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Effects of Cortisone Acetate on Fluid and Electrolyte Balance in Normal Rabbits.* (20036)

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Although the effects of cortisone on the metabolism of sodium, potassium and water have been extensively investigated in diseased man and in the normal rat and dog(1-4), comparable data in the rabbit are lacking. Most studies in immunology, however, have been performed in the latter species of animal. Because of the variability in the response of different species to drugs, a study of the effects of cortisone on fluid and electrolyte metabolism in the rabbit appeared to be desirable.

Recent studies have suggested that immune reactions in the rabbit might be associated with alterations in the distribution of body fluid and electrolytes, and that the adrenal gland may be involved in these changes(5,6). Before the role played by the adrenal gland in the phenomena of immuno-physiology can be interpreted, it is first necessary to determine the effects of adrenal hormones in normal animals. The availability of radioactive potassium (K^{42}) has made possible the direct measurement of the exchangeable potassium content in intact animals. The purpose of the present study was to determine the effects of two different dosages of cortisone acetate on sodium, potassium, and water metabolism in normal rabbits.

Materials and methods. Fourteen normal adult male rabbits of mixed breeds were used. They were kept in individual metabolism cages and were fed a stock diet of compressed pellets. Tap water was given without restriction. In order to acclimate the animals to their new environment, they were placed in the cages one week prior to the initiation of the baseline studies. *Procedure.* Two groups of 7 rabbits were used. External balance studies of sodium and potassium were performed daily for 4 weeks in Group 1 and for 5 weeks in Group 2. Direct measurement of the exchangeable potassium content (Ke) was made with K^{42} at weekly intervals. During the first week, baseline studies were done on each group. Starting on the 8th day

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and continuing every day for 2 weeks, 4 mg of cortisone acetate suspended in one ml of physiologic saline solution was given subcutaneously in the interscapular space to each animal in Group 1. This dose is roughly comparable to that given therapeutically (100 mg daily for a 70 kg man). Starting on the 8th day and continuing every day for 3 weeks, 25 mg of a suspension of cortisone acetate containing 25 mg/ml was given in the interscapular space to each of the 7 animals in Group 2.

External balance of sodium and potassium. Each morning before the rabbits were fed, the body weight, total food consumption, and 24-hour urine volume were measured. Each gram of feed contained 0.275 meq of potassium and .09 meq of sodium. The tap water contained a negligible amount of both ions. The potassium and sodium content of the ingested feed was found to be completely absorbed through the gastrointestinal tract; the amount of these ions in the stool was too low to be detected by flame photometry. The weekly net gain or loss of sodium and potassium from the body, therefore, was calculated on the basis of the difference between the amounts ingested and those excreted in the urine. *Measurement of exchangeable potassium content.* The initial Ke determination was made on the first day of the experiment; the second, on the 7th day. The mean of these 2 determinations was used as the baseline value for each animal in the analysis of the data. Subsequent Ke determinations were made at weekly intervals. The preparation of the potassium solution for injection has been previously described(7). Five ml of the potassium solution, containing 30 μc of K^{42} in 0.5 meq was injected intravenously. The urine was collected for 24 hours; a spot specimen was then obtained by catheterization and its specific activity was determined. The following formula was used to calculate the value for the exchangeable potassium content (Ke) of the body:

$$Ke = \frac{Ki^{42} - Ko^{42}}{Ku^{42} / Ku^{39}}.$$

Ki^{42} = quantity of radiopotassium administered (arbitrary units).
Ko^{42} = quantity of radiopotassium excreted in urine in 24 hr.
Ku^{42} = concentration of radioactivity in the spot specimen.
Ku^{39} = concentration of total inorganic potassium in the spot specimen.
Ku^{42} / Ku^{39} = specific activity of the spot specimen.

The potassium concentration in the urine and in the serum was determined by flame spectrophotometry by a method which has been described previously(8).

Statistical method. The significance of the difference between the means was determined by the use of the "t" test(9). Each animal served as its own control. The differences between the baseline value and the values at the end of the second, third, and fourth weeks in Group 1 (and also at the end of the fifth week in Group 2) were calculated for each animal; the mean difference between the baseline values and each of the subsequent determinations was then obtained. The significance of the mean difference was tested by the following formulae in which d = mean difference, s_d = standard error of the mean difference, S = summation of, and n = number of animals:

$$t = \frac{d}{s_d} \qquad s_d = \sqrt{\frac{Sd^2 - \frac{(Sd)^2}{n}}{n(n-1)}}$$

The calculated value for "t" was compared with the value at (n—1) degrees of freedom, as obtained from standard tables, and expressed as "P". A "P" value of 0.02 or less was considered significant.

Results. Group 1. The daily administration of 4 mg of cortisone acetate resulted in the significant changes shown in Table I. After the administration of cortisone *for 7 days,* an increase in body weight and a reduction in serum sodium concentration had occurred. After *14 days* of cortisone therapy, the mean serum sodium and potassium concentrations were significantly greater than their mean baseline values. The animals voluntarily reduced their intake of sodium although no change in sodium balance occurred. One week after the administration of cortisone was *discontinued,* the animals were in negative sodium balance, primarily because of a reduction in intake. There was no change in potassium balance, although the concen-

TABLE I. Effects of Cortisone Acetate, 4 mg Daily, on Fluid and Electrolyte Balance in Normal Rabbits.

	Wt (kg)	K intake (meq/day)	K excretion (meq/day)	K balance (meq/day)	Na intake (meq/day)	Na excretion (meq/day)	Na balance (meq/day)	Serum Na (meq/l)	Serum K (meq/l)	Exchangeable K (meq)	Exchangeable K (meq/kg)
Baseline values	3.17	34.34	29.24	+5.10	13.32	10.02	+3.20	138.3	4.3	199.4	62.6
Week 1†	+.16*	+5.89	— .03	+5.92	+ .10	—.15	+ .25	—9.9*	+ .5	+13.4	+ .7
2‡	+.12	+ .53	—2.92	+3.45	—1.84*	—.19	—1.65	+7.3*	+ .5*	—25.9	—13.8
3§	—.03	—8.89	—7.05*	—1.84	—4.90*	+.38	—5.48*	+2.8	+1 *	—24.7	— 3.2

* Statistically significant difference when this figure is compared with the baseline value; P = <.02.
† Mean changes after 7 days of cortisone. ‡ Mean changes after 14 days of cortisone. § Mean changes 7 days after discontinuing cortisone.

TABLE II. Effects of Cortisone Acetate, 25 mg Daily, on Fluid and Electrolyte Balance in Normal Rabbits.

	Wt (kg)	K intake (meq/day)	K excretion (meq/day)	K balance (meq/day)	Na intake (meq/day)	Na excretion (meq/day)	Na balance (meq/day)	Serum Na (meq/l)	Serum K (meq/l)	Exchangeable K (meq)	Exchangeable K (meq/kg)
Baseline values	2.40	46.04	19.85	+26.19	15.07	3.51	+11.56	146.2	5.2	168.9	70.5
Week 1†	+.06	—13.95*	+4.53*	— 5.55*	—4.56*	+ .99	—18.48*	+4.26*	— .73	—11.90	—6.95
2‡	—.10	—19.79*	+4.52	— 7.59*	—6.48*	+1.11	—24.31*	+4.84	— .49	—18.61	—5.46
3§	—.34*	—22.91*	+8.11*	—10.12*	—7.49*	+2.62*	—31.02*	+7.11*	—1.11	—30.55*	—3.56
4‖	—.31*	—22.47*	—1.61	— 7.45*	—7.35*	— .10	—20.86*	+4.21	+ .08	—35.88	—7.32

* Statistically significant difference when this figure is compared with the baseline value; P = <.02. † Mean changes after 7 days of cortisone.
‡ Mean changes after 14 days of cortisone. § Mean changes after 21 days of cortisone. ‖ Mean changes 7 days after discontinuing cortisone.

tration of potassium in the serum was increased.

Group 2. The daily administration of 25 mg of cortisone acetate resulted in the significant changes shown in Table II. After the administration of cortisone for *7 days,* the animals were in negative potassium balance, with an increase in the urinary excretion and a decrease in the oral intake of this ion. Sodium intake was decreased and the animals were in negative sodium balance. The mean serum sodium concentration was elevated. After *14 days* on cortisone, potassium and sodium balances were negative and the intake of both ions was spontaneously reduced. After *21 days,* a significant reduction in body weight had occurred. Potassium and sodium balances were still negative, and there was a further increase in the urinary output of both ions and a decrease in their oral intake. The serum sodium concentration was increased still more. Ke was significantly lower than the baseline value. After cortisone had been *discontinued* for a week, body weight was still significantly reduced below the baseline value. Sodium and potassium balances were negative. and the reductions in the intake of sodium and potassium were still significant.

Comment. The results of the present study indicate that the response of normal rabbits to cortisone acetate is dependent both upon the dosage and upon the length of the period of administration. Four mg of cortisone acetate produced at the end of the first week an increase in body weight and a decrease in serum sodium concentration, suggesting a retention of water and dilution of the extracellular fluid phase. The increase in serum sodium and potassium concentrations at the end of the second week indicate hypertonicity of the extracellular fluid space, which in turn suggests overhydration of tissue cells. One week after discontinuation of cortisone, loss of water and sodium through diuresis had produced a reduction in body weight and a negative sodium balance. Although the serum potassium concentration was elevated, no significant change occurred in the potassium balance or in the exchangeable potassium content. The results of the administration of this dosage of cortisone can, therefore, probably

be attributed to changes in the distribution of water.

On the other hand, a daily dosage of 25 mg of cortisone acetate resulted in striking alterations in both sodium and potassium metabolism. After the first week of cortisone administration, the intake of both sodium and potassium was decreased (the animals were not eating) at the same time that the urinary excretion of potassium was elevated. The fact that no significant change in body weight occurred for the first 2 weeks, in spite of this negative potassium and sodium balance, suggests that there was a retention of water. Since the serum sodium concentration was increased at this time, this excess water must have been in the intracellular phase. The changes at 14 days were similar to those at 7 days. After 21 days on this dosage of cortisone, the body weight was decreased and the animals were in negative sodium and potassium balance. A significant reduction in exchangeable potassium content was detected for the first time, and the serum potassium concentration tended to be low. These changes had not been corrected 7 days after the administration of cortisone was stopped. Such changes suggest an increase in the rate of catabolism of tissues with an associated loss of water through the kidneys. It has previously been demonstrated in patients that the administration of either ACTH or cortisone in therapeutic doses may result in a depletion of the body content of potassium(10).

It would, therefore, appear that the earliest change to occur following the administration of a small dose of cortisone acetate to normal adult male rabbits is a retention of water in the extracellular fluid phase. As the administration of the drug is continued, the water is redistributed into the intracellular phase. The administration of a larger dose of cortisone results in depletion of the body's store of potassium, presumably because of the catabolic effects of cortisone as well as the decrease in food consumption. As the administration of cortisone is continued, diuresis eventually takes place.

The results of previous clinical studies dealing with the effects of cortisone on salt and water metabolism are difficult to interpret because of the differences in the physiologic status of the diseased subjects prior to the initiation of cortisone therapy. A fixed dose of the drug may not necessarily represent a physiologic dose in such individuals; furthermore, the response of the tissue cells to cortical hormone may vary with their functional status. Thus, different authors(12) have reported hyponatremia and hypernatremia respectively as the sole effects of cortisone therapy. Contrary to the findings in rabbits, large doses of cortisone have not produced alterations in the serum sodium or potassium concentration of rats, although such dosages did decrease food consumption(11). In dogs, the administration in doses of 5 mg/kg of cortisone daily for one week is reported to have caused a reduction in the serum sodium concentration(12).

The present study does not elucidate the mechanisms whereby alterations in the distribution and concentration of cations and water result from the administration of cortisone. However, it does show that rapid and transitory alterations in the distribution and osmolarity of body fluids may occur even in animals with intact adrenal glands in which hormonal imbalance is induced by the administration of cortisone acetate. The effects of cortisone on fluid and electrolyte balance in disease states must be interpreted in the light of the changes which it may induce in normal animals.

Summary. 1. External balance studies for sodium and potassium and serial isotopic determinations of the exchangeable potassium content were made in 2 groups of normal male rabbits which were given, respectively, 4 mg of cortisone acetate daily for 2 weeks and 25 mg daily for 3 weeks. The administration of 4 mg daily resulted in an increase in body weight and a reduction in serum sodium concentration within 7 days; both serum sodium and serum potassium concentrations were increased in 14 days. One week after discontinuation of cortisone, the animals were in negative sodium balance. The administration of 25 mg daily resulted in a negative sodium and potassium balance with an increase in serum sodium concentration within 7 days. At 21 days, body weight and exchangeable

potassium content were reduced. 2. The results indicate that the response of normal rabbits to cortisone acetate varies with the size of the dose and with the length of the period of administration. The results suggest that the earliest change to occur following the administration of a small dose daily was a retention of water in the extracellular fluid compartment; with continued administration of this dose, water was redistributed into the intracellular phase. The administration of a larger dose of cortisone daily resulted in depletion of the body's store of potassium.

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Effect of Salicylic Acid on Adrenal-Pituitary System.* II. Influence of Aminoacids and Other Compounds. (20037)

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The influence of salicylic acid (SAL) on the adrenal pituitary system of normal rats, as evidenced by a reduction of adrenal ascorbic acid, has been established by several investigators(1-5). This effect is quite specific for many substituted aromatic ortho-hydroxy carboxylic acids(5) and several other types of compounds(1,5). Since the metabolism of SAL involves conjugation with glycine and glucuronic acid, oxidation to gentisic acid and possibly other reactions, and since SAL bloodlevels can be influenced by administration of other substances, (PABA, ascorbic acid)(6-8), the effect of such combinations on the adrenal pituitary system was investigated. The main purpose was to determine whether it might be possible to augment the specific activity of SAL.

Methods. All experiments were made in female Wistar rats in the manner described previously(5). All compounds were administered as the sodium salts (except calcium pantothenate.) The pH of the solutions was not adjusted. The results for the treated animals are expressed in per cent of those for saline controls, since it had been shown that thereby the variations from day to day and from group to group are eliminated.

Results. The substances combined with SAL may be divided into 3 groups: carbohydrate metabolites, aminoacids and miscellaneous compounds. (Table I) None of these 21 acids, at a dose of 150 mg/kg, had any influence upon the ascorbic acid depletion produced by the simultaneous administration of 150 mg/kg of SAL. Particular attention was given to glucuronic and p-aminobenzoic acid because the former conjugates with SAL and both have been suggested for the treatment of rheumatoid arthritis and collagen diseases(9-12). PABA has also been shown to produce higher SAL bloodlevels by interference with renal clearance.

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