phosphorylase *t* cannot be either ignored or explained.

Although others have shown that epinephrine increases rate of recovery of phosphorylase activity ratios in normal rat muscles(11,12), this was not observed in denervated muscle under these conditions. Perhaps if the denervated muscle had been stimulated to exhaustion, the effect of epinephrine on increasing the rate of recovery would have appeared as it did in normal muscle(11,12).

Summary. Following denervation of the rat rectus femoris muscle, phosphorylase *a* and *t* activity and the *a/t* ratio were maintained for 5 days, decreased by 10 days and remained low for 60 days. In muscles denervated for 15 days epinephrine increased the activity of phosphorylase *a* and *t* and the *a/t* ratio, and markedly inhibited the expected decrease in enzyme activity which follows tetanic contraction. Following electrical stimulation, the recovery of activity ratios in both intact and denervated muscles occurred at the same time. In denervated muscle the return of phosphorylase *a* and *t* was delayed as compared to that in normal muscle. Epinephrine showed no effect on the recovery of enzyme activity in the denervated muscle. Changes in muscle phosphorylase activity after denervation atrophy differ from those incurred from other causes.

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Received June 19, 1961. P.S.E.B.M., 1961, v108.

Influence of Adrenal Glucocorticoids on Distribution of Calcium and Phosphorus between Bone and its Surrounding Fluids. * (26899)

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Hypercalcemia has been reported in Addisonian crisis(1-3), following adrenalectomy in patients with Cushing's disease(4), and in adrenalectomized animals(5-8). In many of these instances the elevated serum calcium returned to normal levels following cortisone administration. Hypercalcemia accompanying sarcoidosis and hypervitaminosis D, and idiopathic hypercalcemia in children may be similarly reduced with cortisone(9-11).

Elevated serum phosphorus values have been observed in adrenalectomized rats(12) and monkeys(13), and in patients with Addison's disease(2). On the other hand, ACTH has been found to decrease serum phosphorus in the rat(14), and marked hypophosphatemia has been seen in patients following cortisone treatment(15).

Either a lack of such effects on serum Ca and P concentrations or conflicting results have been reported by other workers(11,14, 16). While these phenomena indicate effects

* This work was supported by grants from Inst. of Arthritis and Metab. Dis., Nat. Inst. Health, U.S.P.H.S.

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of adrenal hormones on serum Ca and P levels, the mechanisms underlying these effects are unknown.

In recent studies in dogs(8) hypercalcemia following adrenalectomy was reversed to normocalcemia with cortisone. A balance study during the hypercalcemic phase revealed no change in the consistently negative Ca and P balance, suggesting that the effects on serum calcium were not mediated through changes in balance. It was therefore suggested that cortisone, in addition to its well-known effects on calcium balance, may also influence the equilibrium distribution of Ca between bone and extracellular fluid in a direction opposite to that of parathyroid hormone and vit D. A study of the possible influence of adrenalectomy and cortisone treatment on this equilibrium using mouse bone *in vitro* is here reported.

Methods. The experimental animals used were 7-8-week-old, male Swiss white mice weighing 40-45 g each. Adrenalectomy was performed one week before sacrifice. 2.5 mg cortisone acetate Merck was injected subcutaneously as described in Table II. On the day after the last dose was given,§ animals were killed by cervical fracture and the calvaria removed and prepared as in other experiments(18). Two calvaria, pooled to give about 80 mg wet weight of bone in each flask, were incubated for 6 hours in a Dubnoff metabolic incubator at 37.8°C in 2 ml of modified Krebs-Ringer media‖ buffered to pH 7.4 with HCO_3^- and 5% CO_2. Penicillin at a final concentration of 5 U per ml and streptomycin 0.01 mg/ml were added. Glucose was used as a substrate at a concentration of 2 mg/ml. Incubations were carried out in an atmosphere of 95% oxygen with 5% CO_2. In such a system at the end of 6 hours incubation Ca and P measured in the medium can be considered to be in steady-state equilibrium with the bone mineral and thus simulate the situation *in vivo* discussed elsewhere(18). For each experiment normal and experimental bone samples were incubated at the same time. All analyses were done by methods currently in use in this laboratory (18).

TABLE I. Effects of Adrenalectomy.

		Ca	P	Ca × P
		mMol/l		
Controls	1	.64	.94	.60
	2	.68	.95	.65
	3	.66	.92	.61
	4	.65	.91	.59
	5	.65	.90	.59
	Mean	.66	.92	.61
Adrenalectomized	6	.58	.93	.54
	7	.65	.89	.58
	8	.62	.89	.55
	9	.65	.93	.60
	10	.65	.93	.60
	11	.71	.97	.69
	Mean	.64	.92	.59

Results. Neither adrenalectomy nor treatment with cortisone (or hydrocortisone) in the dosage used appeared to affect the well-being of the animals. They were active, consumed normal amounts of food and water, fur remained sleek, and they did not lose weight. Data from 2 typical experiments are shown in Tables I and II. Ca and P concentrations maintained in the media surrounding bones from adrenalectomized animals were the same as controls (Table I).

In the cortisone-treated groups (Table II), while Ca concentrations remained unchanged, P concentrations were consistently lower than controls. The decrease in P concentration was marked after only 2 days of cortisone and became more pronounced with longer treatment. The Ca × P products were similarly reduced. Similar results were found with comparable doses of hydrocortisone.

Discussion. The fact that cortisone treatment induced a fall in P concentration, while adrenalectomy had no effect, suggests that the cortisone effect observed may have been the result of an excessive dose. However, although no significant effect on Ca concentration was noted, the changes in P which took place were in a direction which might have

§ This dose of cortisone is comparable to that used in experiments on rats by others(17). It was chosen for these experiments because preliminary experience indicated that it was effective in changing P distribution in the system employed and yet did not appear to affect the well-being of the animals nor cause them to lose weight.

‖ The media contained no Ca. P was present at a concentration of .40 mMol/1.

TABLE II. Effects of Cortisone.

		Ca	P	Ca × P
		mMol/l		
Controls	1	.64	.90	.58
	2	.64	.91	.58
	3	.60	.94	.56
	Mean	.63	.92	.57
Cortisone, 2.5 mg/day				
For 2 days	4	.62	.82	.51
	5	.67	.81	.54
	6	.63	.77	.49
	Mean	.64	.80	.51
For 5 days	7	.60	.74	.44
	8	.62	.80	.50
	Mean	.61	.77	.47
For 8 days	9	.64	.79	.51
	10	.63	.73	.46
	11	.65	.75	.49
	Mean	.64	.76	.49

been expected on the basis of observations from some laboratories of serum P concentrations in patients and experimental animals under the influence of adrenal steroids(14, 15). Our results suggest that one of the mechanisms by which cortisone decreases serum P levels is by changing the equilibrium for this ion between bone mineral and the extracellular fluid. This mechanism would serve to enhance the hypophosphatemic effects of increased glycogen deposition in muscle suggested by Fenn and confirmed by Nichols(19).

The details of the mechanisms involved in distribution of P between bone sample and medium which are influenced by cortisone cannot be determined from available data. Whether they are related to changes in citrate metabolism or changes in permeability or synthesis of the organic matrix requires further investigation.

Why changes in Ca distribution could not be shown to accompany the changes in P is equally unclear. Species differences may have been responsible, or changes in Ca distribution previously noted(1-8) may have been largely the result of phenomena remote from the bone itself such as absorption, excretion, etc.

It is of interest to compare the present results with those obtained previously using exactly the same system(18). It was shown that Ca concentrations were significantly higher when active cellular metabolism was present and following prior treatment with parathyroid hormone. P concentrations, however, were unchanged. In the present study changes were seen in P concentrations, while Ca concentrations were not affected. These findings suggest that cortisone may modify the steady state Ca × P concentration product by a different mechanism than parathyroid hormone.

Summary. Bones from cortisone-treated and adrenalectomized mice were incubated *in vitro* and Ca and P in the medium measured after a steady state was reached. Compared to controls, no significant changes were seen in the adrenalectomized group. In the cortisone-treated group, while Ca concentrations were unchanged, P concentrations were depressed. It is therefore suggested that the depression of serum P levels with cortisone observed in rats and man may be in part a result of a change in distribution of P between bone mineral and extracellular fluid.

The technical assistance of Miss Gretchen Lange and Miss Sylvia M. Zottu is gratefully acknowledged.

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Received June 23, 1961. P.S.E.B.M., 1961, v108.

Blood Volume Parameters of a Poikilothermal Animal in Hypo- and Hyperthermia.* (26900)

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With the current interest in blood volume changes in warm-blooded animals produced by alteration in body temperature (particularly hypothermia) it appears logical that these phenomena be investigated in a poikilothermal animal as a possible source of insight into the nature of physiologic adjustment of blood volume to changes in body temperatures. Several common cold-blooded laboratory animals were considered for this study but due to ease of maintenance, size, and phylogenetic "highness" the alligator is the animal of choice.

Methods. Animals used in this study were 66 American Alligators, *Alligator mississippiensis,* weighing 615-1300 g and measuring 66.5-82.0 cm in length. The animals were obtained from Louisiana, kept in a shallow tank without food, and used within 2 weeks of the time they arrived but not during the first 3 days. Approximately an equal number of males and females were used, and no significant difference between sexes was noted with regard to the parameters studied.

The alligators were anesthetized using 30 mg/kg sodium pentobarbital injected intraperitoneally; anesthesia administered approximately 1½ hours before the experiments were begun proved to be most satisfactory. Cr^{51} tagged red cells were used to determine cell volumes, and I^{131} tagged serum albumin (RISA) was used to measure plasma volumes. The red cells to be tagged were obtained from donor alligators early in the day and tagged according to the technic of Gray & Sterling(1). Donor alligators were used only once although some recovered from this relatively massive bleeding (approximately 18 cc). A suspension of 5 cc of washed, tagged red cells suspended in enough 0.9% saline to approximate the normal hematocrit was given to each animal in the Cr^{51} series immediately after removal of a like volume of their own blood. The tagged blood-cell suspension was washed in with 1 cc of saline and an additional 1 cc of saline was injected slowly during the experimental period (about one hour).

Both withdrawal and injection, as well as subsequent bleeding, were done through an indwelling cardiac needle introduced by puncture. An initial hematocrit determination was made for each animal using the first blood drawn from the heart; for this determination micro-technics were used. Hematocrits were also measured for donor alligators. These hematocrits are subsequently called venous hematocrits although they may represent mixed venous and arterial blood.

In the I^{131} series one cc of a solution of RISA appropriately diluted in 0.9% saline was injected after blood had been withdrawn for hematocrit determinations. The same washing-in technic was used as in the case

* This investigation was supported in part by Research Grant from Nat. Inst. Health.