect that the metabolism of injected thorium followed by zirconium administration should be markedly altered, as is the case with plutonium. It is of fundamental interest, therefore, to learn that zirconium has no significant influence on Th metabolism.

Inasmuch as the citrate salt of zirconium was used in our experiments, it was necessary to study the effects of sodium citrate as a control for the action of the citrate ion. Sodium citrate administration is of value in the treatment of experimental uranium poisoning (5) and also of lead poisoning (7), but it exerts little influence on the metabolism of plutonium and yttrium (4).
TRACER LEVELS IN EXCRETA

EXPERIMENTAL

General Procedures—All injections were made by the intraperitoneal route. Twenty female Sprague-Dawley rats, having weights which fell between 197 and 215 gm., were injected with 1.4 ml. of a solution containing the radioisotopes of Th\(^{230}\) and Sr\(^{89, 90}\). The twenty rats were divided into five groups. To the first group of four rats were administered 4.0 ml. of a zirconium citrate solution containing 12.5 mg. of Zr per ml. ½ hour after injection of the radioelements, while another group of four rats received the same amount of zirconium citrate 3 days later. These groups are referred to as “early zirconium-treated” and “late zirconium-treated,” respectively. In similar fashion a third group of four rats received 4.0 ml. of a 5 per cent solution of sodium citrate ½ hour after the injection of the radioelements, and another group of two rats received the same amount of sodium citrate 3 days later. These groups are referred to as “early citrate-treated” and “late citrate-treated,” respectively. The last group of six rats, the “untreated controls,” received only the radioelement solution. The rats were kept two to a cage. The pooled urine and feces from each cage were collected separately, usually at daily intervals. 8 days after the injection of Th\(^{230} + \text{Sr}^{89, 90}\) the animals were sacrificed with nembutal, and the liver, pancreas, femur, kidneys, spleen, mesenteric lymph node, and the lungs were removed. The rest of the animal constituted the “carcass” portion.

Radioelement Solution—A small volume of a hydrochloric acid solution of the radioelements was added to a solution of citric acid, the pH was adjusted with NaOH, and the solution diluted with distilled water. After dilution the solution used for injection had the following composition: pH 4, citric acid 1 per cent, Na\(^+\) 0.15 M, Sr\(^{89, 90}\) ~15 \(\mu\)c. per ml., and Th\(^{230}\) ~0.64 \(\mu\)c. per ml. Each animal received 1.4 ml. of this solution or a total of \(1.4 \times 10^6\) counts per minute of Sr\(^{89, 90}\) and 0.9 \(\times 10^6\) counts per minute of Th\(^{230}\). Inasmuch as the specific activity of Th\(^{230}\) is about 43,000 disintegrations per minute per microgram or 21,500 counts per minute per microgram at 50 per cent geometry, each rat received a minimum of 0.9 \(\times 10^6/21.5 \times 10^3 = 42 \gamma\) of thorium. Chemical analysis revealed the presence of about 70 \(\gamma\) of Th. This concentration is far below the LD\(_{50}\) value (8). A spectrographic analysis of the injection solution showed that, per ml., it contained less than 2 \(\gamma\) of chemical strontium or of cations other than sodium and thorium.

Zirconium and Sodium Citrate—The solution of zirconium citrate, Na\(^+[\text{ZrO}((\text{C}_6\text{H}_4\text{O}_7))^-\), was prepared as described previously (4). Each animal receiving zirconium was given 4.0 ml. of a 12.5 mg. per ml. solution or a total of 50 mg. as Zr. The sodium citrate-treated rats each received
4.0 ml. of a 5 per cent solution of sodium citrate, \( \text{Na}_3\text{C}_6\text{H}_5\text{O}_7\cdot2\text{H}_2\text{O} \). The amounts of Zr or sodium citrate administered were well below the toxic limits (8, 9).

Methods of Analysis—The excreta and tissues were wet ashed by the use of concentrated \( \text{HNO}_3 \) only. This was accomplished by covering the sample with \( \text{HNO}_3 \) and evaporating to near dryness on a hot-plate. Fresh acid was then added and evaporated as before. This was repeated from three to eight times, depending on the size of the sample, until no organic matter remained, as evidenced by the light yellow to white color of the ash. Finally, the residue was transferred with \( \text{HNO}_3 \) to a volumetric flask and diluted to volume (usually to 100 ml. for tissues and to 2000 ml. for carcasses). The carcasses were dissolved by first being covered with concentrated \( \text{HNO}_3 \), allowed to stand for at least 12 hours, and then treated as described above.

The amount of each radioelement injected into each rat was sufficiently high so that a quantitative measure of its concentration in a sample could be made by direct deposition and subsequent counting of the radiations. Inasmuch as \( \text{Th}^{230} \) is primarily an \( \alpha \) emitter (10) and \( \text{Sr}^{99}, \ 99 \) a \( \beta \) emitter (10), it was not necessary to effect any separation. For counting \( \text{Th}^{230} \) an aliquot (usually 0.25 ml.) of an ashed solution was deposited on a platinum disk, slowly evaporated to dryness by means of an infra-red lamp, and flamed. The activity was determined by counting at about 50 per cent geometry in a proportional \( \alpha \)-counter unresponsive to \( \beta \)-particles. Samples (usually 5 ml.) for \( \text{Sr}^{89}, \ 99 \) assay were deposited in a 10 ml. porcelain capsule, evaporated to dryness with an infra-red lamp, and counted by means of a mica window Geiger tube through a 110 mg. per sq. cm. aluminum absorber. The absorber was used to eliminate activity contributed by the accompanying \( \text{Th}^{230} \) which would otherwise be recorded. The samples were not assayed for \( \text{Sr}^{89}, \ 99 \) until the \( \text{Sr}^{90} \) isotope had regained equilibrium with its 65 hour \( \text{Y}^{90} \) daughter.

The activity in a given sample was always referred to that of samples from the original injection solutions obtained from “dummy” injections into a volumetric flask, which were subsequently mounted and counted under identical conditions. In order to cancel effects due to absorption and other factors, a separate standard for each tissue was prepared by addition of an equivalent amount of an ashed solution of the same tissue from a non-radioactive animal to a sample from the “dummy” injection.

Results

Comparison of Distribution and Excretion of \( \text{Th}^{230} \) and \( \text{Sr}^{89}, \ 99 \) in Untreated Rats—The simultaneous administration of the two radioelements permits a direct comparison of their behavior in the same animal. The
data tabulated in Table I illustrate the striking differences in the excre-
tion and distribution of Th and Sr after 8 days. Sufficient time having
elapsed for the uptake in bone to have reached a maximum (2, 11), it was
found that the concentration of injected Th was considerable in all the
soft tissues, reaching 30 per cent of the injected dose in liver alone, while
that of Sr was negligible, i.e. less than 0.02 per cent. On the other hand,
the concentration of Sr in the bone was 3 times higher than that of Th.

The total urinary and fecal excretion of Sr was about twice that of Th.
Urinary excretion of both radioelements was highest during the first 24
hours after injection. It then dropped sharply during the subsequent
days, especially in the case of thorium. By the 8th day the urinary excre-
tion of strontium had reached about 0.4 to 0.5 per cent of the injected dose
per day, while that of thorium had diminished to about 0.05 per cent.
The day by day urinary excretion of these elements is shown in Fig. 1.
The daily fecal excretion of Sr was quite erratic, while that of Th proceeded
at a fairly constant rate of about 0.5 to 1.5 per cent a day.

Effect of Treatment—The early administration of either zirconium or
sodium citrate caused the urinary excretion of Th to increase from about
9 to about 30 per cent of the injected dose (Fig. 1) and that of Sr from 8
to about 18 per cent during the subsequent 24 hour period (Fig. 1). Some
sustained increase in Sr excretion following early Zr administration, how-
ever, seems to have occurred. Injections of sodium citrate 72 hours after
exposure to Th and Sr had no effect on their urinary output, while zirconium
citrate appeared to have a slight effect on Sr excretion and none on Th
(Fig. 1). It must be kept in mind, however, that a 3-fold increase, for
example, in the urinary excretion of a radioelement shortly after exposure usually constitutes an appreciable fraction of the body content but is relatively unimportant when the normal excretion rate has dropped to very low levels.

The excretion of Sr or Th in the feces was unaffected at any time by the administration of zirconium or sodium citrate.

Since the major part of the increased excretion of the thorium which followed either early sodium or zirconium citrate administration came from the liver, it must be concluded that the citrate ion is responsible for the observed effects (Fig. 2 and Table I). Thorium excretion was also accomplished at the expense of the other soft tissues, namely the kidneys, spleen, mesenteric lymph node, and pancreas, following the administration of sodium citrate. The increased strontium excretion seemed to come from the skeleton primarily. It appears likely that the muscle and skin contributed some of the increased Sr excretion inasmuch as these tissues in toto contain about 35 per cent of tracer Sr 30 minutes after intraperitoneal injection (12).

The results described above are in marked contrast to those obtained with Pu under the same conditions (4). Thus, early zirconium citrate administration increased the urinary excretion of Pu from the normal 1 to 50 per cent of the injected dose during the subsequent 24 hours, while sodium citrate caused a 2-fold increase only. In addition the bone content of Pu was found to be reduced about 6-fold as a result of zirconium treatment, while the soft tissue concentrations were essentially unchanged. Furthermore, the administration of zirconium citrate 3 days after the in-
jection of Pu raised the urinary excretion level from 0.1 to 5 per cent of the injected dose.

**Table I**

*Effect of Zirconium and Sodium on Distribution and Excretion of Simultaneously Injected Thorium and Radiostrontium in Rats*  

<table>
<thead>
<tr>
<th>Tissues</th>
<th>Untreated controls</th>
<th>Early zirconium, citrate-treated</th>
<th>Early sodium, citrate-treated</th>
<th>Late zirconium, citrate-treated</th>
<th>Late sodium, citrate-treated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Th</td>
<td>Sr</td>
<td>Th</td>
<td>Sr</td>
<td>Th</td>
</tr>
<tr>
<td>Carcass</td>
<td>47.4</td>
<td>67.0</td>
<td>48.2</td>
<td>53.0</td>
<td>41.0</td>
</tr>
<tr>
<td></td>
<td>±0.9</td>
<td>±5.4</td>
<td>±6.2</td>
<td>±4.4</td>
<td>±2.8</td>
</tr>
<tr>
<td>Skeleton†</td>
<td>17.8</td>
<td>52.8</td>
<td>20</td>
<td>40.8</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>±3.6</td>
<td>±6</td>
<td>±2</td>
<td>±4.1</td>
<td>±4</td>
</tr>
<tr>
<td>Liver</td>
<td>29.6</td>
<td>0.015</td>
<td>14.4</td>
<td>0.020</td>
<td>9.8</td>
</tr>
<tr>
<td></td>
<td>±6.8</td>
<td>±0.002</td>
<td>±2.3</td>
<td>±0.006</td>
<td>±2.0</td>
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<tr>
<td>Kidneys</td>
<td>1.2</td>
<td>&lt;0.02</td>
<td>1.1</td>
<td>&lt;0.02</td>
<td>0.74</td>
</tr>
<tr>
<td></td>
<td>±0.08</td>
<td>±0.12</td>
<td>±0.12</td>
<td>±0.12</td>
<td>±0.7</td>
</tr>
<tr>
<td>Spleen</td>
<td>1.0</td>
<td>&lt;0.01</td>
<td>0.66</td>
<td>&lt;0.02</td>
<td>0.27</td>
</tr>
<tr>
<td></td>
<td>±0.2</td>
<td>±0.02</td>
<td>±0.02</td>
<td>±0.01</td>
<td>±0.12</td>
</tr>
<tr>
<td>Mesenteric lymph node</td>
<td>0.30</td>
<td>&lt;0.01</td>
<td>0.41</td>
<td>&lt;0.01</td>
<td>0.12</td>
</tr>
<tr>
<td></td>
<td>±0.06</td>
<td>±0.10</td>
<td>±0.08</td>
<td>±0.08</td>
<td>±0.02</td>
</tr>
<tr>
<td>Pancreas</td>
<td>1.0</td>
<td>&lt;0.01</td>
<td>0.61</td>
<td>&lt;0.01</td>
<td>0.33</td>
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<tr>
<td></td>
<td>±0.2</td>
<td>±0.10</td>
<td>±0.08</td>
<td>±0.05</td>
<td>±0.3</td>
</tr>
<tr>
<td>Lung</td>
<td>0.37</td>
<td>&lt;0.02</td>
<td>0.53</td>
<td>&lt;0.02</td>
<td>0.29</td>
</tr>
<tr>
<td></td>
<td>±0.04</td>
<td>±0.04</td>
<td>±0.04</td>
<td>±0.04</td>
<td>±0.10</td>
</tr>
<tr>
<td>Urine</td>
<td>10.6</td>
<td>13.5</td>
<td>27.4</td>
<td>27.1</td>
<td>36.8</td>
</tr>
<tr>
<td></td>
<td>±4</td>
<td>±4</td>
<td>±6</td>
<td>±8</td>
<td>±3</td>
</tr>
<tr>
<td>Feces</td>
<td>7.7</td>
<td>16.7</td>
<td>5.5</td>
<td>19.0</td>
<td>9.6</td>
</tr>
<tr>
<td></td>
<td>±2</td>
<td>±1.5</td>
<td>±2</td>
<td>±2.1</td>
<td>±2</td>
</tr>
</tbody>
</table>

* Values equated to 100 per cent (actual recoveries for Th = 94.5 ± 8.2 per cent and for Sr = 90 ± 4 per cent). The rats were sacrificed 8 days after the intraperitoneal administration of Th**239** and Sr**90. Each value is an average of results obtained from six rats for the untreated controls, four rats each for the early sodium citrate, early zirconium citrate, and late zirconium citrate-treated animals, and two rats for the late sodium citrate group. Further details are given in the experimental sections of the text.

† The concentrations of radioelement in the skeleton were calculated by multiplying the femur content by the somewhat arbitrary factor of 20.

**Discussion**

Action of Sodium Citrate—The action of early sodium citrate administration in minimizing the deposition of thorium in the soft tissues with a resulting increase in the urinary excretion is related to the complexing action of the citrate ion accompanied by a concomitant increase in the
diffusibility of the Th. Most cations in the body exist as a mixture of dif-

cusible and non-diffusible forms. The non-diffusible fractions result from

f a combination of the cation with proteins, or because of the formation of

particles with colloidal properties as a consequence of hydrolysis or reac-
tion with anions to form salts of low solubility. An increase in the dif-

cusible fraction at the expense of the non-diffusible parts is reflected in a

lower deposition in the soft tissues comprising the reticuloendothelial

system and an increase in the urinary excretion because the rate of filtra-
tion through the glomerulus of the kidney is enhanced. These con-
siderations are supported by the fact that citrates form soluble complex
ions with thorium (13) and by ultrafiltration studies in which the pas-

sage of Th from blood serum through Visking membranes was markedly

increased following the addition of sodium citrate.1 Similar concepts

have been proposed to explain the action of sodium citrate and other com-

plexing axions in the metabolism of uranium (14, 15) and lead (16).

Once an element such as thorium, which precipitates from simple in-

organic solution above a pH of 3.5, has been deposited in the body, it is

very difficult to increase its subsequent rate of elimination. This is prob-

ably related to the well known chemical fact that very large (unphysio-

logical) amounts of the citrate anion are needed to dissolve the hydroxide

of an element such as Th once it has formed. This phenomenon explains

the relative ineffectiveness of sodium citrate in promoting the excretion

of Th when administered several days later.

Of further interest is the fact that the concentration of injected Th in

the spleen, mesenteric lymph node, and pancreas was clearly unaffacted

by early zirconium citrate administration; yet early sodium citrate ad-

ministration caused a marked reduction. Such a result is probably re-
lated to the fact that in zirconium citrate the citrate part of the molecule

is not immediately available because it is bound to zirconium. Thus in

the time necessary for the zirconium citrate to be absorbed following

intraperitoneal injection, the mesenteric lymph node, spleen, and pan-

creas are not in contact with free citrate. Subsequent metabolic processes

free the bound citrate, whereupon it is available to exert a solubilizing

effect through complexing action on the Th. Free citrate, however, is

lost in the liver partially by conversion to glycogen (17).

The uptake of strontium and the other alkaline earths as well as the

phosphate ion is most easily interpreted as being an ion exchange reaction

(18–20) with the bone acting as either an anion or cation exchanger con-
taining a variety of functional groups. We can represent the cation ex-

change with strontium by the relation

\[
CaA + Sr^{++} \rightleftharpoons SrA + Ca^{++}
\]

1 Unpublished experiments.
in which, for simplification, we assume that $A$ is a composite anion of all the functional groups in bone with a valence of $-2$. The capacity of bone for cation exchange can be expressed in terms of the total milliequivalents of replaceable calcium. The distribution coefficient, $K_d$, for a given cation adsorbed by bone is given by the expression (21)

$$K_d = \frac{M_s}{M_t} \times \frac{v}{m}$$  \hspace{1cm} (2)

in which $M_s$ and $M_t$ are the fractions of the adsorbed cation in the bone and liquid phases respectively, and $v$ is the volume of solution in contact with $m$ mg of bone. The ratio $M_s:M_t$ is expressed conveniently as ($\%$ adsorbed)/($100 \%$ minus $\%$ adsorbed). By employing the same reasoning used with resinous cation exchangers (21), it can be shown that, to a first approximation, $K_d$ is directly proportional to the mole fraction of exchangeable $\text{Ca}^{++}$ in bone. Therefore $K_d$, and hence the amount of $\text{Sr}^{90}$ exchanged, will remain constant as long as the mole fraction of $\text{Ca}$ and other exchangeable cations in bone remain unchanged. Since a gm. of bone contains about 1 mM of exchangeable calcium, tracer levels of $\text{Sr}^{90}$, i.e. $\sim 10^{-10}$ M, cannot affect $K_d$, regardless of the extent to which the tracer Sr is adsorbed. In the in vitro experiments of Falkenheim et al. (19), 50 mg. of bone ash were equilibrated with 25 ml. volumes of solution-containing $\text{Ca}^{++}$. Their bone sample presumably contained about 0.05 mM of exchangeable calcium. When the contacting solution contained less than $10^{-4}$ M, i.e. $<0.0025$ mM of tracer alkaline earth, no appreciable change in $K_d$ could be expected. However, a solution $10^{-3}$ M, equivalent to 0.025 mM, if adsorbed to the same extent as tracer levels, $\sim 75$ per cent or more as found for $\text{Sr}$ and $\text{Ca}$ (19, 22), would markedly reduce the mM of $\text{Ca}$ in bone, and hence a sharp drop in the per cent adsorption must take place at about $10^{-3}$ M, in agreement with the experiments on bone (18, 19).

The preceding calculations explain why the injection of 0.01 to 10 $\mu$C. of carrier-free $\text{Sr}^{90}$ in the experiments of Murray and Bloom (23) resulted in no change in the per cent uptake. In some experiments of Copp and Greenberg (24) it was thought that the injection of non-radioactive “carrier” strontium might dilute and wash out radioactive strontium. In view of the large amount of exchangeable calcium in bone it can be calculated from equation (2) that it would require about 60 mg. of Sr in a 200 gm. rat to affect the uptake of Sr appreciably. This was, indeed, found to be the case, but inasmuch as this amount of Sr is lethal or near lethal it is of no practical value.

\footnote{We are, of course, disregarding any effects due to the radiation itself.}
Elements such as Th, Pu, and Zr, because of their chemical properties, are most probably retained by bone through an irreversible type of non-specific surface adsorption. Therefore, the uptake and elimination of these elements by bone, in analogy to similar inorganic systems (21), should be unaffected by changes in bone resulting from age, diet, or other ordinary metabolic changes. A fuller discussion of these points together with pertinent experimental data will be given in a subsequent paper.

**SUMMARY**

1. The distribution and daily excretion of tracer levels of injected thorium and strontium were studied in rats receiving zirconium or sodium citrate intraperitoneally ½ hour after the administration of the radioelements ("early" treated) or 3 days later ("late" treated). One group of rats received no other treatment.

2. In contrast to previous studies on plutonium and yttrium, zirconium citrate had no specific effect on Th or Sr metabolism other than that associated with the citrate part of the molecule.

3. The early administration of zirconium or sodium citrate resulted in about a 3-fold increase in the urinary excretion of Th and a 2-fold increase in the urinary excretion of Sr during the following 24 hours. No effect on the fecal excretion was found. The Th concentration in the liver, mesenteric lymph node, pancreas, and spleen was markedly reduced as a result of early sodium citrate administration. No significant changes in the metabolism of Th or Sr were found in the late treated groups.

4. The deposition of Th per gm. was highest in the soft tissues, particularly in the liver, while Sr accumulated mainly in the skeleton.

5. An interpretation of the results in terms of ion exchange, surface adsorption, and complex ion formation was presented.

We wish to express our appreciation to Rosie Hunter for the mounting and counting of many of the numerous samples required by this work.

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