Response of Bone Sodium to Acute Changes in Serum Sodium: Effect of Age.† (27039)

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Previous work in our laboratory has failed to show more than a modest decrease in bone sodium content in response to acutely induced changes in extracellular fluid composition(1, 2,3). Most of the experiments reported in the literature have involved a combination of acidosis and hyponatremia, including those of Bergstrom, who was the first to suggest that sodium could be readily mobilized from bone (4,5). Only a few attempts have been made to study the effects of alteration of serum sodium concentration alone. Acute hyponatremia is reported to result in a drop of 0-7% in bone sodium(1,3,6,7,8,9), though Woodbury(10) reports a drop of 19%, while acute hypernatremia fails to cause a rise. Most of these studies have been done on adult animals, and in no instance was the induced change in serum sodium a large one.

Two considerations suggested the possibility that changes might be produced more readily in young animals. Bergstrom found that a combination of acidosis and hyponatremia produced a larger decrease in bone sodium in younger animals. Forbes *et al*(11) and Munro *et al*(12) reported that radiosodium exchange occurred more rapidly in young bone. We therefore undertook a study of rats of different ages with respect to the effects of acute hypo- and hypernatremia.

Methods. Albino rats aged 20, 40 and 90 days were used. The 40 and 90 day old animals (males) were fasted overnight but allowed water. The 20 day old animals (mixed sexes) were removed from their mothers one day prior to experiment and fed Purina fox chow. They were fasted only during the experiment. An attempt was made to select animals of comparable weight in each group.

Approximately one-third of the animals in each group served as *controls*. One-third were rendered *hypernatremic* by repeated intraperitoneal injections of 2% NaCl (360 mM/l.). The injections were given hourly for 3-4 hours whereupon the animals were killed by decapitation. Two of the 40 day old animals became moribund within 2 hours, and were killed at that time. The volume given at each injection was 10% of body weight, so that the total sodium load was 110-140 meq/Kg, or substantially more than the total sodium content of the animal. The remaining animals were rendered *hyponatremic* by a similar treatment with 5% dextrose solution given intraperitoneally. Some leakage of peritoneal fluid occurred in these animals. Most of the animals appeared moribund at the end of the experiment. Blood for sodium analysis was obtained by decapitation.

Analysis of Bone. The long bones were used. Prior to dissection the lower extremities were dipped in distilled water to remove contamination with peritoneal fluid. The long bones were then removed, cleaned of periosteum and marrow, and the epiphyses clipped off. The resulting sample of marrow-free cortical bone was weighed as soon as possible. Analysis for water, ash, and sodium were made according to previously described methods(3).

Results are set forth in Tables I and II and Fig. I. The magnitude of the induced changes in serum Na (extreme range 66-246 meq/l.) is evident from the figure. In the control animals, bone Na and bone ash content progressively rose with age, while bone H_2O content fell, in accordance with previous work(3,11). Neither bone H_2O nor bone ash content changed significantly in the experimental animals, despite the large loads of water and salt administered.

No attempt was made to calculate average content of total bone sodium for the experimental groups in view of the variations in serum Na concentration which occurred. Rather, a plot of total bone Na against serum Na for each animal is included in Fig. I, to-

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TABLE I. Analytical Data for Bone Composition.

Group	No. Animals	Bone H_2O, %	Bone Na, meq/Kg wet wt Total	Crystal Phase	Bone Ash %	Serum Na meq/l
			20 days, 22-36 g			
Water Load	7	47 (44-49)*		46 ±2†	29.9 ±.8†	
Control	8	48 (43-51)	100 ±3†	36 ±4	28.4 ±.7	134 (131-138)*
Salt Load	6	46 (44-48)		40 ±3	29.5 ±.6	
			40 days, 80-110 g			
Water Load	5	27 (26-28)		126 ±3	48.4 ±.5	
Control	4	31 (27-36)	168 ±4	125 ±3	47.9 ±1.2	141 (137-154)
Salt Load	8	28 (24-32)		126 ±4	47.8 ±.8	
			90 days, 210-260 g			
Water Load	6	18 (15-21)		180 ±8	58.8 ±.8	
Control	6	19 (14-22)	205 ±4	177 ±8	57.7 ±.8	144 (138-148)
Salt Load	6	18 (16-20)		179 ±6	59.4 ±.6	

* range † Standard error

TABLE II. Calculated Data for Bone Composition.

Age Group days	Average Bone H_2O, %	Regression slope	y-axis intercept, meq/Kg	"Available" fraction	"Exchangeable" fraction†
20	46.9	.403 ±.031*	50	.52	.80
40	28.2	.287 ±.034*	125	.25	.64
90	18.5	.156 ±.045*	183	.11	.44

* standard error; $p<.01$. † from previous data(3).

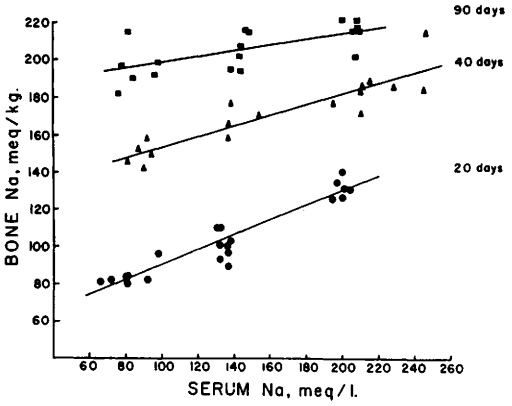

FIG. 1. Plot of bone sodium content against serum sodium for rats of varying age. Calculated regression lines are included.

gether with the calculated regression lines for each group. The bones of the hypernatremic animals contain more Na, and those of the hyponatremic animals less Na than those of the controls in each of the 3 age groups. Moreover, the regression slope becomes progressively less steep with advancing age. Thus the observed change in total bone Na resulting from a given increment or decrement in serum Na is a function of age.

The parameters of the 3 regression equations are listed in Table II. Each of the slopes is significantly different from zero.

The regression slope $\frac{(\triangle \text{ bone Na})}{(\triangle \text{ serum Na})}$ can be taken to represent the sodium "space" of bone; multiplication by 100 yields a value for percentage change in bone Na for each 100% change in serum Na. It is of interest to compare these values with those for bone water content. For the 20, 40 and 90 day old animals, respectively,

Na "space" is 85.9%, 101.8%, and 84.3% (avg. 90.7%) of bone water. The standard error of these values (5.6%) is such as to permit the conclusion that calculated Na "space" does not differ significantly from measured bone water content.

Extrapolation of the regression line to zero serum Na (the y-axis intercept) yields values which agree fairly well with calculated "crystal" phase Na (Table I). "Crystal" phase Na was calculated by subtracting "fluid" Na (serum Na x bone H_2O) from total bone Na. Corrections for serum H_2O content and the Donnan factor were omitted since the 2 nearly cancel out. The chloride "space" of bone was not used since it has been shown that this often exceeds total bone water(3). Dosekun reports that inulin space is 57% of bone H_2O(9). No change occurred in "crystal" phase Na as a result of the experimental procedure; thus, the observed changes in total Na content must be ascribed to those taking place in the "fluid" phase.

A further calculation can be made; namely, the fraction of the total Na of bone which is subject to the effects of alterations in serum Na concentration. On the assumption that the relationship between total bone Na and serum Na is linear, the ratio of calculated sodium "space" Na to total bone Na at normal serum Na concentration is derived from the regression equation for each age group. This ratio is termed the "available fraction" in Table II. The magnitude of this fraction is clearly a function of the age of the animal.

Discussion. We have shown previously that radiosodium exchange is 2-3 times greater in weanling rat bone than in the adult. The present work shows an age effect of similar magnitude for that fraction of bone Na which responds to alterations in serum composition. Table II includes a calculation of radiosodium exchangeability (ratio specific activity bone to serum) for rat bone of comparable water content, based on data previously reported(3). In the older animals only about a quarter of the exchangeable fraction can be classed as readily "available" to the extracellular fluid; the value for the weanlings is about two-thirds.

In the present experiments the observed changes in bone Na were limited to that portion contained in what we have termed the "fluid" phase. There was no evidence for alteration in "crystal" phase Na. The sodium of this latter phase must be tightly bound in some fashion to the bone solid. If it is the hydration shell about the crystals, the concentration must be exceedingly great, as the following calculation will show. For the 3 groups of animals, Na "space" averaged 90.7% of bone water. If all of the "crystal" phase Na is in the remaining water, its concentration would be 1,100, 4,700, and 10,400 meq/l. for the 20, 40 and 90 day animals, respectively. The latter value (61% saline) exceeds the solubility of NaCl in water by a factor of almost two. Values such as these could be used to support the hypothesis that "crystal" phase Na is indeed located on the crystal surface, as Neuman and Neuman have suggested(13).

It should be emphasized that our results pertain only to the effects of *acute* changes in extracellular fluid. In experiments of longer duration, inevitably involving some growth of bone (at least in the rat), changes in "crystal" phase Na might well occur. Such procedures as chronic acidosis, or adrenalectomy, might also produce effects on bone matrix.

In designing this experiment we deliberately set out to provoke extreme changes in serum Na concentration. With these data at hand, it is possible to predict the degree of change to be expected from any given degree of hypo- or hypernatremia. For example, a reduction of serum Na to 120 meq/l would cause a loss of 5.8% of bone Na in weanling animals, but only 1.5% in adults. The latter decrement is too small to detect, and this may account for the fact that some investigators are able to report small changes, and others no change. Most workers, in studying hyponatremia, have usually not lowered serum Na below this level.

Summary and Conclusions. Profound hypo- and hypernatremia were produced in rats of varying age. Resulting changes in bone Na content were much more pronounced in the younger animals, and decreased progressively as the animals became older. Calculated Na "space" of bone averaged 91% of bone water content for the 3 age groups studied. Observed changes in bone Na content could be ascribed entirely to that portion within the "fluid phase" of bone. There was no evidence of a change in "crystal phase" Na. Observed changes in adult

rats were so slight that rather large changes in serum Na would be necessary for their detection.

1. Forbes, G. B., Tobin, R. B., Lewis, A., *Am. J. Physiol.*, 1959, v196, 69.
2. Lobeck, C. C., Forbes, G. B., *Metabolism*, 1958, v7, 133.
3. Forbes, G. B., *J. Pediat.*, 1960, v56, 180.
4. Bergstrom, W. H., Wallace, W. M., *J. Clin. Invest.* 1954, v33, 867.
5. ————, Ruva, F. D., *Am. J. Physiol.*, 1960, v198, 1126.
6. Nichols, G., Jr., Nichols, N., *ibid.*, 1956, v186, 383.
7. Winters, R. W., Whitlock, R. T., De Walt, J. L., Welt, L. G., *ibid.*, 1958, v195, 697.
8. Baker, G. P., Levitin, H., Epstein, F. H., *J. Clin. Invest.*, 1961, v40, 867.
9. Dosekun, F. O., *J. Physiol.*, 1959, v142, 439.
10. Woodbury, D. M., *Am. J. Physiol.*, 1956, v185, 281.
11. Forbes, G. B., Mizner, G. L., Lewis, A., *ibid.*, 1957, v190, 152.
12. Munro, D. S., Satoskar, R. S., Wilson, G. M., *J. Physiol.*, 1957, v139, 474.
13. Neuman, W. F., Neuman, M. W., *Chemical Dynamics of Bone Mineral*, 1958, Univ. Chicago Press.

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Skin Reactivity in Guinea Pigs Sensitized to Flea Bites: The Sequence of Reactions.* (27040)

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It is generally recognized that both humans and animals manifest various types of skin response to bites of hematophagous insects. Some individuals fail to react to bites, some have delayed or immediate skin reaction, while others show both immediate and delayed skin responses. Repeated exposure of an individual to bites of a given species ultimately may completely alter the type of reactivity(1-4,6). Mellanby(3), on the basis of limited clinical observations on humans has shown that, following repeated exposures over varying periods of time, allergic skin responses to mosquito bites are manifested by delayed skin reactions followed by the immediate type. He presented the hypothesis that the skin response of man to mosquito bites occurs in 4 distinct stages: 1) delayed, 2) delayed and immediate, 3) immediate only, 4) non-reactive. However, no experimental evidence was presented for the development of the state of non-reactivity. Generally, in hypersensitivity involving delayed skin reactions, little is known about hyposensitization or desensitization following repeated introduction of antigen. For example, equivocal success has been achieved in desensitization to tuberculin (8). On the other hand, depending on the desensitizing dose, desensitization of guinea pigs to protein antigens has been achieved for periods not exceeding 10 days(9).

The following study indicates that a definite sequence of types of reactivity occurs in guinea pigs in response to repeated exposure to flea bites, and that this sequence terminates in a stage of non-reactivity which lasts for at least 5 months.

Experimental. Three groups of guinea pigs (5 per group) were sensitized to flea bites by daily exposure of each animal to bites of 20 cat fleas, *Ctenocephalides felis felis* (Bouche) according to the method described by Benjamini, Feingold, and Kartman(6). When delayed reactions appeared approximately 5-6 days after the initial exposure, the animals were further exposed twice per week with few exceptions (Table 1) for about 14 weeks. One group was exposed to bites of 20 fleas per

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