Retention and Turnover of Radiocalcium by the Skeleton of Large Rats.* (18444)

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Radioactive calcium has been employed (1-6) under various conditions in studies of calcium metabolism in the rat. Armstrong and Barnum(5) presented evidence that some of the different anatomical forms of the calcified tissues exchange calcium at unequal rates. Norris and Kisieleski reported(6) that the femora and scapulae of rats given radiocalcium by intravenous injection reached their maximum content of the radioelement in approximately 100 minutes after the injection and showed no significant decrease in the amount retained in 6 to 8 days.

This investigation was conducted to obtain data as to the turnover of calcium in selected calcified tissues of the rat over an extended period of time. A second purpose was to determine the fraction of the dose of radiocalcium retained in the animal at various times after the administration of the radioisotope. For these purposes radiocalcium (Ca^{45}) was administered to rats and the animals were sacrificed at intervals following the administration of the isotope.

Experimental. Thirty-six male rats of the Sprague-Dawley strain of comparable age whose weights lay between 250 and 269 g, averaging 265 g, were given intraperitoneal injections of a high specific activity† radiocalcium solution. Each rat received 3 injections of 2 ml of a solution of the isotope in isotonic saline at 2 hour intervals. The average dose of radiocalcium per animal was approximately 8.3 x 10^5 counts per minute, as determined by our methods of sample preparation and of radioactivity measurement. The animals were sacrificed in pairs at intervals varying from 1 to 180 days after the injections with the exception that at 43 days and at 170 days 5 animals were killed. Twenty-four hours before sacrifice the animals were placed in metabolism cages and the urine collected. On 5 occasions fecal collections were made at the same time. While in the metabolism cages the animals were fed a diet devoid of calcium in order to eliminate contamination of the excreta with the calcium of spilled food and to reduce the amount of unabsorbed food calcium in the alimentary tract. The femora, humeri, the 3 lower lumbar vertebrae and teeth were obtained by dissection and freed of soft tissues, a process which was facilitated by boiling the specimens in water. Both epiphyseal ends of the femurs were cut off using a rotating dental disk through recognizable anatomical landmarks. The marrow was removed from the femur diaphyses by scraping and by use of a pledget of cotton on a probe. The 4 epiphyseal ends of the femurs of a single animal were pooled as one sample and the two diaphyses were united as another sample. The humeri were combined as one sample as were the vertebrae. The pooled molar teeth and the pooled incisor teeth were treated as separate samples. The remainder of the animal not present in the above named calcified tissues constituted the carcass residue.‡ The carcass residues of the first 28 animals to be sacrificed were heated

* This study was supported by a grant from the Research Grants Division of the U. S. Public Health Service.

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† Specific activity 80 mc/g Ca. The radiocalcium was obtained from The Oak Ridge National Laboratory on allocation from the U. S. Atomic Energy Commission.

in 200 ml of strong sodium hydroxide solution until disintegration of the soft tissues had been effected. The mixture was then converted into a uniform soap-like suspension in a Waring blendor, a process which reduced the bones to fine fragments, and 2 large weighed aliquots of the suspension were taken for analysis. The carcass residues from the last 8 animals to be sacrificed were wet ashed in concentrated nitric acid(7) and diluted to an appropriate volume. All other samples, including the urine and feces, were dried, ashed at 700°C. dissolved in hydrochloric acid and diluted to known volumes. Some samples of feces ash required fusion with sodium carbonate before solution in hydrochloric acid.

Total calcium was determined by the method of Clark and Collip(8). The calcium in appropriate aliquots of the solutions was precipitated as calcium oxalate monohydrate at pH 5(9). The precipitates were collected as "infinitely thick" samples§ using the apparatus of Armstrong and Schubert(10) and the radioactivity measurements were made with a thin mica window Geiger-Mueller counter. All measurements for radioactivity were corrected for background, resolving time losses, radioactive decay and for changes in counter efficiency. The reproducibility of the radioactive assays is indicated by the results obtained with duplicate aliquots of the carcass residues which agreed within the statistical variation of the count, namely 1.5%. The total calcium content and the fraction of the administered radiocalcium remaining in each animal were calculated from the sum of the results obtained with the carcass residue and the separately examined tissues. The specific activity of each sample describes the results of the division of the percentage of the injected dose of radiocalcium found in the sample by its total calcium content expressed in milligrams.

Results and Discussion. The average result obtained with each of the tissues and with the urine of the animals sacrificed at each time interval is presented in graphic form. The calcium specific activities of the femoral diaphyses, femoral epiphyses, humeri, lumbar vertebrae and total skeletons were considerably different on day 1, but decreased with time to nearly identical values on the 52nd day (Fig. 1). The finding that, in 52 days, there was effected a virtual equality of distribution of the radioisotope in the calcium of the separately examined bones, and within the skeleton as a whole, shows that an intraskeletal redistribution of the exchangeable calcium occurs in the same period.

After the 52nd day the specific activities of the calcified tissues decreased at a much reduced rate (Fig. 1). The decline in specific

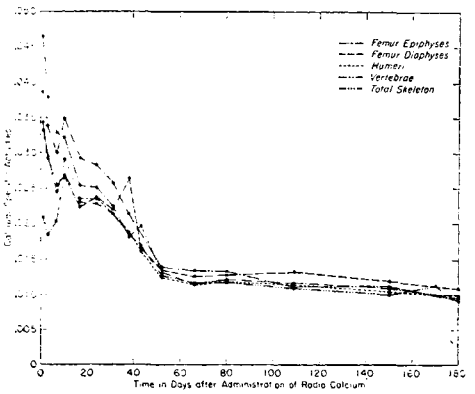

FIG. 1.

Changes in calcium specific activity of selected bones and of whole skeleton of rats following administration of radioactive calcium.

‡ Since the calcium content of extra- and intracellular fluids is very low in comparison with that of the skeleton the calcium of the carcass residues can be considered to have been mainly situated in the bones other than those selected for separate examination.

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§ It has been found in this laboratory that an infinitely thick sample for Ca^{45} beta radiation is 50 mg cm^{-2}. Inert calcium was added, before precipitating calcium oxalate, in those cases in which the amount of this element contained in the aliquot of the solution of the tissue was insufficient to give an infinitely thick layer of calcium oxalate.

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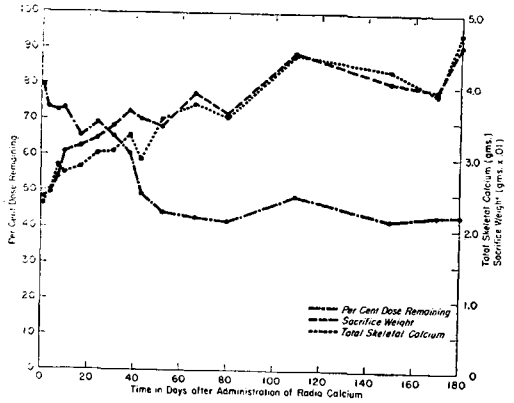

FIG. 2.

Body weight growth and accretion of skeletal calcium in rats. Retention of radiocalcium by rats following intraperitoneal administration.

activities which did occur after the 52nd day was not due, in any important degree, to loss of radiocalcium from the skeleton by excretion since, from the 52nd day onwards, the fraction of the administered dose retained in the animals was maintained very nearly constant at about 42-45% (Fig. 2). Radiocalcium was found in all samples of excreta examined. However, the amount present in 24-hour samples of urine on 8 occasions between the 50th and 180th days averaged only 0.006% and never exceeded 0.013% of the injected dose of radiocalcium. Each of 6 one-day fecal collections made after the 42nd day contained 0.02-0.03% of the administered radioisotope. The reduction in specific activity of the skeleton and its components which occurred between the 52nd and 180th days was due mainly to dilution of the radiocalcium, which remained nearly constant in amount, with the normal isotope of calcium gained by the skeleton as it grew. The decrease in measured specific activity of the whole skeleton after the 52nd day was 26.0% (from 0.0125 to 0.0093) as shown in Fig. 1. In the same time the amount of calcium which accrued to the animals (Fig. 2) was 25.8% of the quantity present at 180 days. The same line of reasoning leads to the conclusion that a part of the rapid reduction in skeletal specific activities occurring before day 52 was also due to bone growth since, as seen in Fig. 2, 34.1% of the skeletal calcium present at day 52 was acquired after day 1. It can be calculated, from this figure and from the fact that 44.4% of the Ca^{45} present on day 1 was excreted by day 52 (Fig. 2), that 29.9% of the measured reduction in skeletal specific activity occurring between days 1 and 52 was due to skeletal growth and the remainder to excretion of radiocalcium. These considerations emphasize the importance of taking into account changes in amount of a tissue constituent, as may occur in a relatively short time in rats by growth, in studies of the turnover of the constituent with tracer isotopes.

The observations reported in this paper indicate that the radiocalcium was deposited in the bones of the animals in forms which differed greatly in their relative abilities to permit mobilization and excretion of the labelled calcium. These forms of incorporation of radiocalcium and the parts of the skeletal calcium associated with each may be referred to as the mobile fraction and the fixed fraction. The fixed radiocalcium is that fraction, amounting to *circa* 45% of the injected dose, which was maintained with only a slight excretion after the 52nd day. It is suggested that the fixed radiocalcium was that which eventually became incarcerated by bone growth, or by rearrangement of the calcium atoms in the bone salt, in positions so remote from the body fluids as greatly to interfere with its mobilization and excretion. Since the specific activity of the several bone types was maintained at quite similar values after the 52nd day (Fig. 1), it can be concluded that equal weights of bone calcium had, by the 52nd day, incorporated fairly uniform amounts of radiocalcium in the fixed form and that this relationship was not disturbed by further bone growth. The amount of radioisotope deposited per unit weight of bone calcium differed originally among the bone types. However, by the 52nd day all of the easily mobile or exchangeable radiocalcium had been excreted‖ or converted into the fixed form, thus reducing the specific activities of all bones to uniform values. After the 52nd day the excretion of radiocalcium occurred only from the difficultly mobilizable bone calcium. The

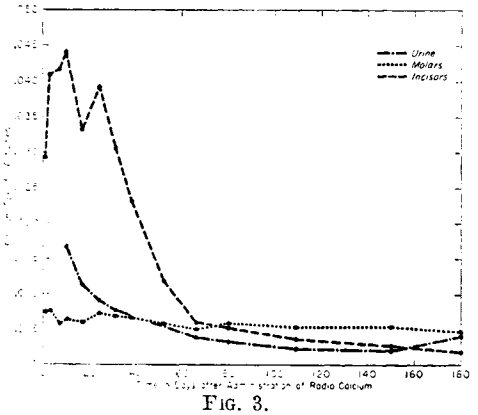

FIG. 3.

Changes in calcium specific activity of teeth and urine of rats following administration of radiocalcium.

results obtained from the urine examinations indicate that this form of bone calcium was excreted at a constant low rate.

Results as to the specific activity of the urine specimens collected before day 10 and on day 38 are not reported because of errors in the determinations of total calcium due in some cases to the very low calcium content of the urine specimens. However, radiocalcium assays demonstrated the presence of an average of 4% of the injected dose in the urine secreted on day 1 and that the average amount in the daily urine rapidly decreased to 0.032% on day 3 and to 0.015% on day 10. From the 66th day onwards the specific activity of the urine was maintained essentially unchanged at a value approximately one-fourth that of the skeleton (Figs. 1 and 3). Since the calcium of the urine is derived from that of the plasma it can be assumed that the specific activity of the calcium of the body fluids, including the saliva, paralleled that of the urine(11).

The specific activities of the molar teeth were low and showed a gradual decline to a value which on day 180 was about 60% of that on day 1 (Fig. 3). It is probable that these non-growing organs acquired some part of their radiocalcium by exchange of calcium between the enamel and the saliva when the saliva had a very high specific activity immediately after the injections. The incisor teeth of rats are known to be worn away at the incisal edge and to be renewed by growth at the root end at approximately 50 day periods(12). On this account the incisor teeth acquired much more radiocalcium than the molar teeth during the period of high specific activity of the calcium of the body fluids. The radiocalcium fixed in the incisor teeth was mainly lost by attrition as is shown by the fact that their specific activities rapidly declined from the 10th to the 66th day, a period of time which is in agreement with the expected time for the replacement of the teeth. After the 66th day the specific activity of the incisor teeth, as they continued to be formed, was maintained very nearly equal to that of the urine and hence to that of the plasma.

As shown in Fig. 2, the calcium content of rats, exclusive of the teeth, is close to 1% of the body weight in animals weighing between 249 and 465 g.

Summary. The calcium specific activities of the femoral epiphyses, femoral diaphyses, humeri, lumbar vertebrae and the remainder of the skeleton of large rats differ soon after the administration of radiocalcium but decrease to a common value after 52 days, following which the specific activities decline at equal but much reduced rates. The fraction of the injected dose of radiocalcium retained in the animals declines to 42-45% on the 52nd day, after which only small quantities of radiocalcium are excreted. The results indicate the existence in large rats of two kinds of skeletal calcium which differ markedly in their rate of turnover and in the length of time over which they fix radiocalcium. The apparent reduction in skeletal calcium specific activity occurring in large rats after the 52nd day following the injection of radiocalcium is due to accretion of calcium to the

[1] The radiocalcium content of the freely exchangeable bone fractions would not be expected to be zero after the 52nd day but would correspond to the specific activity of the body fluids. There is evidence (vide infra) that the latter specific activity was maintained, at least from the 66th day onwards, at a low and uniform value.

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skeleton. Part of the decline in skeletal specific activities which takes place in rats before radiocalcium excretion ceases is also due to skeletal growth.

Data as to the changes of calcium specific activity of the urine and the teeth following the injection of labelled calcium are presented and discussed.

Received November 27, 1950. P.S.E.B.M., 1951, v76.

Toxicity of Glycine for Vitamin B_{12}-Deficient Chicks.*† (18445)

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An unidentified factor which was reported to stimulate growth of chicks fed a vegetable protein diet also lowered the blood non-protein nitrogen level(1). It was later found(2) that a vit. B_{12} concentrate lowered the blood non-protein nitrogen of chicks previously fed a vit. B_{12}-deficient diet. Non-protein nitrogen and urea were found to be higher in zoopherin (vit. B_{12})-deficient rats than in normal rats (3). These and other reports(4,5) indicated that vit. B_{12} is concerned with nitrogen utilization and metabolism. It was the purpose of this experiment to investigate further the vit. B_{12}-nitrogen relationship by feeding chicks a known amount of non-protein nitrogen and observing the effect of vit. B_{12} deficiency.

Procedure. Twenty-eight day-old White Leghorn chicks which had been fed the vit. B_{12}-deficient diet shown in Table I from the day of hatching were used in this experiment. A deficient control group received no treatment, whereas 3 μg of vit. B_{12} per week were injected subcutaneously into the chicks of the supplemented group. The night of the 28th day food was withdrawn from chicks in both groups. The following morning blood was taken from 4 birds of each group by heart puncture, and the birds sacrificed. Protein-free filtrates were prepared from pooled samples of the plasma by tungstic acid precipitation. The filtrates were analyzed for nitrogen by a semi-micro Kjeldahl method and for amino nitrogen using the naphthoquinone-sulfonic acid method(6). Immedi-

TABLE I. Experimental Diet.

	%
Ground yellow corn	43.5
Ground wheat	15
Soybean oil meal (solvent)	37
Dehydrated alfalfa	1
Ground limestone	1.5
Dicalcium phosphate	1.5
Sodium chloride	.5
A and D supplement*	.1
Choline chloride	.1
Methionine	.1
$MnSO_4$	8 g/100 lbs.
	mg per lb.
Riboflavin	4.5
Ca pantothenate	7.5
Niacin	15.0
Thiamin	2.5
Pyridoxine	2.5
Biotin	0.045
Folic acid	0.45
Inositol	4.53
Menadione	4.5
Alpha tocopherol	4.5
Para amino benzoic acid	2.5

* 5,000 I.U. vit. A and 1,000 AOAC chick units vit. D per g.

* This investigation was supported in part by funds provided for biological and medical research by the State of Washington Initiative 171.

† Published as scientific paper 964, Washington Agric. Exp. Stations, Inst. of Agric. Sciences, State College of Washington, Pullman.

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