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Biological Investigations with Radioactive Calcium and Strontium.*

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Radioactive calcium and strontium produced by the bombardment of these elements with 16 million volt deuterons have been used as tracers for mineral metabolism studies. As has been shown by Walke, Thompson and Holt,[1] Ca^{45} is the only radioactive calcium suitable for this purpose. Unfortunately its yield is low, and it emits a very soft beta-radiation (maximum energy 200 kev). Satisfactory measurements have been obtained with a screen wall counter described by Libby,[2] but the activity determinations are extremely time-consuming. This is a serious handicap in biological experiments, where the number of samples to be measured is generally very large. By this method, the fate of radio-calcium introduced into the stomach of a rat has been described by Campbell and Greenberg.[3]

A radioactive form of strontium (Sr^{89}), described first by Stewart, Lawson and Cork,[4] is a very valuable isotope for biological purposes. Its half-life is 55 days, and it emits 1.5 million electron-volt beta-particles, no gamma-rays, and can be produced abundantly by the cyclotron. The yield is about 6 millicuries per milliampere hour when strontium oxide is bombarded with 16 million volt deuterons and slightly higher when metallic strontium is used. At the same time, an appreciable amount of long-lived (about 100 days) radioactive yttrium‡ is produced, *i.e.*, ten milligrams radium gamma-ray equiva-

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1 Walke, H., Thompson, F. C., and Holt, J., *Phys. Rev.*, 1940, **57**, 177.

2 Libby, W. F., and Lee, D. D., *Phys. Rev.*, 1939, **55**, 245.

3 Campbell, W. Wesley, and Greenberg, David M., *Proc. Nat. Acad. Sci.*, 1940, **26**, 3, 176.

4 Stewart, Lawson, and Cork, *Phys. Rev.*, 1937, **52**, 901.

‡ This isotope is discussed in an issue of *Physical Review* (Pecher, Charles, 1940, **58**, 843) which appeared since this paper was submitted for publication.

ient for one milliampere hour of 16 million volt deuterons. It emits a gamma-radiation very similar to that of radium and thus may be substituted for radium. This radioactive yttrium has been independently observed by DuBridge and Marshall[5] in the systematic study of the radioactive isotopes of strontium, yttrium and zirconium.

Radioactive strontium is detected with an electroscope or an electrometer, and the accuracy of the determinations is much better than that for Ca^{45}. The chemical and physiological similarity of the two elements, calcium and strontium, suggested a comparison of their metabolism. Strontium metabolism has been studied with the idea of the possible application of the radioactive isotope to therapeutic bone irradiation.

I. *Uptake of Radioactive Calcium and Strontium in the Tissues.* Doses of 0.1 cc radioactive calcium lactate solution (0.8 mg calcium, 200 counts per sec.) and 0.1 cc of radioactive strontium lactate solution (1.6 mg strontium, 0.8 microcurie) were intravenously injected into adult white mice. After 24 hours, the animals were sacrificed and the distribution of the activity in the various tissues measured.

Fifty-eight percent of the dose of radioactive calcium (average of 30 mice) and 33% of the dose of radioactive strontium (average of 35 mice) were recovered in the skeleton after 24 hours. The activity in the different tissues (average of 15 mice for calcium and 25 mice for strontium) is given in Table I. For comparison, the activities of the tissues after intravenous injection of radio-phosphate are given (average on 5 animals).

TABLE I.
Per Cent of Dose per Gram Wet Weight.

	Bone	Muscle	Skin and Hair	Dig. Tract	Liver	Other Visceræ
Ca^{45}	22	0.33	0.20	0.36	0.12	0.23
Sr^{89}	12	0.17	0.15	0.23	0.07	0.13
P^{32}	5.2	1.4	0.75	1.3	3.0	2.1

The uptake of calcium was greater than the uptake of strontium, but the distribution among the different tissues was almost the same for the two elements. Both of them were concentrated in the skeleton; the activity of the soft tissues was negligible in comparison with that of the bones; the digestive tract had a noticeable activity; that of the liver was extremely low, and there was almost none in the fat. In groups of animals killed at different times after the intravenous injection of radioactive calcium or strontium lactate, it appeared that the activity of the skeleton had nearly reached its maximum at eight

[5] DuBridge, L. A., and Marshall, John, *Phys. Rev.*, 1940, **58**, 7.

hours. When introduced into the stomach of adult mice, 13 to 30% of the ingested calcium and 6 to 14% of the ingested strontium were recovered in the skeleton after 2 days. Uptake of calcium and strontium in the skeleton following intravenous injection was found to be nearly 3 times as great as that following oral administration.

II. *Distribution of Radio-Calcium and Radio-Strontium in the Skeleton.* The distribution of radio-calcium and radio-strontium in the skeleton has been studied by two methods: (1) activity determination of bones or fragments of bones, and (2) autoradiographs of bones (by placing the bone or section of bone on a photographic film).

Activities of shaft and trabecular bone of rats and rabbits recently injected with radioactive strontium lactate have been compared and found to be higher in the trabecular bone. The epiphysis retains more radioactivity than the diaphysis. The epiphysial line is clearly marked on several autoradiographs showing an accumulation of strontium there (Fig. 1). The radio-strontium activity of each bone of the skeleton was measured separately and compared with the weight of inorganic material of each bone. Activity was higher in the dorsal vertebrae (0.030% per mg) than in most of the other bones (average 0.018% per mg). Activity of the inferior maxilla was similar to the activity of the other bones (0.0175%). The average activity of the teeth was higher than that of the bones. The base of the incisor teeth (mice and rabbits) had an exceedingly high activity (0.141% per mg dry weight) (Fig. 3).

A selective concentration of the activity appears during the period of healing of fractured bones. The uptake of radioactive calcium in the healing tibia of a rat (fractured 2 weeks before) was 1.7% of the ingested dose of radio-calcium lactate (32 mg calcium), while the other tibia had an activity of only 0.4%.

III. *Excretion of Radioactive Calcium and Strontium.* Intravenously administered calcium and strontium are excreted both in the feces and in the urine. In mice the larger fraction was generally excreted in the feces. An appreciable activity was found in the contents of the small (.33% of dose) and large (.88% of dose) intestine 4 hours after the intravenous injection of radio-calcium lactate. No activity was detectable in the stomach. The exact ratio between the renal and intestinal excretion of calcium and strontium was difficult to determine exactly in mice because of the difficulty of completely separating the feces and the urine. For this reason rats were used, and the daily activity of feces and urine after subcutaneous injection of radioactive strontium lactate is given in Table II in percent of the dose. On the day following a large subcutaneous dose, most of the

FIG. 1.

A B

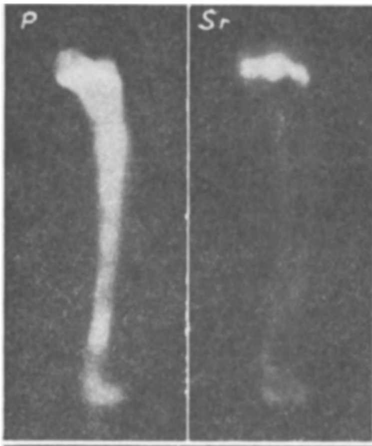
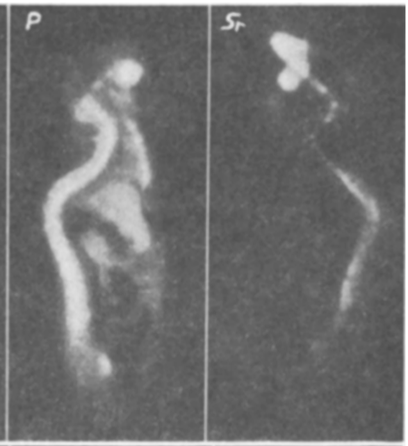


FIG. 2.

A B



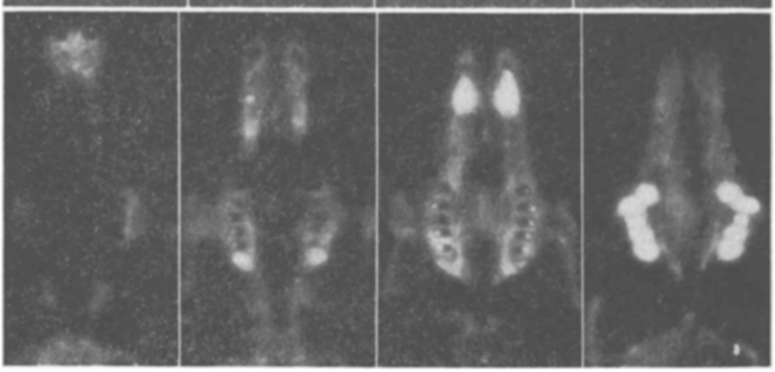


FIG. 3.

FIG. 1. Autoradiographs of the humeri of rabbits five days after intravenous administration of radio-phosphate (A) and of radio-strontium lactate (B). The bone marrow is present in both preparations, and the beta-ray activities of both bones are approximately the same.

FIG. 2. Autoradiographs of whole mice 60 hours after intravenous administration of radio-phosphate (A) and radio-strontium lactate (B). In both pictures the bones and the root of the teeth are strongly radioactive. In A the soft tissues and especially the liver have a high activity. In B the soft tissues contain almost no radioactivity. The intestine contains no radio-phosphorus in A, but in B the colon appears because of the presence of strongly active feces.

FIG. 3. Autoradiographs of successive sections (inferior section to left, superior to right) of the superior maxilla of a rabbit, five days after administration of radioactive strontium lactate showing the distribution of the radioactivity in the various levels in the teeth. The high uptake by the roots is evident.

activity was found in the urine. A few days later about the same amounts were excreted in the feces and urine. The secretion of strontium and calcium in milk will be described in a later paper on the metabolism of strontium during pregnancy.[6]

TABLE II.
Excretion in Feces and Urine of Intravenously Administered Radioactive Strontium Lactate.
(Per cent of dose.)

Rat No.		1st	2nd	3rd	4th	5th	6th	8th day
				Dose: 17 mg Sr.				
47	Urine	18.5	3.36	1.61	.77	.50	.41	.28
	Feces	6.15	7.80	1.85	.63	.54	.45	.20
49	Urine	26.4	4.40	1.37	.71	.33	.45	
	Feces	11.7	13.3	1.52	1.28	.52	.79	
54	Urine	37.8	4.21	2.58	1.38	.95	.64	.26
	Feces	9.65	11.2	5.00	2.22	1.20	.58	.30
				Dose: 2.3 mg Sr.				
50	Urine	13.8	1.6	0.675	.55	.27	.44	.22
	Feces	9.8	6.8	1.67	1.47	1.50	.23	.18
56	Urine	20.8	3.66	0.98	.39	.35	.14	.07
	Feces	7.13	14.0	2.79	.65	.34	.19	.12
58	Urine	20.3	6.17	2.97	.78	.82	.67	.57
	Feces	1.32	6.32	7.21	3.53	1.22	.82	.54

IV. *Comparison of the Uptake of Strontium Chloride, Lactate and Gluconate.* Isomolecular solutions of radioactive strontium chloride, lactate and gluconate were prepared (15 mg strontium per cc). Doses of 0.1 cc of each solution were injected intravenously in 3 groups of 9 adult mice. For 3 other groups of 9 mice, doses of 0.2 cc were introduced in the stomach. After 48 hours, the animals were killed, and the activity of their skeletons measured separately. The average values and the ranges are given in Table III in percentage of the dose fixed in the skeleton.

No significant difference appears whether the strontium is given in chloride, lactate, or gluconate for either method of administration.

Discussion. Tracer studies with radioactive calcium and strontium have shown a close analogy in their metabolism. Although the retained fraction is higher for calcium than for strontium, the distribution of the two elements in different tissues is very similar, the most striking fact being a high uptake in the skeleton. Our observations of a higher concentration in trabecular than cortical bone agrees with the views of Bauer, Aub, and Albright.[7]

TABLE III.
Per Cent of Dose Fixed in the Skeleton in 9 Adult Mice.

	Intravenously			Orally		
	Sr Chloride	Sr Lactate	Sr Gluconate	Sr Chloride	Sr Lactate	Sr Gluconate
Avg	33.1	34.0	33.0	10.2	11.9	10.3
Range	(27.8-40.7)	(22.0-45.5)	(26.6-36.8)	(7.0-15.3)	(3.7-18.0)	(8.5-12.3)

6 Erf, L. A., and Pecher, Charles, in press.

7 Bauer, W., Aub, J. C., and Albright, F., *J. Exp. Med.*, 1929, **49**, 145.

The comparison of the uptake of chloride, lactate and gluconate of strontium shows that the difference, if any, is much smaller than the individual variations. If there is any difference in the uptake of calcium chloride, lactate and gluconate, this difference will probably be of minor importance. This fact can be related to the observation of Greenberg and Gunther[8] that the blood calcium in dogs increases to the same extent whether the same dose of calcium is ingested in chloride, lactate or gluconate. The observation that the uptake in the skeleton is about 3 times higher after intravenous than after peroral administration suggests that approximately one-third of the ingested dose of a calcium or strontium salt is absorbed by the intestine. Strontium and calcium are excreted both in the feces and in the urine.

The selective fixation of radio-strontium in the skeleton, its fairly good yield, the suitable energy of its beta-rays, its convenient half-life and the innocuousness of small doses[9] have given a specific method of irradiation of the skeleton.

Summary. The metabolism of calcium and strontium has been studied on normal animals with the radioactive isotopes of those elements. Uptake in the skeleton of different salts of strontium has been compared. These experiments have furnished two practical findings: (1) a method to irradiate the skeleton selectively for therapeutic purposes, (2) the secondary production of appreciable amounts of a long-lived radioactive yttrium, the most likely among the artificial radioactive elements now known, which may be substituted for radium as a penetrating gamma-ray source.

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Radio-Calcium and Radio-Strontium Metabolism in Pregnant Mice.*

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Studies of calcium and strontium metabolism in mice with

[8] Greenberg, D. M., and Gunther, L., *Arch. Int. Med.*, 1932, **50**, 855.

[9] MacCance, A., and Widdowson. E. M., *Biochem. J.*, 1939, **33** II, 1822.

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