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Research Article

OBSERVATIONS ON ADRENAL INSUFFICIENCY

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The influence of the adrenal cortex upon electrolyte physiology and renal function has been the subject of many recent investigations (1, 2, 3). Improvement in the preparation of cortical extract has been advanced to a state where it is now possible to maintain experimental animals in perfect health indefinitely under the standard conditions of metabolism experiments. This step forward affords an opportunity to make observations over long periods of time on the same animal, and thereby to study repeatedly the results following the termination and the reestablishment of cortical extract or sodium chloride therapy. The present work employs this technique to study the gradual as well as the rapid development of adrenal insufficiency. It includes also the effects of alterations in the sodium intake upon the varying experimental states thus produced.

EXPERIMENTAL

The subject of these observations was a male mongrel beagle hound, apparently in middle age, who lived in the animal colony of this laboratory for two years. The right adrenal gland was removed in April, 1933, and the left in January, 1934. On December 26, 1933, the dog was placed in a metabolism cage where he remained during all the successive periods of observation and was started on a daily diet consisting of 500 grams of raw lean beef and 5 grams of a dried milk preparation in addition to one "Haliver Oil" capsule containing viosterol. The meat was procured in weekly supplies, each new lot being analyzed for Na, Ca, Mg, Cl, P and total nitrogen. The milk was similarly analyzed. These food analyses were limited to those periods of observation in which electrolyte balance studies were made. Varying amounts of sodium chloride were added to the diet. Cortical extract, made according to the extraction method of Pfiffner and Swingle (4), was received in 95 per cent alcohol from the Parke, Davis Laboratories, and the final preparation of the extract was completed in our laboratory. The extract was given, except in a few instances, in two daily subcutaneous injections. In the course of 13 months, during which this cortical extract was employed almost continuously, three lots were obtained which caused severe localized necrotizing lesions. Except for these mishaps, no untoward symptoms followed its administration.

In the balance studies reported,[2] the same technique and precautions were followed as in previous studies, and the methods of chemical analysis employed have been described elsewhere (1). Blood samples were obtained from the jugular vein and delivered under oil. Urea clearance tests were done according to the method of Van Slyke. Despite the removal of 20 to 40 cc. of blood at approximately weekly intervals over a period of 13 months of study, the dog did not develop anemia nor was there any significant decrease in the protein content of his serum.

Routine examination of the urine showed only a very faint trace of albumin at times. No other urinary abnormalities were noted except for a moderate number of white blood cells which appeared following numerous catheterizations.

RESULTS

Relationship between sodium and urea concentrations in serum. It will be seen from Figure 1, that, in a dog maintained on moderate doses of cortical extract, a decrease in blood sodium and an increase in blood urea could be induced either by the withdrawal of salt from the diet, by the gradual reduction of the dosage of cortical extract or by its complete withdrawal, as has been observed by Harrop (2, 3). These changes in blood sodium and in blood urea, whether they followed the withdrawal of salt or the withdrawal of cortical extract, were accompanied by a progressive fall in the phthalein excretion and in urea clearance. However, the velocity and extent of these changes were greater subsequent to the withdrawal of cortical extract than to the withdrawal of salt. When an unusually potent preparation of extract was employed (Periods 30 to 33) reduction in the salt intake failed to influence the blood sodium, the blood urea or the urea clearance. As might

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[2] In the course of these experiments the increased excretion of sodium, accompanying the development of adrenal insufficiency, as previously reported (1, 2), was confirmed. Consequently the electrolyte balances are not presented in this communication.

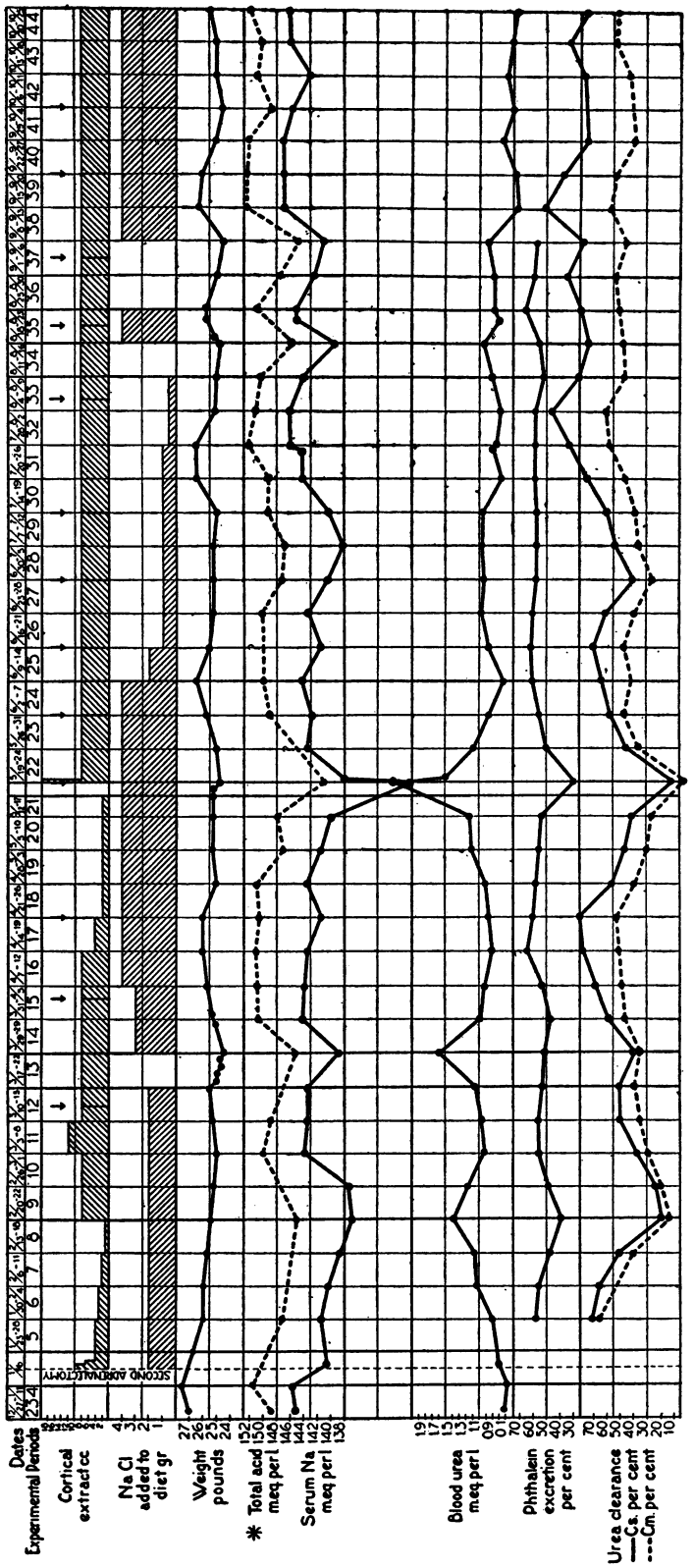

FIGURE 1.

THE EFFECTS OF CHANGES IN DOSAGE OF SODIUM CHLORIDE AND OF CORTICAL EXTRACT IN THE ADRENALECTOMIZED DOG.

↓ Indicates the use of a fresh lot of cortical extract.

? Determination of total acid was omitted.

be anticipated, the withdrawal of salt in this instance had the same effect as in normal animals, i.e., there was loss in body weight without change in electrolyte or urea concentration in the blood.

It has been commonly assumed that the simultaneous increase of urea and decrease of sodium in the blood are correlated disturbances and that the increase in blood urea represents an attempt at "osmolar replacement" of the base lost from the blood. This suggestion seems theoretically untenable. The cell membranes of the body are virtually impermeable to the sodium ion whereas they are readily permeable to urea. Consequently, the concentration of urea in the circulating body fluids cannot influence their osmotic pressure in relation to that of the fluids within the cells. Figure 1 demonstrates the fact that in our studies the fall in sodium and the rise in urea were not quantitatively parallel. For example, during the gradual withdrawal of salt from the diet (Periods 25 and 26, Figure 1), the sodium level remained relatively constant at a normal level whereas the blood urea rose from 0.70 gram per liter to 1.03 gram per liter. Furthermore, the conclusion that these changes in sodium levels represent disturbances which, although frequently simultaneous, are nevertheless, not dependently correlated, is strongly supported by clinical observations in Addison's disease. Table I shows that there may be a marked decrease in the level of sodium in the serum without the commonly associated increase in concentration of nonprotein nitrogen in the blood.

Renal function. Marshall and Davis (5) showed in 1916 that the phthalein excretion after adrenalectomy in dogs is definitely decreased, and they suggested that the adrenal cortex might exert a direct influence on renal function. In Figure 1 it will be seen that a decrease in phthalein excretion followed both the withdrawal of cortical extract or a decrease in the salt intake. The urea clearance decreased even more strikingly under similar circumstances.

It has been shown (6) that the development of acute adrenal insufficiency in Addison's disease secondary to withdrawal of salt is not associated with an increase in urinary ammonia. On the contrary, in the one patient studied, the daily output of ammonia decreased as insufficiency appeared, and the NH_3 excretion rose with the clinical improvement following the administration of NaCl.

In the present study, ammonia excretion was followed as cortical extract dosage was gradually reduced (Table II and Periods 6 to 9 in Figure 1). There resulted in this instance too a decrease in NH_3 excretion from an average of 26.3 m.eq. daily to 21.6 m.eq., as is seen in Table II. Concomitantly, the blood sodium fell from 141.6 m.eq. per liter to 137.0 m.eq. per liter, the blood urea rose from 0.90 gram per liter to 1.48 gram per liter, the phthalein excretion dropped from 60 per cent to 35 per cent, and the urea clearance dropped from 65 per cent to 15 per cent (Figure 1, Periods 6 to 9). These changes took place without the development of vomiting or marked weak-

TABLE I

Showing the lack of correlation between the concentrations of nonprotein nitrogen and sodium in the blood in Addison's disease

Name	Date	Nonprotein nitrogen	Na	K	Cl	HCO_3	Blood sugar	
		mgm. per 100 cc.	*m. eq. per liter*	*m. eq. per liter*	*m. eq. per liter*	*m. eq. per liter*	*mgm. per 100 cc.*	
E.F.....	September 7, 1933	26.7	123.4	5.6	88.9	25.9		After salt poor regime
	September 14, 1933	22.7	138.1	4.9	105.0	25.7	81	After high salt feeding
	October 19, 1933	22.0	136.3	5.0	104.3	24.5	87	
R.C.....	February 15, 1934	35.0	135.5	5.4	101.4	24.0	82	On high salt feeding
	May 10, 1934	37.0	125.0	5.6	93.0	16.3	124	On high salt feeding
	July 26, 1934	34.0	119.8		85.3	25.0		On high salt feeding
	August 14, 1934	29.0	113.3				72	On high salt feeding
R.O.....	April 9, 1934	39.0	127.1	4.7	89.6	24.9		
	April 11, 1934	34.4	119.2	5.3	83.4	25.3	90	After salt poor diet
S.F.....	June 24, 1934	26.0	117.3		91.3			
	August 9, 1934	26.0	135.6		101.8	24.3		After high salt feeding

TABLE II

Effect of the withdrawal of cortical extract upon ammonia excretion

Period	Dates	NaCl added to diet	Cortical extract	Urine NH_3	Urine total N	Urine volume	Total N intake	Fluid intake
		grams	*cc.*	*m. eq.*	*grams*	*cc.*	*grams*	*cc.*
6	Jan. 30 to Feb. 4	2.0	3.0	26.3	16.5	789	17.1	783
7	Feb. 6 to Feb. 11	2.0	2.0	25.9	16.6	896	17.0	848
7	Feb. 13 to Feb. 18	2.0	1.0	21.6	16.6	1073	16.2	1038
9	Feb. 20 to Feb. 22	2.0	8.0	27.1	18.3	1168	16.2	1053

ness. It is impossible to state whether the decrease in NH_3 excretion represents a specific failure of the NH_3 mechanism, dependent upon lack of cortical extract, or whether this change is only another manifestation of a more general cellular disturbance.

Immediate responses to cortical extract in severe adrenal insufficiency. In the presence of adrenal insufficiency certain chemical properties of the blood and urine were studied following the administration of a single large dose of cortical extract. In these experiments, the daily dosage of extract was stopped and insufficiency was allowed to progress until the dog refused food and water and showed definite weakness. After this no food or water was given, and at two hour intervals the dog was catheterized. Blood samples were taken every two to four hours and analyzed for sodium, potassium, urea and water content. The urine samples were analyzed for sodium, urea and ammonia. A massive dose of cortin was injected after the first two-hour interval.

In order to control these studies on the immediate effects of cortical extract, similar observations were made during a period of several hours while cortical extract was withheld, and the dog was allowed to continue in a state of insufficiency. It will be seen from Table III that no consistent changes in the blood serum sodium, potassium or water content occurred in 6 hours following the extract injection, and also that no significant changes occurred in the urea clearance or the hourly excretion of sodium. Despite these nega-

TABLE III

Responses to a single large dose of cortical extract in severe adrenal insufficiency

Date	Hour	Cortical extract	Serum H_2O content	Serum Na	Serum K	Whole blood urea	Urine Na	Urine HN_3	Urea clearance	Remarks
		cc.	*per cent*	*m. eq. per liter*	*m. eq. per liter*	*mgm. per 100 cc.*	*m. eq. per hour*	*m. eq. in 2 hours*	*Cs per cent*	
October 31, 1934	8 a.m.		90.4	133.3	8.1	1.29				After 5 days without cortical extract. Dog very weak
	10 a.m.	25					0.44	0.89	31.3	
	12 m.		91.0	134.1	6.5	1.30	0.33	0.83	37.4	Dog standing in cage and barking
	2 p.m.		90.9	133.8	7.5	1.36	0.18	0.89	32.6	
	4 p.m.		90.9	133.8	7.1	1.36	0.29	0.76	35.0	
November 1, 1934	8 a.m.		91.1	132.1	6.3	0.78				After taking water and food during the night. Dog quite well
	10 a.m.						0.15	1.60	67.4	
January 5, 1935	8 a.m.		90.8	131.8	7.3	1.61				After 5 days without cortical extract. Dog weak
	10 a.m.	20					0.30	0.62	22.5	
	12 m.		91.2	135.9		1.58	0.26	0.56	23.3	Dog walking in cage and barking
	2 p.m.						0.19	0.55	22.5	
	4 p.m.		90.8	134.0	7.6	1.77	0.43	0.66	35.3	
						Control observations				
November 24, 1934	8 a.m.		90.5	137.1	7.2	1.06				After 3 days without cortical extract. Dog moderately weak
	10 a.m.						0.33	1.04	37.2	
	12 m.						0.30	0.97	30.1	
	2 p.m.						0.29	0.81	24.8	
	4 p.m.		90.3	137.6	7.0	1.20				Dog weaker. Urine specimen lost
January 16, 1935	8 a.m.		90.3	131.0		2.31				After 4 days without cortical extract. Dog appears very tired
	10 a.m.							0.08	3.3	Urine volume too small for Na analysis
	12 m.		90.1	129.6		2.61		0.04	1.4	
	2 p.m.							0.01	0.9	Dog practically moribund, experiment discontinued and dog treated with 35 cc. of 4 per cent salt solution and 25 cc. of cortical extract. Recovered and drank 500 cc. of milk and water two hours later
February 6, 1935	8 a.m.		89.9	139.8	6.1	0.89				After 3 days without cortical extract, then one injection of 4 cc. on morning preceding experiment
	10 a.m.						0.85	1.64	68.2	
	12 m.		90.3	140.2	6.4	0.92	0.38	1.00	60.2	
	2 p.m.						0.27	0.75	42.6	
	4 p.m.		90.0	140.7	6.7	0.97	0.20	0.78	33.7	

tive results it is important to emphasize the fact that within 15 to 20 minutes following the injection of cortical extract a striking improvement occurred in the general condition of the dog. Whereas, prior to the injection, the animal was too weak to stand up, soon afterward he climbed up on the door of his cage, and began to bark vociferously. No blood pressure, blood volume, or blood sugar measurements were made in these experiments, and consequently these aspects of recovery cannot be discussed. Nevertheless, it is clear that there is an important and prompt effect of cortical extract which precedes by several hours any objective change in the chemical constituents of the blood or in the physiological functions studied by us.

The behavior of the blood constituents in the control experiments did not appear to differ from those in which cortical extract was injected (Table III). In both series the blood urea rose progressively. However, after the injection of cortical extract, there was a tendency for the urea clearance to rise despite the increase in blood urea. This is in contrast to the striking drop in the urea clearances accompanying an increase in blood urea when cortical extract was not administered.

The rate of ammonia excretion in the control experiments decreased progressively during the period of observation. This change was not consistently observed when cortical extract was injected. These findings are in agreement with the previously mentioned effects upon ammonia excretion following the withdrawal of cortical extract.

Standardization of cortical extract. Many methods of biological assay of cortical extracts have been proposed. One which is in common usage is based upon the amount of cortical extract necessary to prevent an increase of 100 per cent in the blood urea concentration. Figure 1 (Periods 27 and 28) shows that this criterion cannot always be depended upon, since there may be a drop in blood sodium concentration without a rise in blood urea. Moreover, from Table I, it may be seen that in Addison's disease, the nonprotein nitrogen of the blood can be normal even when a striking decrease in the blood sodium level exists. It thus appears that the utilization of the blood urea concentration as the sole criterion of insufficiency of the adrenal cortex would at times lead to erroneous conclusions.

It has been pointed out that changes in the sodium intake influence the concentration of urea and sodium in the blood exactly as do changes in dosage of cortical extract. Consequently, it is obvious that the salt content of the diet must be rigidly controlled in all assay work in which the level of urea or of sodium in the blood, or changes in renal function, serve as criteria of activity.

CONCLUSIONS

1. The decrease in sodium concentration of the blood and the increase in blood urea occurring in adrenal insufficiency are not interdependent disturbances although they are frequently encountered simultaneously.

2. The withdrawal of salt from the diet of an adrenalectomized dog maintained with cortical extract resulted in an increase in blood urea and a decrease in blood sodium, as did the withdrawal of cortical extract. This is a confirmation of experiments reported by Harrop.

3. A marked reduction in salt ingestion failed to influence the concentrations of sodium and of urea in the blood when an unusually potent preparation of cortical extract was employed.

4. Withdrawal of salt from the diet or withdrawal of cortical extract caused a decrease in renal function as determined by the excretion of phenolsulphonphthalein and by the urea clearance test.

5. Ammonia excretion decreased following a reduction in the dosage of cortical extract.

6. The administration of a single large dose of cortical extract in the presence of severe adrenal insufficiency caused striking improvement in the general well-being of the experimental animal before any consistent changes could be determined in the blood sodium, potassium or urea concentrations or in the water content of the serum.

7. A single large dose of cortical extract administered in the presence of severe adrenal insufficiency appeared to inhibit the progressive decrease in renal function as evidenced by the urea clearance and the excretion of ammonia.

8. Standardization of cortical extract on the basis of its effect on blood urea concentration of an adrenalectomized dog is unreliable unless there

is rigid control of the amount of salt ingested. Furthermore, in human beings, severe adrenal insufficiency, as determined by the sodium concentration of the blood, may be present without urea retention.

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