tiple doses of the drug and that injury of this degree was never seen in many other dogs, including 2 littermates of dog 132, which survived a single dose. Serial biopsies showed, in agreement with earlier reports and with our own observations on other dogs, that the primary effect of alloxan was on the tubules. The glomerular changes lagged behind the tubular degeneration and they appeared both in time and in type to be consequent to the latter. The histologic picture suggests that the glomerular damage originated from a failure of drainage, with resulting dilatation of Bowman's space and atrophy of the tuft. The absence of epithelial and endothelial proliferation and the presence of periglomerular fibrosis are consistent with this interpretation.

Summary. In 2 dogs which did not become diabetic following repeated doses of alloxan, serial biopsies of the kidney showed the late development of glomerular lesions. These lagged behind the usual tubular degeneration and appeared both in time and in type to be consequent to the latter. Similar studies on dogs receiving a single dose of alloxan and surviving for comparable periods of time showed only minimal glomerular changes.

Received June 19, 1951. P.S.E.B.M., 1951, v78.

V. Effect of Epinephrine, ACTH and Cortisone on Citrate, Calcium, Glucose and Phosphate Levels in Rabbits.* (18961)

JOSEPH B. PINCUS, SAMUEL NATELSON, AND JULIUS K. LUGOVOY.

From the Departments of Biochemistry, Jewish Hospital of Brooklyn and the Rockford Memorial Hospital, Rockford, Ill.

The effect of administration of glucose, insulin and epinephrine on serum citrate levels in humans has been studied(1-3). Administration of both glucose and insulin resulted in a fall in citrate levels, and administration of epinephrine resulted in a rise in citrate levels for several hours. That administration of glucose will result in a drop in citrate levels has been confirmed(4,5). Evidence has been presented to suggest that epinephrine stimulates secretion of ACTH and in turn the corticoid hormones(6). For this reason it was decided to investigate the changes in citrate levels occurring in blood under the influence of these hormones.

Serum calcium(7) and inorganic phosphate (8) estimations were carried out in addition to citrate(9,10) and glucose(11). In the case of epinephrine potassium levels(12) were also studied. These additional studies were undertaken because a relationship exists between serum calcium and citrate levels and ionic calcium, citrate and phosphate levels due to the formation of poorly ionized calcium complexes(13). Female rabbits weighing be-

* The fifth study on mechanism controlling serum citrate levels. Supported in part by a grant from the Dazian Foundation.

1. Natelson, S., Pincus, J. B., and Lugovoy, J. K., *J. Clin. Invest.*, 1948, v27, 446.

2. Pincus, J. B., Natelson, S., and Lugovoy, J. K., *J. Clin. Invest.*, 1948, v27, 450.

3. Pincus, J. B., Natelson, S., and Lugovoy, J. K., *J. Clin. Invest.*, 1949, v28, 741.

4. Miller, M., Bueding, E., Strach, R. O., Owens, J., and Woodward, H., *Proc. Am. Diabetic Assn.*, 1949, v9, 85.

5. Lundback, K., Posberg, V., and Schonheyder, F., *J. Clin. Invest.*, 1950, v30, 361.

6. McDermott, W. V., Fry, E. G., Brobeck, J. R., and Long, C. N. H., PROC. SOC. EXP. BIOL. AND MED., 1950, v73, 609.

7. Sobel, A. E., and Sobel, B. A., *J. Biol. Chem.*, 1939, v129, 721.

8. Fiske, C. H., and Subbarow, Y., *J. Biol. Chem.*, 1925, v66, 375.

9. Natelson, S., Lugovoy, J. K., and Pincus, J. B., *J. Biol. Chem.*, 1947, v170, 597.

10. Natelson, S., Pincus, J. B., and Lugovoy, J. K., *J. Biol. Chem.*, 1948, v175, 745.

11. Folin, O., *J. Biol. Chem.*, 1929, v82, 83.

12. Natelson, S., *Am. J. Clin. Path.*, 1950, v20, 463.

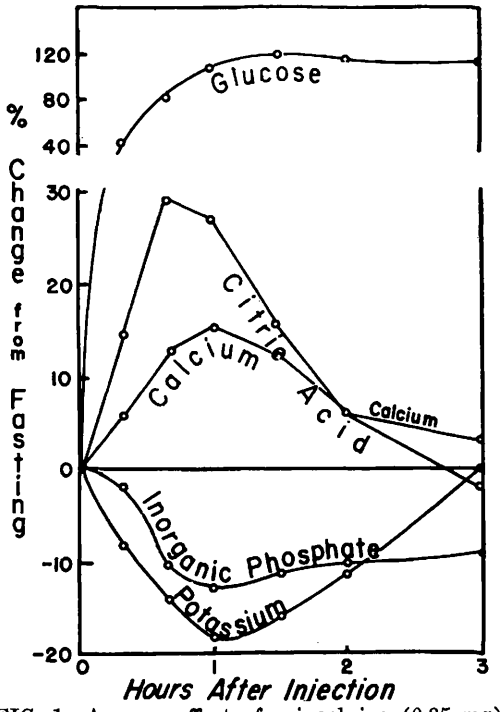

FIG. 1. Average effect of epinephrine (0.25 mg) on serum glucose, citric acid, calcium, inorganic phosphate and potassium.

tween 2-4 kilos were fasted for at least 48 hours to ensure stable initial levels of the various constituents of blood to be studied. Blood citrate levels are affected by food intake and this species is well known for long retention of food in its intestinal tract. The procedure consisted of drawing fasting blood from the ear. The hormone was immediately injected. Blood samples were taken at intervals (Fig. 1-3).

Fig. 1 summarizes the results obtained with epinephrine administered subcutaneously (0.08 mg/kilo). The curves illustrate the average results obtained with 26 rabbits for all values except potassium levels which are averaged for 11 rabbits. The values are the average change from the fasting. The curves for the individual rabbits resembled closely the shape of the average curves.

Fig. 2 shows the results obtained with cortisone injected as a saline suspension intramuscularly. Ten animals were used in these

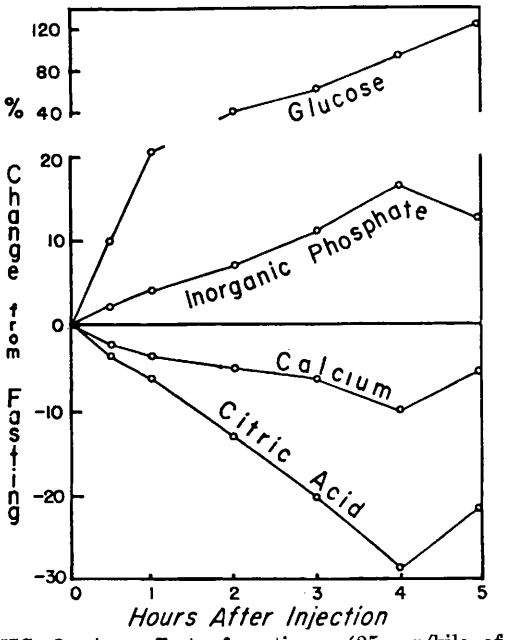

FIG. 2. Avg effect of cortisone (25 mg/kilo of body wt) on serum glucose, inorganic phosphate, calcium and citric acid in 10 rabbits.

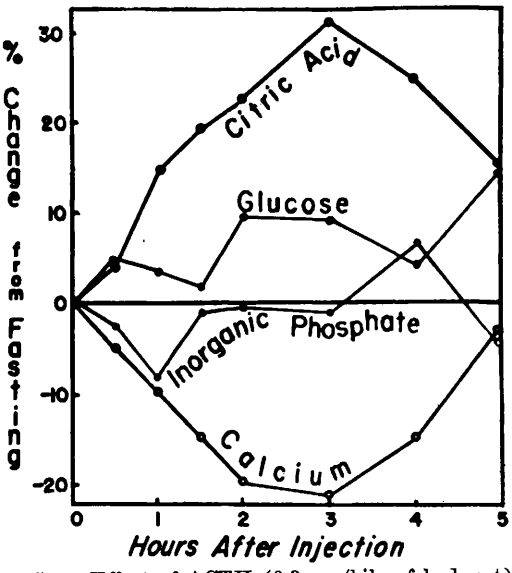

FIG. 3. Effect of ACTH (0.3 mg/kilo of body wt) on serum citric acid, glucose, inorganic phosphate and calcium.

experiments. The response of the individual rabbits was similar to the average curve, maxima and minima occurring at approximately the same times.

Fig. 3 illustrates the results obtained with

13. Wexler, I. B., Pincus, J. B., Natelson, S., and Lugovoy, J. K., *J. Clin. Invest.*, 1949, v23, 474.

TABLE I. Effect of Intramuscular Administration of ACTH (0.6 mg/kilo) on Calcium and Phosphorus Serum Levels in Rabbits After Fasting for 48 Hr.

Rabbit #	mg/100 ml	Fasting	½ hr	1 hr	1½ hr	2 hr	3 hr	4 hr	5 hr
88	P.	3.28	3.28	3.39	3.54	5.04*	—	4.68	3.75
	Ca.	13.6	12.6	12.2	11.9	11.2	—	10.7	10
89	P.	4.07	3.39	3.13	3.19	3.39	3.57	3.60	3.66
	Ca.	12.6	11.9	11.6	11.3	10.2	12	12.4	12.7
90	P.	4.28	4.07	3.68	3.45	3.45	3.54	3.57	3.90
	Ca.	12.1	11.6	9.9	9.1	8.9	10.2	11.8	11.2
91	P.	2.97	3.25	3.36	—	3.39	2.86	2.36	2.26
	Ca.	12.8	12.9	12.8	—	12.5	11.7	10.9	12.6

* Convulsions.

TABLE II. Effect of "Massive" Doses of ACTH on Serum Citrate, Calcium, Phosphate, and Glucose Levels on Fasting (48 Hr) Rabbits.

Rabbit No.	Dose, mg/kilo	Maximum % change from fasting*				Time of death, hr
		Citrate	Calcium	Phosphate	Glucose	
32	.75	+63	—31	——	+82	3
54	1	+43	——	—13	+83	survived
59	1.5	+50	—40	+57	+45	10
33	1.5	+57	—26	——	+110	2
58	1.5	+50	—40	+57	+45	10
67	2	+105	— 2	+240	+10	1½
66	2	+77	—29	+58	+75	survived
68	2	+55	—17	—28	+6	"
69	4	+125	—35	—15	+90	"
70	5	+26	—51	+17	+46	"
71	5	+44	—25	+6	+130	"
72	6.5	+50	— 3	+156	+82	1

* Mean fasting values found in this study (mg/100 ml serum), citric acid 3.67 (66 rabbits), glucose 120 (56 rabbits), calcium 13.8 (42 rabbits), inorganic phosphorus 4.86 (43 rabbits).

ACTH injected intramuscularly in 12 rabbits. The material used was taken from two samples (Armour Standard Clinical La-1A and Armour Animal Lot No. 128-105), dissolved in saline to contain 1 mg/ml and administered in doses of 0.3 mg/kilo. Changes in citrate and calcium levels were consistent and are shown in Fig. 3. Glucose and phosphate changes were somewhat variable. However, the average curve in Fig. 3 for these constituents indicates the general tendency.

Table I lists calcium and phosphorus results for 4 animals on a dosage of 0.6 mg/kilo.

Table II summarizes the results obtained with larger doses of ACTH.

Discussion. Both cortisone and epinephrine (Fig. 1, 2) cause a rapid rise in glucose levels. With respect to calcium, citrate and phosphate levels the response of the two hormones was in opposite directions. The rise in citrate levels on epinephrine administration was similar to that observed in humans(3). Changes in total calcium values were counterbalanced in each case by a change in citrate levels in the same direction. Thus the fall in calcium concentration when cortisone is administered is modified in its effect by a drop in citrate concentration which is known to complex calcium ions. Similarly, the rise in total calcium with epinephrine is also modified by a rise in citrate concentration. The inorganic phosphate moves in the direction of maintaining a constant calcium to phosphate product, the change in phosphate being inverse to the change in calcium in both cases. The animals treated with cortisone were not in distress in doses up to 50 mg/kilo, administered to a few animals.

With ACTH the drop in calcium level was more pronounced than that observed with cortisone and a significant increase in citrate levels took place. Thus calcium levels and citrate levels both moved in the direction which would lower calcium ion concentration. In this respect *the biochemical effects of cortisone and ACTH are quite different.*

The effect of ACTH on glucose levels was markedly different from that observed with

cortisone in rabbits. With cortisone the glucose levels rose promptly and had not reached their peak at the end of 5 hours when the glucose level was twice the fasting level. With ACTH no such dramatic rise was observed (Fig. 2, 3).

The effect of ACTH in causing a reduction in calcium levels prompted us to continue this study on larger doses. In 4 cases only calcium and phosphate determinations were done. By limiting ourselves to these two constituents we hoped to check precisely the effect of ACTH on these levels. It was clearly demonstrated by this set of experiments, checked by triplicate analyses, recoveries and standards, that calcium levels were definitely lowered approximately 30% by a dosage of 0.6 mg/kilo of ACTH. All 4 animals were in distress, breathing rate increased, the animals dropped their heads and became incontinent at the end of one hour. Rabbit No. 88 convulsed at the end of 2 hours and responded with convulsions when approached at the end of 3 hours. This animal survived. It will be noted that in this animal, at the onset of convulsions, the phosphorus level rose to 40% above the fasting level. At the end of 4 and 5 hours this animal still showed an elevated phosphate level. The other 3 animals showed a phosphate minimum. A lowered calcium level accompanied by a lowered phosphate level is unusual in that it is well known that calcium and phosphate levels tend to change inversely so that their product remains constant. Under the influence of ACTH this relationship does not seem to hold. The sudden rise in phosphate level of the animal which had convulsed would only tend to further lower calcium ion concentration along with the rise in citrate which we had reason to believe has taken place at this point (Fig. 3, Table II).

Table II lists the results obtained with higher doses of ACTH. Convulsions and death were observed in 6 out of the 12 animals treated. The 6, who survived, were in severe distress. These animals tended to convulse when stimulated. Protein analysis, not shown in the table, demonstrated that no significant change in protein level had taken place. Thus the rise in citrate and phosphate probably resulted in a marked lowering of calcium ion concentration. A fall in protein level would have a compensating effect on a rise in citrate level as concerns the ionization of calcium.

The effect of the pituitary and adrenal hormones on citrate and calcium levels, demonstrated here, may have some bearing on normal and abnormal calcification and the problem of convulsions.

Summary. 1. Epinephrine subcutaneously causes a rise in calcium, citrate and glucose levels and a fall in phosphate and potassium levels. 2. Cortisone intramuscularly causes a rise in glucose and phosphate levels and a fall in calcium and citrate levels. 3. ACTH intramuscularly causes a rise in citrate and a fall in calcium levels. 4. It is suggested that the balance between ACTH, epinephrine, insulin and cortisone secretion, because of their effect on citrate, calcium and phosphate levels, play a significant role in the maintenance of the calcium ion concentration of the plasma.

Received June 20, 1951. P.S.E.B.M., 1951, v78.