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8. Yosoji Ito, Susumu Tsurufuji, Sadahiko Ishibashi, Morizo Ishidate, Zenzo Tamura, and Harue Takita : Detoxication and Excretion of Radioactive Strontium. III.[1] Effect of Tricarballylic and Lactic Acids.

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Numerous works have already been made on the application of chelating agents to the detoxication and excretion of fission products and fissile materials with satisfactory result in some elements, for instance in ^{91}Y and ^{239}Pu, but there have been as yet very few agents effectively applicable to radioactive strontium. BAL[2] and EDTA[3,4] were tried without any effect, only sodium citrate[5,6] and zirconium citrate[5] being reported as effective.

In order to inhibit the rapid deposition of radioactive strontium on the skeletal tissues and to accelerate its excretion through the kidneys, it is necessary that chelating agents form a soluble chelate compound with strontium in animal body before completion of the deposition of radioactive strontium. However almost all of the known chelating agents tend to combine with calcium more stably than with strontium. Therefore, ionic calcium level in serum decreases, occasionally sufficient to be lethal.

The authors have reported in the preceding paper[1] that several organic acids, which were picked out because of their little difference between calcium and strontium in chelate formation constants, are effective in inhibiting the deposition of radioactive strontium into the skeleton.

In the present experiment, tricarballylic and lactic acids were selected among those acids and examined as to whether they are also effective in activating the excretion of radiostrontium.

Experimental and Results

Toxic Side Effect of Tricarballylic Acid—About 2-month-old male albino rats, weighing 150~210 g., were injected intraperitoneally with sodium tricarballylate (8.5 m. mol./kg. body wt.) 4 times at intervals of 5, 3, and 3 hrs. At the time of injection, tricarballylic acid solution was neutralized to pH 7 with NaOH. Because of the large quantity of the hypertonic solution required, temporary exudation into the peritoneal cavity was observed, but no chronic interference, such as the decrease in body weight, was found and no rat died through the treatment. Histopathological examination of the kidneys showed some cloudy swelling of the tubular epithelium and granular cast in the lumens, but these were regarded as temporary changes caused by the injection of a large quantity of the agent.

Distribution and Excretion of Radioactive Strontium—Three litters of male albino rats, about 2 months old and weighing 130~180 g., were fed on a synthetic diet consisting of corn starch, purified casein, vegetable oil, and mixed salts (NaCl, $MgSO_4$, $FeSO_4$, $CaCO_3$, KH_2PO_4, and KCl) for 1 week before the experiment.

Immediately after the subcutaneous injection of about 5 μc of carrier-free $^{90}Sr(+^{90}Y)Cl_2$ solution, 8.5 m. mol./kg. of sodium tricarballylate to the first group, and 13.5 m. mol./kg. of sodium lactate to the second were respectively given by intraperitoneal injection. Control group was injected with 0.9% NaCl solution. Then the rats were placed in individual metabolism cages and urine

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and feces were collected separately. Volume of the solution injected was 1.5~2.0 cc. After 6 hrs., the second injection was performed in the same way as the first, and the urines from the beginning of the experiment were collected (urine I). Twenty-four and 30 hrs. after the injection of radiostrontium, the third and fourth injections were carried out the same as before. In total, 34 m.mol./kg. of tricarballylate and 54 m.mol./kg. of lactate were given. No one of the animals died through the experiment.

Forty-eight hours after the radiostrontium injection the rats were sacrificed under the anesthesia of pentobarbital sodium by cutting the carotid artery and the blood was drawn from each rat. An aliquot of the serum was used for radioactivity assay and the total serum radioactivity was estimated by using the value of 2.5% of body weight for the total serum volume as reported by Berlin, *et al.*[7] At the time of sacrifice, the urines were collected again (urine II) as well as the feces.

Then the kidneys were removed from the carcass and the rest was divided into two parts, the hard and soft tissues. The feces and each part of the carcass were reduced to ashes in an electric muffle furnace and dissolved in dil. HCl. All radioactivity assays were performed with a G-M counter after radioactive equilibrium between ^{90}Sr and ^{90}Y of counting samples had been reached. The results are summarized in Table I. Concerning the urine I, tricarballylate activated

Table I. Effect of Tricarballylic and Lactic Acids on the Distribution and Excretion of Radioactive Strontium in Rats

Treatment	No. of Animals	Per Cent of Total Activity Injected						
		Urine I	Urine II	Feces	Hard Tiss.	Soft Tiss.	Serum	Kidneys
Control	6	11.5	5.6	9.0	59.2	4.3	0.10	0.011
Lactic acid	6	12.0	5.1	9.2	61.3	4.2	0.12	0.012
Tricarballylic acid	6	30.2	8.3	5.3	45.5	4.0	0.09	0.009

the excretion of radioactive strontium 2.5 times that of the control and lactate groups. Statistical analysis showed the difference between the tricarballylate group and other two groups to be significant at the level of significance of 1%.

The significant difference of the same order was also observed in the urine II, but not so remarkably as in urine I. On the contrary, recovery of radioactive strontium in feces decreased in the tricarballylate group Total excretion of radioactive strontium in the experimental period of 48 hrs. was 43.8% of the injected dose in the tricarballylate group, while only 26.1% was excreted in the control group.

In the hard tissues, radioactivity in the tricarballylate group was about 75% of the control, but in the soft tissues, the serum, and the kidneys, no difference was observed among the groups.

Any litter effect was not observed statistically with the exception of urine I (at the level of significance of 1%). No interaction between the treatments and the litters was recognized.

Total recovery of radioactive strontium was, in an average, 92%.

Discussion

In the preceding paper[1] it was reported that the rats injected with tricarballylate deposited about four times more redioactivity into the kidneys than the control animals, but in the present experiment, no difference in the radioactivity of the kidneys was observed. Therefore, retention of a large quantity of radiostrontium in the kidneys as reported in the preceding paper must be a temporary increase of radiostrontium associated with greatly activated excretion.

Mechanism of accelerating radioactive strontium excretion by tricarballylate is not yet completely followed, but it is most likely that the effect is based on its chelating action. Studies on the metabolism of tricarballylate now in progress in this laboratory shows active excretion of the acid into the urine without any degradation. Therefore, chelate-formation of the agent with strontium appears to be the most probable interpretation.

Comparing the data of urine I and II in the tricarballylate group it is seen that the earlier the injection of the agent, the greater will be the effect of eliminating radiostrontium. Such results are also consistent with the idea that chelating agents must combine with strontium ions before the completion of its deposition into the

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skeleton.

Lactate did not give any significant effect in the present experiment, while it was shown in the preceding paper[1] that in the experiment of short duration lactate was as effective as tricarballylate in inhibiting radiostrontium deposition on the skeleton. It is obscure why lactate is ineffective in activating urinary excretion of radiostrontium.

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Summary

Sodium tricarballylate (8.5 m.mole/kg.) activates the excretion of radioactive strontium injected and reduces its skeketal accumulation. Under a similar condition, sodium lactate (13.5 m.mole/kg.×4) is ineffective. Mechanism of active excretion caused by the injection of tricarballylate was discussed and suggested to be due to its chelating action. Some side effect of tricarballylate injection was observed but it did not produce severe injury.

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9. Jun Hasegawa, Ken Ikeda, and Tai Matsuzawa : Studies on Decomposition and Stabilization of Drugs in Solution. I. Chemical Kinetic Studies on Aqueous Solution of Phenobarbital.

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The unstability of phenobarbital in aqueous solution has been discussed by many investigators, and several studies[1~6] on its degradation and stabilization have been reported. According to these studies, the decomposition of phenobarbital depends on the pH value and temperature of the solution and it was found that the reaction is accelerated very much in the region of high pH value. Many experimental results were obtained from these reports, but theoretical consideration on the degradation has not been published.

The following study was carried out in order to elucidate the mechanism of the reaction of phenobarbital in aqueous solution from the standpoint of chemical kinetics in an accelerated condition.

Theoretical Consideration of the Reaction

The degradation of phenobarbital in alkaline solution, according to Husa,[2] is as shown in Chart 1.

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