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51. Yosoji Ito, Susumu Tsurufuji, Mikio Shikita, and Sadahiko Ishibashi : Detoxication and Excretion of Radioactive Strontium. IV.[1] Effect of Sodium Calcium Citrate and the Mode of Action of Citrate.

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It has been reported in previous papers[1,2] that some organic acids having chelating affinity to strontium inhibit skeletal deposition of radiostrontium, and some of them, such as sodium tricarballylate[1] and sodium citrate,[3,4] have been reported to be effective in accelerating the urinary excretion of radiostrontium. The mechanism of the action of these effective agents seems to be based on their chelating activity, but it has not yet been sufficiently confirmed.

New evidences from the present study support the hypothesis that the effect of citrate in removing radiostrontium from the animal body is attributed to the chelating activity of citrate. It has also been indicated that the parenteral administration of a large quantity of sodium calcium citrate greatly accelerates radiostrontium excretion into the urine without hypocalcemic hazard, contrary to the injection of sodium citrate.

Experimental

Hypocalcemic Toxicity of Citrate—$CaCO_3$ was added to 1/3 mole/L. solution of citric acid in various ratios as shown in Table I. The resulting solution was sucked with a pump to eliminate dissolved CO_2 and neutralized with NaOH to pH 7.2~7.4. As the resulting solution of sodium calcium citrate is supersaturated with calcium citrate, the solution must be injected within 1 hr. of its preparation before precipitation of calcium citrate starts. In order to compare the hypocalcemic toxicity of sodium calcium citrate with that of sodium citrate, 42 male albino rats were divided into 2 groups and injected subcutaneously or intraperitoneally with trisodium citrate (Na_3Cit) or sodium calcium citrate (NaCaCit) as listed in Table I.

Table I. Hypocalcemic Toxicity of Trisodium and Monosodium Monocalcium Citrates

Group No.	No. of animals	Body wt. (g.)	Dose of citrate (m*M*/kg.)	Ratio of (NaCaCit)/(Na_3Cit)	Rate of convulsion (%)	Mortality within 24 hrs. (%)
I	5	165~187	10~12	0	100	100 (2.5)*
	5	170~210	14.3~17.6	0	100	100 (2.5)
II	1	150	6.7	0.25	0	0
	1	230	8.7	0.25	100	0
	5	165~201	10~12	0.25	100	20
	5	127~170	15~23	0.25	100	100 (3.0)
III	13	140~230	20	0.5	0	0
	7	146~192	20	0.5	0	0 (6.0)

* Figures in parentheses show period (in hours) in which animals died.

Outstanding lowering in mortality was observed in the sodium calcium citrate group, especially in Group III of Table I, in which the ratio of NaCaCit to Na_3Cit of the injected solution was 1:2, and no rat died through the treatment.

Level of Plasma Citric Acid—Male Wistar rats, 55 and 57 days old, were injected intraperitoneally with 2 m. mole/kg. of sodium citrate (Na_3Cit). Twenty mins. after the injection, 40 mg./kg. of sodium pentobarbital was given subcutaneously. Seven mins. later, i.e., 27 mins. after the injection of Na_3Cit,

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1) Part III : Y. Ito, S. Tsurufuji, S. Ishibashi, M. Ishidate, Z. Tamura, H. Takita : This Bulletin, **6**, 34(1958).
2) Y. Ito, S. Tsurufuji, E. Murai, S. Ishibashi, M. Ishidate, Z. Tamura : This Bulletin, **6**, 92(1958).
3) J. Schubert, H. Wallace : J. Biol. Chem., **183**. 157(1950).
4) S. Akiya, M. Uchiyama : Seikagaku, **28**, 154(1956).

0.1~0.2 cc.(about 100 units) of heparin solution was injected intravenously to prevent blood coagulation and 3 mins. later, the rats were sacrificed by cutting their carotid artery and blood was drawn from each rat. Collected blood samples were chilled in an ice bath as soon as possible and centrifuged under refrigeration. Plasma citric acid was determined by the method of McArdle.[5]

The results are summarized in Table II. Plasma citrate level of the rats with intraperitoneal injection of sodium citrate was, on an average, about 8.5 times greater than that of the control rats. As their plasma Ca level was found to be in normal range, the decrease of the level of plasma Ca ion is inferred.

Table II. Level of Plasma Citrate of the Animals injected with Trisodium Citrate Intraperitoneally
(Time of blood drawing was 30 mins. after the injection)

Treatment	No. of Rats	Dose of Citrate (m*M*/kg.)	Body Wt. (g.)	Plasma citric acid (mg./100 cc.)	Plasms Ca (mg./100 cc.)
Na_3Cit (intraperiton.)	4	2.0	176~199	39.8 (27.0~57.0)*	9.17~10.05
Na_3Cit**	2	2.0	178~186	4.8 (4.5~5.1)	8.35~10.20
0.9% NaCl (Control)	5	—	174~204	4.7 (3.5~5.7)	9.25~10.40

* Figures in parentheses show ranges of plasma citric acid level.
** Na_3Cit was erroneously injected into the coecum in this case.

Excretion of Radiostrontium—Three litters of male Wistar rats, 52, 61, and 63 days old, were used to investigate the effect of NaCaCit and of Na_3Cit on the excretion of radiostrontium administered parenterally. Number of animals in each litter was 6, and they were divided into three groups, i.e., NaCaCit, Na_3Cit, and control physiological saline groups. The rats were placed in individual metabolism cages for 2 days prior to the injection of radioactive strontium and fed on a suitable synthetic diet consisting of corn starch, purified casein, peanut oil, yeast extract, salt mixture (NaCl, $MgSO_4$, $FeSO_4$, $CaCO_3$, KH_2PO_4, and KCl), and vitamins A and D.

Immediately after the subcutaneous injection of about 3 μc of radiostrontium, the rats were injected with NaCaCit, Na_3Cit, or 0.9% NaCl solution. NaCaCit group was given 20 m.mole/kg. of citrate, in which NaCaCit : Na_3Cit ratio was 1:2. Na_3Cit group was given 2 m.mole/kg. of Na_3Cit. These agents were injected in two ways simultaneously, subcutaneously and intraperitoneally, because of a large volume of the solution. In Na_3Cit group the second injection was performed 1 hr. after the first injection in the same way as the first. Six hrs. after the injection of radiostrontium the rats were sacrificed with blows. Urines for the 6-hr. period were collected and analyzed for the amount of radiostrontium and the excreted citrate. Feces were also analyzed together with the intestines. The results are summarized in Table III. Urinary excretion of radiostrontium was greatly enhanced in the NaCaCit group.

Table III. Excretion of Radiostrontium in Rats treated with Monosodium Monocalcium Citrate or Trisodium Citrate

Litter No.	Age (days)	Treatment	Body wt. (g.)	Citrate: Total urinary excretion (mg. as free acid)	Citrate: Recovery in urine (% of dose)	Recovery of radio Sr (% of dose injected): Urine	Recovery of radio Sr (% of dose injected): Feces + Intestines
I (2)*	61	0.9% NaCl (Control)	187	0.52		16.3	4.6
II (2)	52		153	0.34		7.9	4.7
III (2)	63		196	0.58		16.8	5.8
I (2)	61	NaCit 2 m*M*/kg. 2 times	192	73.6	49.8	27.9	4.7
II (2)	52		158	55.0	45.0	24.2	3.9
III (2)	63		196	67.7	45.8	25.6	5.0
I (2)	61	NaCaCit 20 m*M*/kg.	189	315	42.4	60.9	1.8
II (2)	52		158	262	44.0	52.8	1.3
III (2)	63		188	353	48.5	61.3	1.1

* Figures in parentheses are number of animals.

Discussion

In order to accelerate the urinary excretion of radiostrontium by chelating agents it is necessary that following terms be satisfied.

1) Strontium forms a complex with a chelating agent *in vivo* and the concentration of strontium ion falls significantly without hypocalcemic hazard.

5) B. McArdle : Biochem. J., **60**, 647(1955).

2) Strontium-chelate compound is water soluble and able to pass through the kidneys with ease.

3) The chelating agent has not any severe toxicity.

Strontium chelated by citrate dissociates as follows : $SrCit^- \rightleftarrows Sr^{2+} + Cit^{3-}$

The ratio (R) of complexed strontium to strontium ion will be

$$R = \frac{[SrCit^-]}{[Sr^{2+}]} = K_{Sr} \cdot [Cit^{3-}] \quad (1)$$

where K_r represents chelate formation constant of citrate with strontium. The larger the concentration of Cit^{3-}, the larger the R in equation (1) will become, but citrate also chelates with blood calcium to reduce plasma calcium ion concentration. From the equilibrium between calcium and citrate

$$[Cit^{3-}] = \frac{[CaCit^-]}{K_{Ca} \cdot [Ca^{2+}]} \quad (2)$$

$[Ca^{2+}]$ must be near 10^{-3} mole/L.[6] in mammalian blood plasma, and therefore, if sodium citrate alone is injected, $[Ca^{2+}]$ would decrease towards the lethal range. Therefore, injection of a large amount of sodium citrate is supposed to be impossible.

Substituting $10^{3.2}$ for K_{Ca} and 10^{-3} for $[Ca^{2+}]$ in equation (2), and rearranging

$$\frac{[CaCit^-]}{[Cit^{3-}]} = 10^{3.2-3.0} = 1.6 \quad (3)$$

Therefore, if $[Cit^{3-}]$ in blood plasma could be elevated while keeping the constant ratio of $[CaCit]/[Cit^{3-}]$ at 1.6, concentration of plasma strontium ion would be effectually reduced without any change of $[Ca^{2+}]$ in plasma.

Na_3Cit injected into mammalian body would dissociate completely in body fluid of pH 7.3, and trivalent Cit anions equivalent to injected citrate would be produced. When the amount of injected citrate is sufficient to disregard the interference of magnesium and other metal ions, and a ratio of injected NaCaCit to Na_3Cit is 1.6, $[Cit^{3-}]$ would be elevated enough to get a high value of R in equation (1) without hypocalcemic hazard. However, because of the ability of animals to regulate blood calcium level in a normal range and their tolerance against low blood calcium, ratio of NaCaCit to Na_3Cit of 1.6 may not be necessary. In fact, the ratio of 1:2 was found to be satisfactory as indicated in Table I.

When a mixture of NaCaCit and Na_3Cit is given, plasma citrate can be written as

$$[\text{Total Cit}] = [CaCit^-] + [Cit^{3-}] \quad (4)$$

where [Total Cit] represents molar concentration of total plasma citrate, which is measurable.

From equation (2) and (4) we have

$$[CaCit^-] = \frac{K_{Ca} \cdot [Ca^{2+}] \cdot [\text{Total Cit}]}{1 + K_{Ca} \cdot [Ca^{2+}]} \quad (5)$$

Substituting equation (2) for $[Cit^{3-}]$ in equation (1) and rearrangement

$$R = \frac{[SrCit^-]}{[Sr^{2+}]} = \frac{K_{Sr}}{K_{Ca}} \cdot \frac{[CaCit^-]}{[Ca^{2+}]} \quad (6)$$

Substituting equation (5) for $[CaCit^-]$ in equation (6) and rearrangement

$$R = \frac{[SrCit^-]}{[Sr^{2+}]} = \frac{K_{Sr}}{K_{Ca}} \cdot \frac{K_{Ca} \cdot [\text{Total Cit}]}{1 + K_{Ca} \cdot [Ca^{2+}]} = \frac{K_{Sr} \cdot [\text{Total Cit}]}{1 + K_{Ca} \cdot [Ca^{2+}]} \quad (7)$$

Supposing that $[Ca^{2+}]$ in the blood plasma is $10^{-3.0}$ mole/L. and from the determination

6) F.C. McLean, A.B. Hastings : Am. J. Med. Sci., **189**, 601(1935).

of total citric acid in the plasma, ratio of chelated strontium to ionic strontium could be estimated by equation (7) and results of the computation are listed in Table IV.

TABLE IV. Percentage Dissociation on Radiostrontium in Rat Plasma

Total citrate concn. in plasma (mole/L.)	10^{-2}	2×10^{-3} *	10^{-3}	2.5×10^{-4} **
(mg./100 cc.)	192	38.4	19.2	4.8
$[SrCit^-]/[Sr^{2+}]$	31.6	0.613	0.316	0.079
Dissociation (%)	24	61	76	93

* The value corresponds to citrate level 30 mins. after the injection of 2 m. mole/kg. of Na_3Cit intraperitoneally (see Table II).

** The value corresponds to normal control level of plasma citrate.

When 2 m.mole/kg. of sodium citrate was injected, plasma citric acid level of rats was found to increase to the level 8.5 times as high as that of the normal. In this condition, equation (7) shows that the concentration of strontium ion in blood plasma would diminish to about 2/3 of untreated animals, and urinary excretion of radiostrontium was found to become about twice as shown in Table III. Together with increased urinary radiostrontium, nearly 50% of the injected citrate was excreted into the urine within 6 hours of parenteral administration. The results shown in Table III indicate that when citrate injected becomes larger, the excretion of radiostrontium into the urine becomes greater with increased urinary excretion of citrate. In these conditions urinary excretion of citrate increased to the amount $10^2\sim10^3$ times as much as that in the normal control rats. Injection of a rather small amount of Na_3Cit into the rat produces severe hypocalcemic toxicity, while injection of very much larger amount of NaCaCit was observed to be harmless.

From these results and considerations it is concluded that the effect of citrate in increasing the urinary excretion of radiostrontium is attributed to its chelating ability.

Summary

After the injection of radioactive strontium, a mixture of trisodium citrate and monosodium monocalcium citrate was injected into rats and the effect of monosodium monocalcium citrate injection in accelerating the excretion of radiostrontium was compared with the effect of the injection of trisodium citrate only. Greatly enhanced excretion of radiostrontium into urine was found in the rats treated with sodium calcium citrate, without any symptom of hypocalcemia. When citrate was injected into rats in the form of either trisodium salt or sodium calcium salt, citrate level in blood plasma was greatly elevated and nearly one-half of injected citrate was found in the urine within six hours. Mechanism of the action of citrate on the excretion of radioactive strontium is discussed and attributed to its chelating ability.

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