

## STUDIES ON THE SUPRARENAL CORTEX

### IV. THE EFFECT OF SODIUM SALTS IN SUSTAINING THE SUPRARENALECTOMIZED DOG\*

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A number of papers during the past few years, particularly those of Stewart and Rogoff (1), of Marine and Baumann (2), and of Loeb and his coworkers (3), have shown clearly the close relations which exist between the suprarenal glands and the metabolism of salt and water. The recent studies of Loeb are of fundamental interest since they provide for the first time a rational explanation of the mechanism of suprarenal insufficiency, which this author suggests may be due to a primary loss of sodium through the kidney.

The results which follow total ablation of the suprarenal glands in the dog have been confirmed by studies on the effects of withdrawal of injections of the cortical hormone from the suprarenalectomized dog which had been previously maintained with healed wounds in a normal state of health and nutrition (4). Following the withdrawal of the hormone injections it has been shown that there is an increased urinary excretion of sodium, chloride, and of water, relative to the intake, which is presently reflected in a fall in the concentration of

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A preliminary report of portions of this work has been published (*Tr. Assn. Am. Physn.*, 1934, **49**, 153).

these substances in the blood plasma. It would seem probable that an explanation of the prolonged survival which has been reported in suprarenalectomized hibernating animals (5) may be found in the lessened urinary excretion during the winter sleep, with a resulting diminished loss of sodium. It is possible that some additional mechanism for the conservation of sodium may be in part responsible for the prolonged survival also reported in suprarenalectomized animals during estrus or pregnancy (6).

The problem now arises as to whether this regulation of salt and water metabolism is the primary function of this cortical hormone obtained by extraction of beef suprarenal glands with lipoid solvents (7), or, on the other hand, whether there are other physiological derangements produced by removal of the suprarenal glands in the dog, which develop more slowly, and for this reason are usually masked by the earlier, more rapidly fatal effects of the dehydration associated with the loss of electrolytes.

The prolongation of the life of suprarenalectomized animals by injection of various fluids containing glucose or salts has been attempted by a number of workers, but the earlier experiments have never been entirely convincing.<sup>1</sup> The first demonstration of this effect of infusion was made by Brown-Séquard who showed in 1856 (8) that transfusion of blood from a normal into a suprarenalectomized animal will delay death for a considerable period. Marine and Baumann (2) in 1927 found that daily intraperitoneal injections of various sodium salts, for example normal sodium chloride solution, Ringer's solution, and isotonic sodium acetate, prolonged the lives of suprarenalectomized cats to about three times the span of untreated totally suprarenalectomized controls. They found further that sodium glycerophosphate was only slightly less effective. On the other hand, hypertonic saline solution, isotonic glucose solution, and glycerol had very little effect. They concluded that chloride is not an important factor even though a decrease in blood chlorides regularly occurred after suprarenalectomy. Although they pointed out the specific value of sodium salts in sustaining the animal they concluded that the action is only palliative, as all of the animals ultimately died of suprarenal insufficiency. The survival period did not exceed 15 days in any case.

Banting and Gairns (9), who observed a fall in blood urea after the injection of hypertonic saline (100 cc. 5 per cent NaCl), concluded that death could not be prevented even if blood urea, non-protein nitrogen, and chlorides are maintained at nearly normal levels.

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<sup>1</sup> The earlier work is summarized by S. W. Britton (*Physiol. Rev.*, 1930, 10, 617).

Stewart and Rogoff (1), on the theory that suprarenal insufficiency is brought about by a severe and progressive intoxication, attempted the use of Ringer's solution, using 100 cc. per kilo per day, injected intravenously. Out of seventeen animals so treated, eight lived to the 18th day or longer, and of these three survived 30, 40, and 54 days respectively. Nausea, vomiting, and death eventually terminated all of their experiments. They concluded that "if it were practicable to wash out all of the poison it is conceivable that the animals would survive indefinitely," but that salt solution cannot "substitute" for the missing hormone.

In a recent paper Swingle and his collaborators (10) have studied the effect of feeding sodium chloride upon the life span of suprarenalectomized dogs. They found that intraperitoneal injections were ineffective in severe insufficiency, and so chose the oral route. Seven animals were used, but only three survived over 14 days. Of the three, one lived 19 days and one 21 days. The third animal was maintained for 50 days in excellent condition and only showed signs of insufficiency when the experiment was terminated, by cessation of the daily feeding of sodium chloride. Swingle concluded that it is not possible to sustain suprarenalectomized dogs without extract. The salt in his experiments was mixed with the food, and eventually the animals seem to have refused to eat the extremely salty rations.

The current view appears to be that salt and water are not effective substitutes for the cortical hormone (11). Some other factor seems to be regarded as essential, the nature of which is not clearly established.

The problem is important for an understanding of cortical function, and we have undertaken a series of experiments in which sodium has been supplied, both with and without added chloride, to the suprarenalectomized dog deprived of hormone injections. An attempt has been made to sustain so far as possible, the proper plasma level of the sodium ion. Under such circumstances, it was thought that the relatively uncontrolled renal excretion of sodium might be satisfied by an augmented exogenous supply, leaving the stores in the plasma and body fluids substantially intact, and hence, presumably, leaving undisturbed the normal conditions of tissue hydration. Granted the possibility that such proper electrolyte levels might be sustained by administration of added sodium and chloride ions alone, and with hormone injections completely withheld, we thought it possible that other physiological abnormalities due to cortical suprarenal deficiency, pointing to other essential functions of the suprarenal cortex or medulla might then be unmasked. Disorders of carbohydrate storage or of its mobilization might be disclosed, or nutritional deficiencies resulting

from the absence of the suprarenal cortical hormone, might be uncovered. It was thought possible that the increased plasma concentrations of potassium and of magnesium, which appear during suprarenal insufficiency, might progress unabated after withdrawal of hormone injections and at length prove toxic should they be phenomena occurring independent of the changes in sodium and chlorides.

On the other hand, the continued well-being of the suprarenalectomized animal when the level of plasma sodium is properly maintained without injections of the suprarenal cortical hormone would offer substantial evidence that regulation of electrolyte metabolism is its most essential function in the adult male dog.

It is necessary at the outset in such experiments to make sure that the amounts of sodium and chloride ions given to the animal are sufficient to repair the losses due to urinary excretion. This can best be ascertained by following the concentration of these electrolytes in the blood plasma at sufficiently frequent intervals. The results of our work indicate that suprarenalectomized adult male dogs may be sustained for prolonged periods without any cortical extract and without any apparent ill effect, provided that a balance be effected between intake and outgo of sodium and chloride ions so that no appreciable loss occurs at any time, and that no hemoconcentration takes place.

Following the withdrawal of injections of cortical hormone from the suprarenalectomized dog which is being given our usual diet of table scraps and occasional added beef stew and bones but without added salt, the excretion of urinary sodium exceeds that of chloride, and the fall in the plasma concentration (in milli-equivalents) is greater. From this observation it has seemed desirable that sodium should be supplied in excess of chloride to make up for this difference in the rate of excretion. That is, a mixture of sodium chloride plus sodium bicarbonate might be more effective in maintaining the normal plasma ion concentration in the suprarenalectomized animal over prolonged periods than sodium chloride alone. Such we have found to be the case. Indeed, adequate administration of sodium ion alone, as will be shown presently, can maintain proper blood concentration (ratio of plasma to cells) even though the plasma chloride drops to a low level. Eventually, however, such animals utterly refuse to eat and analysis

of the gastric juice after alcohol stimulation<sup>2</sup> shows a complete absence of free acid. It is possible that the low plasma chloride level may

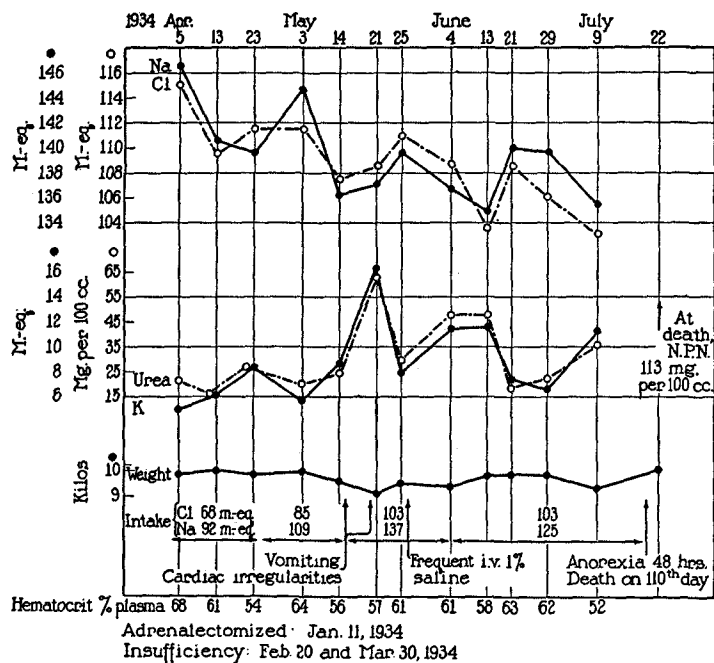


CHART 1. Dog 1-29. Adrenalectomized animal sustained 110 days (Apr. 5 to July 23) on sodium chloride and sodium bicarbonate only. Between May 14 and 25, vomiting and cardiac irregularities required additional intravenous saline. Thereafter the animal continued very well until July 9. The concentrations of sodium and chloride were low on July 9 (97th day) and hemoconcentration was indicated as well by the hematocrit reading. This indication for raising the intake of salt was unfortunately disregarded and the animal died 13 days later on July 22 after 72 hours of anorexia. Proper administration of sodium chloride and water would undoubtedly have prolonged this experiment indefinitely.

interfere with the secretion of gastric juice and of free hydrochloric acid in the stomach and that this may have a bearing on the proper

<sup>2</sup> 50 cc. of 10 per cent ethyl alcohol are administered by stomach tube and the gastric contents are aspirated at the end of 15 and of 30 minutes. Practically no gastric juice may be obtained from the fasting animal without such stimulation. The use of histamine is not safe in suprarenalectomized dogs.

maintenance of appetite. At any rate administration of sodium chloride in such animals immediately restores appetite coincidental

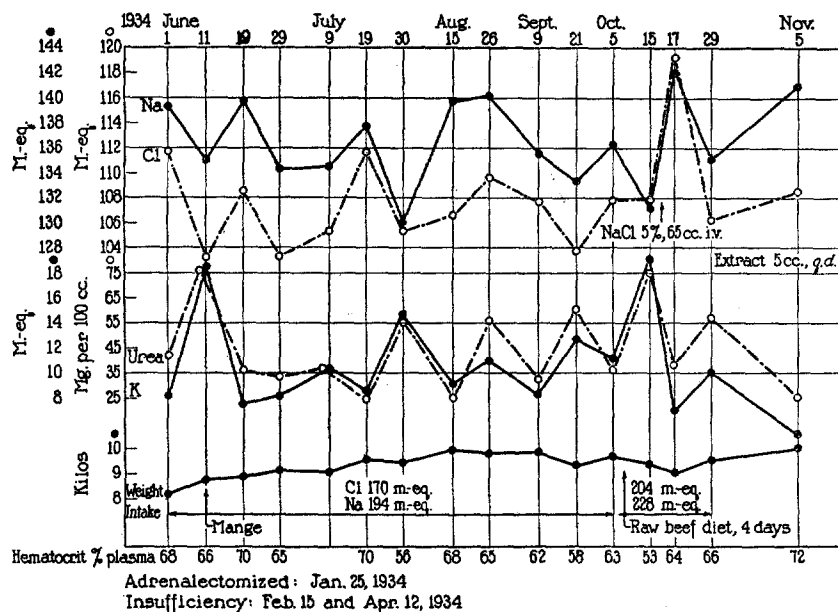


CHART 2. Dog 1-32. Adrenalectomized animal sustained for 152 days (June 1 to Oct. 29, 1934) on sodium chloride and sodium bicarbonate only. The diet was shifted on Oct. 9 to raw beef, resulting in anorexia, fall in plasma sodium and chloride, and rise in urea and potassium. The intravenous injection of 65 cc. 5 per cent sodium chloride on Oct. 15 restored the electrolyte pattern to approximately normal values with restoration of appetite and return of weight. The experiment was discontinued Oct. 29 on the 152nd day, when injections of cortical hormone were resumed. At this point the condition of the animal was excellent. At the end of 7 days (Nov. 5) the potassium had dropped and the sodium risen to normal values, with rise of 0.6 kilo in weight, indicating the more accurate electrolyte regulation possible only with injections of the cortical hormone. Both hormone and salt administration was then stopped, and the animal promptly went into insufficiency. Infection with mange, producing changes in pattern on June 11, was completely gone June 19.

with a rise in the plasma chloride level and the reappearance of free acid in the gastric juice.

Since appreciably greater amounts of sodium than of chlorides are lost, the result is that when sodium chloride (and hence equivalent

amounts of sodium and of chloride ions) alone is supplied in small quantities to the animal after the injections of the cortical hormone are stopped, a relatively greater loss of urinary sodium occurs producing a greater lowering of the plasma sodium level and hence a relative chloride acidosis. This acidosis may become uncompensated, and its occurrence may also have contributed to the failure of earlier attempts to maintain animals beyond a limited period by the administration of saline solutions alone.<sup>3</sup>

We report a group of experiments on suprarenalectomized dogs sustained by the oral administration of sodium and chloride ions for periods of 100 to 150 days (Charts 1 and 2, Table I). At the end of the period of experimentation, when properly conducted, the dogs do not differ in weight or activity from normal dogs or from suprarenalectomized dogs adequately sustained with injections of the cortical hormone. They appear to exhibit normal sexual activity, but the maintenance of normal fertility was not studied. Insufficiency has followed only when the salt administration was inadequate.<sup>4</sup> A careful technique is required which has been developed after considerable experimentation.

#### *Methods*

Daily intravenous infusions, such as were made by Stewart and Rogoff (1), or intraperitoneal infusions, such as were employed by Marine and Baumann (2) over prolonged periods, were considered impractical for our purpose. In the latter case, the use of alkaline salts such as sodium bicarbonate intraperitoneally proved very irritating and sometimes fatal to our suprarenalectomized dogs. We turned, therefore, to the administration of the salts by mouth. If mixed with the diet, the large amounts that are frequently required resulted, as in Swingle's experience, in refusal to eat. Accordingly they were given by stomach tube dissolved in small amounts of water (5 per cent solution) in divided doses, twice a day, at 6 or 7 hour intervals, and never in association with the food. The animals were kept on the table for 20 to 30 minutes after gavage to prevent regurgitation.

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<sup>3</sup> Unpublished data supplied by Dr. K. Stuart Hetzel.

<sup>4</sup> Postmortem study of animals sustained by sodium salts over prolonged periods without cortical hormone injections has shown no accessory cortical tissue present. No definite abnormalities were demonstrable in the pituitary, such as have been described by H. B. Schumacker and W. M. Firor (*Endocrinology*, 1934, **18**, 676) in their adrenalectomized dog maintained with inadequate doses of the hormone.

TABLE I  
 Dog 1-22. Suprarenalectomy Jan. 11, 1934. Insufficiency Mar. 1, 1934, following withdrawal of extract injections. Prompt recovery with extract and salt.

Date	Day of experiment	Weight kg.	Non-protein nitrogen mg. per 100 cc.	Urea mg. per 100 cc.	Sugar mg. per 100 cc.	Plasma sodium per liter m.-eq.	Plasma potassium per liter m.-eq.	Plasma chlorides per liter m.-eq.	Plasma bicarbonate per liter m.-eq.	Hematoctrit plasma per 100 cc.	Remarks
1934 Mar. 16		8.0	48	30	65	143.1	8.1	110.5	25.0	66.0	Injections of cortical hormone stopped Mar. 15. 5 gm. NaCl daily <i>per os</i>
	10	8.3	52	28	52	138.8	8.7	107.9	20.3	62.5	5 gm. NaCl + 2 gm. NaHCO <sub>3</sub> daily <i>per os</i>
Apr. 2	17	8.1	34	18	52	145.5	5.6	111.8	26.7	68.0	5 gm. NaCl + 2 gm. NaHCO <sub>3</sub> daily <i>per os</i>
	12	8.2	49	32	67	143.4	6.7	109.4	23.0	69.0	5 gm. NaCl + 2 gm. NaHCO <sub>3</sub> daily <i>per os</i>
	23	7.7	48	31	66	143.9	7.0	112.1	22.5	66.5	5 gm. NaCl + 2 gm. NaHCO <sub>3</sub> daily <i>per os</i>
May 7	52	7.8	52	39	65	139.7	9.7	108.4	27.0	71.5	5 gm. NaCl + 2 gm. NaHCO <sub>3</sub> daily <i>per os</i>
	17	7.7	44	26	82	139.1	6.2	106.8	26.0	70.0	6 gm. NaCl + 2 gm. NaHCO <sub>3</sub> daily <i>per os</i>
	31	7.8	50	30	65	139.4	8.9	102.6	25.1	66.5	6 gm. NaCl + 2 gm. NaHCO <sub>3</sub> daily <i>per os</i> Animal in excellent condition. Sustained from this point with NaHCO <sub>3</sub> alone
June 8	84	7.9	39	22	60	140.3	6.3	99.9	37.0	67.5	No NaCl, 6 gm. NaHCO <sub>3</sub> daily <i>per os</i> 100 cc. 5% NaHCO <sub>3</sub> injected intravenously on this and on subsequent removal of blood samples for electrolyte study 6 gm. NaHCO <sub>3</sub> daily <i>per os</i>



18	94	7.5	66	45	67	139.6	9.8	108.2	24.3	60.8	6 gm. NaHCO <sub>3</sub> daily per os Some vomiting and diarrhea. Animal otherwise well NaHCO <sub>3</sub> administration stopped Typical insufficiency, spasticity, low temperature
22	98	8.0	50	30	78					64.0	
25	101	7.7	100	69	45	134.9	13.7	104.0	25.6	60.0	
28	104	7.3	164	130	67	127.2	22.3	94.2	15.7	53.6	

Animal sustained 104 days (Mar. 16 to June 28, 1934) on sodium chloride and sodium bicarbonate only. From June 1 (77th day) the animal was sustained on sodium bicarbonate only, but the sodium chloride given in the food was evidently sufficient to maintain a proper plasma chloride concentration, except on June 8. From June 22 to 25 there was some vomiting and diarrhea. On June 25 (101st day) sodium bicarbonate administration stopped, anorexia appeared, and animal went into typical insufficiency during the following 72 hours. It was promptly restored to normal by the usual measures.

TABLE II  
*The Effect of Insufficient Salt Administration on the Suprarenalctomized Dog Maintained without Extract. Its Partial Repair with Intravenous Salt Infusions*

Dog 1-32.

Date	Food intake per day	Weight	Non-protein nitrogen	Urea	Sugar	Plasma sodium per liter	Plasma potassium per liter	Plasma chlorides per liter	Plasma bicarbonate per liter	Plasma volume	Plasma proteins	Salt per os		Remarks	
												NaCl	NaHCO <sub>3</sub>		
	gm.	kg.	mg. per 100 cc.	mg. per 100 cc.	mg. per 100 cc.	m.-eq.	m.-eq.	m.-eq.	m.-eq.	per cent.	mg. per 100 cc.				
1934															
May 4		8.6	47	32	65	145.1	7.0	119.6	17.8	75	6.2	5	2		
5	540	8.7										5	2		
6	600	8.7										5	2		
7	600	8.6										5	2		
8	210	8.7										5	2		
9	430	8.5										5	2		
10	450	8.5										5	2		
11	500	8.6										5	2		
12	520	8.7										5	2		
13	50	8.4										5	2		
14	240	8.1	108	81	71	131.2	15.8	91.4	15.6	57	7.8	5	2	Vomited Anorexia 24 hrs. 250 cc. normal saline intravenous	
15	320	8.3										5	2		
16	280	8.3	70	43	60	139.1	12.3	108.4	20.4	64	7.0	5	2	130 cc. normal saline intravenous	
17	40	8.2										5	2		
18	330	8.2	70	50	80	137.2	12.0	108.3	21.2	66	6.3	5	2	Appetite poor. 175 cc. normal saline intravenous	
19	310	8.1										5	2		

20	290	7.9																	On May 19 appetite poor, and no food eaten May 20. Staggers. Pulse 40. Vomiting. Restored with extract and saline
21	20	8.0	112	75	80	134.2	17.3	106.3	17.8	60	7.0	5	2	5	2				

The animal developed insufficiency on May 14 due to the administration of insufficient amounts of salt, the plasma sodium being sustained better than the chloride due to the larger ingestion of sodium. Infusion of 0.9 per cent sodium chloride lowered the potassium and urea concentration, reduced the hemoconcentration, and temporarily restored appetite. Eventually severe insufficiency occurred, however, because the salt ingestion by mouth was not adequate. In a subsequent experiment this animal was sustained with an adequate salt intake for 5 months without cortical extract (Chart 2).

Vomiting and diarrhea were avoided completely, and animals which did not retain the gavigated material readily were rejected. Failure in our preliminary studies was due primarily to the use of insufficient amounts of salt (Table II, Chart 3). The quantity required varies with the individual dog, and a proper dosage is necessary from the outset. This was regulated by the results of plasma electrolyte studies. The electrolyte pattern of the arterial blood plasma was determined at 7 to 10 day intervals, together with blood counts and hemoglobin, hematocrit, urea, and plasma protein estimations.<sup>5</sup> Care was always taken to

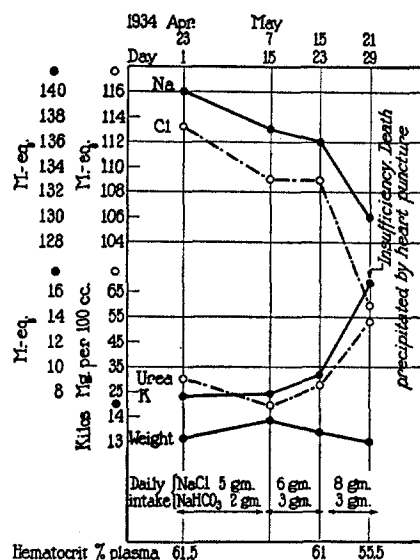


CHART 3. Dog 1-34. Suprarenalectomy Feb. 1, 1934. Typical changes in the electrolyte pattern, and the production of hemoconcentration resulting from an insufficient dosage of sodium salts in a suprarenalectomized dog receiving no extract.

replace the blood withdrawn by the intravenous injection of two or three times the quantity of 1 per cent salt solution. Occasionally further intravenous injec-

<sup>5</sup> The methods employed for the various chemical analyses have been previously described (4), with the following exception.

*Plasma Potassium Method.*—The method of S. E. Kerr (*J. Biol. Chem.*, 1926, 67, 689) was used for the deproteinization of serum and the estimation was made by the method of Kramer and Tisdall as described by J. P. Peters and D. D. Van Slyke (*Quantitative clinical chemistry. Volume II, Methods*, Baltimore, The Williams & Wilkins Co., 1932, 748).

tions of saline were given for a day or more when required, as indicated by the blood studies. Careful attention was paid to proper exercise, to proper food, to the avoidance of infections or parasites, and to cleanliness and proper shelter. The methods for the maintenance of our colony of suprarenalectomized dogs have been previously described (13).

TABLE III

*Hypoglycemia without Insufficiency Produced by Fasting in a Suprarenalectomized Dog Receiving 4 Cc. Cortical Extract Daily and 0.5 Gm. Sodium Chloride*

Dog 1-22. Suprarenalectomy Feb. 2, 1934. Repeatedly in insufficiency.

Date	Weight	Non-protein nitrogen		Urea	Plasma sodium per liter	Plasma potassium per liter	Plasma chlorides per liter	Plasma bicarbonate per liter	Plasma volume	Plasma proteins	Sugar	Remarks
		mg. per 100 cc.	mg. per 100 cc.									
1934	kg.	mg. per 100 cc.	mg. per 100 cc.	m.-eq.	m.-eq.	m.-eq.	m.-eq.	per cent	mg. per 100 cc.	mg. per 100 cc.		
Aug. 23	7.7	32	18		137.9	5.9	112.1	24.5	71.0	5.4	65	No food after Aug. 22. Given 4 cc. cortical extract daily. 0.5 gm. NaCl per os
25	7.4	24	13								55	
27	7.1	22	13								45	
29	6.7	24	10								65	
31	6.5	22	10								62	
Sept. 2	6.3	28	14								57	
4	6.1	24	14								50	Very weak. Experiment terminated. No convulsions. Alert on injection of intravenous glucose
5	6.0	24	14		143.0	5.7	113.0	22.7	70.9	5.6	35	

The weight loss is greater in the animal receiving minimal amounts of salt, than in the animal sustained with salt alone (Table IV). There is no change in the percentage of plasma or in the plasma protein concentration. No evidence of dehydration or alteration of plasma electrolyte pattern when the experiment was terminated.

Mention has been made by Swingle (10) of the hypoglycemia observed in his experiments upon salt-fed dogs. This is often noted when the salt administered is inadequate in amount, and particularly when anorexia develops. Anorexia in animals treated with adequate amounts of cortical hormone may result in fatal hypoglycemia. The effect is clearly shown in fasted suprarenalectomized dogs sustained

either with cortical hormone together with minimal amounts of salt, or by means of adequate salt administration alone (Tables III, IV). In either case severe hypo-

TABLE IV

*Hypoglycemia without Insufficiency Produced by Fasting in a Suprarenalectomized Dog. Sustained with Salt Mixture but Given No Cortical Extract*

Dog 1-41. Suprarenalectomy May 4, 1934. Insufficiency June 1, 1934, and July 2, 1934.

Date	Weight kg.	Non-protein nitro-	Urea	Plasma sodium per	Plasma potassium	Plasma chlorides	Plasma bicarbon-	Plasma volume	Plasma proteins	Sugar	Remarks
		gen		liter	per liter	per liter	ate per liter				
		mg. per 100 cc.	mg. per 100 cc.	m.-eq.	m.- eq.	m.-eq.	m.- eq.	per cent	mg. per 100 cc.	mg. per 100 cc.	
1934 July 30	8.8	46	25	143.8	8.4	112.2	18.8	70.5	6.5	62	No food after July 29. Given 6 gm. NaCl + 2 gm. NaHCO <sub>3</sub> daily per os
Aug. 1	8.5	32	15							75	
3	8.6	25	11							68	
5	8.4	30	14							60	
7	8.0	30	17							68	
9 a.m.	7.8	30	17							38	Dog very dull. Pulse slow 35 to 40, ir- regular
9 p.m.		28	15	143.6	5.4	114.0		78.3	4.8	20	Experiment termi- nated. No convul- sions. Lies in cage. Does not respond. Immediately after injection of 100 cc. 5% glucose animal became alert and active

As a result of fasting and salt feeding the percentage of plasma increased markedly and the plasma protein concentration dropped sharply. The plasma electrolyte pattern was not essentially altered.

glycemia occurs in 7 to 12 days, but without extracellular fluid loss. The plasma electrolyte pattern and the blood concentration are not altered. While either cortical hormone and salt, or salt alone, therefore, in proper amounts

will sustain the fasted animal and prevent hemoconcentration, neither will prevent the occurrence of severe hypoglycemia. The suprarenalectomized animal cannot form glycogen to maintain its blood sugar level from its own endogenous metabolism. When the glycogen supply is exhausted by fasting, hypoglycemia results. This, however, has not yet been established. It is a fact that the development of hypoglycemia is slower in the fasted, hormone-treated animal, but the differences, compared to the animal sustained with salt alone, are not very striking. This cortical hormone which influences mineral salt and water metabolism does not enable the dog to form carbohydrate from its own tissues. Hypoglycemia may be effectively prevented in animals sustained by salt alone by the daily use from the outset of certain lipoids, particularly cottonseed oil, or by the use of glucose, administered by stomach tube. Aside from the lack of food, an important cause of hypoglycemia in suprarenalectomized dogs is unusual physical exertion such as is brought about by fighting. Such an accident has caused sudden death in several of our animals, sustained without suprarenals over many months. As others have found, and as we have previously indicated, hypoglycemia is neither constant nor characteristic of suprarenal insufficiency in the dog, but is clearly related to the duration and extent of anorexia. The injection of glucose alone, or of glucose together with adrenalin, even though it raises the blood sugar level, does not relieve suprarenal insufficiency, nor influence the characteristic changes in the blood plasma in the male adult dog.

#### RESULTS

The maintenance of an approximately normal plasma concentration of sodium and chloride by the administration of adequate amounts of sodium and chloride ions is necessary for sustaining suprarenalectomized dogs without extract. This in turn sustains proper tissue hydration, that is to say, a sufficient supply of extracellular water, so that cell metabolism is unimpaired, the transfer of materials across cell membranes proceeds normally, and the intrinsic stores of intracellular water and dissolved substances are not interfered with. When this is effected there is no rise in plasma potassium nor urea concentration, no hemoconcentration, and, under proper conditions, no anorexia. When such proper tissue hydration is interfered with for a period, producing alteration in cellular permeability and a rise in plasma potassium and urea concentrations, adequate injections of sodium chloride may restore the normal plasma urea and potassium levels by facilitating urinary excretion of the excess. When the loss of sodium and the hemoconcentration is allowed to become extreme it is difficult and eventually impossible, without the use of the cortical hormone, to restore the animal to its normal state. When cell damage

TABLE V  
*High Plasma Sodium and Chloride Values in the Stage of Insufficiency Due to Concentration of the Plasma with Marked Loss of Weight*

Date	Weight kg.	Non- protein nitrogen mg. per 100 cc.	Urea mg. per 100 cc.	Plasma sodium per liter		Plasma potas- sium per liter		Plasma chloride per liter		Plasma bicar- bonate per liter		Plasma volume	Remarks
				m.-eq.	m.-eq.	m.-eq.	m.-eq.	m.-eq.	m.-eq.				
1934 Feb. 19	8.2	48	36	137.6	8.5	111.8	18	64	Injections of extract stopped Feb. 18. 4 gm. NaCl + 2 gm. NaHCO <sub>3</sub> daily per os				
26	8.1	47	36	133.0	8.7	106.8	20	62	4 gm. NaCl + 2 gm. NaHCO <sub>3</sub> daily per os				
Mar. 6	8.1	75	56	133.4	18.5	102.7	21	60.5	5 gm. NaCl + 2 gm. NaHCO <sub>3</sub> daily per os				
13	8.0	76	53	134.0	18.9	104.6	18.6	58.5	5 gm. NaCl + 2 gm. NaHCO <sub>3</sub> daily per os. Marked anorexia. Loss of weight				
17	7.3	76	50	150.1	18.3	129.2	17.0	60	Classical symptoms with stagger in gait. Pulse 40, heart block. T. 95°F.				
26	8.0	30	25	Restored with extract and intravenous saline and glucose and salt by mouth.		5.7	70	Injections of extract stopped Mar. 25. 4 gm. NaCl + 2 gm. NaHCO <sub>3</sub>					
Apr. 4	8.0	48	25	141	9.0	112.4	18.5	61.5	+ 20 mg. daily vitamin C intravenously				
12	7.1	81	60	151.3	15.2	128.3	14.5	62	T. 98°F. Stagger. Anorexia. Bradycardia				
13	6.95	40	26	142.1	5.8	114.2	22.8	77	Restored with extract, glucose and saline solution				
14	7.6	40	26	142.1	5.8	114.2	22.8	77					

Two cycles of insufficiency, the first of 27 days, the second of 16 days. In both cases marked hemoconcentration and loss of weight (water) associated with rise in concentration of sodium and chloride and rise in urea and potassium. Revival with extract, salt solution, and glucose produced lowering of sodium and chloride to normal levels. Treated with extract and restored to prime condition.



has proceeded to an advanced stage a fatal outcome cannot be prevented by any treatment.

In a considerable proportion of animals which are fed salt in amounts insufficient to sustain proper tissue hydration, an actual terminal rise in the concentration of plasma sodium and of chloride occurs (Table V). Such animals have generally been maintained for several days on salt alone, the intake of fluids has been reduced, and urinary excretion is diminished. Hemoconcentration has taken place. The mechanism here involved may be analogous to that pointed out by Hartman (12), who observed that when urine excretion is too scanty, as in anhydremia due to diarrhea, the total electrolyte concentration in the plasma occasionally reaches enormous values. It may also be due to the administration of sodium chloride at too rapid a rate for the kidney of the suprarenalectomized animal to discharge with the water available for its excretion. In suprarenal insufficiency, coincidental with such increases in plasma concentration of sodium and of chloride, the urea and potassium values are also greatly increased. In each instance this rise in sodium and chloride concentrations was associated with a marked loss of body weight.

The question now arises as to the relative importance of the loss of sodium and of chloride in producing the dehydration and the associated symptoms, after withdrawal of the hormone injections. Experiments have been carried out in which the plasma sodium concentration was sustained by the use of sodium salts other than the chloride. For this purpose sodium bicarbonate in the quantity necessary proved unsatisfactory because it produced distention, vomiting, and diarrhea. Sodium gluconate and sodium lactate by mouth caused diarrhea. It was finally possible to use daily injections of suitable quantities of intravenous molar sodium N-lactate solution, prepared as directed by Hartman (14). Animals so sustained, without cortical hormone, maintained normal hydration, as indicated by hematocrit observations and protein studies (Table VI). A rapid fall in the plasma chlorides occurred, but the level was sustained when it reached about 90 m.-eq. per liter. No further fall occurred so long as the plasma sodium level was properly maintained. In these experiments an unsalted lean meat diet was used. Eventually anorexia appeared. The anorexia was associated with a diminution in the secretion of

TABLE VI  
*Experiment with Intravenous  $m/1$  Sodium Lactate. Sustained 18 Days until Anorexia Caused Marked Loss of Weight. Diet of Unsalted Raw Beef*

Date	Weight kg.	Non- protein nitrogen <i>mg. per 100 cc.</i>	Urea <i>mg. per 100 cc.</i>	Plasma sodium per liter <i>m.-eq.</i>	Plasma potas- sium per liter <i>m.-eq.</i>	Plasma chlorides per liter <i>m.-eq.</i>	Plasma bicar- bonate per liter <i>m.-eq.</i>	Plasma proteins <i>gm. per 100 cc.</i>	Plasma volume <i>per 100 cc.</i>	Remarks
1934										
Nov. 15	9.7	60	40	136.6	7.3	105.8	25.4	6.4	66.1	Extract and sodium chloride stopped Nov. 14
19	9.4	112	100	135.0	9.0	99.8	26.2	7.1	62.0	50 cc. $m/1$ sodium lactate daily by vein + 3 gm. $NaHCO_3$ per os
23	9.5	120	90	141.1	7.8	94.2	35.0	6.4	65.9	100 cc. $m/1$ sodium lactate daily by vein + 3 gm. $NaHCO_3$ per os
27	9.5	108	82	137.8	8.4	98.0	28.0	6.5	68.0	150 cc. $m/1$ sodium lactate daily by vein + 3 gm. $NaHCO_3$ per os. Anorexia
Dec. 3	9.0	44	30	138.1	4.9	93.0	34.5	6.0	63.6	No free hydrochloric acid in gastric juice. Anorexia. Given 4 gm. NaCl per os. Blood sugar 75 mg. per 100 cc.
4	9.4	49	34	139.2	7.8	108.5	30.5	6.5	72	Free acid present in gastric juice. Ate 350 gm. beef 12 gm. NaCl + 2 gm. $NaHCO_3$ per os. Diarrhea, Dec. 9-10
11	9.1	88	64	130.9	12.6	103.0	25.3	7.2	64.2	

On Dec. 1 anorexia developed, but administration of increasing quantities of sodium lactate served to sustain the plasma sodium level. A lowered urea and potassium concentration resulted. The sodium concentration is sustained during the sodium lactate injections. On the evening of Dec. 3, no free acid was present in gastric contents. The dog was given 4 gm. sodium chloride by mouth, and free acid was present on Dec. 4, 14 hours later, coincidental with the rise to normal in plasma chloride concentration. The diarrhea on Dec. 9-10 was due to the cathartic action of the prolonged large doses of sodium chloride and sodium bicarbonate by mouth, and produced a condition of suprarenal insufficiency. The animal was restored with cortical extract and sodium chloride.

gastric juice and a disappearance of free hydrochloric acid. These studies afford further evidence that the sodium ion and not the chloride ion primarily governs tissue hydration in the suprarenalectomized dog, but that the concentration of plasma chloride has an intimate bearing upon the secretion of free hydrochloric acid in the stomach of the dog.

#### DISCUSSION

The experimental data which are presented indicate that an approximately normal concentration of both plasma sodium and chloride are required for the maintenance of the bilaterally suprarenalectomized dog. If the chloride falls anorexia appears and hypoglycemia eventually results. Fall in plasma sodium is accompanied by dehydration and hemoconcentration. Under the influence of the intact suprarenal, the sodium and chloride concentrations in the plasma (and of the extracellular fluid), are regulated with a high degree of constancy. It is not to be expected that in the absence of this regulatory mechanism it should be possible to supply additional sodium and chloride ions in amounts just sufficient to repair the body deficit resulting from their uncontrolled excretion, and thus to permit the salt and water metabolism to be maintained with the efficiency which it possesses in the normal presence of the natural hormone. Nevertheless the duration of the experiments which have been carried out should be sufficient to evoke other metabolic or nutritional disabilities resulting from the withdrawal of the cortical hormone and none has been demonstrated during experiments lasting from 3 to 5 months. Also it has been found in the treatment of Addison's disease by means of salt, that it is not possible in certain cases to maintain the normal plasma electrolyte levels by means of sodium salts alone, but that such a result is possible when injections of the hormone are given simultaneously (15). Similarly, it is only by the exhibition of both extract and salt in adequate amounts that entirely normal plasma electrolyte levels may be sustained in the totally suprarenalectomized dog.

Not only is the presence of the suprarenal cortex necessary for the maintenance of the constant normal concentration of plasma sodium in the dog when the intake is barely adequate or deficient, but the administration of excessive amounts of sodium, in the absence of sufficient

water available for its excretion, raises the plasma concentration of this ion appreciably above the normal level in the suprarenalectomized animal. The capacity of the normal kidney, depending upon the needs of the organism, to excrete sodium over a rather wide range is restricted with respect to the water simultaneously available for excretion. The level of this electrolyte in the plasma, and of the dissolved substances dependent upon it, in the animal sustained by salt alone, are, therefore, to a significant extent conditioned by the intake. Since the water available as a vehicle for the urinary excretion of dissolved substances, in the absence of the suprarenal cortical hormone, is particularly claimed by sodium and chloride, the excretion of other materials probably is impeded.

The fall in the concentrations of plasma sodium and chloride are usually accompanied, in these salt-treated animals, by a coincidental rise in the concentrations of plasma potassium and of urea, and conversely. These changes are usually parallel to each other (Charts 1, 2) and appear, as might be expected, to be at least approximately related to the extent of hemoconcentration. Infusion of sodium chloride solution, raising the plasma levels of sodium and of chloride, tends at the same time to restore the proper plasma concentrations of urea and potassium, by facilitating the urinary excretion of the excess. The effect of these reciprocal alterations in the plasma (extracellular fluid) concentrations must be to counteract or dampen the serious alterations in the osmotic pressure of the extracellular fluids attendant upon the disordered excretion of sodium and chloride. Such a buffering effect must make for stability of the fluid stores within extracellular and intracellular compartments.

We have elsewhere pointed out the probable relationship of the greatly increased potassium content of the plasma to the occurrence of cardiac arrhythmias during suprarenal insufficiency in the dog (16).

The possibility that various toxic effects due to potassium poisoning make their appearance during suprarenal insufficiency has been suggested by Hastings and Compere (17).

#### SUMMARY

1. A group of experiments is reported in which bilaterally suprarenalectomized adult male dogs have been maintained in apparently

normal condition over prolonged periods, up to 5 months, without the use of any suprarenal gland preparation or extract and by the administration of sodium chloride and sodium bicarbonate alone. Withdrawal of the salts then produced typical suprarenal insufficiency.

2. The relation of the absence of free hydrochloric acid in the gastric juice of suprarenalectomized animals, in addition to, or independent of the factor of dehydration, for the production of anorexia and hypoglycemia, is discussed.

3. Further evidence is presented in these experiments in support of the view that the suprarenal cortical hormone in the adult male dog is concerned with the regulation of sodium excretion by the kidney, and thus eventually with the proper maintenance of water balance in the organism. It has no direct influence on carbohydrate metabolism.

4. The reciprocal changes in the plasma concentrations of urea and of potassium which take place as the concentrations of plasma sodium and chlorides vary, are pointed out as furnishing a mechanism whereby abrupt alterations in osmotic pressure are dampened, and the volumes of fluids in extracellular and intracellular compartments more efficiently stabilized.

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