Strontium/Calcium Ratios in Parts of Rat Femur.* (24127)

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Behavior of strontium in the animal body has become of particular interest in relation to possible hazards from atomic fission debris. The body burden of radiostrontium in terms of body calcium, attained after ingestion of contaminated material over long periods of time, will be governed by Sr/Ca ratio of the intake and by the over-all discrimination between strontium and calcium. Of the many metabolic processes in which such discrimination occurs, this investigation deals only with the discrimination observed in particular parts of a long bone, as studied in rats using double tracer experiments with Ca^{45} and Sr^{89}.

Methods. Young female rats of Wistar strain, weighing 160-200 g, and grown on normal stock diet, were given a single intraperitoneal injection of a mixture of Ca^{45} and Sr^{89}. The 0.5 ml dose contained about 8 microcuries each of Ca^{45} (carrier-free) and Sr^{89} (containing less than 10% Sr^{90}), and 2.8 mg of Ca as $CaCl_2$. Animals were killed at intervals of 15 minutes, 30 minutes, 1 hour, 2, 4, 8, 24, and 48 hours after injection, with 3 to 6 rats in each group. A terminal caval blood sample was taken with most rats. Both femurs were removed, cleaned of adherent muscle, cut transversely into thirds, pooled to make comparable samples of proximal ends, distal ends, and shafts for each group of animals. After weighing, each bone sample was dried at 105°C for 24 hours and then ashed at 600°C for 24 hours in a muffle furnace. The weighed ash was ground and aliquots taken to prepare oxalated samples with a plastic-tube-and-cup assembly as described by Comar(1). The 2 isotopes were measured by usual methods for radioassay of mixtures based on differential absorption of the beta emission. An aliquot of a certain dilution of the injection solution was oxalated with 40 mg of normal bone ash and carriers exactly like the experimental samples and constituted the standard against which all measurements were compared. All samples were counted after a 21-day delay to allow the unknown amount of Sr^{90} present to reach equilibrium. Blood serum calcium was determined by standard volumetric permanganate titration methods adapted for our purposes, the sample re-oxalated and a radioassay done. Radioactivity in serum was recorded as counts/minute of Ca^{45} and Sr^{89}/mg of serum calcium. The results are expressed as strontium/calcium Observed Ratio (OR bone/injection solution) as suggested by Comar *et al.*(2): OR bone-injection sol. $= \frac{Sr^{89}/Ca^{45} \text{ in bone}}{Sr^{89}/Ca^{45} \text{ in inj. soln.}}$.

Results. The Observed Ratios of the 3 parts of femur, *viz.*, distal ends, proximal ends, and shafts, are shown in Table I and graphically illustrated in Fig. 1. A maximal strontium content appeared early in all samples with the shaft samples, indicating actual discrimination against calcium. During the following 48 hours, the OR's gradually fell in all bone samples but remained consistently higher in samples of the shaft. Specific activity of both Ca^{45} and Sr^{89} (counts/minute of Ca^{45} or Sr^{89}/mg stable Ca) reached a maximum in distal and proximal growth areas between 4 and 8 hours after injection, and was always higher than in samples of shaft bone.

In the right portion of Fig. 1, sharpest rise in OR was in femur shaft and serum, with drop for serum a rapid one from 15 minute to 4 hour interval, while the shaft OR showed a second peak at 4 hours before it gradually fell off. The initial peak in the shaft OR curve at 30 minutes is questionable since the S.D. for that group of animals was large; actually the curve could have been a gradual rise from 15 minutes to 4 hours, with the fall beginning at the 4 hour interval.

The Observed Ratio appeared to fit a straight line with time from 4 to 48 hours. Accordingly, straight lines were fitted and

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TABLE I. Sr^{89}/Ca^{45} Observed Ratios in Pooled Femur Parts, and in Blood Serum at Intervals after a Single Intraperitoneal Injection of Ca^{45} and Sr^{89}.

Time	Distal ends		Shafts		Proximal ends		Serum	
15 min.	*.820	.116	.864	.112	.820	.115	.732	
30	.901	.031	1.157	.328	.896	.081	1.000	.954
1 hr	.794	.064	.940	.063	.788	.115	.938	.036
2	.735	.097	.907	.074	.734	.138	.887	.100
4	.651	.014	1.026	.019	.627	.050	.460	.114
8	.588	.069	.975	.034	.627	.034	.406	.054
24	.576	.003	.835	.148	.565	.044	.444	.063
48	.487	.088	.67	.070	.516	.040	.334	.018

* Mean and stand. dev. of mean.

the following slopes (with upper and lower 95% confidence limits for the true slope) were recorded (Table II).

A straight line was not a good fit to Sr^{89}/Ca^{45} for blood serum, the data indicating slight evidence for a decrease in Sr^{89}/Ca^{45} with time: but the decrease was not significant because of the variability. Slopes and confidence limits for bone show that there was a significant negative decrease of Sr^{89}/Ca^{45} with time for distal femurs, femur shafts, and proximal femurs. The slope in all cases represented the average decrease in Sr^{89}/Ca^{45} for each hour from 4 to 48 hours. The decrease for femur shafts was significantly more (at 95% level) than that for proximal femurs and was very nearly significantly more than that for distal femurs and whole femurs.

Discussion. Accumulated experimental evidence (Bauer *et al.*(3); Comar, *et al.*(4)) indicates that with regard to skeletal accretion and exchange, there is no marked difference between calcium and strontium. The differences in retention which appear in both single and multiple dose studies were due to preferential excretion of strontium by the kidneys (Talmadge(5), MacDonald(6)). The observed ratios reported in the present single dose experiments show an expected discrim-

TABLE II.

	Lower 95% confidence limit	Slope	Upper 95% confidence limit
Distal femur	−.0045	−.0032	−.0019
Femur shafts	−.0120	−.0081	−.0043
Proximal femurs	−.0040	−.0027	−.0014

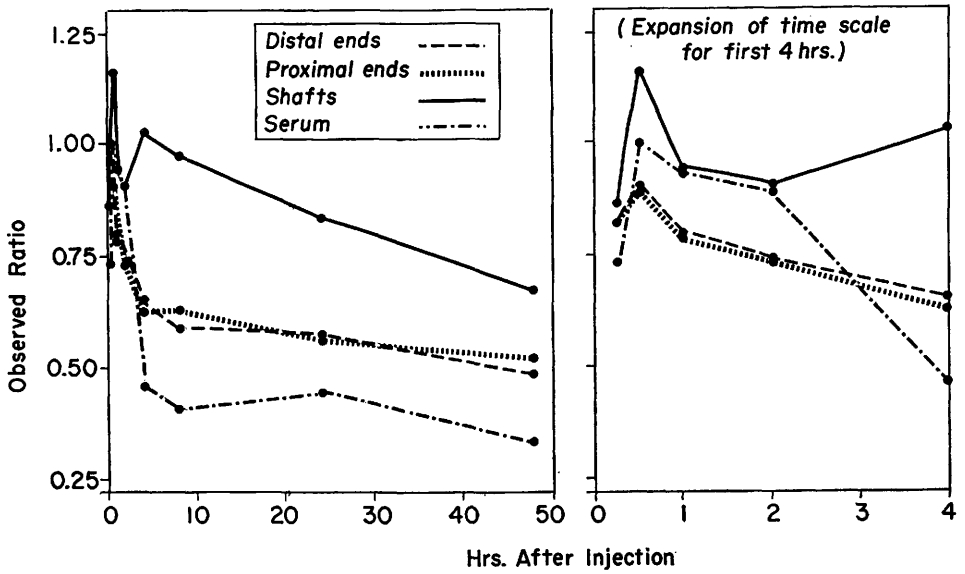

FIG. 1. Sr^{89}/Ca^{45} observed ratios for parts of femur and for blood serum plotted against time after a single inj. of a mixture of Sr^{89} and Ca^{45}.

ination against strontium with time. It is inferred that the observed discrimination occurs in the kidney and that endogenous excretion *via* the gut is negligible in this case. The point to which attention is directed is the differential observed between parts of long bones in the rat under the experimental conditions used. The more rapid turnover of the radioisotopes in processes of accretion, exchange, and resorption in the epiphyseal ends of the femur as contrasted with the less actively growing shaft leads to a preferential loss of strontium in the ends of the femur as compared with the shaft. It is conceivable that vascular arrangements in the epiphyses as compared with the shaft play a role in the discrimination observed.

Summary. After intraperitoneal injection of a mixture of Ca^{45} and Sr^{89} in the rat, the Sr/Ca Observed Ratio in parts of a long bone, the femur, was obtained 15 minutes to 48 hours after injection. A maximal Sr content appeared in bone within 30 minutes, with the shaft showing discrimination against Ca; during the next 48 hours the Observed Ratios fell but remained consistently higher in shaft portions of the bone. Data on blood Sr/Ca ratios are also presented.

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Effect of Phenethylbiguanide on Adrenal Function and Responsiveness as Measured by ACTH Test.* (24128)

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The quest for an oral hypoglycemic agent has received considerable impetus since reintroduction of the aryl-sulfonylureas by Franke and Fuchs(1), extending the work that Janbon(2) and Loubatieres(3) had begun 13 years earlier. Unger, Freedman and Shapiro (4), reviewing a group of potentially hypoglycemic drugs, discovered N B′ Phenethylformadinylimourea (DBI)†, a biguanide. DBI has been used by Pomeranze(5,6), Williams, *et al.*(7), and in our own clinic (unpublished) in treatment of hyperglycemia in man. It lowers blood sugar concentration in patients with diabetes mellitus but the moderately severe side effects of nausea and anorexia have precluded its continued use in many. The mechanism by which biguanides act is unknown. Since adrenal steroids can affect blood glucose level by increasing gluconeogenesis and decreasing glucose utilization, it was believed that one action of DBI might involve the blockade of adrenal steroid production. Our studies were designed to measure the effect of DBI on adrenocortical secretion of hydrocortisone and demonstrate either a decrease in or a blockade of adrenal cortical activity by comparing baseline plasma hydrocortisone levels with levels after DBI, and by comparing baseline responses of adrenal gland to pituitary stimulation (ACTH test) to responses to ACTH following DBI therapy.

Methods. The 6 subjects, all on wards of St. Luke's Hospital had recognized diabetes mellitus for 1 month to 20 years and were well controlled on 20 to 80 units of insulin/day. These patients received DBI for 8 to

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