A STUDY OF THE MECHANISM OF SECRETION OF THE SODIUM-RETAINING HORMONE (ALDOSTERONE) [1, 2]

By JOHN H. LARAGH and HERBERT C. STOERK

(From the Department of Medicine, College of Physicians and Surgeons, Columbia University, the Presbyterian Hospital in the City of New York and the Merck Institute for Therapeutic Research, Rahway, N. J.)

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Aldosterone, which has been isolated from the adrenal cortex and chemically characterized, is the most powerful sodium-retaining hormone among the known naturally occurring adrenal steroids (1). As such, it seems probable that it plays a dominant part in the regulation of sodium and potassium metabolism.

There is considerable evidence that aldosterone may be unique among adrenal steroids in that its rate of secretion is largely independent of anterior pituitary control. This is suggested by the absence of atrophy of the zona glomerulosa in rats and dogs after hypophysectomy (2, 3), by the persistence of aldosterone in the adrenal venous blood of hypophysectomized dogs (4) and by the observation that some patients with acute pituitary insufficiency induced by total hypophysectomy conserve sodium normally (5) and excrete normal amounts of salt-retaining hormone in the urine (6).

In 1950, Luetscher, Neher, Wettstein, and Curtis (7, 8) first reported increased sodium-retaining activity in the urine of patients forming edema. In subsequent studies this group has identified the active substance in the urine as aldosterone. In addition, Luetscher and Axelrod (9) have reported increases in the excretion of hormone in normal subjects in response to short periods of sodium deprivation. The increase occurs without apparent alteration in the serum sodium concentration.

The present study was designed to examine the effects of changes in the dietary intake and of associated changes in the serum concentration of sodium and potassium ions upon the urinary excretion of the salt-retaining hormone. Certain aspects of the relationship between the distribution of sodium and potassium ions have been utilized to produce alterations of serum K and Na. Earlier studies have demonstrated that the administration of potassium to sodium-depleted dogs and human subjects produces a marked and sustained hyperkalemia which is not observed in subjects in normal electrolyte balance (10, 11).

The present report demonstrates that potassium ingestion, when accompanied by a rise in the serum potassium, may be associated with a pronounced increase of sodium-retaining activity in the urine. A fall in the serum sodium concentration *per se* does not appear to produce a comparable effect.

METHODS

The experiments were carried out on trained, unanesthetized mongrel dogs housed in metabolism cages. The animals were fed a synthetic diet of fixed electrolyte content containing casein, lard, dextrin, dextrose, vitamin and mineral supplements and agar-agar to provide approximately 80 calories per kilogram per day. The basal diet contained less than 1.8 mEq. of Na and less than 0.44 mEq. of K per day. When KCl was given it was administered either as a 20 per cent solution by stomach tube or incorporated into the diet. Urine was collected daily and pooled in 48-hour lots. After removal of 10-ml. aliquots for estimation of Na, K and Cl, the urines were stored at $-76°$ C. Stool specimens were not saved for analysis, but none of the dogs had diarrhea at any time. Methods for the estimation of serum and urinary sodium and potassium and chloride have been described previously (10).

For estimation of sodium-retaining activity, the thawed urine specimens were adjusted to pH 1 with 6 N HCl and extracted continuously for 24 hours with 350 ml. of redistilled methylene chloride in a Wolfe-Hirschberg extractor. The methylene chloride extract was washed successively five times with 50-ml. portions of 0.1 N NaOH and five times with 50 ml. of distilled water and taken almost to dryness at reduced pressure in an atmosphere of nitrogen. The extracts were dried further over P_2O_5 in a vacuum desiccator at room temperature and then stored at $-4°$ C. At the time of assay the dried

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extract was first dissolved in 4 ml. of 50 per cent ethanol and dilutions of 1 : 16 and 1 : 80 were made.

The bioassay was carried out on male rats, adrenalectomized 24 hours before the test. After adrenalectomy the rats were offered distilled water ad libitum and a low sodium diet (Na 0.001 per cent, K 0.3 per cent). At the start of the assay 5 ml. of normal saline were injected intraperitoneally, and the bladder was emptied by electric shock. The animals were injected subcutaneously with either desoxycorticosterone acetate (DOCA) standards or unknowns in 0.25 ml. of 30 per cent ethanol, and placed in groups of three rats in metabolism cages. Duplicate groups of three rats were used for each DOCA standard (2, 10 and 50 micrograms) and for each unknown sample in 1 : 16 and 1 : 80 dilution. Human urine samples were handled similarly except that 24-hour collections were used, and extracts were assayed at 1 : 16, 1 : 80 and 1 : 400 dilutions. When samples were strongly active at all dilutions throughout the range tested, appropriate additional five-fold dilutions were employed until a suitable endpoint could be obtained. Further dilutions of inactive samples were tested since, with excessive amounts of aldosterone (near or greater than 10 micrograms per rat), sodium retention may not occur with this procedure.

At the end of a five-hour collection period the rats were forced to void by electric shock, the total urine volume was measured (4 to 10 ml. per group), and the amount of sodium excreted determined by flame photometry. The amounts of sodium excreted by the groups injected with DOCA were plotted on an arithmetic scale against the logarithm of the dose of DOCA (2, 10, 50 μg.). The standard curve thus obtained serves for estimation of the sodium-retaining equivalents of the unknowns. In this assay, 100 μg. of DOCA and 1.0 μg. of aldosterone have approximately equivalent activities. Using three groups of 3 rats each, the lambda is 0.23 (Lambda = standard deviation ÷ slope). Compounds B and F, in doses up to 1,500 μg., do not cause sodium retention nor interfere with the sodium-retaining activity of DOCA or aldosterone in this assay. In our hands, normal humans on unselected diet excrete 1 to 4 micrograms per 24 hours.

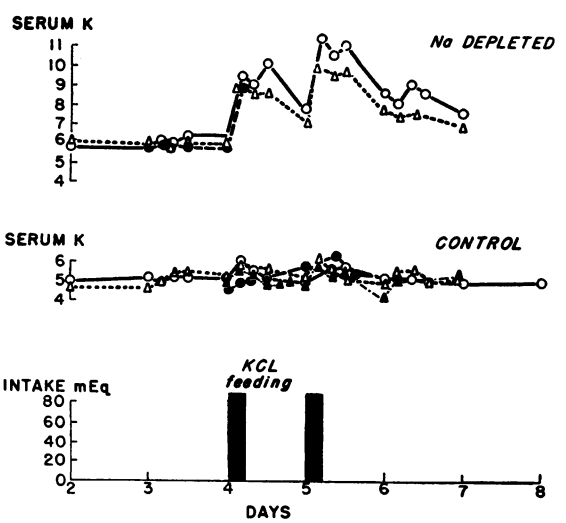

FIG. 1. HYPERKALEMIA RELATED TO SODIUM DEPLETION

Dogs rendered hyponatremic by peritoneal dialysis develop sustained hyperkalemia after feeding KCl. The same dose of KCl does not increase the serum potassium of animals not sodium depleted.

Active urine extracts were chromatographed on paper (12). The region of aldosterone was eluted and reassayed. With the larger amounts present in human urine, activity has been consistently confirmed in the eluate, and fluorescence in ultraviolet light and reduction with blue tetrazolium also gave results consistent with aldosterone. The active, crude extracts of urine consistently have been found to possess activity indistinguishable from pure aldosterone. Other groups have reported similar results (13).

Figure 1 illustrates the effect of orally administered KCl on the concentration of serum potassium in normal and sodium-depleted dogs. In four control dogs receiving 2 gm. of NaCl daily, the serum K was not appreciably

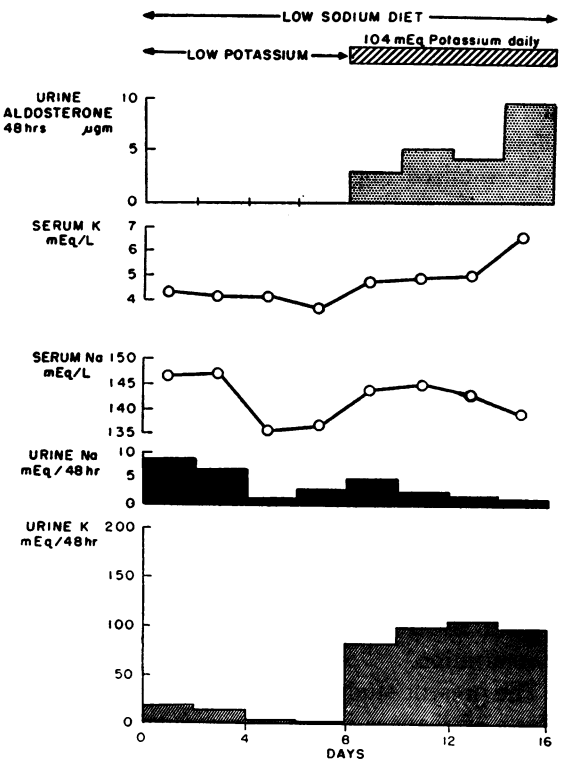

FIG. 2. EFFECT OF LOW AND HIGH POTASSIUM INTAKE UPON ALDOSTERONE, SODIUM AND POTASSIUM EXCRETION IN DOG N ON A LOW SODIUM DIET

No detectable sodium-retaining activity of urinary extracts was observed with a diet virtually free of sodium and potassium. When dietary KCl supplements were given, hyperkalemia developed and aldosterone-like activity appeared in the urine. No concurrent change in Na balance or serum Na was observed.

TABLE I

Feeding experiment in normal dogs

Dog	Experimental period	Consecutive days of balance period*	Daily intake Na (mEq.)	Daily intake K (mEq.)	Daily intake H_2O† (ml.)	Observed serum‡ Na (mEq./L.)	Observed serum‡ K (mEq./L.)	Aldosterone activity 48 hr. (Est. μg.)	Average daily urinary excretion§ Na (mEq.)	Average daily urinary excretion§ K (mEq.)	Average daily urinary excretion§ Vol. (ml.)
N	Control diet	7, 8	51	104	450	147	4.2	0	44	94	400
	Na and K free diet	1, 2	0	0	450	147	4.3	0	8.5	16.2	550
		3, 4	↓	↓	↓	148	4.2	0	6.1	10.6	470
		5, 6				136	4.2	0	1.8	3.0	480
		7, 8				137	3.8	0	2.2	1.8	525
	KCl added daily from day 9	9, 10	0	104	450	143	4.9	2.8	4.7	82.0	405
		11, 12	↓	↓	↓	145	5.1	5.2	2.1	98.0	415
		13, 14				142	5.3	4.6	1.7	103.0	425
		15, 16				139	6.7	9.0	1.2	92.0	410
W	Control diet (KCl added) on day 7, 8	5, 6	51	80	450	146	4.4	0	40	77	485
		7, 8	51	230	450	151	4.9	0	37	221	590
	Na free	1, 2	0	67	450	143	4.8	0	18	74	555
		3, 4	↓	67	↓	145	4.5	0	5.1	54	380
		5, 6		104		147	4.7	0.5	4.4	113	367
		7, 8		104		148	5.8	0.7	3.6	86	375
	Na and K free diet	9, 10	↓	0	↓	145	4.6	0	0.5	16.8	510
		11, 12		↓		144	3.6	0	0.4	2.4	455
		13, 14				144	4.3	0	1.8	0.9	480
		15, 16				143	3.8		0.5	0.7	478

* Refers to days from beginning of the particular diet.
† Distilled water given daily by stomach tube throughout.
‡ Refers to serum levels obtained in fasting state at end of the corresponding 48-hour balance period.
§ The average of two consecutive 24-hour measurements.

increased by KCl feeding. In contrast, dogs previously depleted of Na by peritoneal dialysis, so that the serum sodium was reduced to about 120 mEq. per L., uniformly developed a marked hyperkalemia when given the same amounts of KCl. Two dogs (M and R) died after receiving a single oral dose of 10 gm. of KCl when severely depleted of sodium. These dogs had tolerated even higher doses of KCl when on normal sodium intakes. This phenomenon has been utilized as a means of producing relatively sustained hyperkalemia.

The present studies were performed on 1) normal dogs, 2) dogs with diabetes insipidus, and 3) a single human subject with chronic congestive heart failure and edema.

1) Normal dogs

Three types of experiments were performed on these animals:

(*a*) *Feeding experiment* (*Table I, Figure 2*). One of two dogs (N) received a constant intake of a sodium and potassium-free diet for 8 days, and in the succeeding 8-day period KCl was added. In the other dog (W) this procedure was reversed, KCl being added in the first and absent in the second experimental period. Distilled water (450 ml.) was given daily by stomach tube.

(*b*) *Depletion experiment* (*Table II, Figure 3*). The dogs were first depleted of sodium chloride by peritoneal dialysis, according to the method of Darrow and Yannet (14), and then maintained on a constant diet free of sodium. Repeated dialysis was necessary in several instances to achieve significant hyponatremia. Dogs R and M received 18 mEq. of KCl on this diet whereas dogs W and E were kept on a K free as well as a Na free regimen. Distilled water (400 to 500 ml.) was given daily and, after a control period, KCl was added for two successive days by stomach tube.

(*c*) *Dilution experiment* (*Table III*). The serum sodium and potassium were reduced by a combination of forced hydration (1000 to 1500 ml. water by tube daily) and 2.5 units of Pitressin Tannate in Oil® injected twice daily. Sustained hemodilution and hypervolemia can be achieved in this way with reduction in serum sodium and potassium (15).

2) Dogs with diabetes insipidus (*D.I.*) (*Table IV*)

Diabetes insipidus was produced by electrocoagulation of the hypothalamic tracts by Dr. R. C. deBodo of the Department of Pharmacology, New York University College of Medicine. These dogs excreted from two to six liters of urine per 24 hours when having free access to food and water. They were studied during three successive periods: 1) unselected or stock diet, 2) sodium and potassium-free diet, and 3) with the addition of KCl

TABLE II

NaCl depletion experiments in normal dogs

Dog	Experimental period	Consecutive days of balance period	Daily intake Na (mEq.)	Daily intake K* (mEq.)	Daily intake H_2O† (ml.)	Observed serum Na (mEq./L.)	Observed serum K (mEq./L.)	Aldosterone activity 48 hr. (Est. μg.)	Average daily urinary excretion Na (mEq.)	Average daily urinary excretion K (mEq.)	Average daily urinary excretion H_2O (ml.)
R	Control diet	3, 4	51	107	500	148	4.0	0	46	101	525
	Balance study started 8 days after a dialysis	1, 2	0	18	500	128	4.2	1.5	0.5	8.8	460
		3, 4	|	105	|	137	6.9	11.2	2.3	85.0	560
		5, 6	|	18	|	130	6.2	16.8	1.3	27.5	435
		7, 8	↓	18	↓	129	4.5	3.6	0.7	14.2	490
	A second study started 6 days after another dialysis	1, 2	0	18	500	139	5.1	6.8	0.3	11.7	482
		3, 4	|	105	|	141	7.1	11.4	1.6	99.5	570
		5, 6	|	18	|	138	5.9	4.8	0.5	19.1	480
		7, 8	|	18	|	137	5.0	2.0	0.4+	13.5+	415+
		9, 10	↓	18	↓	137	4.8	0.8	0.4	11.7	445
M	Study started 7 days after dialysis	1, 2	0	18	400	134	5.8	5.1	0.4	15.4	265
		3, 4	|	132	|	131	6.8	9.6	2.2	107.0	355
		5, 6	↓	18	↓	129	5.0	5.0	0.4	27.0	322
W	Stock diet	1, 2			500	143	4.5	0	105	62	550
	Study started the day after a dialysis Na and K free diet	1, 2	0	0	500				0.5	15.1	325
		3, 4	|	|	|	134	4.5	0.5	0.3	4.5	410
		5, 6	|	|	|	133	4.3	0	0.3	2.9	480
		7, 8	|	↓	|	132	4.4	0.8	0.3	2.1	462
		9, 10	|	94	|	132	5.5	0.5	6.5	86.6	462
		11, 12	↓	0	↓	133	4.7	0.5	0.2	9.4	540
C	Stock diet	1, 2			500	145	4.2	0	42.4	46.3	412
	Balance study started 1 day after a dialysis	1, 2	0	0	500				0.8	27.4	490
		3, 4	|	|	|	123	4.6	1.1	0.5	20.0	455
		5, 6	|	|	|	125	5.2		0.7	14.0	525
		7, 8	|	↓	|	124	5.3	5.9	0.8	22.4	525
		9, 10	|	67	|	135	6.0	9.6	3.3	56.4	530
		11, 12	↓	0	↓	127	5.4	2.2	2.6	15.4	495

* K was given as KCl by stomach tube as a 20 per cent solution. The figures given are the averages of two doses for each two-day balance period.

† Represents total daily free water intake and does not include water used in mixing diet batches.

in amounts similar to those used in the normal dogs. Distilled water was allowed ad libitum in all periods.

3) A patient (Figure 5) with rheumatic heart disease

The patient was admitted to the metabolism ward and studied while on a constant regimen. He was a 52-year-old man with mitral insufficiency, auricular fibrillation and chronic, right-sided congestive failure. He had taken digitalis preparations daily for fifteen years and Mercuhydrin® injections, as often as twice a week, for over ten years. He had resorted to a low-sodium diet for at least ten years. Maintenance digitoxin was continued in the hospital. The sodium intake was kept constant throughout the study (12 mEq. daily). The rates of aldosterone excretion on a low (16 mEq. per day) and relatively high (140 mEq. per day) potassium intake were compared.

RESULTS

The results of the animal studies are summarized in the accompanying tables and in Figures 1–4.

Feeding experiments

In the experiments with two normal dogs (N and W, Table I) and two dogs with diabetes insipidus (T and E, Table IV), it was not possible to detect any sodium-retaining activity in the urine during periods of stock diet intake, or when KCl was given without simultaneous sodium deprivation. The KCl supplements ranged from 67 to 230 mEq. per day.

Dog N was kept on a diet free of sodium and potassium for a period of 8 days. During this

TABLE III

Dilution experiments in normal dogs

Dog	Experimental period	Consecutive days of balance period	Daily intake Na (mEq.)	Daily intake K (mEq.)	Daily intake H_2O* (ml.)	Observed serum Na (mEq./L.)	Observed serum K (mEq./L.)	Aldosterone activity 48 hr. (Est. μg.)	Average daily urinary excretion Na (mEq.)	Average daily urinary excretion K (mEq.)	Average daily urinary excretion H_2O (ml.)
N	Pitressin® 2.5 units b.i.d.	3, 4	0	0	ad lib.	142	4.4		5.0	4.8	380
	on days 5, 6, 7, 8	5, 6	0	0	1,125	122	3.6	0	8.1	3.5	615
		7, 8	0	204†	900	129	6.2	2.6	24.5	190.9	578
W	Pitressin in Oil® 2.5 units	3, 4	0	0	ad lib.	143	4.5	0	5.9	10.7	390
	b.i.d. on days 5, 6	5, 6	0	0	1,225	128	3.6	0	10.9	11.6	460

* Distilled water given by stomach tube daily or ad libitum as indicated.
† Dog vomited 200 ml., and this amount of K was subtracted from balance.

time, no sodium-retaining activity was detected in the urinary extracts. When 104 mEq. of potassium were added to the diet, significant hyperkalemia developed and salt-retaining activity appeared in appreciable amounts in the urine (Figure 2).

In a second normal dog (W), the administration of KCl did not produce as great an elevation of serum potassium, and insignificant amounts of sodium-retaining activity were detected in the urinary extracts.

Table IV presents data from similar feeding experiments in two dogs with diabetes insipidus. These animals also, as stated above, appear to excrete little or no aldosterone-like material when on a stock diet or on a diet free of sodium and potassium. When potassium chloride was added to the diet, an increase in the sodium-retaining activity of the urinary extracts was observed in both dogs. Water balance was not detectably altered, and sodium balance did not change appreciably. A consistently low urinary specific gravity throughout the experiment affords further evidence that hydration was adequately maintained. In the dogs with diabetes insipidus, the serum potassium concentration was maintained at slightly higher levels than in the normal dogs during both the control and experimental periods.

Depletion experiments

Table II summarizes five balance studies in dogs depleted of sodium by peritoneal dialysis. In three of the four animals, detectable amounts of salt-retaining hormone appeared in the urine at some time during the study. The amounts excreted did not correlate well with the degree of hyponatremia, nor were they related to a further loss of body sodium or water, but appear to be more directly related to the level of the serum potassium. It is noteworthy that the largest amounts of hormone were generally excreted on the days when potassium intake was greatest (R, M, C).

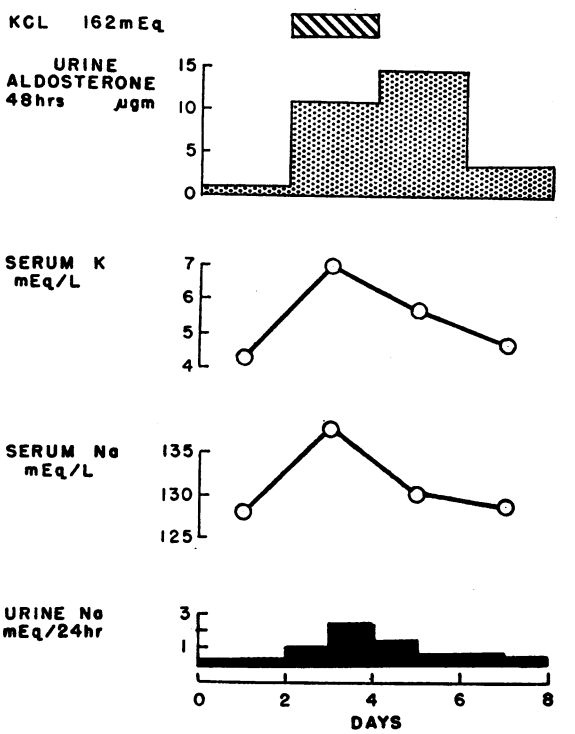

FIG. 3. EFFECT OF POTASSIUM FEEDING ON ALDOSTERONE EXCRETION AND SERUM POTASSIUM DURING SODIUM DEPLETION IN DOG R

Demonstration that sodium depletion with hyponatremia did not increase urinary aldosterone-like activity until dietary potassium was increased. No change in overall sodium balance was observed.

TABLE IV

Feeding experiments in dogs with diabetes insipidus

Dog	Experimental period	Consecutive days of study	Daily intake* Na (mEq.)	Daily intake* K (mEq.)	Daily intake* H_2O (ml.)	Observed serum Na (mEq./L.)	Observed serum K (mEq./L.)	Aldosterone activity 48 hr. (Est. μg.)	Average daily urinary excretion Na (mEq.)	Average daily urinary excretion K (mEq.)	Average daily urinary excretion H_2O (ml.)
T	Stock diet	1, 2			5,200	152	5.2	0	180	86	4,475
	Na and K free diet beginning on day 3	3, 4	0	0	2,050				15	18	1,780
		5, 6			1,850	152	5.0	0	17.5	14.5	1,800
		7, 8			1,150	156	4.9	0.24	7.0	12.0	1,070
		9, 10	↓	↓	1,450	152	5.8	0	6.0	12.0	1,450
	KCl added daily beginning on day 11	11, 12	0	67	2,150	153	4.8		6.0	70.0	1,520
		13, 14		67	1,475	152	6.0	0.7	2.2	60.4	1,140
		15, 16		67	1,475	146	6.7	0.5	2.4	66.0	1,310
		23, 24	↓	134†	2,550	150	7.6	2.0	3.8	122.0	2,300
E	Stock diet	1, 2			3,300	148	5.4	0	174.0	65.4	3,270
	Na and K free diet beginning on day 3	3, 4	0	0	1,325				15.9	24.3	1,375
		5, 6			1,225	151	5.4	0.01	6.5	7.6	865
		7, 8			1,185	150	5.6	0.7	4.9	6.5	1,322
		9, 10	↓	↓	1,800	149	5.4	0	2.7	3.9	1,595
	KCl added daily beginning on day 11	11, 12	0	67	2,500				3.8	46.8	2,275
		13, 14		67	2,150	143	5.6	2.4	3.6	66.0	2,125
		15, 16		67	2,275	152	8.0	6.0	2.1	56.9	1,945
		23, 24	↓	134†	3,315	149	7.7	3.0	7.0	120.4	3,180

* Water intake is the average of two days of ad libitum intake of distilled water.
† From day 16 on dogs received 134 mEq. of KCl and no Na in the daily ration.

Two of the dogs (R, M) received small amounts of KCl (18 mEq.) as part of the daily diet after dialysis. These animals excreted appreciably more of the active hormone than did the two dogs (W, C) given K-free diets. In dog W, hormone excretion remained low throughout the study, despite serum sodium levels as low as 132 mEq. per L. and serum potassiums as high as 5.5 mEq. per L. It is of interest that this is the same dog that failed to excrete hormone in the feeding experiment described previously (Table I).

Dilution experiments (Table III)

Two dilution studies were performed primarily for comparison with the data obtained in the depletion experiments. These also show that a reduction of serum sodium is not invariably associated with a change in urinary salt-retaining activity. In contrast to the dialysis depletion studies, the dilution procedure maintains the total body sodium more nearly at its normal level and hyponatremia is produced, at least in part, by excessive water retention. A parallel reduction in serum potassium also occurs. In one instance (N), when hypokalemia was corrected by feeding KCl, increased salt-retaining activity was noted in the urine. This occurred with hyperkalemia and an associated increase in total body water, as suggested by the fluid balance.

Figure 4 presents a graphic correlation between the levels of serum potassium and the excretion of aldosterone-like material in all of the animal studies. The response in a given animal appears to be qualitatively reproducible, as evidenced by the data obtained with dogs W and R.

Observations on a cardiac patient

The possible relationships between the levels of serum Na and K and the excretion of aldosterone have been explored in a man with heart failure and edema (Figure 5). The serum sodium concentration remained at about 120 mEq. per L. during the entire study. As in the case of the sodium-depleted dog, the serum potassium tended to be high, *i.e.*, 6 to 7 mEq. per L., when he was on a moderately high potassium intake. The serum potassium was reduced to 2.7 mEq. per L. over a 24-hour period by a single 2-ml. dose of

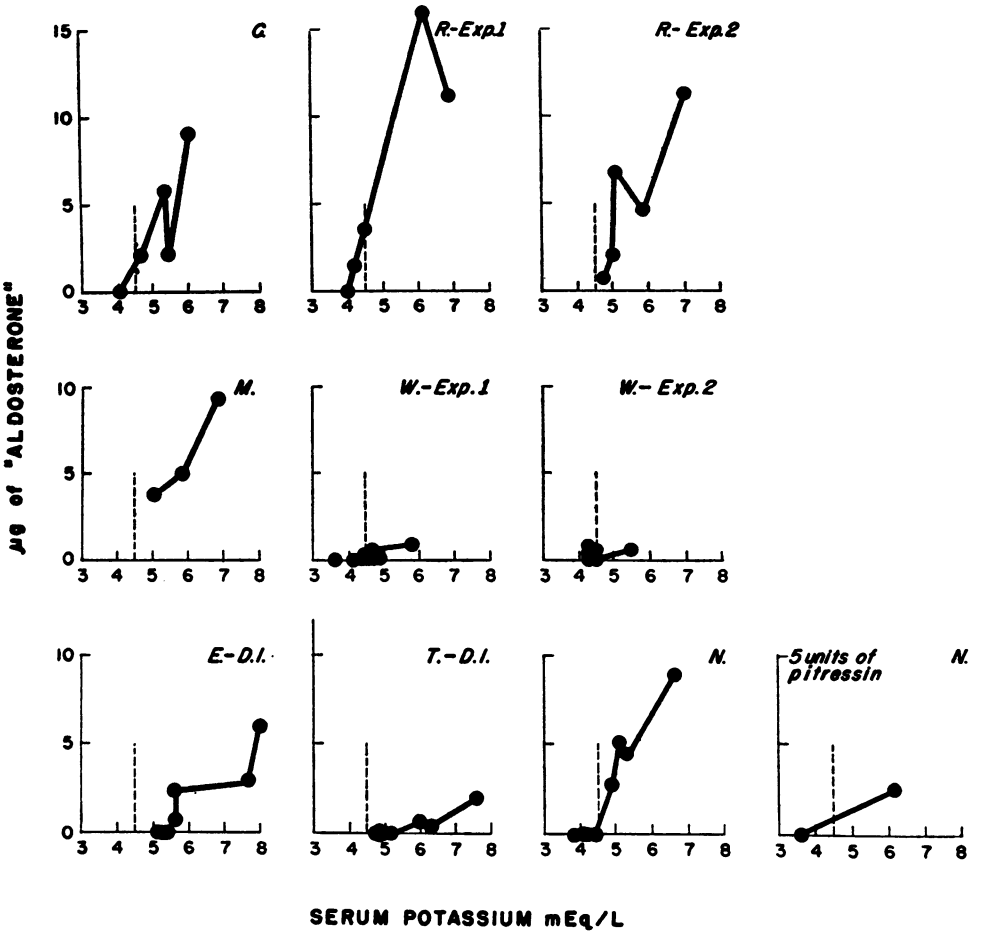

FIG. 4. CORRELATIONS BETWEEN THE CONCENTRATION OF SERUM POTASSIUM AND THE EXCRETION OF ALDOSTERONE-LIKE MATERIAL IN DOGS C, R, M, W, E, T, AND N

Mercuhydrin® in conjunction with the institution of a low potassium diet. As shown in Figure 4, the daily aldosterone excretion was initially high (300 µg.) and fell to a minimal value of 35 µg. per day after the serum K was reduced. This rate of excretion is still considerably above that observed in normal individuals on low sodium diets (9). Subsequently, the administration of K restored the serum K to higher levels and the excretion of aldosterone increased to a peak of 630 µg. per day. In this study, the estimates of aldosterone were confirmed by re-assay after chromatography and by U-V absorption and reduction with blue tetrazolium.

DISCUSSION

In this study, the amount of salt-retaining activity of urinary extracts has been considered to be an index of the rate of aldosterone secretion. It should be appreciated that probably only a small fraction of the total amount secreted by the adrenal cortex actually appears in the urine (8). The activity of the urinary extracts is undoubtedly affected by variations in the rate of renal excretion and metabolic degradation.

Among the factors suggested thus far as promoting the secretion of aldosterone are sodium deprivation (9), potassium administration (16, 17) and a reduction in body water (17, 18). There is general agreement that sodium restriction results in an increase in urinary aldosterone (8, 17) and, conversely, that the administration of sodium in sufficiently large quantities suppresses the excretion of aldosterone (8, 17, 19). Luetscher and Johnson (20) have reported an inverse relationship between urinary sodium and aldos-

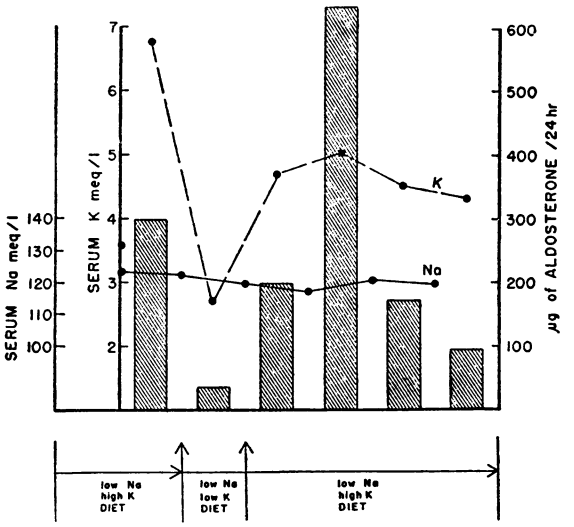

FIG. 5. METABOLIC BALANCE STUDY OF A PATIENT IN CONGESTIVE HEART FAILURE, WITH APPARENT RELATIONSHIP BETWEEN SERUM POTASSIUM LEVEL AND ALDOSTERONE EXCRETION.

Serum Na level did not change appreciably.

terone content. The effects of potassium administration have been reported by several groups (16, 17). It is of interest that Falbriard, Muller, Neher, and Mach (21), who administered potassium to normal human subjects, found an increase in urinary aldosterone which was not necessarily correlated with a reduced urinary sodium content. Further evidence that potassium may be important in aldosterone secretion has been obtained in the following experiments. Vogt (22) has reported an increase in the cold survival activity of the adrenal effluent during perfusion with solutions high in potassium which was not observed with perfusion of solutions low in sodium. In similar experiments utilizing the perfusion of calf adrenals, Rosenfeld, Rosemberg, Ungar, and Dorfman (23) demonstrated an actual increase in aldosterone and attributed this response to a lowering of the Na/K ratio in the perfusing fluid. Singer and Stack-Dunne found a reduction in adrenal venous aldosterone in rats depleted of potassium (24). Stoerk, Knowlton, and Loeb have found a correlation between the serum potassium and the size of the zona glomerulosa of rat adrenal cortex (25).

Bartter, Liddle, Duncan, and Delea (17) on the basis of studies involving water-loading or dehydration by various means, have stressed the dominant role of the volume of the extracellular fluid in governing aldosterone secretion. These investigators have been unable to establish any correlation between the electrolyte composition of the extracellular fluid and the rate of aldosterone elaboration.

From the data presented in this communication it appears that the administration of potassium has little effect on aldosterone excretion unless there is a simultaneous elevation of the serum potassium. It also has been shown that potassium administration in sodium depleted subjects causes a significant and sustained hyperkalemia (10, 11). Therefore, it may be anticipated that sodium deprivation, in the absence of potassium deprivation, may lead to some elevation of serum potassium. Consequently, changes in the rate of aldosterone secretion occurring in the course of sodium deprivation may not be entirely referable to alteration in sodium balance. In this connection, it is of interest that in the two subjects reported by Luetscher and Axelrod (9), the increases in urinary aldosterone induced by sodium withdrawal were accompanied by rises in the serum potassium of 0.7 to 0.9 mEq. per L., while the concentration of serum sodium remained relatively constant. More recently, this group has found with sodium deprivation that the extent of increase in hormone is directly related to the concurrent K intake (26).

In the present studies it is worthy of note that changes in aldosterone excretion did not appear to correlate well with any observable change in the level of serum sodium, sodium balance, or the state of hydration.

In edematous states, the administration of sodium (17, 19) or potassium deprivation, as reported here in one patient, may reduce urinary aldosterone but to values which remain well above normal. Furthermore, in experimental edema characterized by increased urinary aldosterone (27) a sustained large increase in plasma volume and in body water does not lead to sodium excretion as it does in normal subjects (15), nor is there evidence of a contracted volume in clinical edema. Thus, the increased urinary aldosterone of edematous states does not appear to be entirely accounted for by any of these known mechanisms.

SUMMARY

Metabolic balance studies have been carried out on dogs, some of which were depleted of sodium by peritoneal dialysis. Normal dogs did not exhibit any detectable salt-retaining activity in 48-hour urinary extracts when on diets containing normal amounts of sodium. Potassium administration in this situation did not induce appreciable hyperkalemia and was not associated with any detectable hormone activity of the urine.

Dietary restriction of sodium did not lead to appreciable hormone activity of urine extracts unless potassium was provided in the diet. With the feeding of potassium supplements the hormone activity increased.

Reduction in the serum sodium by peritoneal dialysis was associated with only slight to negligible hormone activity unless potassium was provided in the diet.

Two dogs with diabetes insipidus exhibited responses similar to the normal dogs.

The sodium-retaining activity of the urinary extracts in this study can be correlated with an induced increase in the serum potassium ion concentration and does not correlate well with reduction in the serum sodium ion concentration.

The data support the concept that an increase in the concentration of the serum potassium ion is a stimulus for the secretion of aldosterone.

REFERENCES

1. Simpson, S. A., Tait, J. F., Wettstein, A., Neher, R., v. Euw, J., Schindler, O., and Reichstein, T., Die Konstitution des Aldosterons. Helvet. chim. acta, 1954, **37**, 1200 [über Bestandteileder Nebennicrenrinde und Verwandte Stoffe].
2. Deane, H. W., and Greep, R. O., A morphological and histochemical study of the rat's adrenal cortex after hypophysectomy, with comments on the liver. Am. J. Anat., 1946, **79**, 117.
3. Lane, N., and deBodo, R. C., Generalized adrenocortical atrophy in hypophysectomized dogs and correlated functional studies. Am. J. Physiol., 1952, **168**, 1.
4. Rauschkolb, E. W., Farrell, G. L., and Koletsky, S., Aldosterone secretion after hypophysectomy. Am. J. Physiol., 1956, **184**, 55.
5. Maclean, J. P., Li, M. C., Lipsett, M. B., Ray, B., and Pearson, O. H., The physiological role of adrenal salt hormone (aldosterone) in man. J. Clin. Invest., 1955, **34**, 951.
6. Luetscher, J. A., Jr., and Axelrod, B. J., Sodium retaining corticoid in the urine of normal children and adults and of patients with hypoadrenalism or hypopituitarism. J. Clin. Endocrinol. & Metab., 1954, **14**, 1086.
7. Luetscher, J. A., Jr., Neher, R., and Wettstein, A., Isolation of crystalline aldosterone from the urine of a nephrotic patient. Experientia, 1954, **10**, 456.
8. Luetscher, J. A., Jr., and Curtis, R. H., Aldosterone: Observations on the regulation of sodium and potassium balance. Ann. Int. Med., 1955, **43**, 658.
9. Luetscher, J. A., Jr., and Axelrod, B. J., Increased aldosterone output during sodium deprivation in normal men. Proc. Soc. Exper. Biol. & Med., 1954, **87**, 650.
10. Laragh, J. H., The effect of potassium chloride on hyponatremia. J. Clin. Invest., 1954, **33**, 807.
11. Laragh, J. H., and Capeci, N. E., Effect of administration of potassium chloride on serum sodium and potassium concentration. Am. J. Physiol., 1955, **180**, 539.
12. Bush, I. E., Methods of paper chromatography of steroids applicable to the study of steroids in mammalian blood and tissues. Biochem. J., 1952, **50**, 370.
13. Liddle, G. W., Cornfield, J., Casper, A. G. T., and Bartter, F. C., The physiological basis for a method of assaying aldosterone in extracts of human urine. J. Clin. Invest., 1955, **34**, 1410.
14. Darrow, D. C., and Yannet, H., The changes in the distribution of body water accompanying increase and decrease in extracellular electrolyte. J. Clin. Invest., 1935, **14**, 266.
15. Davis, J. O., Howell, D. S., and Hyatt, R. E., Effect of chronic Pitressin administration on electrolyte excretion in normal dogs and in dogs with experimental ascites. Endocrinology, 1954, **55**, 409.
16. Laragh, J. H., and Stoerk, H. C., On the mechanism of secretion of the sodium-retaining hormone (aldosterone) within the body. J. Clin. Invest., 1955, **34**, 913.
17. Bartter, F. C., Liddle, G. W., Duncan, L. E., and Delea, C., The role of extracellular fluid volume in the control of aldosterone secretion in man. J. Clin. Invest., 1956, **35**, 688.
18. Beck, J. C., Dryenfurth, F., Giroud, C., and Venning, E. H., Observations on the regulatory mechanisms of aldosterone secretion in man. Arch. Int. Med., 1955, **96**, 463.
19. Gordon, E. S., The role of aldosterone in congestive heart failure. J. Lab. & Clin. Med., 1955, **46**, 820.
20. Luetscher, J. A., Jr., and Johnson, B. B., Observations on the sodium-retaining corticoid (aldosterone) in the urine of children and adults in relation to sodium balance and edema. J. Clin. Invest., 1954, **334**, 1441.
21. Falbriard, A., Muller, A. F., Neher, R., and Mach, R. S., Étude des variations de l'aldostéronurie sous

l'effet de surcharges en potassium et de déperditions rénales et extrarénales de sel et d'eau. Schweiz. med. Wchnschr., 1955, **85**, 1218.
22. Vogt, M., Cortical secretion of the isolated perfused adrenal. J. Physiol., 1951, **113**, 129.
23. Rosenfeld, G., Rosemberg, E., Ungar, F., and Dorfman, R. I., Regulation of the secretion of aldosterone-like material. Endocrinology, 1956, **58**, 255.
24. Singer, B., and Stack-Dunne, M. P., The secretion of aldosterone and corticosterone by the rat adrenal. J. Endocrinol., 1955, **12**, 130.
25. Stoerk, H. C., Knowlton, A. I., and Loeb, E. N., The correlation between serum potassium and the weight of the adrenal glomerulosa in rats. J. Clin. Invest., 1955, **34**, 965.
26. Mulrow, P. J., Lieberman, A. H., Johnson, B. B., and Luetscher, J. A., Jr., Potassium to sodium ratio as an index of aldosterone output. J. Clin. Invest., 1956, **35**, 726.
27. Goodkind, M. J., Davis, J. O., Pechet, M. M., and Liddle, G. W., Aldosterone-like activity in urine from dogs with cardiac failure and with thoracic inferior vena cava constriction and ascites. Am. J. Physiol., 1955, **183**, 620.

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