1950, v185, 201.

5. Callow, N. H., Callow, R. K., and Emmens, C. W., *Biochem. J.*, 1938, v32, 1312.

6. Brown, H., Willardson, D. G., Samuels, L. T., and Tyler, F. H., *J. Clin. Invest.*, 1954, v33, 1524.

7. Sandberg, A. A., Nelson, D. H., Glenn, E. M., Tyler, F. H., and Samuels, L. T., *J. Clin. Endocrinol. and Metab.*, 1953, v13, 1445.

8. Nelson, D. H., and Samuels, L. T., *ibid.*, 1952, v12, 519.

9. Bush, I. E., *Ciba Found. Colloquia*, 1955, 203.

10. Zaffaroni, A., and Burton, R. D., *J. Biol. Chem*, 1951, v193, 749.

11. Sweat, M. L., *Analytical Chem.*, 1954, v26, 1964.

Received January 23, 1956. P.S.E.B.M., 1956, v91.

Steroid-Induced Adrenal-Pituitary Hypofunction II. (22255)

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It has been demonstrated that daily administration of hydrocortisone to rats lowers their tolerance to intraperitoneal injection of potassium chloride(1). Since this observation suggested adrenal-pituitary hypofunction, efforts were made to determine if these animals could be protected by treatment with various hormones. Intravenous administration of ACTH or aldosterone immediately prior to the injection of potassium chloride increased the tolerance of steroid-treated animals to KCl stress(2). It was observed that the same response was obtained if animals were given 1% NaCl to drink for 18 hours prior to injection of potassium chloride(3).

The protective action of sodium chloride suggested that the mortality of non-protected animals was not due to a hypofunctional adrenal or pituitary but rather to an electrolyte disturbance as a result of steroid treatment. The present experiments were designed (1) to measure the amount of sodium and potassium excreted in the urine during steroid administration, and (2) to determine the tolerance to potassium stress in rats depleted of sodium and potassium.

Methods. 1. Male rats, averaging 160 g body weight were placed in metabolism cages (2/cage) and fed Ingle's liquid medium carbohydrate diet *ad libitum*(4). Following a 2-week control period, these animals were injected once daily for 10 days with either 16% gelatin, 4 mg, hydrocortisone (F) in 16% gelatin, or 6 mg 11β, 17α-dihydroxy-4-pregnene-3,20-dione (21-desoxy-F) in CMC.* Urine volumes were recorded daily and urinary sodium and potassium were determined in a flame photometer. 2. Male rats were given intraperitoneal injections of 8 mg Thiomerin sodium (Wyeth), 5 mg Neohydrin (Lakeside), or 6 mg Diamox (American Cyanamid) in 0.2 cc aqueous solution. The rats were placed in metabolism cages, fasted for 18 hours and then injected with 0.16 M potassium chloride (5 cc/100 g body weight). The 18-hour urine collections were analyzed for sodium and potassium.

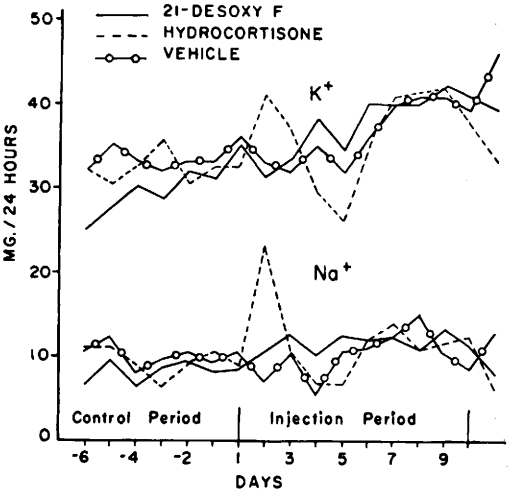

FIG. 1. Effects of hydrocortisone and 21-desoxy F on urinary sodium and potassium excretion.

* 0.5% carboxymethyl cellulose, 0.4% Tween 80, 1.5% benzyl alcohol and 0.9% sodium chloride.

TABLE I. Effects of Various Diuretics on Urinary Excretion of Sodium and Potassium.

No. rats	Treatment	% survivals*		Sodium, mg/18 hr	Potassium, mg/18 hr
49†	4 mg F/day/10 days	19	—	—	—
15	Controls	73	S‡ 11	10.4 ± 4.73	33.0 ± 3.20
			D 4	8.62± 3.87	34.6 ± 4.13
15	Thiomerin, 8 mg I.P.	73	S 11	13.5 ± 3.87	37.7 ± 7.01
			D 4	10.37± 2.80	35.75± 1.69
15	Neohydrin, 5 mg I.P.	53	S 8	14.3 ± 4.85	40.7 ± 9.03
			D 7	15.5 ± 7.09	43.8 ± 9.56
15	Diamox, 6 mg I.P.	66	S 10	23.8 ± 6.13	43.9 ± 8.73
			D 5	24.6 ± 6.63	42.0 ± 9.54

* Stressed with 0.16 M KCl, 5 cc/100 g body wt. † Collins(1). ‡ S = Survivals; D = Deaths.

Results. Fig. 1 demonstrates the pattern of sodium and potassium excretion during daily administration of F or 21-desoxy-F for 10 days. Urinary excretion of sodium and potassium increased during the 24-hour period following the injection of F. This increased excretion was not maintained. The animals showed a decrease in the excretion of both sodium and potassium during the third day of treatment. Decreased urinary excretion of sodium and potassium coincided with a period of decreased food intake. By the sixth day, food intake was again normal and urinary electrolyte concentrations paralleled that of control animals.

Animals injected with 21-desoxy-F demonstrated a more gradual and sustained increase in sodium and potassium excretion. However, the increased urinary concentration of these ions was not too different from the values observed in control animals.

Table I gives the effects of various diuretics on the urinary excretion of sodium and potassium as well as the results following potassium stress. Urinary excretion of sodium and potassium in the Diamox-treated group is significantly higher than the control group. However, within any given group, there is no significant difference between the animals that survived the stress and those rats in which the potassium chloride proved fatal. Fifty-three per cent of the Neohydrin-treated animals survived after potassium stress. However, this survival rate is higher than the survival rate for hydrocortisone-treated animals.

Discussion. Dietary potassium enters the blood stream and becomes available to the cells via the extracellular fluid. When potassium is presented to the cells, the amount taken up is dependent upon cellular requirements. If the supply exceeds cellular demands, the excess potassium is presumably eliminated by renal excretory mechanisms. Increased concentrations of extracellular sodium dilutes extracellular potassium and increases renal elimination of potassium which results in a loss of intracellular potassium. These actions of sodium make it useful in the treatment of potassium intoxication. Conversely, depletion of sodium enhances the possibility of potassium intoxication(5-7).

Although the mechanism of potassium stress in animals with hypofunctional adrenal cortices has not been defined, a hydrocortisone-induced increase of sodium excretion is suggestive of a decreased tolerance to a potassium load. However, the present data indicate that marked sodium depletion does not decrease the resistance of rats to potassium stress if adrenal-pituitary function is not impaired. The sodium losing effect of hydrocortisone is of 1-3 days duration and is not sustained during chronic administration. This is in agreement with the observations of Ingle *et al.*(8). The data which have been presented above indicate that decreased resistance to a potassium load results from a decrease in adrenal-pituitary function and not from steroid-induced changes in electrolyte composition of the organism.

Summary. Data are presented which in-

dicate that marked sodium depletion does not alter the resistance of intact rats to a potassium load. Early but transitory urinary electrolyte changes were observed during chronic hydrocortisone treatment. The decreased resistance to potassium stress in animals treated with hydrocortisone is probably due to hypofunction of the adrenal-pituitary axis rather than to steroid-induced alterations of electrolyte balance.

1. Collins, E. J., PROC. SOC. EXP. BIOL. AND MED., 1955, v89, 443.

2. ———, 1955, in press.

3. ———, unpublished data.

4. Stafford, R. O., Bowman, B. J., and Olson, K. J., PROC. SOC. EXP. BIOL. AND MED., 1954, v86, 322.

5. Merrill, J. P., Levine, H. D., Somerville, W., and Smith, S., *Ann. Int. Med.*, 1950, v33, 797.

6. Finch, C. A., Sawyer, C. G., and Flynn, J. J., *Am. J. Med.*, 1946, v1, 337.

7. Luetscher, J. A., Ciba Foundation, *Colloquia*, 1952, vIV, 430.

8. Ingle, D. J., Sheppard, R., Oberle, E. A., and Kuizenga, M. H., *Endocrinology*, 1946, v39, 52.

Received January 25, 1956. P.S.E.B.M., 1956, v91.

Effect of Choline, Heparin and Aureomycin on Fatty Livers of Dogs.* (22256)

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Gyorgy, *et al.*(1,2) demonstrated that Aureomycin exerts a lipotropic and anti-cirrhotic effect in rats fed a high fat, low protein, choline deficient diet. Baxter and Campbell (3) noted prevention of severe renal injury and some diminution of liver fat after Aureomycin therapy. More recently the amount of fat in livers has been reduced in cholesterol-fed rabbits(4) and rats(5) by the daily administration of heparin. It therefore became of interest to compare the effectiveness of choline, Aureomycin and heparin in a different species, the dog, and to ascertain whether the mechanism of lipotropic action of Aureomycin was related to the choline sparing effect of this drug(3,6).

Methods. Healthy mongrel dogs, previously maintained on Purina laboratory chow were fed 14 g per kg body weight of a high-fat, low-protein, choline-deficient diet‡(7) for 21 days. Eight of these animals received 0.5 g of Aureomycin§ (Chlortetracycline) in capsule form in the morning and late afternoon simultaneously with the feeding of the diet. Seven animals received daily oral supplements of 1% choline chloride.‖ Nine other animals were injected twice a day with heparin§ 2 mg/kg body weight intramuscularly. On the 21st day, some animals were killed by rapid intracardiac air injections and liver slices were made by a Martin slicer(8). Liver slices were incubated with radioactive phosphate for one hour, with and without the *in vitro* addition of choline chloride (1 mg/cc of medium). Composition of medium and isolation of phospholipides have been described(6). From the specific activity of the inorganic phosphate of the medium and the total radioactivity in the phospholipide, the *in vitro* rate of phosphatide synthesis was calculated. The remaining dogs were sacrificed after the 3 week experimental period. Phospholipide phosphorus was determined on an

* This investigation was supported, in part, by grants from Lipotropic Research Fund and National Heart Institute of National Institutes of Health, Public Health Service, Grant No. (H-1238).

† This study was carried out during tenure of Public Health Service Predoctoral Research Fellowship of National Institutes of Health, Public Health Service.

‡ The diet was composed of 38% lard, 44% sucrose, 8% vitamin test casein, 3% cod liver oil and 5% Cowgill's salt mixture. Any animal that did not consume entire diet upon feeding was force-fed.

§ Kindly donated by Lederle Co., Pearl River, N. Y.

‖ Kindly donated by Merck and Co.