SALT AND WATER LOSSES IN DIURETIN DIURESIS AND THEIR RELATION TO SERUM NON-PROTEIN NITROGEN AND ELECTROLYTE CONCENTRATIONS

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Gruenwald (1) in 1909 in experiments on rabbits showed that the repeated administration of diuretin caused an excessive chloride loss in the urine and that after the fourth or fifth administration of diuretin the animal died in a paralytic comatose state with strikingly low blood chloride values. Since simultaneous saline administration with each dose of diuretin saved the lives of the rabbits, he felt he had in this way excluded a possible toxic effect of the drug. In repeating this experiment Bilbao and Grabar (2) in Blum's clinic observed that a high blood urea concentration accompanied the low blood chloride and that rabbits supplied with salt solution did not develop azotemia. They concluded that the appearance of azotemia was connected with the fall in blood chloride. Blum (3) in considering the etiology of azotemias in patients without anatomical kidney lesions, assumed that the elevated blood urea was a compensatory mechanism preventing an osmotic deficit in the blood in the presence of low blood chloride. Hartmann and Darrow (4) also argued the existence of such an adjustment. This idea of an etiological relation between low blood chloride and high blood urea concentrations has profoundly influenced the continental literature of the past 5 years. Many clinical and experimental non-nephritic uremias following diarrhea, vomiting, or salt losses through other channels have been reported as "azotemias because of the lack of salts," Blum (3), Ambard (5), Rathery (6), "uremias of chloride want" Strauss (7), Glass (8), and "hypochloremic comas" Porges (9). The fact that administration of saline solution to such patients reduces the azotemia and restores the urea concentrating

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capacity of the kidneys, which Chabanier (10), Glass (8), Meyer (11), and others had observed to decrease with the low serum chloride, is used to support the belief in the etiological relationship between chloride level and nitrogen retention.

Against this view may be cited (see Table III) various conditions produced experimentally or by disease in which an apparently non-nephritic azotemia exists in the presence of a normal or even a much elevated plasma chloride and, conversely, in which large reductions of plasma chloride unaccompanied by nitrogen retention are found. The Blum theory rests almost entirely on measurements of chloride and non-protein nitrogen levels in the plasma. Further and more comprehensive study of water and electrolyte metabolism in relation to impaired nitrogen excretion is therefore desirable. We have accordingly undertaken to repeat Gruenwald's (1) experiment with the purpose of simultaneously following the metabolism of sodium, potassium, chloride, nitrogen, and water and the changes in the serum concentrations of these substances.

Methods

Rabbits weighing between 1730 and 2230 gm. were used. In the earlier experiments the animals were fed according to Gruenwald's (1) original procedure with maize, ground and washed thoroughly with distilled water to reduce the salt content. Since little of this ration was taken, the animals in later experiments were fasted. Every day or, in some instances, every other day, 0.5 gm. of diuretin per kilo of body weight, dissolved in distilled water, was administered with a stomach tube. The animals had free access to water, the amount taken being accurately measured. All blood samples were drawn from the ear veins 24 hours after the preceding administration of water, diuretin, or salt. Care was taken to avoid excitement or movement of the animals as exercise of only short duration has been shown to depress the bicarbonate and cause considerable rise in the fixed base content of the blood serum (Csapó and Kerpel-Fronius (12)).

The urine was collected in 24 hour specimens under toluene, the bladder being emptied by careful manual pressure at the end of each collection period. The analytical methods were as follows: sodium in blood serum and urine according to Butler and Tuthill (13); potassium according to Fiske (14); chloride by Fiske and Lin (15); bicarbonate, manometrically (16); N.P.N. colorimetrically with Nesslerization; and total protein and nitrogen by the Kjeldahl method.

EXPERIMENTAL RESULTS

Fig. 1 presents for each of five fasting animals over 9 day periods the total, not daily, amount of sodium, potassium, and chloride excreted in the urine above the

amount of each administered and also the weight loss, all values being divided by the body weight to reduce them to a standard 1 kilo animal. The values of the scale on the left of the figure represent milli-equivalents, the values of the scale on the right represent weight loss in grams. The losses of each substance in the different experiments are grouped together in the figure. The first two columns of each group are from Experiments 1 and 2 on fasting controls. Column 3 in each group represents the loss in Experiment 3 from a fasting animal receiving four doses of diuretin. Column 4 represents the loss in Experiment 4 from a fasting animal receiving four doses of diuretin plus forced water amounting to 150 to 200 gm. daily in one single dose. Column 5 in each group represents the loss in Experiment 5 from a fasting animal receiving four doses of diuretin plus 70 cc. of saline intraperitoneally on the days of diuretin administration. The weight loss attributable to the nitrogen excretion in the urine was estimated by assuming that each gram of nitrogen represents 29.5 gm. of tissue. The weight loss due to this destruction of protoplasm is represented in Fig. 1 by the darkened portion of each weight column. The extent of the columns above the darkened portions measures the sum of the other factors of the total weight loss. These are: (1) loss of glycogen; (2) loss of body fat; (3) the portion, probably small, of destroyed protoplasm represented by nitrogen excreted in the stools, which was not measured; and (4) water withdrawn from the body above that accompanying the destruction of protoplasm, *i.e.* the fraction of the total weight loss which can be credited to dehydration.[1] The first three factors may be assumed to have approximately the same total value in all of the experiments, since all of the animals were fasted and since no difference in the quantity or consistency of the stools was observed. The extent to which the undarkened portions of the columns in Experiments 3, 4, and 5 are above the average value found in the two control experiments will, therefore, provide a rough measurement of the dehydration produced in these experiments beyond that due to fasting.

From Fig. 1 it can be seen that there is an excessive sodium and chloride loss in Experiments 3 and 4. The administration of saline in Experiment 5 markedly reduced the loss of these two ions, the actual sodium loss being no greater than in the control animals. The potassium loss was increased above the control level in all three diuretin experiments. The administration of saline did not protect the rabbit of Experiment 5 from a potassium loss double that of the controls. The weight loss attributable to tissue destruction is only slightly higher in the diuretin experiments than in the controls. The weight

[1] The term dehydration as used in this paper means, then, loss of water above that attributable to protoplasm destruction as measured by nitrogen excretion. Thus it includes loss of intracellular fluid from tissue without cell destruction, loss of extracellular fluid, and insensible fluid loss.

loss attributable to dehydration is markedly greater in the diuretin experiments, being the greatest in Experiment 3 where the sum of

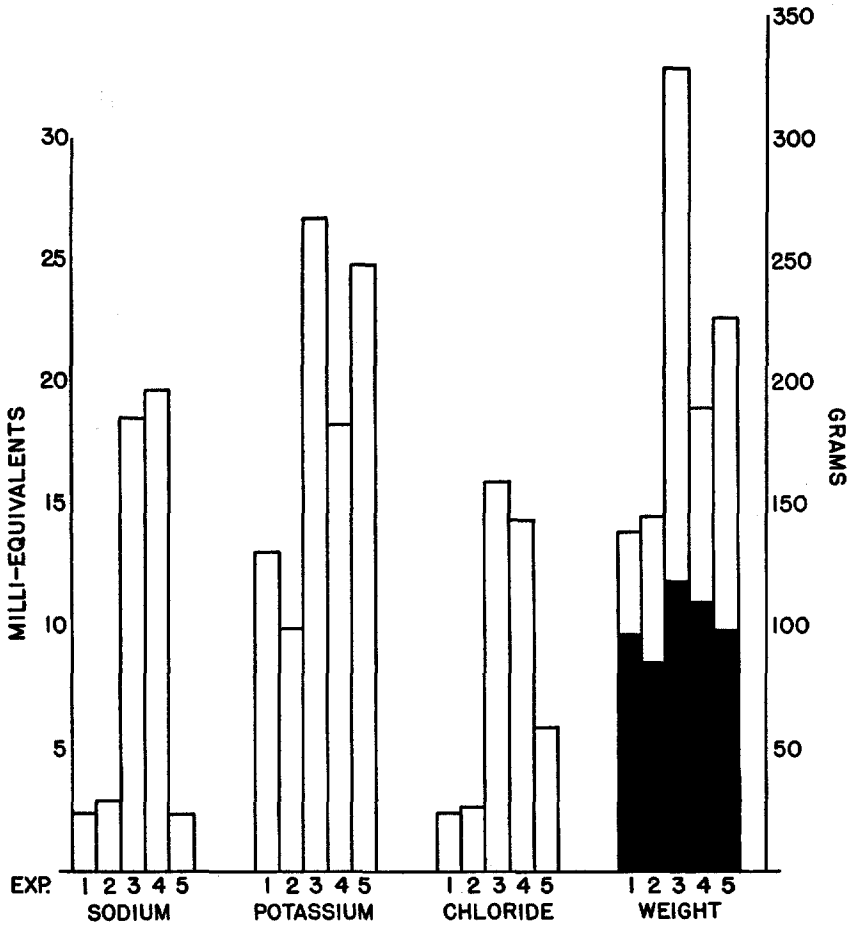

FIG. 1. Urinary excretions of sodium, potassium, and chloride above amount of each ingested and weight losses of fasting animals over a 9 day period. Darkened portion of each weight column represents weight losses attributable to protoplasm destruction as calculated from urinary nitrogen excretion. Experiments 1 and 2, fasting controls. Experiment 3, fasting rabbit receiving diuretin. Experiment 4, fasting rabbit receiving diuretin plus forced water. Experiment 5, fasting rabbit receiving diuretin plus saline.

sodium plus potassium loss is largest. The markedly lower dehydration weight loss in Experiment 4 as compared to Experiment 3 is

associated with both a slightly lower potassium loss and a retention of water relative to substances producing a dilution of body fluid concentrations, as will be shown later by the data presented in Fig. 3. Taking the concentration of sodium in the extracellular fluids as 140 milli-equivalents per litre, the much larger body fluid loss in Experiment 3 as compared with Experiment 5 can be approximately accounted for by the loss of 16 milli-equivalents more of sodium in Experiment 3. Evidently the fluid loss in Experiment 5 is related chiefly to the loss of potassium which the administration of sodium chloride solution does not prevent.

Confirmation of this finding may be seen in Fig. 2 which presents the results of two diuretin experiments, one with and the other without the administration of saline, together with the two control experiments.[2] The experiments were on fasting animals over a 5 day period. In Experiments 6 and 7, diuretin was given on 4 days. In Experiment 7 each dose of diuretin was supplemented by 70 cc. of saline. The results are in agreement with those obtained in Experiments 3 and 5, Fig. 1. The fact that the data in Fig. 2 give slightly higher losses per day than those presented in Fig. 1 may be ascribed to the larger per day dose of diuretin and to the well established fact that losses per day in fasting decrease with time.

The behavior of several serum values in the presence of the losses of water and salt produced by diuretin was studied in two experiments, Nos. 8 and 4, and the results are presented in Fig. 3.

In the lower section of the figure the data describing the daily intake of water and the excretion of water, sodium, and chloride in the urine are recorded by means of pairs of columns, the total height of the left hand one measuring the water intake and that of the right hand one the volume of the urine. The relationship of the two values is thus easily seen. The measurements of excretion of sodium and of chloride are also laid off on these columns, sodium left and chloride right. In the upper part of the figure the measurements of daily nitrogen excretion in the urine and those of body weight are recorded, together with the several serum concentration values determined; *viz.*, protein, non-protein nitrogen, sodium, and chloride. The procedure in Experiment 8 consisted in giving a maize-fed animal five doses of diuretin dissolved in 100 cc. of distilled water in the course of a 9 day period of study at the intervals indicated at the bottom of the figure. In Experiment 4 the animal was fasted and received in the course of a 10 day period five doses of diuretin dissolved in 100 cc. of distilled water and in addition was given

[2] The controls are the first 5 days of the control Experiments 1 and 2, Fig. 1.

daily by stomach tube 150 to 200 cc. of distilled water in a single administration. The animals in both experiments had free access to water and the amounts taken were recorded.

From the results of Experiment 8 one observes a steady decline in weight and an approximately uniform relation between water intake and urine volume up to the last 3 hours of the experiment. In Ex-

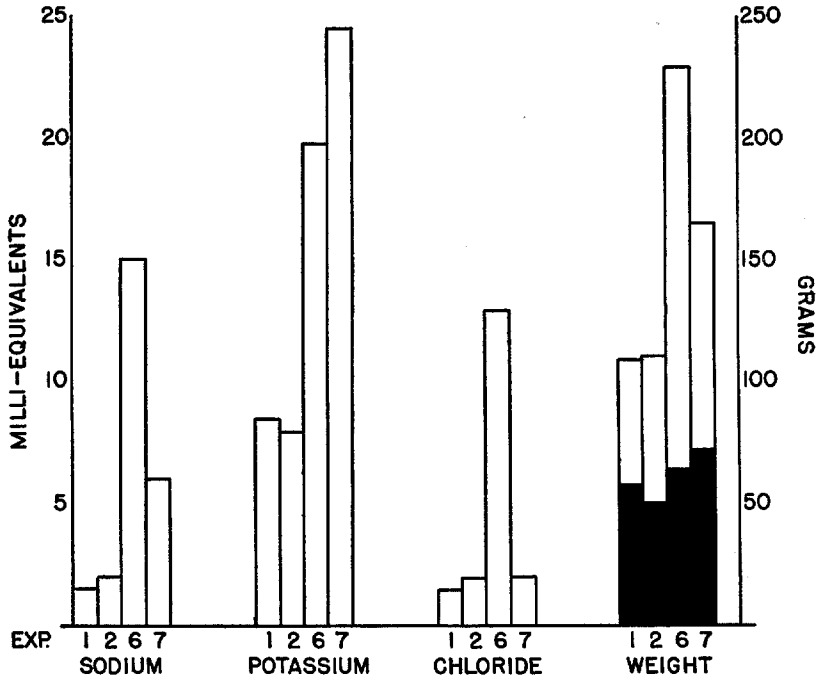

FIG. 2. Urinary excretions and weight losses, as described for Fig. 1, over a 5 day period. Experiments 1 and 2, fasting controls. Experiment 6, fasting rabbit receiving diuretin. Experiment 7, fasting rabbit receiving diuretin plus saline.

periment 4 there is a marked irregularity in the weight curve which is coincident with the marked irregularity in water intake-urine volume relationship. It is evident from the data that this results from the large difference between water intake and urine volume on the diuretin-free days and the large water diureses on the diuretin days. The data of the last day represent the 3 hours preceding death.

The serum concentrations found in Experiment 4, representing

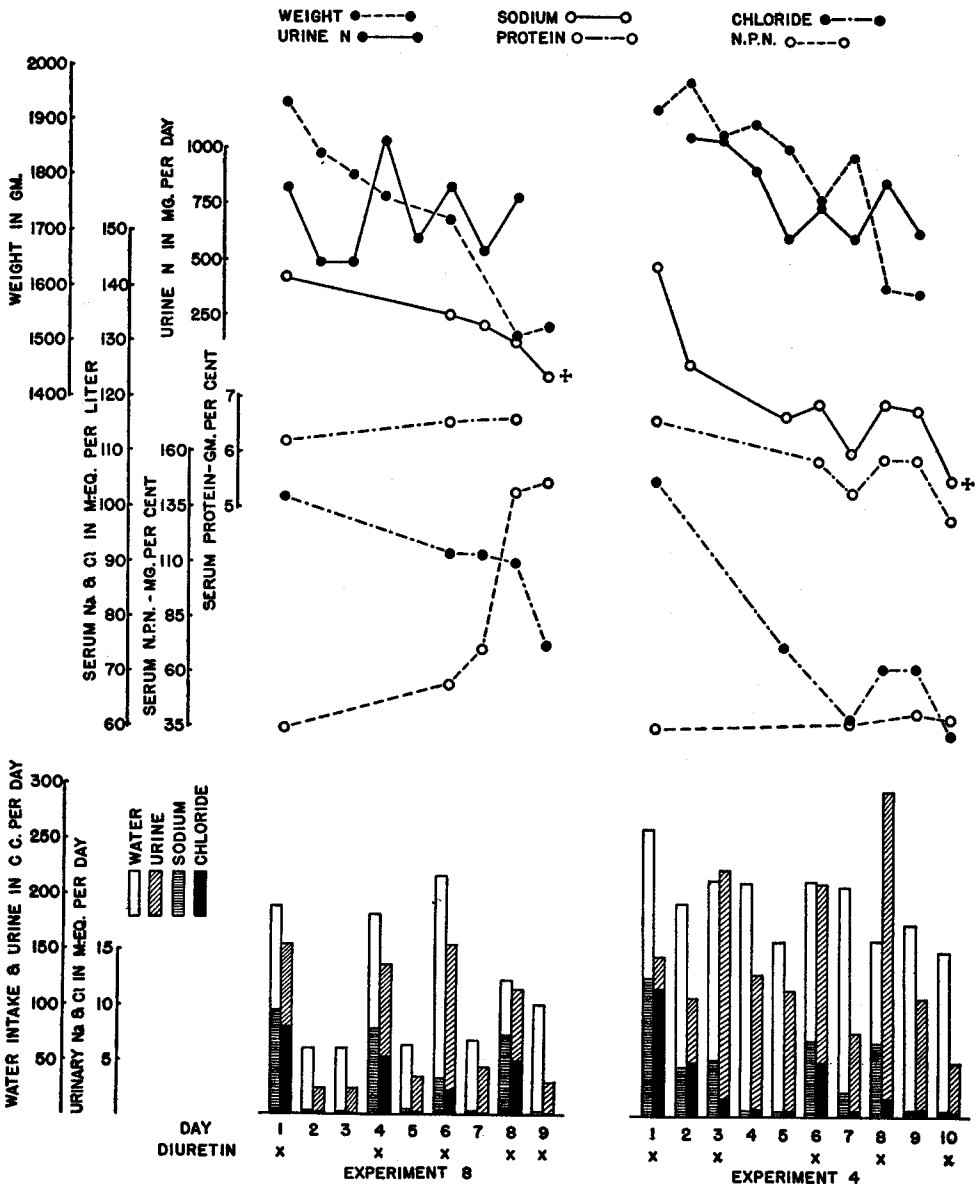

FIG. 3. Experiment 8, maize-fed rabbit receiving diuretin and water as desired. Experiment 4, fasting rabbit receiving diuretin, water as desired, and 150 to 200 cc. of additional water in single daily doses.

analyses of serum drawn 24 hours after the last administration of water, reflect the disturbance in water balance. For the first 5 days there was a large fall in serum concentrations as compared to those recorded from Experiment 8. The changes in serum sodium concentrations over the remainder of the experiment show an inverse relation between water retention and sodium concentration in spite of a large loss of sodium in the urine on the diuretin days. In so far as serum protein and chloride determinations are available, they confirm this effect of excessive water retention on serum concentrations. The fall in serum sodium and chloride concentrations on the last day of Experiment 8 reflects a similar influence of retained water. Until this last day of the experiment the loss of sodium and chloride incident to the diuretin is accompanied by water to such extent as results in but slight changes in serum concentration values as compared with those found in Experiment 4.

The failure of the total nitrogen excretion in Experiment 8 to increase in the presence of marked increase in serum N. P. N., we feel, reflects a diminution in the efficiency of kidney function as measured by nitrogen clearance. This deduction seems permissible on the basis of total nitrogen excretion, instead of the preferable non-protein nitrogen excretion, because it may be assumed that the protein nitrogen in the urine would tend to increase during the course of the experiment. Hence an error introduced by using total nitrogen excretion will increase and not decrease nitrogen clearance. Throughout the course of Experiment 4 the N. P. N. remained approximately constant and there is no significant divergence between nitrogen retention and excretion.

Thus the data from Experiments 8 and 4 contradict the existence of a direct relationship between hypochloremia and azotemia.

Table I presents data from other experiments which confirm the findings presented in Fig. 3. The data from Experiment 11 (Table I) particularly confirm the findings presented from Experiment 4 and discussed above. In this experiment, in which the animal was maize-fed, dehydration was minimal, as reflected by the small weight loss and excessive fall in serum concentrations, and no rise in N. P. N. occurred. The data further show that the degree of azotemia in the presence of similar dehydration is inversely proportional to the urine

TABLE I

Daily Urine Excretion, Serum Concentrations, and Weight in Diuretin-Fed Rabbits

Experiment No.	Day of experiment	Dose of diuretin	Water intake	Urine excretion				Serum concentrations					Weight
				Volume	N	Na	Cl	Na	Cl	HCO_3	N.P.N.	Protein	
			cc.		*gm.*	*m.-eq.*	*m.-eq.*	*m.-eq./liter*	*m.-eq./liter*	*m.-eq./liter*	*mg. per cent*	*gm. per cent*	*gm.*
10	0							142	104	22	32	6.38	2368
	1	1	255	198	0.455	13.5	13.5						
	2	2	173	140	0.859	6.2	4.7	135	95	21	38	6.70	2225
	3		50	50	0.533	0.3	0.1						
	4		50	50	0.533	0.3	0.1						
	5	3	170	165	0.783	5.2	2.5	133	89		104	6.50	
	6		30	25	0.297	0.1	0.1		89		200		2070
	7	4*	100	0	0	0	0	124	60		214	5.72	2130
3	0							146	111	24	35		1730
	1	1	296	326	0.919	21.5	18.1						1585
	2		109	84	0.737	0.2	0.2						1535
	3	2	315	256	0.696	2.2	1.9		104				1501
	4		89	109	0.960	0.1	0.1	132	100		50		1415
	5	3	310	279	0.630	4.5	4.4	135	101				1350
	6		93	90	1.039	0.1	0.±						1295
	7		69	47	0.543	0.1	0.1				54		1275
	8	4	291	250	0.856	3.3	2.9						1220
	9		93	57	0.760	0.1	0.1	130	97		60		1200
	10	5*	106	60		0.±	0.±	126	82	34			
6	0							144	99	25	35		2000
	1	1	289	312	Total = 4.158	13.6	11.9						1865
	2	2	305	310		2.9	2.2						1795
	3	3	247	231		2.4	2.2						1695
	4		58	25		0.1	0.1	137	88		58		1680
	5	4	242	222		12.2	9.0	135	78		75		1545
	6	5*	40	11		0.2	0.1	132	74	14	75		
11	0							141	105		38	6.3	2200
	1	1	281	208	0.707	11.9	8.9						
	2		160	56	1.082	0.8	0.6						
	3	2	211	232	0.696	10.2	6.2						
	4		160	46	0.984	0.5	0.4	115	88		40	5.6	2030
	5	3	160	214	0.941	7.7	5.2						1850
	6		210	54	0.729	0.5	0.2	109	70			5.4	1935
	7	4*	150	30		0.6	0.4	101	62		42	4.3	2035

Rabbit 10. Maize-fed and 1 gm. of diuretin in 100 cc. H_2O as specified.

Rabbit 3. Fasting and 0.75 gm. of diuretin in 100 cc. H_2O as specified.

Rabbit 6. Fasting and 0.75 gm. of diuretin in 40 cc. H_2O as specified.

Rabbit 11. Maize-fed, 1 gm. of diuretin as specified, and 175 cc. forced water daily.

* Animals died a few hours after this dose of diuretin.

volume; see Experiments 10, 3, and 6, Table I, and Experiment 8, Fig. 3. In other words the relationship of azotemia to the limitation of urine volume by dehydration is clearly indicated.

Table II presents data from the control experiments on fasting animals and fasting animals given diuretin and simultaneously salt solution. The spontaneous water intake of the fasting controls shows that the water given in the forced fluid experiments was not excessive, whereas the spontaneous water intake of the diuretin experiments without forced fluid was in the diuretin-free intervals rather low. No significant blood changes occurred either in the fasting controls or in

TABLE II

Average Daily Water Intake, Urine Volume, and Serum Concentrations at Beginning and End of Experiments

Experiment No.	Régime	Water intake	Urine volume	Na	Cl	HCO_3	N.P.N.	Day of experiment
		cc.	*cc.*	*m.-eq.*	*m.-eq.*	*m.-eq.*	*m.-eq.*	
1	Fasting	178	144	142	110	20	32	1
				137	98	19	49	9
2	Fasting	136	99	141	107	25	32	1
				138	95	23	30	9
5	Diuretin and salt	229	199	144	108	23	35	1
				140	100	23	35	9
7	Diuretin and salt	157	145	142	97		35	1
				138	99		30	5

the controls given diuretin and salt solution. The metabolism data of these animals are presented in Fig. 1, Experiments 1, 2, and 5, and Fig. 2, Experiments 1, 2, and 7.

DISCUSSION

The experiments reported here, as well as confirming the withdrawal of large amounts of chloride in the urine following the administration of diuretin, as reported by Gruenwald (1), also show a large withdrawal of sodium and potassium incident to diuretin administration. The urinary losses of sodium and chloride in our experiments

are equivalent to between three or four times the salt content of the initial plasma volume as estimated from Utheim's data (17). These losses are approximately equal to the salt losses reported by Gamble and McIver (18) in rabbits dehydrated by pyloric obstruction. The average urinary potassium loss in the diuretin animals was double that in the fasting controls. Taking the potassium content of protoplasm from Goto's (19) analysis of muscle as 8 milli-equivalents per 100 gm., computation from the data represented by the potassium columns and the darkened portion of the weight columns in Figs. 1 and 2 shows that for a protoplasmic loss of 100 gm. there is an average loss of potassium corresponding in the control animals to 170 gm. and in the diuretin animals to 300 gm. of protoplasm, indicating in the diuretin experiments a much greater removal of potassium above that ascribable to tissue destruction. Such an event has been observed in fasting children by Gamble, Ross, and Tisdall (20) and in children with severe diarrhea by Butler, McKhann, and Gamble (21). Large losses of potassium have been reported following the use of acid-producing salts in rabbits by Goto (19) and moderate losses in human patients by Gamble, Blackfan, and Hamilton (22).

The sodium losses, described above, are accompanied in the experiments where the animals received water as desired by such losses of body water as to result in but small serum sodium dilutions. In other words, the tendency of the organism to maintain normal plasma base concentration, as emphasized by Gamble (23), is evident. In those animals given additional water in single large daily doses the sodium losses were not accompanied by such an excretion of water as to prevent large serum dilutions. Curtis (24) has shown that there is a delay in the onset of the diuresis following the administration of diuretics, if large amounts of water are given. And Darrow and Yannet (25) have observed in experiments of short duration low serum electrolyte concentrations and oliguria following the intraperitoneal administration of isotonic glucose solution which withdraws electrolytes into the peritoneal cavity and thus produces a fall of concentrations in the blood plasma. In our experiments over a period of several days the presence of serum dilution did not result in an excretion of water relative to salt to an extent sufficient to correct the low sodium and protein concentrations of the serum.

Comparison of the sodium and potassium losses above that ascribable to tissue loss which may be derived from the data shown in Figs. 1 and 2, with the dehydration portion of the corresponding weight losses, suggests not only the observed dilution of serum but also a dilution of tissue or intracellular potassium. The loss of sodium plus potassium in excess of the loss of water on the basis of their concentrations respectively in extracellular and intracellular fluids is particularly striking in Experiment 4, Fig. 1, where forced water was given with the diuretin and where a marked fall in serum concentrations was observed, Fig. 3.

TABLE III

Data Illustrating a Lack of Direct Relation between Serum Electrolyte Concentrations and Azotemia

Source	Total base	Na	Cl	N.P.N.	Δ
	m.-eq.	*m.-eq.*	*m.-eq.*	*mg. per cent*	*°C.*
Dog—withdrawal of pancreatic juice (29)	160		94	200	
Withdrawal of gastric juice (8)			69	34	
Same (8)			53	196	
Dog—adrenalectomized (28)	144	133	100	185	
Puppies—dehydrated by concentrated milk (26)	236		160	192	0.95
Puppies—carbohydrate-rich, salt-free diet (30)	120		67	15	0.41
Infants—diarrheal dehydration (27)	156		104	74	0.57
Same (27)	147		91	94	0.55
Rabbit—diuretin (present paper)		130	90	143	
Same		101	62	42	0.42

The quantitative data with which a calculation of intracellular dilution in these experiments might be made are too rough to warrant an estimation. That such dilutions of intracellular potassium occur is confirmed by Goto's (19) figures, which show not only a withdrawal of muscle potassium from acid-fed rabbits but also a 20 to 30 per cent reduction in the potassium concentration of muscle.

Table III presents data from the literature (8, 30) confirming the lack of relation between azotemia and hypochloremia observed in our experiments. In dehydration coincident with the loss of approximately equivalent amounts of base and chloride, such as follows the loss of salt by withdrawal of pancreatic juice (29) or intestinal secre-

tions (27) or through the kidney, as in adrenal insufficiency (28) and in these diuretin experiments, azotemia may be present with but slight lowering of the serum chloride concentration. The observations of one of us (Kerpel-Fronius) in experiments on puppies dehydrated by feeding concentrated milk (26) illustrate an azotemia with dehydration in the presence of increased serum base and chloride concentrations. Coincident with the dehydration in his experiments there occurred an impairment in kidney function as evidenced by the fall in urine volume and urine nitrogen and chloride concentrations in the presence of increasing serum concentrations (31).

The ascribed dependence of azotemia on hypochloremia seems to have resulted from the frequency with which dehydration follows the loss of gastric secretions, in which the loss of chloride above the loss of base produces marked lowering of the serum chloride concentrations.

The data presented in our experiments together with those summarized in Table III indicate a consistent relation between azotemia and dehydration when of sufficient degree to greatly reduce urine volume. The effect of reduction of urine volume on nitrogen excretion is probably related to the dependence of urea clearance on urine volume as described by Van Slyke and coworkers (32). The *modus operandi* of dehydration in reducing nitrogen excretion, aside from a resulting oliguria, is not indicated by our experiments. Reasonably, however, nitrogen retention may be regarded as a result of other changes produced by dehydration such, for instance, as decrease in blood volume (Marriott (33) and McIntosh, Kajdi, and Meeker (34)), increase in blood viscosity (Surányi and Sonnauer (35) and observed qualitatively by us), and fall in blood pressure (Bottin (36) and Swingle *et al.* (37)), changes which together affect blood flow through the kidney and hence urine volume and urea excretion (Van Slyke (38)). From the evidence reported here the beneficial effect of salt solution in reducing the N. P. N. of non-nephritic azotemias is dependent upon an augmentation of urine volume rather than upon the restoration of serum electrolyte concentrations. The reduction of N. P. N. by administration of water without salt depends upon a temporary increase in urine and blood volumes at the possible risk of a hypotonicity of the blood which may approach that observed in water intoxication. Furthermore such water administration does not per-

manently remove the underlying cause of the nitrogen retention, namely the dehydration. It is incidentally interesting that the results of Experiment 4 demonstrate, in agreement with those of Darrow and Yannet (25), that the volume of the plasma may be defended at the expense of a fall in electrolyte concentrations in the presence of a presumably normal renal activity.

Another interesting point in these experiments is the clinical picture of the animals preceding death. The paralysis of the extremities, which extends from below upwards and ascends until death appears, is described in detail by Gruenwald (1). Having ruled out a toxic effect of the diuretin on the kidneys by histological examination and having been able to protect the animals from death by the simultaneous administration of salt with the diuretin, which removed the hypochloremia, Gruenwald was led to ascribe the final symptoms to hypochloremia. In our experiments death in the usual manner occurred after the fifth dose of diuretin even when the serum chloride concentration was but slightly below normal and it also occurred in the case of a fasting animal given salt with each dose of diuretin. Since we observed that the maize intake of salt-supplied animals was two to three times that of animals given diuretin without salt, we suggest that death in the last mentioned experiment was due to the marked loss of potassium which probably did not occur in Gruenwald's rabbits fed salt and maize.

SUMMARY

The losses of sodium, potassium, chloride, nitrogen, and water following the administration of diuretin to rabbits over 5 to 9 day periods together with the changes in serum concentrations of sodium, chloride, N. P. N., and total protein occurring simultaneously with these losses are described.

The circumstances responsible for the presence of azotemia in the animals were investigated in particular and the dependence of nitrogen retention upon dehydration and the modification of this dependence by variation in urine volume were demonstrated. It was clearly shown that no direct relationship exists between the azotemia and the coincident hypochloremia. It was found that nitrogen retention can be removed by the administration of water without salt, and the extent

to which serum electrolyte and protein concentrations can be lowered by this procedure was also observed.

The withdrawal from the body of large amounts of potassium as well as of sodium and chloride following the administration of diuretin, and also the inefficacy of sodium chloride solution in preventing the potassium loss was demonstrated.

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