CHRONIC SODIUM CHLORIDE TOXICITY IN THE ALBINO RAT

II. Occurrence of Hypertension and of a Syndrome of Edema and Renal Failure

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Plates 6 and 7

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The need for common salt in the diet is an ancient, inveterate cognition, deeply rooted in human mores, to such an extent that little thought has been given to the chronic toxicity of sodium chloride. A search of the literature reveals a number of papers which describe toxic effects of sodium chloride in fowl (1), but a paucity of information relative to mammals. Campbell (2) reported the possibility of kidney damage in the rat fed a diet containing more than 5 per cent of sodium chloride. Mosier (3) studied the adrenal glands in rats on diets very high in sodium chloride. Sapirstein, Brandt, and Drury (4) and Gross (5) produced hypertension in rats fed diets high in sodium chloride and restricted in the amount of fluid. This was accomplished by substituting saline solution for drinking water. Binet, Dejours, and Lacaisse (6) raised rats on a high sodium chloride regimen and described a resulting renal hypertrophy and functional adaptation. A few experiments of a chronic nature have been made using the mouse (7), the albino and the kangaroo rat (8), the dog (9), and the cow (10).

Recently Meneely, Tucker, and Darby (11) reported on the growth of rats fed for 20 weeks on purified diets containing seven different levels of sodium chloride. The present paper describes certain pathologic manifestations of chronic sodium chloride toxicity which were observed in these animals. Abstracts of these findings have appeared (12).

Materials and Methods

The experimental regimen has been described (11). Male, albino, Sprague-Dawley rats, weighing an average of 119 gm., were placed on purified rations and demineralized water *ad libitum*. The purified rations contained seven different levels of sodium chloride as follow: ration I (low Na diet), about 0.01 per cent NaCl; ration II (control diet), 0.15 per cent NaCl; ration III, 2.8 per cent NaCl; ration IV, 5.6 per cent NaCl; ration V, 7.0 per cent NaCl; ra-

tion VI, 8.4 per cent NaCl; ration VII, 9.8 per cent NaCl. Eleven rats were placed on ration I and thirty or thirty-one rats on each of the other six diets. The experiment was designed to cover the life span of the rat. Systolic blood pressure measurements were made by the cuff method of Kersten *et al.* (13).[1] At the time of sacrifice the mean arterial blood pressure in a number of rats was determined manometrically by direct cannulation of the carotid artery under diallyl barbituric acid with urethane (Ciba Pharmaceuticals Products, Inc., Summit, New Jersey) anesthesia. A few animals developed intercurrent disease, usually respiratory infection, and died or were sacrificed. Other animals for sacrifice were chosen at random from each group. Autopsies were performed as soon after death as possible, immediately in the case of all sacrificed animals. The tissues were fixed in 10 per cent formaldehyde solution and blocked, sectioned and stained by standard histologic techniques except for those prepared by frozen section for fat stains.

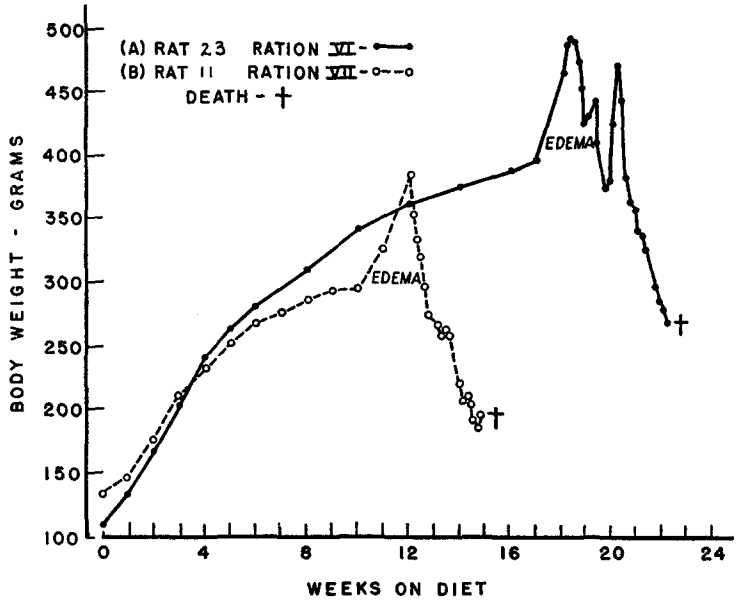
RESULTS

Edema.—As previously reported, all seven groups of rats tolerated the diets and gained weight, although the animals on sodium restriction (ration I) and those on excessive salt gained less rapidly than did the controls (ration II). After the 2nd week the rats all appeared healthy upon inspection. During the ensuing 2 months the rats on the higher levels of salt intake consumed large amounts of water and maintained an impressive polyuria, approximately proportional to the amount of sodium chloride in the diet. Beginning in the 3rd month there appeared sporadic instances of rapidly developing, massive edema among those three groups eating the highest levels of salt (from 7.0 to 9.8 per cent). This occurred with moderate frequency until the 6th month. Since that time edema has been observed but rarely.

Two typical weight curves of edematous rats are shown in Text-fig. 1. During the phase of edema the weight of the animal usually was unstable, fluctuating in the manner seen in curve (A). Most of the rats which developed edema died or were sacrificed, but those that passed through the edematous episode exhibited a sudden and precipitate fall in weight, became emaciated, cachectic, and died. Two rats entered a terminal cachectic phase directly without ever exhibiting detectable edema. The total number of rats that showed these changes was sixteen, which is 18 per cent of the ninety initially eating the three high salt diets (Table I).

Examination of these edematous animals revealed quite uniform findings. Interstitial fluid was greatly increased, as evidenced by the appearance of the animal before death (Fig. 1) and by the obvious edema of all the tissues and organs at autopsy (Fig. 2). Measurements of the sodium space with the radioisotope Na^{24} revealed apparent volumes of extracellular fluid as high as 57,5 per cent of body weight (Table II); this value may be contrasted with an average of 23.8 per cent among the controls at that time. The plasmas from these edematous animals were lipemic in the gross. The total serum proteins were greatly diminished, in some instances to values as low as 3.5 gm. per

[1] The photoelectric tensometer used in this experiment was manufactured by Metro Industries, Long Island City, New York.



TEXT-FIG. 1. Growth curves for two rats which developed massive edema. A. Solid line. Rat 23 on ration VI (8.4 per cent NaCl). B. Broken line. Rat 11 on ration VII (9.8 per cent NaCl).† Death.

TABLE I

Data on Rats Which Became Edematous from the 7th until the 23rd Week of the Experiment

Rat No.	Ration	Time of onset of edema	Weight at onset of edema	Maximum weight	Time of death	Weight at death	Anemia	Cachexia	Died or sacrificed	Lowest total serum proteins	Lowest hematocrit reading	Remarks
		wk.	*gm.*	*gm.*	*wk.*	*gm.*				*per cent*	*per cent*	
54	VII	7	241	346	12	113	+	+	Died	4.62	24.4	
24	VII	7	259	457	10	427	+		Sacrificed	4.13	17.0	See Figs. 1 and 2.
22	V	8	231	315	10	278	+		Died	—	19.1	
51	VI	9	286	350	10	270	+		"	3.52	27.5	
52	V	9	294	440	12	380	+		Sacrificed	—	—	
42	VII	10	320	363	15	178	+	+	Died	5.89	—	
11	VII	11	295	384	15	196	+	+	"	5.10	—	See Text-fig. 1.
17	VI	13	324	406	15	375	+		"	4.15	—	
23	VI	18	395	489	23	267	+	+	Sacrificed	—	30.0	NPN, 77–175 mg. per cent. See Text-fig. 1.
64	VII	20	410	430	29	294	+	+	"	—	—	Slight edema.
6	VII	21	396	427	25	330	+	+	Died	—	38.0	NPN, 82 mg. per cent. Slight edema.
18	VII	23	425	549	27	305	+	+	"	—	—	

cent. A severe anemia was present, the hematocrit reading falling as low as 17.0 per cent in one instance. Azotemia, as indicated by elevation of the plasma non-protein nitrogen, was observed terminally in animals on rations V through VII. Blood pressure measurements, when made, revealed hypertension in most of the edematous rats. At autopsy, the organ weights were obtained, but the edema obscured their interpretation. Therefore they are not presented at this time.

Microscopic examination of the tissues of animals in this stage showed spectacular lesions of the kidneys in every instance. All components of the nephron were affected, but the glomeruli were most uniformly and severely damaged. The tufts were enlarged but appeared almost bloodless, and overall cellularity seemed diminished. Individual cells, both endothelial and epithelial, were swollen, and the cytoplasm was empty or vacuolated. Nuclei

TABLE II

Na^{24} Space Expressed as Per Cent of Gross Body Weight

	Sample	Rations	Per cent NaCl	N	Mean Na^{24} space	S.D.
From 2–4 mos.	Controls	II	0.15	7	23.8	2.00
	High salt (without edema)	V, VI, VII	7.0, 8.4, 9.8	11	26.1	2.03
	High salt (with edema)	V, VII	7.0, 9.8	2	57.5	—
From 10–11 mos.	Controls	II	0.15	8	21.6	0.96
	High salt (without edema)	V, VI, VII	7.0, 8.4, 9.8	20	25.8	2.87

were misshapen and often pyknotic, but some were vesicular. The basement membranes were greatly swollen, frayed, degenerated, and tended to fuse with the cellular elements so that capillary structure was virtually obliterated. Staining with sudan IV demonstrated large quantities of lipid throughout the glomerular tufts. This occurred in both fine and coarse droplets and was largely intracellular. It appeared that the endothelial cells uniformly were more affected, but even the capsular epithelium was not exempt. The convoluted and straight tubules were generally dilated and contained protein precipitate varying in amount and density. The lining epithelium was of pale granular appearance with abundant sudanophilic lipid in the cytoplasm. The renal arterioles were uniformly diseased, and many small arteries were affected. Stainable lipid formed vacuoles in smooth muscle cells, some of which were necrotic. In many arterioles the elastic lamina was greatly swollen, frayed, and intensely eosinophilic; the lumina were narrowed or even oc-

cluded (Figs. 3 and 4). Extrarenal arterial lesions occurred only occasionally in this group of animals and were usually less severe than those in the kidney.

In the rats on rations V, VI, and VII, which survived only 6 to 9 months and which did not become edematous, the kidneys again showed widespread lesions with somewhat more severe tubular degeneration. Among these animals visceral arterial lesions were fairly constant and were almost invariably present in the heart, pancreas, testis, and gastro-intestinal tract. Vascular lesions in the heart were associated with small areas of scarring. In none was the aorta sclerotic. The microscopic structure of the adrenal glands was not different from that of control animals.

Hypertension.—Most of the rats (134 out of 193) forming the initial seven groups in this study are still alive after 14 months, and the experimental regimen continues. With few exceptions the animals appear healthy and alert

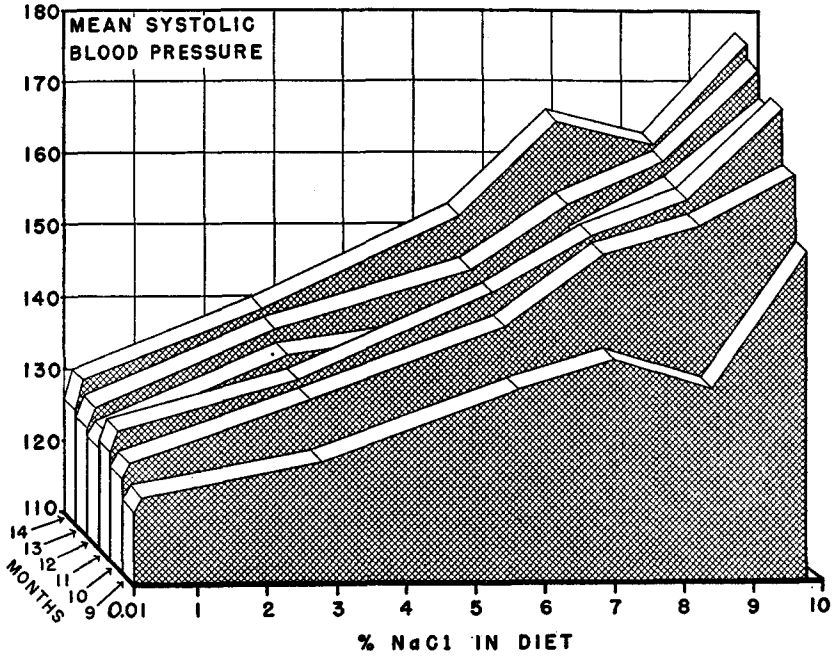
TABLE III

Mean Body Weight

Ration	Per cent NaCl	N	Mean weight		S.D. + 1 yr.
			Start	+ 1 yr.	
I	0.01	8	113	614	44
II	0.15	25	120	670	73
III	2.8	25	121	592	50
IV	5.6	27	118	566	41
V	7.0	21	119	543	40
VI	8.4	18	125	538	50
VII	9.8	18	120	491	41

with sleek coats. Several of the control animals and some of those on rations I, III, and IV are actually obese (Table III). None of the animals on rations I through IV has shown significant visceral lesions. Despite their generally healthy appearance, many of the rats are not normal. Practically all the rats on rations V, VI, and VII have sustained arterial hypertension, and most of the rats on rations III and IV have significant elevations in blood pressure. Reliable determinations of blood pressure on the colony as a whole have been obtained beginning in the 9th month of the experiment. Previous to the 9th month the rats were insufficiently trained. Text-fig. 2 shows that striking changes in blood pressure have appeared among the animals eating rations III through VII. In this figure the means of the systolic blood pressures at monthly intervals are plotted for each group. All the data obtained from the beginning of the 9th month until the end of the 14th month of the experiment are included in this graphic summary. Part of the intragroup fluctuation is due to sacrifice or death of some animals.

The animals on diets V, VI, and VII, sacrificed while in apparent good health, showed renal lesions which resembled those described above but which were somewhat less severe and much less uniform. In fact many nephrons appeared uninjured while neighboring units were effectively destroyed. The arterial lesions, both renal and general visceral, were less impressive and less constant, usually consisting of medial hypertrophy. However, in the continuing study some of the hypertensive rats which were sacrificed at about 14

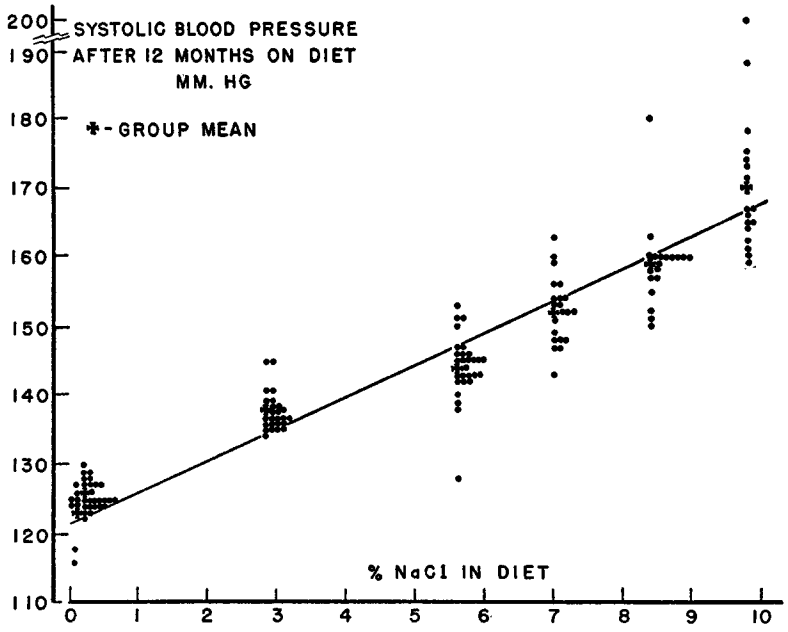


TEXT-FIG. 2. The oblique lines represent the means of the systolic blood pressures of the seven different groups at monthly intervals.

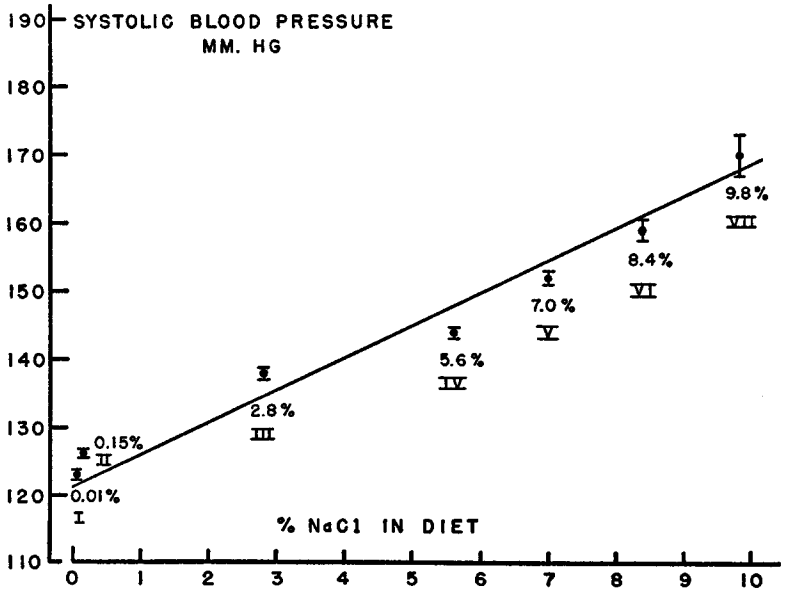
months and were manifestly declining before death, exhibited both renal and arterial disease equal to that observed in the edematous group.

DISCUSSION

Hypertensive disease was provoked in the rat by increase in the ration of sodium chloride. The diet fed was essentially cholesterol-free. The rats were housed in suspended steel wire cages; hence they had only limited access to their feces. After several months of the dietary regimen, a sustained arterial hypertension developed in the rats eating the high salt rations. The average blood pressure was related in a linear fashion to the amount of sodium chloride (Text-figs. 3 and 4). Within a given salt intake group the distribution of



TEXT-FIG. 3. Scatter diagram of systolic blood pressure values after 1 year of the dietary regimen. Identical values are arrayed to the right of the perpendicular.



TEXT-FIG. 4. The mean systolic blood pressure and the standard error of the mean after 1 year of the dietary regimen.

blood pressures showed some scatter; the group means, however, fell close to a straight line. No more than random departure from linear regression was indicated. The product moment coefficient of correlation was $+0.91 \pm 0.08$. These effects occurred without restricting the amount of drinking water, although others have claimed that it is necessary to restrict water intake to produce hypertension with salt (4).

The measurements of blood pressure were made with a standardized photoelectric device (13). In a number of rats which were sacrificed, these observations were confirmed by direct measurement of the blood pressure in the cannulated carotid artery.

Rats which developed marked edema on the three highest rations of salt (7.0 to 9.8 per cent) evinced similar signs and strikingly similar alterations of their organs in the gross and upon microscopic examination. The syndrome of massive edema, lipemia, anemia, hypoproteinemia, and azotemia resembles nephrosis as seen in human beings, but might differ in other respects from the human disease. Neither the pathogenesis of salt injury in rats nor the pathogenesis of human nephrosis is known. The renal lesion observed in these rats is different from that seen in human nephrosis principally in that there is initial and severe involvement of the glomeruli in the rat kidney. The severity of hypertension in these rats may well account for the similarity of the arteriolar lesion in these animals to the arteriolar lesion seen in malignant hypertension in man. Also it may be noted that the experimental syndrome developed mainly among the rats while they were young, that is, during the first one-fifth of the usual life span, an age which corresponds to the time of occurrence of nephrosis in man.

Some animals went into terminal renal failure without exhibiting detectable edema. In these the histologic picture was indistinguishable from that seen in rats which passed through an edematous phase. It seems that the clinical course exhibited by an individual rat depends upon a balance of those factors which favor salt and water retention as opposed to those which lead to salt and water loss. In all cases impairment of renal function inevitably predisposed to an early death.

Owing to the fact that the initial reading of blood pressures dates from the 9th experimental month, by which time segregation of groups with respect to blood pressure had occurred (Text-figs. 2 to 4), the earliest time of origin of the hypertension cannot be stated.

Pathologic changes have not been found as yet by usual staining techniques in the animals of groups III and IV, although mild hypertension has developed in both of these groups. It is possible that recognizable lesions will appear later in the course of the experiment. This suggests that physiologic changes resulting in hypertension occur before there is any readily demonstrable anatomic lesion in the kidney or elsewhere.

Except for edematous rats, the Na^{24} space measured in rats fed high salt diets for 2 to 4 months was not significantly greater than in control rats. However, after approximately 10 months a significant difference was observed (Table II). This apparent increase in volume of extracellular fluid probably is accounted for in part by differences in total body fat; some of the control rats have become obviously obese (Table III).

SUMMARY

Sustained arterial hypertension developed in male, albino rats chronically fed diets rich in sodium chloride with demineralized drinking water available *ad libitum*. After 12 months of the experimental regimen a positive, linear correlation ($r = 0.91$) was found between the systolic blood pressure and the concentration of sodium chloride in the diet.

A syndrome of edema and renal failure was observed in 18 per cent of the group fed at the level of 7.0 to 9.8 per cent of sodium chloride. Significant histologic changes occurred in the kidneys and certain other organs in rats consuming rations containing these levels of NaCl.

The relative volume of the radiosodium space was increased in the rat by high dietary sodium chloride.

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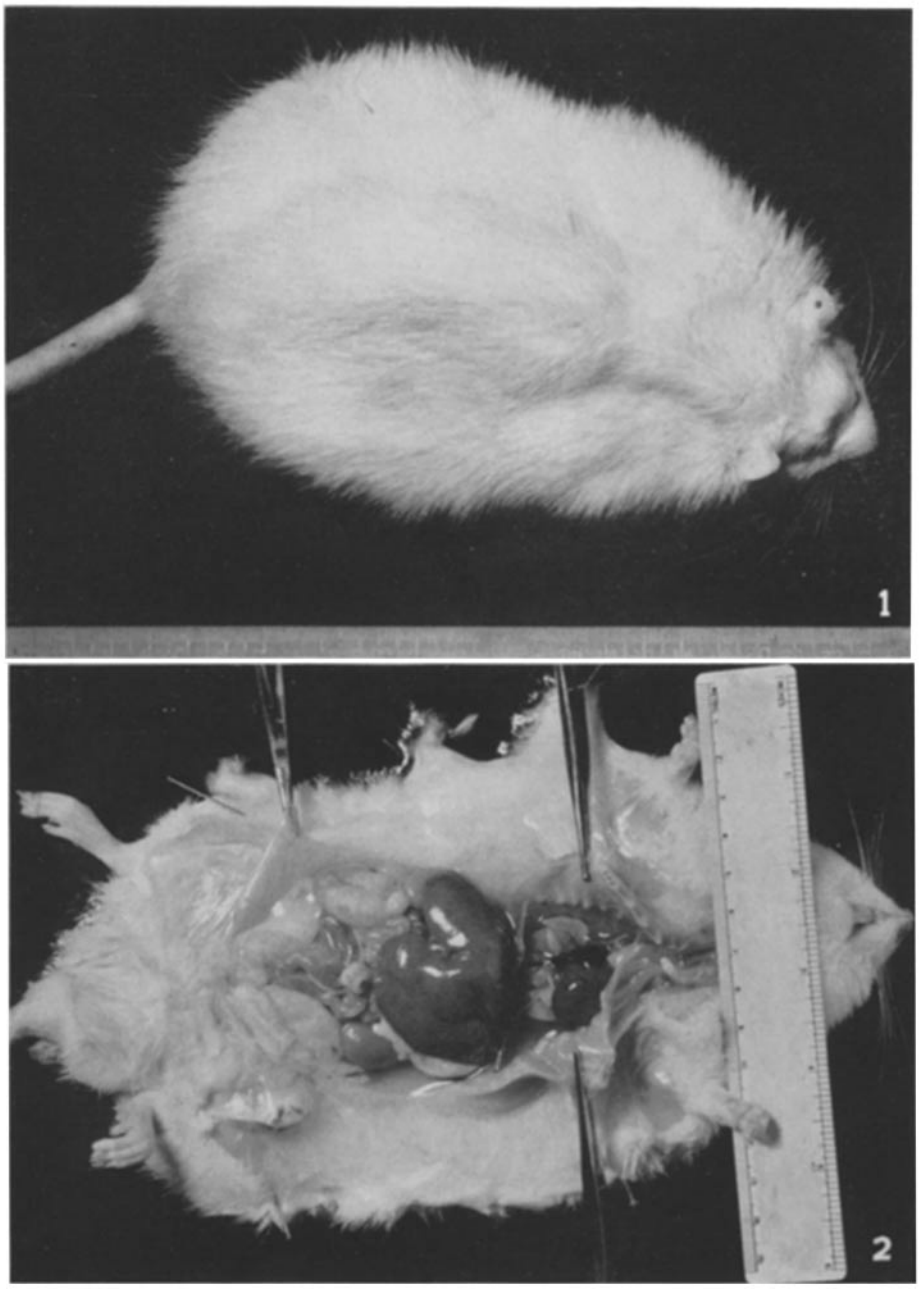
EXPLANATION OF PLATES

PLATE 6

FIG. 1. Rat 24. After eating ration VII (9.8 per cent NaCl) for 8 weeks, the rat developed massive edema. This photograph was taken in the 10th week when the body weight was about 440 gm. The pit on the nose was produced by finger pressure.

FIG. 2. Appearance of edematous rat 24 (ration VII) at autopsy. This photograph was taken shortly after Fig. 1.

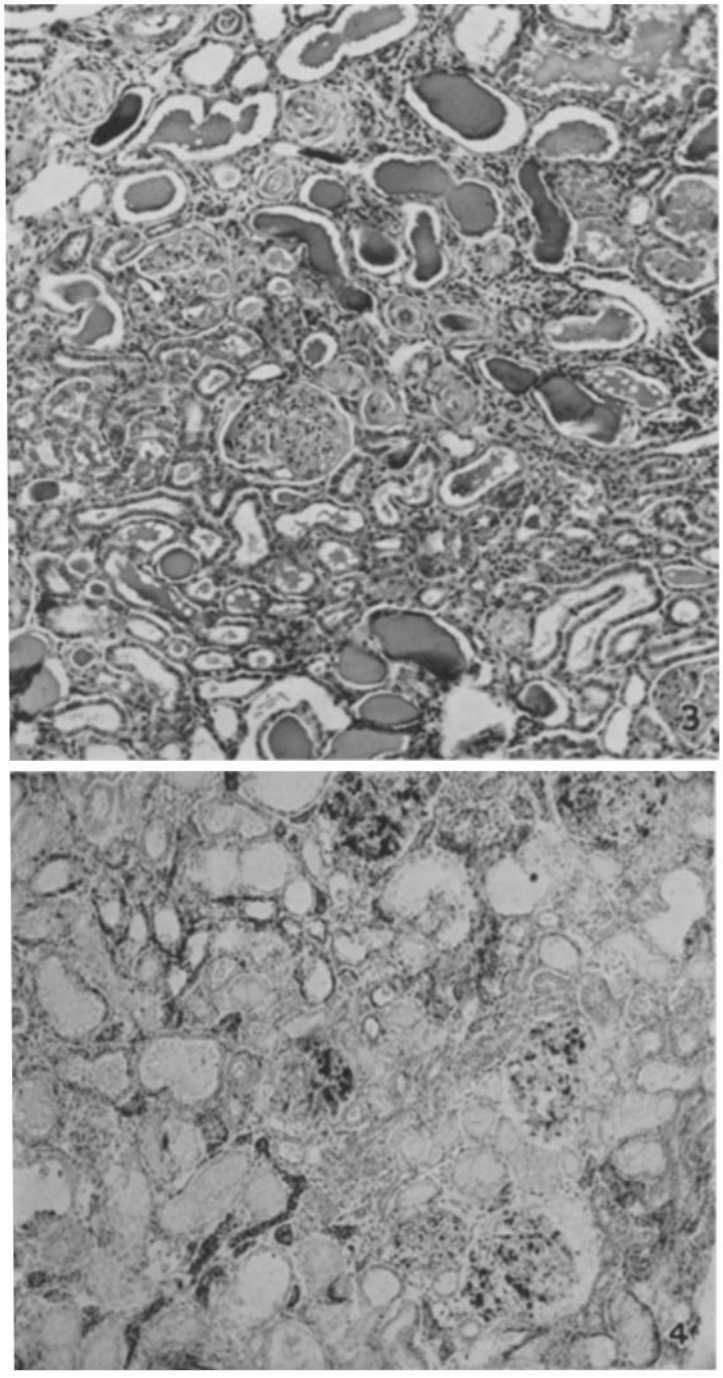


(Meneely *et al.*: Sodium chloride toxicity. II)

PLATE 7

FIG. 3. Rat 24 kidney stained with hematoxylin and eosin. × 100. This rat (on ration VII) was sacrificed in the edematous phase. All elements of the nephron tissue are involved. Extensive arteriolar disease is also present.

FIG. 4. Rat 24 kidney stained with sudan IV. × 75. This photomicrograph shows accumulation of lipoid in the glomeruli, the tubules and the arterioles in the same kidney illustrated in Fig. 3.



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