THE DISTRIBUTION OF BODY WATER AND ELECTROLYTES IN ADRENAL INSUFFICIENCY [1]

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The mode of action of the hormone of the adrenal cortex necessary for life has been the subject of much discussion. Among the prominent hypotheses for explaining the symptoms of adrenal cortical insufficiency are loss of sodium and chloride with accompanying changes in the distribution of body water (1, 2, 3); toxic effects of potassium (4, 5, 6, 7); disturbance in the distribution of body water not clearly related to loss of sodium or retention of potassium (8); and disturbances in metabolism or carbohydrate (9). Since it seems, at present, bootless to attempt to relate the anomalies in carbohydrate metabolism to the disturbances in metabolism of water and electrolyte, this aspect of the function of the adrenal cortex will not be discussed. The present investigation was undertaken to determine by tissue analyses the actual distribution of water and electrolyte in the tissues of adrenalectomized rats in relation to the toxic action of potassium and the therapeutic effect of sodium salts.

EXPERIMENTAL PROCEDURE

Healthy male albino rats weighing approximately 200 grams were adrenalectomized under ether anesthesia via the usual lumbar route. The majority of the animals were fed a low potassium diet of the following composition: lactalbumin 15 per cent, sucrose 25 per cent, dextrin 32 per cent, crisco 22 per cent, cod liver oil 1 per cent, yeast powder 2 per cent, bone ash 2 per cent and NaCl 1 per cent. One group of animals was fed a ration containing 1.5 grams of K_2HPO_4 per 100 grams of the above diet. Diet 1 on analysis, was found to contain 0.03 per cent potassium whereas Diet 2 contained 0.7 per cent potassium. The animals were given distilled water, and the food and water intakes were measured daily.

The adrenalectomized animals were divided into groups and treated as follows. The animals of the first group were sacrificed when symptoms of severe adrenal insufficiency were manifest. The time of onset of symptoms was exceedingly variable on Diet 1 so that the rats could not be sacrificed at stated intervals after operation if comparable results were to be obtained. The symp-

toms used for the diagnosis of adrenal insufficiency were anorexia, loss of weight, bloody nasal discharge, diarrhea, muscular weakness and finally, marked diminution of the intake of water. It was found that the rats almost invariably died within 24 hours after they ceased drinking, so that it was possible by waiting for this symptom to obtain a series of animals for analysis in a similar state of severe insufficiency.

The animals of the second group were allowed to reach the same stage of adrenal insufficiency as those of Group 1 and were then treated by intraperitoneal injections of a potent adrenal cortical extract.[2] The amounts given were sufficient to cure the symptoms although the animals were fasted and given no sodium except that contained in the extract used. These rats were sacrificed at 24 and 48 hour intervals after therapy was instituted.

The animals of the third group when in a similar state of adrenal insufficiency were treated by daily intraperitoneal injections of 10 cc. of a solution containing 250 mm. NaCl and 50 mm. $NaHCO_3$ per liter. This treatment was continued until symptoms of insufficiency disappeared and the animals were thought to be in normal condition again. The animals were then sacrificed. These rats received no cortical extract.

The control animals were male rats from the same colony and of the same weight as the adrenalectomized rats. Since rats developing adrenal insufficiency refuse to eat, the control animals were likewise fasted before being sacrificed. A number of animals were treated as controls of caloric intake by giving only as much food as the adrenalectomized rats ate. This procedure was found unnecessary, however, and subsequent controls were uniformly fasted for 7 days and then sacrificed for analysis.

A number of intact animals were also fed both the low and the high potassium diets and then analyzed. These showed no differences and served as fed controls.

METHODS OF ANALYSIS

Since sufficient blood and tissue for a complete analysis could not be obtained from a single rat, the animals were pooled for analysis into groups of 2 to 4 animals. The rats were etherized and as much blood as could be obtained was withdrawn from the abdominal aorta without exposure to air. A portion of the blood was collected in a bottle containing potassium oxalate to prevent clotting,

[2] We are indebted to Dr. G. F. Cartland and the Upjohn Co. for a generous supply of cortical extract.

while the remainder was allowed to clot, centrifuged, and the serum separated.

Immediately after sacrificing the animals, the livers were removed and a sample of muscle was dissected off rapidly and freed of fat and connective tissue. The skin was removed *in toto* and the remainder of the carcass treated separately. All of the tissues were put into covered receptacles, weighed and then placed in an oven at 105° C. and dried until constant weight was attained.

The dried tissues were ground to a powder in a small food chopper. The dried powdered tissues were kept in glass stoppered flasks and aliquots taken for analysis.

One cubic centimeter samples of serum were accurately weighed in platinum dishes and the water content obtained by drying 24 hours in an oven at 105° C. The dried residue was ashed in a furnace at 500° C. for 2 hours.

Serum chloride was determined by the method of Van Slyke and Sendroy (10). The tissue chloride was determined after ashing the material at 450° C. in the presence of a large excess of sodium carbonate. The ash was dissolved in water with the aid of a little dilute nitric acid and then the usual Volhard titration was carried out. This method was found exceedingly satisfactory and accurate recoveries could be obtained. Sodium was determined by the Butler and Tuthill modification of the Barber and Kolthoff method (10) after separation of potassium by precipitation as the chloroplatinate. Potassium in serum and tissues was determined by a modification of the chloroplatinate precipitation in which the chloride of the K_2PtCl_6 was titrated by the Volhard technique. This method will be described in detail elsewhere. The nonprotein nitrogen of the whole blood was determined by Folin's Nesslerization method (10). Tissue phosphorus and nitrogen were determined by the Fiske and Subbarow (11) and Kjeldahl methods respectively. The tissue fat was determined by extracting the tissues with alcohol-ether mixture, and after evaporation to dryness, re-extracting the residue with petroleum ether. The petroleum ether soluble residue was weighed.

EXPERIMENTAL RESULTS

The effect of the potassium content of the diet on the survival of adrenalectomized rats

The results of the present experiments agree with previous reports that a high intake of potassium accelerates the onset and aggravates the symptoms of adrenal insufficiency. Of the 19 adrenalectomized rats on Diet 2 (potassium content 0.7 per cent) all but one showed symptoms of adrenal insufficiency within 5 days after operation. On the other hand, of 64 animals on Diet 1 (potassium content 0.03 per cent) 29 (45 per cent) survived 10 days or more without showing symptoms; 14 (22 per cent) lived 15 days or

longer without symptoms of adrenal insufficiency. When 8 of the 14 animals surviving more than 2 weeks were changed to a stock diet (Purina Dog Chow) which contains 0.6 per cent potassium and 1.5 per cent sodium chloride, all developed symptoms of adrenal insufficiency within 6 days.

The concentration of serum electrolytes

As shown in Table I, rats in adrenal insufficiency show a constant but moderate reduction in

TABLE I

Serum water and electrolyte

	Water	Chloride	Sodium	Potassium	Nonprotein nitrogen
	grams per 100 cc.	mM. per liter	mM. per liter	mM. per liter	mgm. per 100 cc.
Adrenal insufficiency					
Maximum	94.0	101	140	8.3	168
Minimum	93.6	94	131	6.4	50
Average	93.8	97	136	7.4	96
Cortical extract,* 24 hours	93.3	92	132		60
Cortical extract,† 48 hours	94.2	99	134	11.9	64
Cortical extract,‡ 48 hours	94.3	101	143	5.5	22
Saline treated	94.5	105	143	6.1	40
Saline treated	94.1	102	143	5.7	28
Control					
Maximum	94.2	107	146	5.9	45
Minimum	93.6	102	140	4.5	29
Average	93.9	105	144	5.2	35

* Single injection of 5 cc.
† Two injections totalling 9 cc.
‡ Three injections totalling 12 cc.

concentrations of sodium and chloride in serum but no significant change in total water. The concentration of potassium in serum is moderately increased. The marked increase in nonprotein nitrogen is invariably found in rats exhibiting symptoms of adrenal insufficiency. The decreases in sodium and chloride are considerably less than those reported in adrenalectomized dogs (1, 12) and cats (13), and human patients with Addison's disease (14, 15). This explains the failure of Rubin and Krick (16) to find an increased urinary loss of sodium and chloride in adrenalectomized rats.

It should be recalled that the animals treated with extract received only water by mouth and no sodium chloride except the small amount contained in the cortical extract. Within 24 hours after a single injection of cortical extract, symptomatic recovery is obvious although the concen-

tration of sodium and chloride in serum remains low. When cortical extract is given for 48 hours with only water by mouth, the concentrations of sodium and chloride approach the normal figure. This restoration of the concentration of sodium and chloride is explained by the loss of water and tissue with retention of these ions. With prolonged intensive treatment normal concentrations of potassium and nonprotein nitrogen are found.

Intraperitoneal administration of hypertonic solutions of sodium chloride and sodium bicarbonate restores to normal the concentrations of all the serum constituents studied.

The total electrolyte of muscle, liver and total animal

In order to make the various figures comparable, the data in Table II are expressed per kilogram

TABLE II

Water and electrolyte of muscle, liver and the total animal

(The results are expressed per kilogram of fat-free tissue.)

	Adrenal insufficiency	Cortical extract	Saline treated	Fasted controls	Fed controls
Number of animals	22	8	9	20	11
MUSCLE					
Water, grams	779	778	770	774	774
Potassium, mM	117	106	109	109	106
Chloride, mM	15.0	14	15.4	16.4	14.5
Sodium, mM	18.8	21	23.6	23.6	23.3
Phosphorus, mM	80	77	77.7	79	78
LIVER					
Water, grams	755	753	757	746	739
Potassium, mM	92	95	98	95	95
Chloride, mM	34	34.2	35.6	31.4	31.3
Sodium, mM	34.6	32.0	28	30.5	29.0
Phosphorus, mM	117	121	110	113	114
TOTAL ANIMAL					
Water, grams	731	717	723	729	732
Potassium, mM	85	79	79	77	76
Chloride, mM	37	36	40	39	37
Sodium, mM	50.5	53	56	55	52
Phosphorus, mM	235	256	256	249	233

of fat-free tissue. In each case the result given is the average for the number of animals indicated at the top of the column.

When compared with the saline treated and the fasted, control animals, the total bodies of rats

in insufficiency show a slight decrease in chloride and sodium but a definite increase in potassium. No consistent change in water is demonstrated. A decrease in chloride is not shown when compared with fed controls, but since animals in insufficiency refuse to eat, the fasted controls are probably a better standard of comparison. The animals treated with cortical extract show a persistence of the low concentration of chloride and sodium. However, the striking feature is restoration of potassium to practically normal figures in the groups treated with either cortical extract or saline. The rats treated with salt or extract are the only ones showing relative loss of water.

The findings in muscle are probably chiefly responsible for the changes in the animals as a whole, although similar changes cannot be excluded in any of the tissues not separately examined. In the muscle, the concentration of potassium is strikingly elevated in the animals showing adrenal insufficiency. In the adrenalectomized rats treated with either sodium chloride or cortical extract, the muscle potassium returns to normal. In the animals with adrenal insufficiency the concentrations of water and chloride in muscle are unaltered while that of sodium is definitely reduced. Moreover, in rats treated with cortical extract, the sodium and chloride are essentially the same as in the untreated ones.

The livers are quite different from the muscles in that no increase in potassium occurs in adrenal insufficiency. Apparently, the irregular values for sodium and chloride in the liver depend to a large extent on the amount of blood in the organs so that comparisons are probably not valid.

The concentrations of protein and electrolyte in intracellular water

Since the data in Table II do not separate the changes in the tissues as a whole from those within the cells, the data were calculated for Table III so as to show the concentrations of cell solutes per 1000 cc. of intracellular water. The method of calculation is that described by Harrison, Darrow and Yannet (17) based on the premise that the chloride, except for the red cells, is practically exclusively an extracellular ion and therefore the volume of extracellular fluid in the muscle can be estimated from the total amount of muscle chloride divided by the concentration of

TABLE III

The concentration of potassium, magnesium, phosphorus, and protein in the intracellular water of muscle and liver

(The figures are expressed in terms of 1000 cc. of intracellular water)

(1) Number of animals	(2) Group	Muscle					Liver		
		(3) Potassium	(4) Phosphorus	(5) Magnesium	(6) Protein	(7) Adjusted potassium	(8) Potassium	(9) Phosphorus	(10) Protein
		mM.	mM.	mM.	grams	mM.	mM.	mM.	grams
4	Adrenal insufficiency	186	128		345	186			
2	Adrenal insufficiency	189	128	20	347	189			
3	Adrenal insufficiency	185	128	18	346	185	183	237	451
3	Adrenal insufficiency	180	122	20	316	197	173	221	467
3	Adrenal insufficiency	177	121	21	312	196	189	244	433
	Average	183	125	20	333	191	182	234	450
2	Cortical extract*	154	114	20	299	178	174	226	407
3	Cortical extract†	163	123	17	340	166	178	242	455
3	Cortical extract‡	167	118		350	165	183	228	434
	Average	161	118	18.5	330	169	178	232	432
4	Saline	167	121	24	345	167	177	202	343
5	Saline	173	123		346	173	169	190	306
	Average	170	122		346	170	173	195	325
5	Fasted controls	173	128		348	172			
5	Fasted controls	164	119	24	346	164	175	210	423
5	Fasted controls	171	124		343	171			
5	Fasted controls	168	119	22	349	167	182	239	474
	Average	169	123	23	347	168	179	225	449
4	Fed controls	159	125	21	338	162	175	212	399
2	Fed controls	161	118	20	341	164	180	220	359
5	Fed controls	172	121	21	332	179	173	208	348
	Average	164	121	21	337	168	176	213	369

* Single injection of 5 cc.
† Two injections totalling 9 cc.
‡ Three injections totalling 12 cc.

chloride in extracellular fluid. In the liver, the analogous calculations are based on the sodium content of the tissue since, in rat livers, perhaps owing to the large amount of red cells included, the chloride values are higher than those of the sodium, indicating that there is an excess of chloride beyond that accounted for by the extracellular fluid. The concentration of protein of the cells is estimated by subtracting the non-protein nitrogen from the total nitrogen and multiplying the resulting nitrogen by the conventional factor 6.25. This amount of protein may then be assumed to be present in intracellular water without gross error.

When the data are presented after these calculations (Table III), the concentrations of potassium, phosphorus and protein in the intracellular water of normal muscle are found to be remarkably constant. In the fasting animals the concentration of protein in intracellular water is slightly higher than that of the fed controls. These findings suggest that, during starvation, the loss of water proceeds more rapidly from muscle cells than the loss of nitrogen, producing a concentration of cellular protein.

The outstanding finding in the muscle of rats with adrenal insufficiency is the invariably increased concentration of potassium in intracellular water. This increased concentration of base in intracellular water is all the more striking when it is recalled that the concentration of base in extracellular fluid is reduced in these animals. Furthermore, the increase of potassium within the cell is not associated with any change in the concentration of magnesium which is the other base found within the cells in considerable amounts. If the concentration of proteins within the cells can be used as a measure of cell dilution, it is seen that in 3 of the groups of animals with adrenal insufficiency no dilution of the muscle cells has occurred, whereas in 2 groups a definite shift of water from the extracellular to the intracellular phase is indicated by the decrease in concentration of protein.

All the animals treated either with cortical extract or with hypertonic saline solution show a reduction of the concentration of potassium in the water of muscle cells to the values found in the control animals. In the group sacrificed within 24 hours after a single injection of cortical extract, the concentration of protein in intracellular water of muscle is low, indicating that recovery may occur although dilution of the cell persists.

Column 7 of Table III shows the hypothetical concentrations of potassium which would obtain if the concentrations of intracellular protein were constant. For this purpose, the concentration of protein in the muscle cell water of the fasted control animals was taken to be the standard. This calculation eliminates the effects of changes in the amount of intracellular water on the concentration of potassium. Column 7 shows, therefore, the actual change in content of cellular potassium. The average "adjusted" concentrations of potassium in the intracellular water of muscle are practically identical in animals showing no symptoms of adrenal insufficiency, whereas in the muscle of animals in severe adrenal insufficiency, the concentration of potassium is increased about 14 per cent.

The concentrations of muscle phosphorus of the animals in adrenal insufficiency are slightly

higher than those of the controls or the treated animals. However, the considerable scattering of the values does not permit much emphasis to be placed on these differences.

In the liver, the results are quite in contrast to those of the muscles. The concentrations of potassium, phosphorus and protein in intracellular water of liver are essentially the same in the animals showing symptoms of adrenal insufficiency and in the fasted controls. An incidental finding of some interest is the effect of starvation on the protein concentration in the liver cell. The fasted animals, whether adrenalectomized or fasted controls, show an increased concentration of protein in the water of the cell indicating a relative loss of water from the cell. The concentrations of protein in the liver cell of adrenalectomized animals treated with saline are comparable to those of the fed animals. The explanation is presumably the fact that return of appetite was used as one of the criteria of cure in the case of adrenalectomized rats treated by daily injections of saline. Hence, the animals treated with saline were not sacrificed until after they had resumed eating for at least one day. Whatever the effect of adrenal insufficiency on the livers, no change in concentration of potassium in the liver cell is found, although the normal concentration of potassium is slightly higher than that of muscle.

The effect of changes in concentration of sodium in extracellular water on intracellular hydration

Having shown that in adrenal insufficiency the concentration of base in intracellular water of muscle increases while the concentration of base in extracellular water decreases, it seemed important to determine what effect the changes in the relative concentrations of extracellular and intracellular base have on the water content of the cell. Since the movement of water into the cells results in a lowered concentration of solids in the intracellular water, the concentration of solids in the intracellular water may be used as a measure of cellular hydration. The intracellular water of muscle tissue can be calculated as previously described, and the fat free solids of muscle tissue are assumed to be entirely intracellular. The previous experiments gave data showing a moderate variation in concentration of

extracellular electrolyte but in order to increase the variations in adrenalectomized animals, depletion of extracellular electrolyte by the technique of Darrow and Yannet (18) was carried out on adrenalectomized rats. In order to produce deficit of extracellular electrolyte without adrenal insufficiency, the same procedure was carried out on normal rats.

In the two groups of normal rats, 10 cc. of 5 per cent glucose solution per 100 grams of body weight was injected into the peritoneal cavity. After an interval of 5 hours, a volume of ascitic fluid equal to that injected was removed from the peritoneal cavity. One group of animals was sacrificed immediately while the other group was sacrificed after 48 hours during which only water by mouth was given. These intact animals showed no obvious ill effects following the injection of the glucose solution and its subsequent removal.

Two groups of adrenalectomized rats showing *early* evidences of adrenal insufficiency were injected with 7.5 cc. of 5 per cent glucose solution per 100 grams of body weight. These animals rapidly went into collapse and at the end of 3 hours all were moribund. At this time, the peritoneal fluid was removed and the animals sacrificed.

Two other groups of adrenalectomized rats were similarly treated but at the time of the injection of the glucose solution, 5 cc. of cortical extract was also injected into each animal. This treatment protected the rats from prostration and death although they still manifested some symptoms of adrenal insufficiency. After 3 to 4 hours, the peritoneal fluid was removed. One group was sacrificed at this time while each rat of the other group received 3 cc. of cortical extract and was sacrificed 24 hours later. The blood and tissues were in all cases analyzed as in the previous experiments.

The correlation between the concentration of solids in intracellular water of muscle and the concentration of sodium in extracellular water is graphically shown in Figure 1. Each point represents the pooled analysis of a group of 2 to 5 animals. Although there is considerable scattering, a definite relationship of the concentration of intracellular solids to the concentration of sodium in extracellular water is demonstrated.
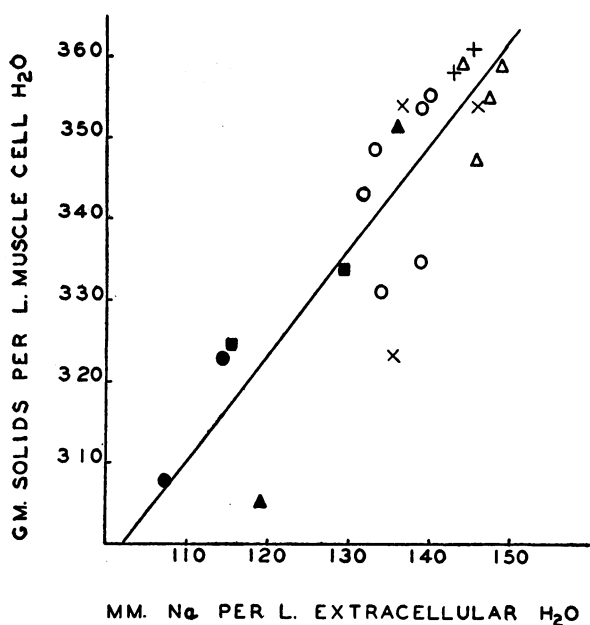
FIG. 1. RELATION OF CONCENTRATION OF SOLIDS IN MUSCLE CELL TO CONCENTRATION OF SODIUM IN EXTRACELLULAR WATER

△ Fasted control rats.
O Rats in adrenal insufficiency.
+ Adrenalectomized rats treated with saline.
✕ Adrenalectomized rats treated with cortical extract.
   Control rats depleted of sodium.
● Adrenalectomized rats depleted of sodium (moribund).
   Adrenalectomized rats depleted of sodium and protected with cortical extract.

In other words, as the concentration of sodium in extracellular water decreases, water diffuses into the cells in order to maintain osmotic equilibrium. The values for the intact, adrenalectomized and the treated adrenalectomized rats all fall along the same line indicating that the concentration of extracellular base is the determining factor for concentration of intracellular solids in both intact and adrenalectomized rats. Since the total body water of the rats represented in Figure 1 remained unchanged, the extent of the shift of water into the cells also measures the degree of depletion of the volume of extracellular fluid.

The injection of adrenal cortical hormone does not affect the distribution of body water except through changes in concentration of sodium in extracellular fluid, since the untreated and the treated groups of adrenalectomized rats having

the same depletion of sodium by intraperitoneal injection of glucose show the same degree of dilution of the cells, although the untreated group was moribund and the group treated with cortical hormone was protected from immediate death. Furthermore, the intact animals depleted of extracellular electrolyte show as marked dilution of the cells as the adrenalectomized rats, as is indicated by the similar reduction in concentration of intracellular solids. Nevertheless, this same degree of hydration of the cells is accompanied by no obvious symptoms in intact rats while in the adrenalectomized rats a moribund state is induced. These experiments show that the concentration of extracellular electrolyte is the chief factor controlling movement of water into and out of the cells and that changes in volume of either intracellular or extracellular fluid are not essential aspects of adrenal insufficiency in rats nor necessary factors explaining the curative effect of cortical extract. Furthermore, the increased concentration of intracellular base in muscle of rats in adrenal insufficiency plays no appreciable rôle in the movement of water through the muscle cell membrane.

DISCUSSION

In a series of papers Swingle and coworkers (19, 20, 21, 22) have offered evidence that, in dogs, adrenal insufficiency is associated with hemoconcentration and lowered plasma volume. These investigators suggest that the chief physiological disturbance is depletion of the volume of extracellular fluid owing to shift of water from extracellular spaces into the cells (8, 23). Harrop (3) demonstrated that in adrenalectomized dogs in collapse the volume of extracellular fluids is actually diminished. The mechanism of the loss of extracellular volume was thought to be a shift of water from the extracellular to the intracellular compartments similar to that demonstrated by Darrow and Yannet (18) to be produced as the result of depletion of extracellular electrolyte by intraperitoneal glucose injection.

In the present experiments on rats, the movement of water into and out of the cell is found to depend chiefly on the concentration of base in the extracellular water. Since rats in adrenal insufficiency show only slight reduction of the

concentration of sodium in the extracellular water, little or no changes in water distribution are found. The therapeutic action of cortical hormone in the adrenalectomized rat may thus be entirely independent of any changes in the volume of intra- or extracellular water. In contrast to rats, the greater reduction of concentration of sodium in the serum of dogs (1, 12), cats (13) and humans (14) with adrenal insufficiency may bring about an appreciable increase of intracellular water and a considerable reduction in the volume of extracellular fluid.

Nevertheless, although changes in distribution of body water may occur in adrenal insufficiency in certain animals, evidence is accumulating that reduction of the volume of extracellular water does not play a deciding rôle in the genesis of the symptoms following adrenalectomy. Swingle, Parkins, Taylor and Hays (23) observed that adrenalectomized dogs improved rapidly when given large amounts of cortical extract even though no change in concentration of sodium in the serum was found. Conversely, an adrenalectomized dog was found to develop severe symptoms and signs of adrenal insufficiency following withdrawal of cortical extract, although because of anuria there was no appreciable reduction of the concentration of sodium in the serum (8). Their hypothesis that adrenal cortical hormone controls the movement of water from the cells to the extracellular spaces independently of the concentration of sodium in the extracellular water is untenable. In the recovery experiments, if water left the cells to increase the volume of the extracellular fluids, an actual decrease of the sodium and chloride concentrations in the serum should have been found. Similarly, in the animal going into adrenal insufficiency while anuric, an incease of the concentration of serum sodium and chloride should have taken place if water entered the cells and thus reduced the volume of extracellular water. The assumption of Swingle, Parkins, Taylor and Hays (8) that sodium and chloride diffuse out of or into the cell together with water, thus maintaining the concentrations in the extracellular fluid unchanged, is not a likely one. Sodium and chloride have not been found within the tissue cells in sufficient quantities (24, 25, 26, 16) to permit such an assumption. When dogs are treated with cortical extract without sodium chloride, evidences of increase in plasma volume do not necessarily indicate an increase in the volume of extracellular fluid but rather a shift of extracellular fluid from interstitial spaces into the blood vessels. These changes might be secondary to improved circulation and vascular tone. The available data indicate that in other animals as in the rat, the action of adrenal cortical hormone is not primarily a regulation of the distribution of body water.

The present data support and amplify previous work indicating the importance of potassium in adrenal insufficiency. In adrenalectomized rats, the aggravation of symptoms and the acceleration of their onset by diets high in potassium confirms previous work in cats (6), dogs (5) and patients with Addison's disease (7). As in dogs and humans, a diet low in potassium protects adrenalectomized rats from adrenal insufficiency. Furthermore, adrenalectomized rats frequently show a high concentration of potassium in serum. The increased concentration of potassium in serum in adrenalectomized animals is apparently the result of changes in renal function since Harrop, Nicholson and Strauss (27) have shown that excretion of potassium is diminished in animals suffering from adrenal insufficiency and that recovery from adrenal insufficiency following the injection of cortical hormone is accompanied by the excretion of considerable quantities of potassium. Even in normal animals, injection of cortical hormone produces a transitory loss of potassium in the urine (28). However, both the amount of potassium retained during development of adrenal insufficiency and the amount excreted during recovery are too large to be contained in the extracellular fluids. The suggestion of Zwemer and Truszkowski (29) that the increased concentration of potassium in the serum is brought about by increased diffusion of potassium from the cells is not supported by tissue analyses. Although these workers found that the concentration of potassium in the muscle of adrenalectomized cats was reduced, this finding is explained by the increase in muscle water and not by loss of potassium. If the potassium concentration is expressed per unit of solids, an appreciable increase in muscle potassium is demonstrated. Hegnauer and Robinson (30) also showed that the concentration of potassium in muscle is cer-

tainly not reduced in adrenal insufficiency in cats and may be increased. In the rat and presumably in other animals, the retention of potassium after adrenalectomy can be accounted for by increased concentration in muscle cells as well as increased concentration in the extracellular fluid.

It is not possible to consider adrenal insufficiency as a special case of potassium intoxication owing entirely to increased concentration of potassium in extracellular water. In many examples of severe adrenal insufficiency the concentration of potassium in serum is only slightly elevated. In occasional cases of chronic nephritis in man (31, 32) and in experimental roentgenray nephritis (33) in dogs, the concentration of serum potassium may rise in the terminal stages to much higher levels than that seen in Addison's disease in crisis or in adrenalectomized dogs. Ingle, Nilson and Kendall (34) have demonstrated that cortical hormone prolongs the life and capacity for work of rats from which both the kidneys and adrenals have been removed. In such animals given cortical hormone, the concentration of potassium in serum may be higher than in animals dying of adrenal insufficiency. Furthermore, no regular correlation between the concentration of potassium in serum and the severity of the symptoms has been observed in adrenal insufficiency.

Since in the rats not subject to adrenal insufficiency the concentration of potassium in muscle cells is quite constant, the higher concentration in adrenal insufficiency may be assumed to indicate important and profound changes in the muscle cell. Although at present one cannot correlate increased concentration of muscle potassium with definite physiological functions, certain features of adrenal insufficiency point to disturbed function of the muscle. Rapid muscular fatigue is an outstanding phenomenon in all adrenalectomized animals (35, 36) as well as in patients with Addison's disease. Fenn and Cobb (37) have found that, normally, muscular activity is associated with changes in the content of potassium in muscle cells since muscles stimulated repeatedly lose potassium and, during the process of recovery, potassium diffuses back into the muscle cell. It will be important to determine whether, in adrenal insufficiency, the concentration of cell potassium is increased in cardiac and smooth muscle and in the kidneys, as these tissues as well as skeletal muscle show marked disturbances of function.

The excess of potassium in the muscle cells in adrenal insufficiency must be present in an almost entirely unionized or osmotically inactive form. Otherwise, it would be impossible for increased concentration of base in intracellular water to exist concomitantly with decreased concentration of base in extracellular water unless cell membranes were impermeable to water. That the membranes of muscle cells in adrenal insufficiency are as freely permeable to water as those of normal animals is demonstrated by the comparable dilution of cellular solids when the concentration of sodium in extracellular water is decreased. Since so little is known of the actual combinations of muscle potassium, the mechanism by which intracellular potassium becomes osmotically inactive cannot be discussed.

It has been conclusively demonstrated that administration of relatively large amounts of sodium salts may protect adrenalectomized animals for long periods (28, 39, 40). In spite of the relatively slight decrease in concentration of sodium in serum in adrenalectomized rats, sodium salts have been found just as effective in prolonging life in adrenalectomized rats as in adrenalectomized .dogs in which decrease in concentration of sodium is much greater. In the experiments reported above, hypertonic solutions of sodium chloride and sodium bicarbonate were given for 3 to 4 days. Improvement was never as rapid as following the injection of cortical hormone, but after 3 to 4 days apparent recovery occurred even when food was withheld. Presumably, following each injection, the slight deficit of sodium and chloride was replaced, but apparent recovery only occurred when large amounts of sodium chloride were given over a considerable period. On analysis the animals cured with sodium salts showed normal concentrations of potassium in the intracellular water of muscle as well as normal concentrations of sodium, chloride and potassium in extracellular fluids. Hence, one aspect of the curative effect of sodium salts may be facilitation of the release of potassium from muscle cells and the excretion of potassium in the urine.

The mechanism by which changes in the concentration of sodium in extracellular fluid may

influence the concentration of potassium within the muscle cell is unknown. However, it has long been known that the effectiveness of adrenal cortical extract is enhanced by the simultaneous administration of sodium chloride. Moreover, reduction of the concentration of sodium in extracellular fluids reduces the effectiveness of the hormone. This fact was demonstrated in the experiments in which adrenalectomized rats were further depleted of sodium by intraperitoneal injection of glucose solution. It had previously been found that 5 cc. of cortical extract sufficed to produce a rapid cure of all symptoms in rats which had been allowed to progress to the terminal stages of insufficiency. However, in the adrenalectomized rats further depleted of sodium chloride, the same amount of extract merely protected the animals from prostration and immediate death since symptoms such as diarrhea and muscular weakness persisted and the blood nonprotein nitrogen remained elevated. Furthermore, muscle potassium was not restored to normal as was always the case with adrenalectomized rats not further depleted of sodium and chloride. Future work may show an interrelationship between the regulation of the concentration of potassium within the cell by the adrenal cortical hormone and the control of the concentration of sodium and other solutes in the extracellular water.

SUMMARY

By analysis of the tissues, the distribution of body water and electrolyte was studied in rats in the terminal stages of adrenal insufficiency and in rats cured of adrenal insufficiency.

Following adrenalectomy, rats show diminished concentrations of sodium and chloride in the serum and increased concentrations of potassium and nonprotein nitrogen. The changes in the concentrations of sodium and chloride are less marked in rats than in dogs, cats and man in adrenal insufficiency.

The acceleration of the onset of symptoms of adrenal insufficiency by a high intake of potassium previously found in dogs, cats and humans is confirmed in adrenalectomized rats.

The concentration of potassium in the muscle cells is constantly increased in rats showing marked symptoms of adrenal insufficiency, whereas in animals cured of adrenal insufficiency either by adrenal cortical extract or by a hypertonic solution of sodium chloride and sodium bicarbonate, the concentrations of potassium in the muscle cells are identical with those found in unoperated controls. No changes in the potassium content of the liver cells are found. These findings may be associated with the functional changes of muscle in adrenal insufficiency.

In rats with adrenal insufficiency, as well as control animals, the water content of the muscle cell is inversely related to the concentration of sodium in the extracellular water. The adrenal cortical hormone does not regulate the movement of water into and out of the cell except as it influences the concentration of sodium in the extracellular water. In the rat, the symptoms of adrenal insufficiency are not related to changes in the distribution of body water.

BIBLIOGRAPHY

1. Loeb, R. F., Atchley, D. W., Benedict, E. M., and Leland, J., Electrolyte balance studies in adrenalectomized dogs with particular reference to the excretion of sodium. J. Exper. Med., 1933, 57, 775.
2. Loeb, R. F., Atchley, D. W., and Stahl, J., The rôle of sodium in adrenal insufficiency. J. A. M. A., 1935, 104, 2149.
3. Harrop, G. A., The influence of the adrenal cortex upon the distribution of body water. Bull. Johns Hopkins Hosp., 1936, 59, 11.
4. Hastings, A. B., and Compere, E. L., Effect of bilateral suprarenalectomy on certain constituents of the blood of dogs. Proc. Soc. Exper. Biol. and Med., 1931, 28, 376.
5. Allers, W. D., Nilson, H. W., and Kendall, E. C., Studies on adrenalectomized dogs: The toxic action of potassium. Proc. Staff Meet., Mayo Clin., 1936, 11, 283.
6. Zwemer, R. L., and Truszkowski, R., The importance of corticoadrenal regulation of potassium metabolism. Endocrinology, 1937, 21, 40.
7. Wilder, R. M., Kendall, E. C., Snell, A. M., Kepler, E. J., Rynearson, E. H., and Adams, M., Intake of potassium, an important consideration in Addison's disease. A metabolic study. Arch. Int. Med., 1937, 59, 367.
8. Swingle, W. W., Parkins, W. M., Taylor, A. R., and Hays, H. W., The influence of adrenal cortical hormones upon electrolyte and fluid distribution in adrenalectomized dogs maintained on a sodium and chloride free diet. Am. J. Physiol., 1937, 119, 684.
9. Britton, S. W., and Silvette, H., On the function of the adrenal cortex—general, carbohydrate and circulatory theories. Am. J. Physiol., 1934, 107, 190.

10. Peters, J. P., and Van Slyke, D. D., Quantitative Clinical Chemistry, Volume II. Methods. Williams and Wilkins Co., Baltimore, 1932.

11. Fiske, C. H., and Subbarow, Y., The colorimetric determination of phosphorus. J. Biol. Chem., 1925, 66, 375.

12. Harrop, G. A., Soffer, L. J., Ellsworth, R., and Trescher, J. H., Studies on the suprarenal cortex. III. Plasma electrolytes and electrolyte excretion during suprarenal insufficiency in the dog. J. Exper. Med., 1933, 58, 17.

13. Baumann, E. J., and Kurland, S., Changes in the inorganic constituents of blood in suprarenalectomized cats and rabbits. J. Biol. Chem., 1926–27, 71, 281.

14. Loeb, R. F., Chemical changes in the blood in Addison's disease. Science, 1932, 76, 420.

15. Allot, E. N., Chemical changes in the blood in Addison's disease and their alteration in response to treatment. Lancet, 1936, 1, 1406.

16. Rubin, M. I., and Krick, E. T., The salt and water metabolism of adrenal insufficiency and partial starvation in rats. J. Clin. Invest., 1936, 15, 685.

17. Harrison, H. E., Darrow, D. C., and Yannet, H., The total electrolyte content of animals and its probable relation to the distribution of body water. J. Biol. Chem., 1936, 113, 515.

18. 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.

19. Swingle, W. W., Pfiffner, J. J., Vars, H. M., Bott, P. A., and Parkins, W. M., The function of the adrenal cortical hormone and the cause of death from adrenal insufficiency. Science, 1933, 77, 58.

20. Swingle, W. W., Pfiffner, J. J., Vars, H. M., and Parkins, W. M., The effect of hemorrhage on the normal and adrenalectomized dog. Am. J. Physiol., 1934, 107, 259.

21. Swingle, W. W., Vars, H. M., and Parkins, W. M., A study of the blood volume of adrenalectomized dogs. Am. J. Physiol., 1934, 109, 488.

22. Swingle, W. W., Parkins, W. M., and Taylor, A. R., Experiments on intact and adrenalectomized dogs subjected to sodium and chloride depletion by intraperitoneal injections of glucose. Am. J. Physiol., 1936, 116, 430.

23. Swingle, W. W., Parkins, W. M., Taylor, A. R., and Hays, H. W., Relation of serum sodium and chloride levels to alterations of body water in the intact and adrenalectomized dog, and the influence of adrenal cortical hormone upon fluid distribution. Am. J. Physiol., 1936, 116, 438.

24. Peters, J. P., Body Water, C. C. Thomas Co., Springfield, 1935, Chapter 6.

25. Fenn, W. O., Electrolytes in muscle. Physiol. Rev., 1936, 16, 450.

26. Hastings, A. B., and Eichelberger, L., The exchange of salt and water between muscle and blood. I. The effect of an increase in total body water produced by the intravenous injection of isotonic salt solution. J. Biol. Chem., 1937, 117, 73.

27. Harrop, G. A., Nicholson, W. M., and Strauss, M., Studies on the suprarenal cortex. V. The influence of the cortical hormone upon the excretion of water and electrolytes in the suprarenalectomized dog. J. Exper. Med., 1936, 64, 233.

28. Harrop, G. A., and Thorn, G. W., Studies on the suprarenal cortex. VI. The effect of suprarenal cortical hormone upon the electrolyte excretion of the intact normal dog. A proposed method of comparative assay. J. Exper. Med., 1937, 65, 757.

29. Zwemer, R. L., and Truszkowski, R., Potassium: A basal factor in the syndrome of corticoadrenal insufficiency. Science, 1936, 83, 558.

30. Hegnauer, A. H., and Robinson, E. J., The water and electrolyte distribution among plasma, red blood cells and muscle after adrenalectomy. J. Biol. Chem., 1936, 116, 769.

31. Rabinowitch, I. M., On the relative proportions of sodium, potassium, calcium and magnesium in blood plasma in renal disease. J. Biol. Chem., 1924–25, 62, 667.

32. Nelken, L., and Steinitz, H., Über den Gehalt des Blutserums an Calcium und Kalium bei Nierenkrankheiten. Ztschr. f. klin. Med., 1926, 103, 317.

33. Bolliger, A., and Breh, F., Über die Mineralstoffveränderungen des Blutes bei experimenteller Nephritis, mit spezieller Berücksichtigung des Kalium-Kalziumspiegels im Serum. Zentralbl. f. inn. Med., 1928, 49, 825.

34. Ingle, D. J., Nilson, H. W., and Kendall, E. C., The effect of cortin on the concentrations of some constituents of the blood of adrenalectomized rats. Am. J. Physiol., 1937, 118, 302.

35. Hales, W. M., Haslerud, G. M., and Ingle, D. J., Time for development of incapacity to work in adrenalectomized rats. Am. J. Physiol., 1935, 112, 65.

36. Everse, J. W. R., and DeFremery, P., On a method of measuring fatigue in rats and its application for testing the suprarenal cortical hormone (Cortin). Acta brev. Neerland., 1932, 2, 152.

37. Fenn, W. O., and Cobb, D. M., Electrolyte changes in muscle during activity. Am. J. Physiol., 1936, 115, 345.

38. Harrop, G. A., Soffer, L. J., Nicholson, W. M., and Strauss, M., Studies on the suprarenal cortex. IV. The effect of sodium salts in sustaining the suprarenalectomized dog. J. Exper. Med., 1935, 61, 839.

39. Rubin, M. I., and Krick, E. T., Effect of adrenalectomy on salt metabolism in rats. Proc. Soc. Exper. Biol. and Med., 1933–34, 31, 228.

40. Allers, W. D., The influence of diet and mineral metabolism on dogs after suprarenalectomy. Proc. Staff Meet., Mayo Clin., 1935, 10, 406.