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Effect of Low Sodium Diet on Sodium Content and Radiosodium Exchange in Rat Bone.* (23971)

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Skeletal tissues are rich in sodium, a part of which readily exchanges with radiosodium. In recent years a number of workers have been interested in studying the degree of lability of this skeletal component under a variety of experimental situations. Among these is the effect of a low sodium diet.

Orent-Keiles, Robinson and McCollum(1) noted that the bones of rats reared on a low Na diet were soft in consistency. Bergstrom and Wallace(2) found that the bones of young rats kept on a low Na diet for 2 weeks contained only half as much Na as the controls. More recently Munro, Satoskar and Wilson (3) in a study of adult rats observed a decrease of 10% in bone Na content during a 14 week period of low Na feeding. The latter workers also determined radiosodium exchangeability in the bones of their animals, and finding no change concluded that the Na lost from bone came from the non-exchangeable fraction.

The present study was undertaken to investigate this problem.

Experimental. Twelve 20-day-old male albino rats of the Rochester strain were fed Big Red dog pellets and tap water for 10 days, at which time they were divided into 2 equal groups according to a table of random numbers. The flip of a coin then decided which group was to receive the low Na diet. The diet was constructed by blending Crisco 300 g, Lonalac 600 g, rolled oats 2070 g, brewer's yeast 30 g, $CaCl_2$ 12 g, $CaHPO_4 \cdot 2H_2O$ 11.4 g, and polyvisol 1.8 cc. On analysis it was found to contain 0.011% Na, 0.7% Cl, 0.42% K and 0.44% Ca. For the control group 60 g of $NaHCO_3$ was added, bringing the Na concentration to 0.55%. Distilled water was provided for drinking.

Since preliminary trials had indicated that food consumption was poor on the low Na diet, it was decided to pair-feed the 2 groups. Records of food consumption were kept, and the control group offered only as much food as the average consumed by the low Na group.

The experimental period lasted 4 weeks. The animals were then given radiosodium^{22} (2.5 μc/100 g) by intraperitoneal injection, and 2½ to 4 hours later killed by decapitation. The long bones were dissected free of adherent muscle and periosteum, the ends clipped off, and the marrow removed. Bone water was determined by drying *in vacuo* at 70°C. The dried samples were then ashed at 525°C and the ash taken up in a small amount of 2 N HCl. Sodium content was determined in this ash solution by means of a Baird internal standard flame photometer equipped with a Na III-Li II filter and a manufactured gas burner, with natural gas as a fuel. Using this apparatus we found that interfering substances present in bone produce a small, and constant, error in sodium readings over a concentration range of 0.1-0.4 meq/l. Serum sodium was determined by flame photometry, and chloride by Volhard titration. Radiosodium was determined in a scintillation counter.

In view of the uncertainty regarding the position of Cl in bone, the total Na con-

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tent of bone was partitioned into 2 phases —*fluid* Na and *crystal* Na—on the basis of bone water content rather than the calculated Cl space(4). By this method of calculation, fluid Na = serum Na x bone H_2O x 0.942/0.93, and crystal Na = total Na – fluid Na. where 0.942 is the Donnan factor and 0.93 the assumed serum H_2O content. Radiosodium values were treated in a similar manner, and on the assumption that fluid Na of bone had the same specific activity† as serum, the specific activity of the crystal Na fraction could be calculated. Radiosodium exchangeability was then expressed as the ratio: S.A. bone crystal Na /S.A. serum Na.

Results. Table I lists body weights for the 2 groups of rats. The low Na group grew more slowly than controls, and by end of the third week had stopped growing altogether. Values for various measured constants in bone and serum are listed in Table II. There was a modest decrease in concentration of sodium in both serum and bone. This decrease involved both calculated fluid Na and crystal Na phases. Radiosodium exchangeability also decreased in response to the low Na diet and it should be noted that percentage decrease in exchangeability was equal to decrease in crystal Na content. Thus the observed change in crystal Na involved only the exchangeable fraction, and there was no detectable change in the non-exchangeable fraction.

TABLE I. Body Weight, g.

Age (days)	Control group	Low Na group
30	69 ± 13*	74 ± 10
50	135 ± 15	102 ± 14
58	150 ± 13	106 ± 13

* Stand. dev.

Discussion. It has long been known that a certain minimum intake of Na is necessary for normal growth in the rat(1). Evidently the degree of Na restriction in our experiments was sufficient to interfere with growth.

The principal question which these experiments were designed to answer was whether the newly forming bone in the young, rapidly growing animal would contain less than normal amounts of Na, and further to decide whether exchangeable or non-exchangeable fraction of bone crystal Na would be involved in this change.

The magnitude of the change in bone Na content was comparable to that observed by Munro *et al.*(3) in older rats fed a low Na diet, but distinctly less than that observed by Bergstrom and Wallace(2) in young rats. Changes in serum Na were comparable in all 3 investigations. In constructing their low Na diet the latter authors substituted NH_4^+ ion for Na in the incorporated salt mixture, and perhaps this accounted for the larger decrement in bone Na in their animals.

From our data it would appear that the difference in bone Na content in the 2 groups of animals can be accounted for in the exchangeable fraction of crystal Na. This is quite in keeping with observations made in our laboratory on bone Na changes during diabetic acidosis, in which decrease in crystal Na was matched by a decrease in radiosodium exchangeability(5). Munro *et al.*(3) on the

TABLE II. Serum and Bone Composition.

Constituent	Control group	Low Na group	Difference (%)	"P"
Serum Na, meq/l	145 ± 1 *	133 ± 2	− 8	
" Cl, "	101 ± 1	93 ± 2	− 8	
Bone H_2O, %	29.2 ± 2.2	27.4 ± 2.2	− 6	
" Cl, meq/g wet wt	.0205± .0028	.0195± .0028	− 5	
Total bone Na, meq/g wet wt	.168 ± .005	.155 ± .006	− 8	<.01
" " " " dry "	.237 ± .003	.214 ± .006	−10	
Crystal Na, meq/g dry wt	.176 ± .007	.163 ± .007	− 7	"
S. A. crystal Na/S. A. serum Na	.49 ± .04	.43 ± .02	− 6	"
Exch. crystal Na, meq/g dry wt	.086	.070	−19	
Non-exch. " " " " "	.090	.093	+3	

* Stand. dev.

† counts/min./meq. Na.

other hand observed no change in radiosodium exchangeability in their animals, and reached a conclusion quite different from ours—namely that the decrement in bone Na was due to a change in the non-exchangeable fraction. Munro *et al.* used a 24-hour period for radiosodium equilibration whereas we employed a much shorter time.

On the basis of our data it would seem that the division of bone crystal Na into two phases—exchangeable and non-exchangeable—has a physiological justification. In the present experiments, and those of Lobeck and Forbes(5) on diabetic acidosis, it was shown that Na lost from bone came from the exchangeable fraction.

Summary. Young rats, reared on a low Na diet, manifested growth failure, and their bones contained 8-10% (depending on method of calculation) less Na than pair fed controls. Radiosodium exchangeability declined by a similar amount, and it is concluded that the change in Na content involved the exchangeable fraction.

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Effect of Low Potassium Diet on Cyclopropane-Epinephrine Ventricular Tachycardia and Arterial Plasma Potassium in Dogs.* (23972)

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Meek *et al.*(1) observed that epinephrine in doses which cause only occasional ventricular extrasystoles in unanesthetized dogs produced ventricular tachycardia during cyclopropane anesthesia. O'Brien *et al.*(2) found that the onset of arrhythmia was associated with a rapid and marked rise in arterial plasma potassium. Since liver is the primary source of potassium mobilized by epinephrine (3,4), O'Brien *et al.*(5) eliminated the hepatic circulation surgically and found a smaller rise in potassium as well as protection from arrhythmia in 17 of 18 dogs.

This study was undertaken to seek a non-surgical method for reducing the rise in arterial plasma K that normally results from an epinephrine injection and to determine whether or not any K changes that might be found could be related to the occurrence of cyclopropane-epinephrine ventricular tachycardia.

Methods. After administering cyclopropane to dogs for 30 minutes, 0.005 mg/kg epinephrine in 5 ml isotonic sodium chloride was injected intravenously at a constant rate of 1 ml per 10 seconds. Two control determinations of the occurrence and duration of ventricular tachycardia were made one week apart and with the second determination, the changes in arterial plasma potassium concentration were studied. Effects on cardiac rhythm were determined from electrocardiographic records. Blood was rapidly withdrawn from the femoral artery into heparinized syringes before and at intervals of 20, 40, 60, 80, 100, 120 and 150 seconds after the beginning of the epinephrine injection. Plasma was analyzed for potassium with the Beckman flame spectrophotometer(6). After control studies, 20 dogs were placed on a low potassium diet for 2 to 3 weeks following which the effects of epinephrine on arterial plasma potassium and cardiac rhythm were again noted.

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