Effect of Adrenocortical Steroids on Bone Electrolyte Metabolism.* (26557)

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Bone contains about 40% of the total body sodium. It therefore has the potential of being an important factor in electrolyte metabolism.

Harrison(1) concluded from studies on normal rats and rats with various disorders of calcification, that bone sodium varies with calcium and that the calcium:sodium ratio in bone remains constant. Bergstrom and Wallace(2) subsequently demonstrated that bone sodium could vary independently from the calcium in acidosis associated with peritoneal dialysis(2,3,4,5) and following dietary salt restriction(2,3).

Studies of the role of the adrenal gland in bone electrolyte metabolism are conflicting. Adrenalectomy has been reported to cause bone sodium depletion in presence of a normal diet(6,7) a low salt diet(8) or with salt added to the drinking water(9). Other investigators, however, have reported that adrenalectomy failed to change bone sodium when combined with either a normal diet(10) or with salt added to drinking water(8).

Workers investigating adrenal insufficiency by balance studies in dogs found a greater loss of sodium from the extracellular fluid than could be accounted for by external losses(10,11). They suggested that the sodium was sequestered in the bone. However, White, *et al.*(6) were unable to confirm these findings in adrenalectomized rats.

The present investigation was carried out in an attempt to clarify the role of the adrenal gland in bone electrolyte metabolism.

Materials and methods. Female Sprague Dawley rats were used for all experiments. At time of sacrifice the animals were given pentobarbital 6 mg/100 g I.P. Blood was drawn from the right ventricle and the serum separated. Sera were pooled to obtain samples for electrolyte determinations.

The femurs with cartilage removed but with marrow present were weighed in the fresh state and again after drying for at least 48 hours at 105°C. The dry bones from 3 or 4 rats were ground together in a mortar and 2 samples of the powder were taken. These were prepared for sodium and potassium determinations by the method of Bergstrom and Wallace(2). Our technic yielded 95% of the Na and 90% of the K present in a mixture of various salts approximately the composition of bone.

Sodium and potassium concentration in bone and serum samples was determined by flame photometry, using lithium as an internal standard.

Adrenalectomies were done through bilateral subcostal incisions and the rats were maintained on Purina Laboratory Chow and tap water until the fourth postoperative day when they were sacrificed. Half the adrenalectomized rats were controlled by simultaneous measurements on unoperated rats selected at random from the same group. The other half were compared to controls of similar weight but not measured simultaneously. There was no difference between the subgroups and they are grouped together for statistical analysis.

A number of different steroid-administration experiments were done with simultaneous controls selected from the same group. All these animals were maintained on Purina Chow. Groups A and B were both given saline instead of tap water for drinking during the experiment. Group C received tap water. Each animal in subgroup A steroid-treated received 5 mg desoxycorticosterone trimethylacetate i.m. at the start plus 1.0 mg desoxycorticosterone acetate (DOCA) daily for 4

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TABLE I. Bone and Serum Electrolytes of Normal and Adrenalectomized Rats.

			Mean serum electrolytes (meq/l)			Mean bone electrolytes (meq/kg fresh bone)			
Group	No. of rats	Mean wt at sacrifice, g	No. of measurements	Na	K	No. of measurements	Na	K	Bone water conc., %
Control	22	132	11	139	5.1	12	132	49	40
S.E.		1.3		1.0	.7		2.8	2.3	
Adrenx	20	119	11	132	6.2	10	92	45	40
S.E.		2.5		1.4	1.7		8.7	1.9	
p		.01		.1	.2		$<.01$	$<.2, >.1$	

days, and were sacrificed 18 hours after the last dose. Each in subgroup B steroid-treated received 1.0 mg DOCA daily for 4 days, then was sacrificed. Subgroup C steroid-treated animals each received one injection of 12.5 mg desoxycorticosterone trimethylacetate and were sacrificed 2 weeks after injection. (Serum electrolytes on different animals in this laboratory on this regimen show the expected fall in potassium concentration with no change in sodium.)

All measurements for bone electrolytes are in units of mEq per kg fresh whole femur. No attempt was made to calculate "chloride space sodium" or "free sodium" since our interest was in the net sodium or potassium balance of whole bone.

Results. Our measurements of adrenalectomized compared to control rats are summarized in Table I. Weights given are those at time of sacrifice and reflect dehydration of the adrenalectomized animals. Bone sodium concentration decreased significantly in the adrenalectomized rats. The potassium appeared to decrease slightly but the change was not statistically significant for the number of measurements.

The results of steroid treatment are reported in Table II. Neither bone sodium nor bone potassium changed significantly, although in each experiment, average potassium values for steroid-treated rats were slightly lower than the controls.

Discussion. This investigation confirms the results of others showing that adrenalectomy causes a decrease in bone sodium(6,7, 8,9). It does not support Stern, Cole, Bass, and Overman's(10) conclusions of no change. These workers, however, actually found a fall in mean bone sodium levels in their dogs

TABLE II. Bone Electrolytes of Normal and Steroid Treated Rats.

Group	No. rats	Mean wt, g	No. measurements	Na (meq/kg)	K (meq/kg)	Bone water conc., %
Control A	7	149	4	145	45	36
S.E.		3.3		3.6	2.8	
Steroid A	7	146	4	156	43	35
S.E.		4.5		9.8	2.1	
p				.3	.9	
Control B	8	171	4	155	42	36
S.E.		6.1		9.5	2.8	
Steroid B	8	168	4	150	38	36
S.E.		5.6		3.0	.6	
p				.6	$<.3, >.2$	
Control C	11	172	6	—	40	37
S.E.		2.9		—	2.0	
Steroid C	12	180	6	—	38	37
S.E.		3.3		—	1.4	
p					$<.2, >.1$	

from 246 to 229 mM/kg fresh midfemur. Because of the large variation between measurements and small number of measurements made, this fall was not statistically significant.

Munro, Satoskar, and Wilson(8) did not demonstrate a fall in bone sodium in adrenalectomized rats maintained on 0.9% saline when compared to normal rats on a regular diet and tap water. They did find a fall in adrenalectomized rats on a rice diet and distilled water, but had already shown(3) that a rice diet and distilled water can lower bone sodium in normal rats. They did not report results of adrenalectomized rats on a standard diet and water or normal rats on saline. Nichols and Nichols(12) have shown that salt alone, in large quantities, could actually raise bone sodium.

The effects of adrenal steroid administration on bone electrolyte composition have not been thoroughly studied. Dosekun(9) found that cortisone treatment prevented the fall of bone sodium concentration in adrenalectomized animals. Borle, *et al.*(7), demonstrated that prednisolone did not change bone Na or K concentrations in intact rats. Woodbury(13) and Green *et al.*(14) using a radioactive sodium technic found that mineraloactive corticoids did not elevate bone sodium concentration above normal. Using a direct measurement technic and various mineraloactive corticoid regimens, we were unable to produce a significant change in bone Na or K concentrations in intact rats.

Summary. The effects of adrenocortical hormones on bone electrolyte metabolism have been investigated by 2 series of experiments. In the first, femur sodium and potassium concentrations were compared in normal and adrenalectomized rats. Sodium concentration decreased a significant amount. In the second series, normal rat femurs were compared to femurs from rats given varying amounts of NaCl in the diet and various mineraloactive steroid drugs. In each of these experiments sodium concentration was unchanged and potassium fell an insignificant amount.

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