STUDIES ON THE SUPRARENAL CORTEX

III. Plasma Electrolytes and Electrolyte Excretion during Suprarenal Insufficiency in the Dog

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In the preceding papers (1, 2), we have described the phenomena produced by the cessation of injections of suprarenal cortical extract in the suprarenalectomized adult dog, which has been maintained in a normal state of nutrition, with well healed incisions, for a period of several weeks after suprarenalectomy. We have pointed out that death in such an animal, if injections of the hormone are not resumed, is due to a condition of shock produced by loss of body fluid. The observed sequence of events is: hemoconcentration, which becomes more and more marked; progressive loss of body weight; anorexia; lowered body temperature and basal oxygen consumption; muscular weakness; vomiting and diarrhea; and ultimate failure of the circulation, as indicated by diminished blood flow and fall in blood pressure. These symptoms are associated with a progressive rise in blood nonprotein nitrogen (urea) concentration, a drop in plasma chlorides, and, as we have recently shown (1), a fall in plasma total base. The present communication is concerned with an analysis of the factors producing this hemoconcentration and of the train of phenomena which then follows it.[1]

[1] We wish to acknowledge the assistance of Dr. Mary Buell in organizing the chemical procedures involved in the manufacture of the cortical extract and in the selection and setting up of analytical methods used in the studies herein reported.

We are indebted to Miss Margaret Strauss who has made lactic acid, inorganic phosphorus, and calcium estimations on a series of animals before and during insufficiency.

We acknowledge the important assistance of Dr. Oliver Kamm, of Parke,

It is well known that changes in the distribution of body fluids are often accompanied by disturbances in the normal electrolyte structure of the blood plasma. Loeb and his coworkers have made an important contribution to the elucidation of this problem in experimental suprarenalectomy (19). They have shown that the sodium concentration of the blood decreases both in suprarenalectomized dogs and cats and in patients suffering from Addison's disease. By balance studies they have shown that a striking loss of sodium occurs due to loss of sodium in the urine and that the behavior of the chloride ion follows that of the sodium, but the loss is not equivalent. They discuss the possibility that the suprarenal glands have a regulatory effect upon the sodium metabolism and upon renal function. They have previously shown that similar changes occur in the blood of patients with Addison's disease which may be materially modified by the administration of sodium chloride. We have, therefore, determined the concentrations of the plasma electrolytes during the cycle of events which takes place following the cessation of injections of extract as the animal goes into suprarenal insufficiency, and during the progress of recovery when injections of extract are resumed.

Procedure

Samples of (femoral) arterial blood were taken for urea estimations and study of the electrolyte pattern. They were obtained with an oiled syringe by direct puncture of the vessel. We have found that adequate protection from the air is secured by delivery of the blood through a bent glass tube into 15 cc. centrifuge tubes, provided with a drop of purified heparin (6 per cent solution), and a flat glass bead. Each centrifuge tube is filled to the top and immediately capped with a rubber stopper, such as is used for vaccine bottles, through which a needle is inserted, the tip just emerging at the lower end. This permits the escape of air bubbles, followed by any excess of blood. The tubes are vigorously turned end for end for a minute or two to secure complete mixing of the blood and heparin. On centrifuging, a clear non-hemolyzed plasma invariably results. Carbon dioxide determinations are first made by removing the stopper, and inserting the measuring pipette to remove the sample from the lower half of the supernatant plasma, without disturbing the red cell mass below. The duplicate is taken from a second centrifuge tube, and the plasma is then immediately separated from the cells.

Davis and Company, who has generously supplied us for the past 2 years with the beef suprarenal glands from which we prepare our cortical extract.

Because the operation for suprarenalectomy is technically difficult in larger animals and the maintenance extract requirement is proportionally greater, we have used male dogs exclusively, weighing between 7 and 10 kilos. Owing to the rather large quantities of blood required for repeated analyses, we have restricted our studies, in one group of experiments, to estimations of total base, bicarbonates, chlorides, sodium, and urea (or non-protein nitrogen) (Chart 1, Tables V, VII). Studies of calcium, magnesium, and potassium, inorganic phosphates, lactic acid, and total proteins, with the partition of the ratio of albumin to globulin, were usually done in separate groups of experiments (Tables I and II).[2]

The results show that during the course of suprarenal insufficiency induced by cessation of injections of extract, a fall occurs in the plasma total base concentration, the extent depending in great measure on the degree of insufficiency into which the animal is allowed to lapse. This is associated with a considerable rise in the concentration of magnesium and potassium, but without marked increase in that of calcium. Since the total concentration of plasma base fell, and since that of the other constituents rose, the results of our earlier experiments pointed to a decided drop in the plasma sodium concentration. This

[2] *Methods of Analysis.*—

Estimations of *total base* were made by the method of Stadie and Ross (*J. Biol. Chem.*, 1922, **51,** 55), with certain modifications; of *sodium* by that of Butler and Tuthill (*J. Biol. Chem.*, 1931, **93,** 171); of *potassium* (plasma only) by that of Taylor (*J. Biol. Chem.*, 1930, **87,** 27); *calcium* and *magnesium* by that of Kramer and Tisdall (*J. Biol. Chem.*, 1921, **47,** 475); *carbon dioxide* by that of Van Slyke and Neill (*J. Biol. Chem.*, 1924, **61,** 523); *oxygen capacity* by that of Sendroy (*J. Biol. Chem.*, 1931, **91,** 307); *inorganic phosphate* by that of Fiske and Subbarow (*J. Biol. Chem.*, 1925, **66,** 375); *non-protein nitrogen* by direct nesslerization (Wong, S. Y., *J. Biol. Chem.*, 1923, **55,** 431); *urea* by decomposition with urease and direct nesslerization, using gum ghatti as a stabilizing colloid (Folin, O., *J. Biol. Chem.*, 1929, **81,** 231); *plasma chlorides* by the method of Van Slyke and Sendroy, (*J. Biol. Chem.*, 1923, **58,** 523); *lactic acid* by that of Friedman *et al.* (*J. Biol. Chem.*, 1927, **73,** 335, and *J. Biol. Chem.*, 1929, **82,** 23); *plasma proteins* by difference of the results of total nitrogen and non-protein nitrogen concentration estimated by the Kjeldahl method; and the *albumin-globulin ratio* by Howe's Kjeldahl method (*J. Biol. Chem.*, 1921, **49,** 109).

Standard methods were employed for estimations of urine constituents: *total nitrogen* by macro Kjeldahl digestion and titration; *creatine* and *creatinine* by Folin's method (*J. Biol. Chem.*, 1914, **17,** 475); *ammonia* by aeration into acid and titration of the excess (Folin, O., *J. Biol. Chem.*, 1910, **8,** 497).

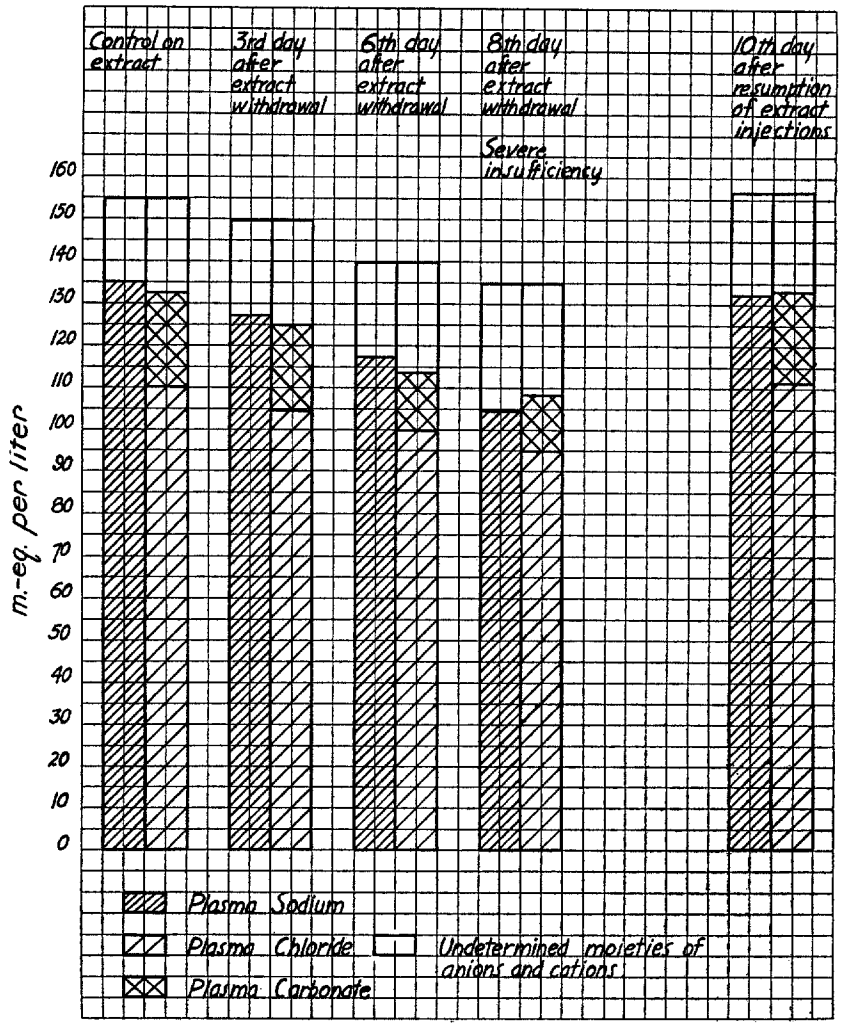

CHART 1. Plasma electrolyte pattern during the course of suprarenal insufficiency following withdrawal of extract from the suprarenalectomized dog.

we have confirmed directly. The change in concentration may amount to 20 m.-eq. per liter or even more.[3] Since the concentration

[3] The fall in plasma sodium, which is accompanied by an appreciable rise in plasma potassium concentration, produces a marked alteration in the ratio nor-

TABLE I

Plasma Cations and Total Base Values in Normal Dogs and in Dogs with Suprarenal Insufficiency

Normal animals							Suprarenal insufficiency						
Dog No.	Plasma calcium per liter	Plasma magnesium per liter	Plasma potassium per liter	Plasma total base per liter	Plasma sodium* per liter	Plasma sodium/potassium	Dog No.	Plasma calcium per liter	Plasma magnesium per liter	Plasma potassium per liter	Plasma total base per liter	Plasma sodium* per liter	Plasma sodium/potassium
	m.-eq.	*m.-eq.*	*m.-eq.*	*m.-eq.*	*m.-eq.*			*m.-eq.*	*m.-eq.*	*m.-eq.*	*m.-eq.*	*m.-eq.*	
6-0	6.1	1.5	4.7	157.0	144.7	30.6	6-0†	7.5	2.3	4.9	147.8	131.1	26.6
7-0	7.2	1.3	4.7	150.6	137.4	29.2	7-0	7.9	2.4	9.8	139.0	118.9	12.1
1-9	6.2	1.5	6.2	154.4	140.5	22.6	1-9	5.8	1.7	10.2	150.4	132.7	13.0
5-6	6.2	1.4	6.2	152.6	138.8	22.2	5-6	8.2	3.8	13.9	146.8	120.9	8.7
6-8		1.5	5.5	152.2	138.2‡	25.1	6-7	7.7	4.1	10.0	134.6	112.8	11.3

* Sodium values obtained by subtraction. Total base $-$(Ca + Mg + K).

† Mild insufficiency only.

‡ Calcium lacking. Sodium estimated for assumed normal calcium of 7.0 m.-eq.

TABLE II

Blood Lactic Acid and Plasma Inorganic Phosphate Concentration in the Suprarenalectomized Dog

Dog W-1-3.

Date	Lactic acid	Blood non-protein nitrogen	Plasma inorganic phosphate	Clinical condition
1931	*mg. per 100 cc.*	*mg. per 100 cc.*	*mg. per 100 cc.*	
Feb. 25	8.8	60		Rather marked symptoms
Feb. 26	13.1	84		Severe insufficiency
Mar. 2	7.6	40		Active. Condition excellent
Mar. 9	7.3	44	5.1	Condition good
Mar. 13	9.0	50	5.7	Condition good
Mar. 16	14.6	82	8.2	Insufficiency. Staggering gait
		45		Extract given
Mar. 17	11.3	65	7.1	Improved
Mar. 23	6.5	45		Active, alert
Mar. 30	6.3	43	4.4	Active, eating well
Apr. 24	7.6	56	5.2	Condition good
May 7	6.0	36	4.6	Condition good
May 18	8.7	140	10.1	11th day after extract withdrawal severe insufficiency

of total base is believed to delimit the total concentration of plasma electrolytes, this latter quantity must be greatly lowered, and will account for the diminished plasma conductivity long ago noted by Stewart (7). There is also a progressive and steady fall in the concentration of bicarbonates and chlorides, the sum of the change being roughly equal to that of the sodium. The bicarbonate concentration drops before that of the chlorides, but the fall in the latter in severe insufficiency exceeds that of the bicarbonate. This is associated in the later stages with a rise in the concentration of inorganic phosphates. We have not made sulfate analyses, but the experiments of Swingle and Wenner indicate a similar rise in sulfate concentration (4). The increases in the concentrations of plasma proteins and of calcium may be explained as due to the plasma concentration itself. There is no increase in the concentration of lactic acid during the progress of insufficiency; no animals were studied, however, during convulsions (Table II).

The rate of recovery of the normal relations of the several plasma constituents following the readministration of potent cortical extract depends (*a*) on the degree of the insufficiency produced before measures for resuscitation are undertaken, and (*b*) on the extent to which accessory measures, namely the use of fluids, sodium chloride, and glucose, are employed in addition to the injections of cortical extract. Where large amounts of extract are injected the recovery is rapid, unless the animal has been allowed to become moribund, in which case it cannot be restored to the original condition (1).

Studies of the inorganic constituents of the blood and plasma of normal and suprarenalectomized cats under ether anesthesia, by the use of modern methods, were made by Baumann and Kurland (5) in 1926. They observed a drop in the proportion of plasma in the blood (65 per cent control, 57 per cent in insufficiency), and an increase in plasma solids. A fall of 15 per cent occurred in the sodium concentration and 9 per cent in that of chlorides. They further observed an increase in the concentration of potassium and magnesium, but little change in that

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mally existing between these ions in the plasma (Table I), and, it may be anticipated, in the tissue fluids as well. We are studying the possibility that this change may in part account for the weakness and spasticity of the voluntary muscles during the later stages of insufficiency, as well as for the curious disturbances of the cardiac rhythm (1) and the eventual heart failure.

of plasma calcium. They found no noteworthy change in the inorganic or total acid-soluble phosphates. Lucas (6) as well as Stewart and Rogoff (7) found evidence of similar blood concentration in dogs, and the former reported the decrease in blood chlorides in the dog. Baumann and Kurland pointed out the probable importance, but unknown significance, of the changes in the ratio of sodium to potassium in the blood of the suprarenalectomized cat. Hastings and Compere (8), following the changes in the blood serum of dogs after suprarenalectomy, found a progressive fall in the bicarbonate content, a marked and consistent fall in lactic acid, and an increased concentration of serum proteins and calcium, the latter effects being explained by the hemoconcentration. They stressed the marked increase in serum potassium, which they found attained at death the very high value of 20 m.-eq. per liter. No changes were observed in the creatine or creatinine concentration until a terminal rise took place commencing 24 hours before death.

We have previously indicated (2) that the loss of plasma volume during the progress of the insufficiency can be explained by increased permeability of the capillaries, and hence loss into the tissues; by loss through the gastrointestinal tract, due to vomiting or diarrhea; or, finally, by excretion of fluid through the urine. We have advanced reasons in our previous paper to show why it cannot be satisfactorily explained by drainage from the capillaries into the extravascular space, a process which is said to occur in traumatic shock, and following injections of histamine. We have also pointed out that it cannot be lost by vomiting, as in intestinal obstruction, nor by diarrhea, as in the condition of hemoconcentration and shock seen in cholera, since these effects do not occur until very late during insufficiency.[4]

We turn therefore to the consideration of loss of body fluids through the kidneys. We have previously pointed out that the volume of urine is well maintained until the blood pressure falls, late during the course of the insufficiency. During the days immediately following extract withdrawal the output is usually increased. Since a loss of body

[4] During the final stages of suprarenal insufficiency, when the animal is in deep "shock," it is possible that both loss into the tissues by increased capillary permeability, and by vomiting and diarrhea, may account for the disappearance of water as well as electrolytes from the plasma. Proof is entirely lacking that either of these routes constitutes an important channel for the loss of the water and electrolytes which initiate the hemoconcentration.

water is commonly accompanied by a loss of the electrolytes with which it is associated in the tissues (9), balance experiments with measurements of fluid and food intake, and urine and stool output, have been carried out to ascertain what fluid and electrolyte changes may occur.

The dogs have been maintained in a well lighted room heated with thermostatic control at 70–78°F. Metal wire metabolism cages were used. The sides and floor were washed down with distilled water at the beginning of the experiment and between the periods of urine collection. The wash water was analyzed for its constituents, and the values found added to the total excretion for the period. Stools were removed and analyzed separately. Fecal excretion of sodium and of chlorides cannot be neglected. By reason of the impossibility of exact separation into periods it constitutes an uncertain source of error. The dogs were catheterized at 9.30 a.m. at the beginning of each control period, and daily during the stage of extract withdrawal and recovery. The weight was determined on silk scales to 0.05 kilos, immediately after catheterization. After removal of the urine, the bladder was washed out with a measured volume of sterile distilled water, which was added to the specimen. The urine specimens were collected under toluol with precautions against evaporation, measured daily, and kept on ice. Rectal temperatures were taken and observation made of the blood pressure by the method previously described (2). The water intake was measured and corrected for evaporation by subtracting the loss from a similar container suspended beside the cage.

The animal under study was given its food at noon and trained to consume it within 5 hours, during the fore periods. Complete consumption of food during the period when extract is withdrawn is sometimes difficult to secure. The uneaten food was thus weighed back with a minimal loss from evaporation since it was exposed only during this 5 hour period. Where salt mixtures were given in addition, the material was enclosed in a bolus of the food which was placed at the back of the dog's mouth. The muzzle was held shut until the animal was induced to swallow. Half of the prescribed dose was given at noon and half at the end of 5 hours.

The food intake for the balance experiments was given in two forms: (*a*) a proprietary canned dog food, (cooked salted horse meat), the chloride and sodium content of which was uniform as checked by control analyses, and (*b*) ground raw lean beef muscle to which definite amounts of sodium chloride were added as described above. The material was passed twice through the electric grinder to insure thorough mixing, weighed out in oiled paper, and preserved by refrigeration in ventilated tins until needed. Aliquot portions were taken for analyses for water, nitrogen, sodium, and chlorides. The chloride and sodium content of the canned dog food, as well as its nitrogen content, proved uniform within 5 per cent. The fresh beef muscle contained variable amounts of fat and was somewhat more

irregular in chloride and sodium. Both were low, however, in comparison to the extra sodium chloride administered, and hence this error was not great in proportion to the total intake.

We have been constantly engaged in making balance experiments for the past 2 years. The technique upon suprarenalectomized dogs is difficult and the results presented are selected from a group of successful experiments to show the typical changes.

During the control periods the animals were injected subcutaneously with cortical extract in divided doses, twice daily. The same batch of extract was used throughout the experiment. Our extract is now so standardized that its strength, as determined by assay, is fairly constant (10).

TABLE III

Effect of Salt-Free Diet in Accelerating Suprarenal Insufficiency after Extract Cessation

Dog 2-9. Right suprarenalectomy, Mar. 28, 1932. Left suprarenalectomy, Apr. 15, 1932. Death in insufficiency, Feb. 11, 1933.

Extract discontinued, Apr. 21	Insufficiency on 7th day	Salted diet
Extract discontinued, May 11	Insufficiency on 10th day	Salted diet
Extract discontinued, Nov. 9	Insufficiency on 9th day	Salted diet
Extract discontinued, Dec. 2	Insufficiency on 10th day	Salted diet
Salt-free diet from Feb. 1 (weight 8.1 kg.)		
Extract discontinued, Feb. 7	Insufficiency on 2nd day	Salt-free diet
Death on 4th day. Could not be revived		

It was observed early that the salt intake of a suprarenalectomized animal has an important bearing on its behavior after withdrawal of extract. Dog 2-9 illustrates this fact (Table III).

This animal was known among our laboratory group as a "10 day" dog, because under the usual dietary regime with ample salt intake, it regularly went into marked suprarenal insufficiency, on the 8th to the 10th day following cessation of injections of the extract. Such individual regularity of behavior after extract withdrawal, when conditions are uniform, is characteristic of the suprarenalectomized dog. When this animal was then given a salt-poor (lean muscle meat) diet for several days, abrupt stoppage of extract injections then produced symptoms of severe insufficiency in 48 hours, and death, in spite of all efforts to revive the animal, 36 hours later.

The salt content of the food also has a significant bearing on the extract requirement. Dog 7-0 illustrates this fact (Table IV).

TABLE IV

Dog 7-0. Right suprarenalectomy, Dec. 13, 1932. Left suprarenalectomy, Dec. 22, 1932. Removal of salt from the diet where the extract dosage is at the maintenance level produces insufficiency. Conversely extract is required even where the diet is adequate in sodium.

Date	Weight	Extract per day	Remarks	Plasma total base per liter	Plasma sodium per liter	Plasma chlorides per liter	Plasma non-protein nitrogen
1933	*kg.*	*cc.*		*m.-eq.*	*m.-eq.*	*m.-eq.*	*mg. per 100 cc.*
Feb. 11 to 17	9.0 9.9	2	Mixed diet. Dog in excellent condition, gains 0.9 kg. in 6 days. Temperature, 101°	152.8	143.1	111.2	37
Feb. 17 to 22	9.9 8.8	2	Lean raw beef muscle without added salt. Loss of 1.1 kg. in 5 days. Feb. 22, dog in insufficiency. Can hardly stand, will not eat. Temperature, 99.3°. Given 5 gm. NaCl—intravenous and intraperitoneal infusions	144.6	132.7	105.1	113
Feb. 23	8.5	2	Somewhat improved. Ate small amount of food. Salt given by mouth, intravenously and intraperitoneally				
Feb. 24	8.5	2	Ate very well				43
Mar. 3	9.2	2	Maintained on this dosage of extract in good condition until Mar. 17, gaining 0.9 kg. in weight on normal salted diet	154.1	143.2	111.2	37
Mar. 17 to 27	9.4	2	Lean raw beef muscle with 1.5 gm. added salt. The salt protects the animal although on the identical diet and extract dosage which previously threw him into insufficiency (Feb. 17 to 22)	153.3	140.9	108.9	38
Mar. 27 to 31	9.4 8.8	No extract	Extract removed Moderate insufficiency. Blood pressure not lowered	147.9	132.1	107.8	64

This animal was placed upon a low salt (whole chopped beef muscle) diet following the use of a well salted mixed diet, (mixed meat scraps, potato, bread, and vegetables from the hospital kitchen), on which it had been well maintained on an established level of extract dosage for weeks. After 4 days upon the low salt

TABLE V

Effect of Salt Content of Diet on Fall in Electrolyte Concentration and Production of Suprarenal Insufficiency after Cessation of Extract Injections. On Low Salt Diet the Symptoms of Insufficiency Appear Much Earlier and the Changes in the Plasma Electrolyte Pattern Are More Marked

Dog 8-4. Right suprarenalectomy Jan. 26, 1932. Weight 9.7 kilos. Left suprarenalectomy Feb. 2, 1932.

Date	Weight	Non-protein nitrogen	Plasma total base per liter	Plasma sodium per liter	Plasma chlorides per liter	Plasma bicarbonate per liter	Remarks
1933	*kg.*	*mg. per 100 cc.*	*m.-eq.*	*m.-eq.*	*m.-eq.*	*m.-eq.*	
Feb. 13	10.0	34	154.8	140.0	116.8	23.6	Mixed diet, well salted
	Extract injections stopped Feb. 14						
Feb. 17	9.9	50		137.1	112.8		
Feb. 19	9.3			131.0	106.0	17.2	
Feb. 21	8.9	96		128.2	103.0	14.2	Severe insufficiency. Temperature, 98°. Vomits, staggers. Blood pressure 55/0
Feb. 22	Revived with cortical extract and intravenous saline solution—8th day after cessation of extract						
Mar. 6	9.7	40		143.0	112.0	23.7	
Mar. 8	Extract injections stopped (weight 9.6 kg.)						Salt-free diet (ground lean meat) from Mar. 3, + 1.5 gm. NaCl
Mar. 11	8.8	100		131.5	102.6	15.8	Marked insufficiency
Mar. 13	8.4	160		121.9	95.6	13.6	Severe insufficiency. Appears almost moribund
Mar. 13	Revived with cortical extract and intravenous saline solution—5th day after cessation of extract						
Apr. 6	9.4	33		140.1	112.2	22.9	

diet, this constant dose of extract from the same lot proved inadequate for maintenance, and it lapsed into insufficiency. The dog was restored to its normal condition by the usual measures and was then replaced on the same low salt diet (whole chopped beef muscle), but with the addition of 1.5 gm. of sodium chloride daily. On this regime the original dosage taken from the identical lot of extract maintained it in excellent condition.

The magnitude of the salt intake introduces a new variable into the assays of cortical extract upon the suprarenalectomized dog. Our

TABLE VI

Dog 2-9. Right suprarenalectomy Mar. 28, 1932. Left suprarenalectomy Apr. 15, 1932 (Penick). Balance experiment—canned cooked salted horse meat.

Day	Cortical extract per day	Weight	Urine volume per day	Nitrogen balance per day	Sodium balance per day	Chloride balance per day	Creatine per day	Creatinine per day	Total creatinine per day	Total phosphates per day	Blood non-protein nitrogen	Food intake per day	Clinical condition
	cc.	*kg.*	*cc.*	*gm.*	*m.-eq.*	*m.-eq.*	*gm.*	*gm.*	*gm.*	*gm. P*	*mg. per 100 cc.*	*gm.*	
1	5	7.0											
2	5												
3	5					Average per day							Control period
4	5		145	−0.1	−0.7	−0.3	0.12	0.25	0.37	0.16		300	
5	5												
6	5	7.1									34		
	Extract injections discontinued beginning Nov. 10, 1932												
7	0		218	−0.1	−27.9	−25.3	0.15	0.31	0.46	0.14		300	Condition excellent
8	0		244	+0.1	−35.6	−27.0	0.25	0.29	0.54	0.14		300	
9	0	7.0	247	0.0	−27.7	−22.0	0.24	0.28	0.52	0.14		300	
10	0		167	+0.5	−7.5	−4.1	0.22	0.23	0.45	0.15		300	
11	0	6.8	185	+0.5	−3.7	+1.0	0.19	0.25	0.44	0.13		300	
12	0		230	+0.1	−3.1	+1.0	0.22	0.27	0.49	0.16	90	300	Quite active. Eats
13	0		179	−0.4	−5.0	−2.5	0.23	0.23	0.46	0.12		200	food promptly
14	0	6.7	158	−0.8	−10.9	−6.1	0.16	0.21	0.37	0.11		95	
	Extract injections resumed Nov. 18, 1932												
15	15	6.6	290	−3.2	+5.0	+8.4	0.30	0.31	0.61	0.34	115	100	Very weak, but can stand. Temperature 98.4°.
16	10		202	−3.1	+1.5	+1.4	0.21	0.20	0.41	0.23		70	Much more active
17	10	6.3	170	−0.7	+3.9	+7.7	0.28	0.31	0.59	0.27	45	300	but ate very little
18	5					Average per day							
19	5		147	+0.1	+3.9	+2.9	0.15	0.28	0.43	0.19			
20	5	6.6									40	300	Condition excellent
21	5												
22	5					Average per day							
23	5		184	0.0	+7.1	+0.5	0.13	0.26	0.39	0.16		300	
24	5	6.8									27		

previous work was done upon animals whose food intake was weighed daily. The salt intake was ample but it was not measured. It is

evident that the salt intake per kilo of body weight must be given close consideration. It is clear that the dosage may be reduced to a fraction of its original amount when a high salt diet is substituted for one low in salt content.

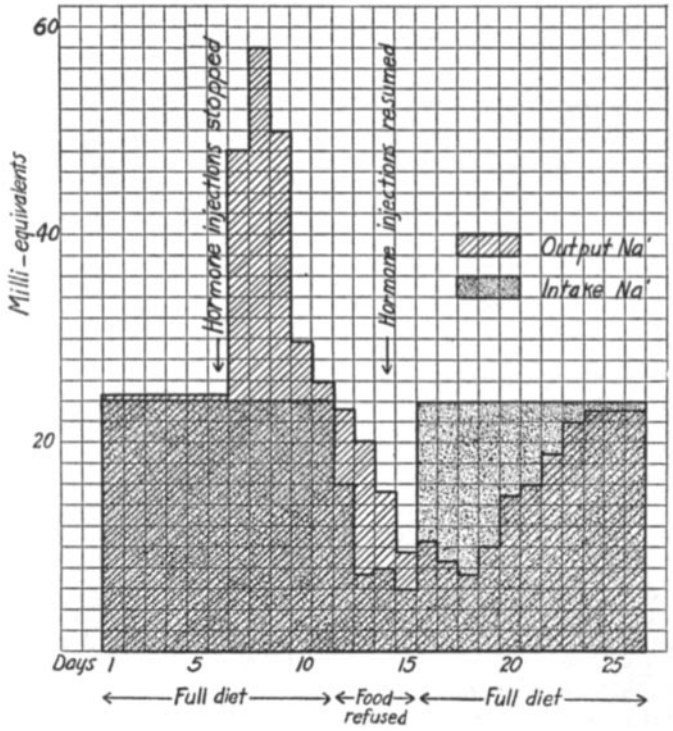

CHART 2. Sodium intake and output following cessation of cortical extract injections in a suprarenalectomized dog. The return to approximate balance on the 10th day is due to the exhaustion of the stores of extracellular water, and the fall in intake is due to refusal of food during the later stages of insufficiency. The sodium retention following resumption of the cortical extract injections about equals the loss during the earlier cessation period. Salted meat diet; no parental fluids given. Weight at beginning of experiment regained at its conclusion.

The results of these balance experiments (Tables VI, VII), indicate that after cessation of the injections of cortical extract in an animal on a measured diet and sodium chloride intake, an abrupt rise in the excretion of sodium, chloride, and water occurs. The sodium

TABLE VII

Dog 8-4. Right suprarenalectomy Jan. 26, 1932. Left suprarenalectomy Feb. 2, 1932. Balance experiment—raw lean beef muscle 450 gm. + 2.3 gm. sodium chloride daily.

Day	Cortical extract per day	Weight	Urine output per day	Fluid intake per day*	Fluid balance per day	Nitrogen balance per day	Phosphate urine per day	Sodium balance per day	Chloride balance per day	Plasma volume	Oxygen capacity	Plasma total base per liter	Plasma sodium per liter	Plasma bicarbonate per liter	Plasma chlorides per liter	Blood non-protein nitrogen	Plasma urea nitrogen	Plasma total protein	Albumin-globulin ratio	Food
	cc.	*kg.*	*cc.*	*cc.*	*cc.*	*gm.*	*gm. P*	*m.-eq.*	*m.-eq.*	*per cent*	*vol. per cent*	*m.-eq.*	*m.-eq.*	*m.-eq.*	*m.-eq.*	*mg. per 100 cc.*	*mg. per 100 cc.*	*gm. per 100 cc.*		*gm.*
1	2.5	9.2			Average per day															450
2	2.5		458	670	+212	+0.7	0.66	+1.1	+4.6											450
3	2.5	9.2																		450
4	5.0									62.8	16.9	156.6	142.1	22.9	112.2	48	33	5.26	1.0	450
5	5.0				Average per day															450
6	5.0		422	683	+261	+0.9	0.65	+3.8	+0.1											450
7	5.0	9.3																		450
		Extract injections discontinued																		
8	0	9.3	353	423	+70	+0.4	0.33	−26.3	−27.4				146.3	22.0	112.2		32			250
9	0	9.0	685	563	−122	+0.2	0.67	−61.0	−53.1											450
10	0	8.7	529	668	+139	−0.4	0.52	−12.3	−10.1				134.9	18.0	106.0	56	45			330
11	0	8.6	450	570	+120	+0.2	0.33	−3.2	+6.8											200
		Extract injections resumed																		
12	20.0	8.5	508	593	+75	−6.6	0.93	+24.3	+10.9	51.5	21.7	143.4	128.2	15.7	103.2	102	90	6.46	0.9	150
13	15.0	8.3	573	958	+385	0.0	0.78	+23.6	+23.4		20.3	144.6	134.2	21.0	105.2	40	23			450
14	8.7	8.6	484	678	+194	+0.6	0.77	+16.6	+13.9											450

15	4.4	8.8	Average per day							64.0	17.0	150.6	137.7	23.3	111.7		18			450
16	4.4	8.9	424	703	+279	+1.1	0.65	+8.2	+19.4											450
17	0.9	8.9										152.8	143.1	23.6	114.8		14	5.24	1.1	450
18	0.9		Average per day																	450
19	0.9	9.0	434	660	+226	+0.1	0.67	+0.4	+0.7											450

* Fluid intake includes water content of food. The fluid balance does not take account of water loss through feces, expired air, or skin.

excretion is at its maximum during the following 48 to 72 hours. The excretion then falls with the drop in the concentration of these ions in the blood plasma, but the animal may remain on a negative balance even at the lowest plasma level until it is restored by injections of extract. Sodium is still excreted in appreciable amounts in the urine at a plasma sodium level of 106 m.-eq. per liter and chloride at a plasma chloride level as low as 85 m.-eq. per liter. The concentration of sodium in the urine increases following extract cessation. Notwithstanding the increased urinary volume, the concentration of this ion for the first 2 or 3 days may be nearly doubled.

The loss of sodium and of chloride through the urine in excess of the amounts ingested during the period of extract cessation is much greater than can be accounted for by the loss due to the fall in their plasma concentration, and it is evident that the excess must come from the tissues. Thus during the 48 hours following extract cessation (Dog. 8-4, Table VII, days 8 and 9), the excretion of urinary sodium in excess of that of the control period amounted approximately to 85 m.-eq. During the same period the fall in plasma sodium was 11.4 m.-eq. per liter. Even assuming the blood volume to be 1000 cc. and the plasma volume 600 cc., the loss from the plasma alone could account for but 6.8 m.-eq. of sodium ($600/1000 \times 11.4$). The rest, over 90 per cent of the whole, or 78.2 m.-eq. must be withdrawn from the water depots outside of the blood, during this period. It is true that a certain absolute shrinkage occurs in the plasma volume, but it is small during these early stages. It is clear that in suprarenal insufficiency, as has been elsewhere observed, the changes in the concentrations of plasma electrolytes do not necessarily reflect, either in a qualitative or in a quantitative sense, the shifts in water and electrolytes which may be taking place in the depots of body water, or in the water and electrolyte balance of the body as a whole. It will be noted that the increased excretion of sodium takes place chiefly during the days immediately following extract cessation and that the excretion then falls off abruptly (Chart 2). We interpret this fall as due to the exhaustion of the stores of interstitial body fluid. Once depleted, further supplies of sodium are obtained from the intracellular fluids with greater difficulty and probably with more serious consequences to the organism. The increased sodium output in the urine, corrected

for the amounts yielded by the blood plasma during this period, may well define the total volume of interstitial fluid so lost from the body.

During the recovery period, there is a positive balance with retention of sodium and chloride, a phase which may continue over several days after injections of extract are resumed. When the total loss of these electrolytes during the cessation period is compared with the retention during the recovery period it is found that these values approximate each other, provided the body weight returns to its original value. At the conclusion of the after period, the animal is again in sodium and chloride equilibrium and the plasma electrolyte pattern is restored approximately to its original form.

Immediately after the resumption of injections of cortical hormone, an increased excretion of urine is observed for a period of 24 to 72 hours. Simultaneously the urinary nitrogen is greatly augmented. During this time the body weight continues to fall although the animal may obviously be improving. Such a lag or even further loss of weight nearly always occurs before the body weight begins to return to its original level with the use of adequate injections of the cortical extract.

The sodium chloride intake in the food must be adjusted at a rather low level in order to demonstrate clearly the effects of removing the injections of cortical hormone. Where ample salt is supplied in the food to compensate for the loss through the kidneys, the body stores of sodium are not drained and no immediate pronounced effect is noted on the balance. On the other hand, for the purpose of demonstrating the loss of body salt after removal of the extract, the salt intake must not be too low, since in such circumstances a state of insufficiency (Table V, March 8 to 11) may appear abruptly, with vomiting and diarrhea, which ruin the observations.

It has been observed repeatedly during assay studies that animals which continue to eat can be maintained on lower amounts of extract for longer periods than those whose appetite is capricious. The effect may readily be explained as due to the lowered salt intake and consequent increased drain upon the endogenous salt stores of the animals whose food intake is low, and hence whose salt intake is greatly reduced.[5]

[5] During the summer of 1932 we were unable to explain the high blood nonprotein nitrogen values which we found in our suprarenalectomized dogs. For

Neither the nitrogen nor the phosphate excretion is materially increased during the period of extract cessation in spite of the loss of weight. On the contrary there is often a fall in excretion which becomes marked when food is refused. We have shown previously that during this phase a marked rise in blood urea concentration also takes place, notwithstanding the continued excretion of an equal or greater volume of urine. In the after period, following the injection of effective amounts of extract, there is an immediate increase in the urinary excretion of nitrogen, and the urea fraction is particularly affected. This is coincident with the return to normal of the blood urea level. As the concentration of blood urea is an accurate index of the concentration throughout the tissues of this freely diffusible substance, it is reasonable to suppose that the increased urinary excretion of urea is derived largely from this source. Coincident with it there is also an increased excretion of urinary phosphorus. This is interpreted as being derived from the protein destruction resulting from cellular disintegration during the period of insufficiency.

DISCUSSION

Loeb and his coworkers (19) have just published a paper upon the electrolyte balance in three dogs during an extended control period,

weeks they were maintained clinically in an active healthy condition with blood non-protein (urea) nitrogen levels of 80 to 120 mg. per 100 cc. (65 to 100 mg. per 100 cc. urea nitrogen), a concentration which is ordinarily associated with severe insufficiency. Injections of large amounts of the cortical hormone produced relatively little effect upon this blood level. We were at first inclined to attribute it to some unknown substance which came through the various lipid extractions in the manufacture of our extract due to the fact that the glands were exposed to unusually hot weather at the abattoir before use. One animal, apparently in excellent condition, which also received 30 cc. daily of cod liver oil showed a constant elevation for several weeks up to 200 mg. per 100 cc. of non-protein nitrogen. It becomes evident, however, on reviewing the protocols during that period, that the diet of the animals consisted principally of boiled unsalted beef. Since the water in which it was cooked was discarded, much of the soluble sodium chloride was undoubtedly extracted and lost. A ration very low in salt content was thus given. The seasonal variation in the survival period following bilateral suprarenalectomy in various animal forms has been noted by many observers, as well as the fact that hibernating forms survive longer than those which are active. We have not had convincing evidence, however, that the extract requirement of our animals, maintained in healthy condition, is any greater in the warm summer months than it is during the winter.

followed by suprarenalectomy. The animals were then observed until death in suprarenal insufficiency. The magnitude of the disturbance in the electrolyte balance which we have found in the suprarenalectomized animal following cessation of injections of the cortical hormone, and during the subsequent repair of the process following resumption of its use, is quite comparable to that reported by these authors. It is evident that the effects on the electrolyte balance immediately following suprarenalectomy and those due to removal of injections of the cortical hormone are identical, in the dog. Loeb reaches the conclusion that the suprarenal glands have a regulatory effect upon sodium metabolism and upon renal function.

The reciprocal relations existing between the plasma concentrations of sodium and chlorides, and of urea, during both the period of extract cessation and that of recovery following its resumption, recall the theory, recently again revived by Blum and his coworkers (11), and by Hartman and Darrow (12), that urea nitrogen retention may be a compensation for the loss of electrolytes, in an effort to maintain osmotic equilibrium.[6] However doubtful such an explanation may be, the changes are so striking that the existence of some significant relationship seems probable.

It is evident that the retention of nitrogen during insufficiency cannot be due to a reduction of water available for urea excretion, nor can the accumulation be merely secondary to circulatory failure and fall in blood pressure (20). As pointed out above there is an actual increase in urinary volume following extract withdrawal, and the collapse of the circulation occurs only after the rise in blood nonprotein nitrogen is marked. The evidence points clearly to a direct regulatory influence exerted by the hormone upon the renal excretion of the several substances under discussion.

The consequences of the loss of sodium, chlorides, and water through the kidneys in suprarenalectomized dogs, as a result of lack of the

[6] The changes in the electrolyte pattern in suprarenal insufficiency do not affect the plasma pH (colorimetric method of Cullen and Sendroy (*J. Biol. Chem.*, 1922, **52**, 501), except in the later stages, when a fall occurs which may be large at death. The condition, therefore, during suprarenal insufficiency is that of a compensated alkali deficit which in the advanced stages becomes uncompensated (personal communication from Dr. K. Stuart Hetzel).

cortical hormone, recall particularly the studies of Gamble and his coworkers in the dog concerning loss of pancreatic juice due to drainage from a Pawlow fistula (16, 17). The similarity in the symptoms of the animals which they studied to those observed during the course of insufficiency in the suprarenalectomized dog is evident. The greater rapidity of the appearance of serious symptoms on a meat diet without added salt, in which the fixed base is in large part potassium, and hence unsuited to the repair of plasma and interstitial fluid loss was striking and also in keeping with the effects in the suprarenalectomized dogs deprived of extract.

Since the loss of sodium and of chlorides must come chiefly from the body fluids in which they principally occur, namely the blood plasma and interstitial tissue fluid, it follows that the water loss from the parenchymatous tissues, in which potassium is the principal base, should be relatively slight. Such appears to be the case in the animal dying in insufficiency. There is no appreciable reduction in the water content of liver or of muscle tissue in the suprarenalectomized dog or rat dying of insufficiency, despite the marked loss of weight. Analyses of such tissue which we have made from three suprarenalectomized dogs and nine suprarenalectomized rats, killed during severe insufficiency following the cessation of extract, when compared with like tissues obtained from a control series composed of equal number of normal animals, shows an average difference in water content of less than 2 per cent.

The increased excretion of urinary nitrogen and phosphorus, which follows resumption of injections of cortical extract, indicates an actual destruction of protoplasm and a reduction in its total mass during insufficiency. Destruction of muscle tissue is suggested by the changes in creatine and creatinine excretion as well.

The question now arises as to whether the dehydration, used in its broader sense, to embrace not only loss of water, but loss of electrolytes from the interstitial body fluids and blood plasma, is a sufficient explanation for the failure of the several physiological processes which we have described in our earlier papers (1, 2), and the ultimate cause of death in suprarenal insufficiency. We cannot at the present time accept this explanation as proven, but we believe the evidence presented indicates its importance and renders superfluous the various theories

of intoxication which have been so frequently put forward. Studies of the effect of sodium chloride in the acute insufficiency of Addison's disease support this view (18).

The experience of numerous authors indicates clearly that all attempts to support the life of suprarenalectomized animals by sodium chloride alone have been unsuccessful. The cortical hormone, at least in minimal amounts, is indispensable for this purpose. It is possible that in the particular species which we have studied, the dog, the changes due to dehydration occur early, and mask other equally important changes due to lack of the cortical hormone, which make their appearance more slowly in this animal.

SUMMARY

A characteristic alteration in the electrolyte structure of the blood plasma of the suprarenalectomized dog occurs when injections of cortical extract are stopped. This alteration progresses during the course of the suprarenal insufficiency, parallel with the hemoconcentration and the loss in weight. When injections of cortical extract are resumed, the electrolyte structure returns to its original form, the alterations paralleling the dilution of the blood and the return of the body weight to its original level.

The hemoconcentration, with the resulting physiological changes which take place in the suprarenalectomized dog after the cessation of cortical extract injections, is associated with a loss of sodium and chloride, accompanied by their proper complement of body water, by way of the kidney. Since this effect is produced in the suprarenalectomized animal, well nourished and in excellent condition, solely by cessation of injections of the cortical hormone, and since the reverse process of repair of the electrolyte and water losses can be effected solely by resumption of extract injections, it follows that all of the observed phenomena are due to this cause, and to this alone. It can be concluded that one function of the cortical extract in the suprarenalectomized dog is that of participation in the regulation of the sodium and chloride metabolism, and consequently, of the balance and distribution of water. The loss of water, in the absence of the cortical hormone, is sustained partly by the blood plasma, but to a far greater extent by the interstitial body fluid. The available evidence points

to the kidney as the locus of this regulatory function of the cortical hormone.

REFERENCES

1. Harrop, G. A., and Weinstein, A., *J. Exp. Med.*, 1933, **57,** 305.
2. Harrop, G. A., Weinstein, A., Soffer, L. J., and Trescher, J. H., *J. Exp. Med.*, 1933, **58,** 1.
3. Stewart, G. N., *J. Pharmacol. and Exp. Therap.*, 1926, **29,** 373.
4. Swingle, W. W., and Wenner, W. F., *Physiol. Zool.*, 1928, **1,** 37.
5. Baumann, E. J., and Kurland, S., *J. Biol. Chem.*, 1927, **71,** 281.
6. Lucas, G. H., *Am. J. Physiol.*, 1926, **77,** 114.
7. Stewart, G. N., and Rogoff, J. M., *Proc. Soc. Exp. Biol. and Med.*, 1925, **22,** 394.
8. Hastings, A. B., and Compere, E. L., *Proc. Soc. Exp. Biol. and Med.*, 1931, **28,** 376.
9. Gamble, J. L., Ross, G. S., and Tisdall, F. F., *J. Biol. Chem.*, 1923, **57,** 633.
10. Harrop, G. A., Pfiffner, J. J., Weinstein, A., and Swingle, W. W., *Proc. Soc. Exp. Biol. and Med.*, 1932, **29,** 449.
11. Blum, L., Grabar, P., and van Coulaert, *Ann. mêd.*, 1929, **25,** 34.
12. Hartman, A. F., and Darrow, D. C., *J. Clin. Inv.*, 1929, **6,** 127.
13. Banting, F. G., and Gavins, S., *Am. J. Physiol.*, 1926, **77,** 100.
14. Marine, D., and Baumann, E. J., *Am. J. Physiol.*, 1927, **81,** 86.
15. Gamble, J. L., *New England J. Med.*, 1929, **201,** 909.
16. Gamble, J. L., and McIver, M. A., *J. Exp. Med.*, 1928, **48,** 859.
17. Gamble, J. L., and Ross, S. G., *J. Clin. Inv.*, 1925, **1,** 403.
18. Harrop, G. A., Weinstein, A., Soffer, L. J., and Trescher, J. H., *J. Am. Med. Assn.*, 1933, **100,** 1850.
19. Loeb, R. F., Atchley, D. W., Benedict, E. M., and Leland, J., *J. Exp. Med.*, 1933, **57,** 775.
20. Swingle, W. W., Pfiffner, J. J., Vars, H. M., Bott, P. A., and Parkins, W. M., *Science*, 1933, **77,** 58.