

THE RENAL LESIONS OF ELECTROLYTE IMBALANCE*

III. THE EFFECT OF ACUTE CHLORIDE DEPLETION AND ALKALOSIS ON THE RENAL CORTEX

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PLATES 98 TO 100

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Two previous studies (1, 2) have described the structural changes in the kidneys of rats that occur as a response to the alterations in electrolyte balance which accompany potassium deficiency. The effect of potassium deficiency *per se* (1) was found to be principally localized to the collecting tubules and consisted of three separate changes: a general hyperplasia of the tubular epithelial cells in the outer zone of the medulla, a specific hyperplasia of the intercalated cells in the same area, and a development of droplets in the cells of the papillary portion of the collecting system which had been previously noted by Spargo (3, 4). These changes were associated with a defect in ability to concentrate urine, and further studies of this relation have been described (5, 6). The effect of massive phosphate loading in producing extensive changes in the kidney consisting of necrosis and calcification of the terminal segment of the proximal convolutions was first described by MacKay and Oliver (7). The same lesion was found to develop with a modest phosphate intake which of itself produced no change in normal animals when potassium deficiency was present (2); this added lesion then existed in association with the lesions of potassium deficiency.

In the original description of the changes noted in potassium deficiency varying degrees of cellular hyperplasia were noted in some proximal convolutions. In the experimental design of that study a diet had been used that was low in chloride content. Extensive change had been noted previously in the cortex of rats acutely depleted of chloride although the locus of this change was not certain (8).

The present study explores the conditions under which this renal cortical change develops. In brief, it has been found that it consists of extensive cell

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damage and hyperplasia in the proximal convolutions and develops in response to acute chloride depletion. Potassium deficiency is not essential to its development, but there is evidence that potassium deficiency increases the severity of the change. Amino-aciduria was not demonstrable and impairment in concentrating function was minimal.

Studies of the effects of phosphate intake also have been made. As was the case in potassium deficiency (2), chloride depletion predisposes to the development of the renal change due to phosphate loading at levels of intake which produce no lesions in controls.

Methods

A general experimental design was used in all experiments to effect chloride depletion (9). Male Wistar strain rats weighing 250 to 350 gm. were used. The animals were placed on a diet low in chloride, sodium, potassium, and phosphorus. The drinking water generally contained 30 mM/liter KHCO_3 ; where indicated it also contained NaHCO_3 . After 2 to 3 days each animal was given 10 ml. per 100 gm. body weight of a specified fluid by intraperitoneal injection. In 2 to 4 hours the fluid in the peritoneal cavity was removed through a small incision in an amount roughly equal to that injected. The animal was then given access to the low electrolyte diet and a drinking solution indicated specifically in each experiment.

Three days later the animals were killed, the kidneys obtained for histological study and microdissection, and serum and muscle samples taken for chemical analyses (2).

The first study consisted of three experimental groups with appropriate controls. The first group was depleted of both sodium and chloride by the dialysis technique in which 3 per cent glucose solution containing no electrolyte was injected intraperitoneally. The average concentration of chloride in the fluid removed was 83 m.eq./liter and the quantity removed equally 22 per cent of total body chloride. The controls were dialyzed with no loss of chloride and no gain or loss of sodium. Both groups of animals were continued on the diet and given distilled water to drink for the subsequent 3 days.

The second group was depleted of chloride by the dialysis technique in which 0.3 M NaHCO_3 was injected intraperitoneally and the fluid withdrawn in 2 to 4 hours. No net sodium loss occurred; instead a net gain of sodium bicarbonate was effected since hypertonic sodium bicarbonate had been injected. An average of 27 per cent of total body chloride was removed. The controls were similarly dialyzed with 0.2 M NaHCO_3 and 0.11 M NaCl so that there was no net loss of chloride. The animals were continued on the diet and given 0.15 M NaHCO_3 to drink in the succeeding 3 days.

A third group was also studied that was similar to the second in all respects except that both in the dialysis fluid and in the drinking solution 50 mM/liter KHCO_3 was substituted for 50 mM/liter NaHCO_3 . In this way an abundant amount of potassium was provided.

The volumes removed and the concentrations of chloride in the fluid injected and that removed in the three experimental and the control groups are given in Table I.

RESULTS

The over-all results are summarized in Table II. The first group, depleted of both sodium and chloride, and its controls had a normal concentration of chloride and bicarbonate in serum and a normal content of potassium in muscle at the time of sacrifice. In the second group alkalosis, hypochloremia, and potassium deficiency developed in both the chloride-depleted group and the

controls. The changes were less extreme in the controls in which excessive sodium bicarbonate was given and no chloride deficit incurred. In the third group, in which bicarbonate was exchanged for chloride but extra potassium was given, alkalosis and hypochloremia comparable to that noted in the second group, was effected. The abundant intake of potassium ameliorated, but failed to prevent, a deficit of muscle potassium.

In all three groups that were depleted of chloride, extensive structural al-

TABLE I
Amount and Composition of Fluid Injected and Fluid Removed During Peritoneal Dialysis

Group		Solution	Volume of fluid		Cl concentration of fluid		Net Cl removed
			In-jected	Re-moved	In-jected	Re-moved	Total body Cl
			<i>ml./100 gm. body wt.</i>		<i>m.eq./liter</i>		<i>per cent</i>
1	Na and Cl-depleted	3 per cent glucose	10.0	8.4	0	83	22
	Controls	0.03 M NaHCO ₃ 0.11 M NaCl 1 per cent glucose	10.0	9.5	110	111	1
2	Cl-depleted	0.3 M NaHCO ₃ 1 per cent glucose	10.0	11.7	0	74	27
	Controls	0.2 M NaHCO ₃ 0.11 M NaCl 1 per cent glucose	10.0	11.3	100	108	2
3	Cl-depleted alkalosis K added	0.25 M NaHCO ₃ 0.05 M KHCO ₃ 1 per cent glucose	10.0	11.8	0	72	28

terations were noted in the cortex of the kidney in virtually all animals. No changes were noted in the controls. The affected kidneys had a white mottled appearance but were not significantly increased in size.

On low powered microscopic examination scattered areas composed of contiguous cross sections of tubules were seen which stained more lightly than those which formed the normal background of cortical tubules (Fig. 1). On higher magnification these islands of lighter tubules showed a progression of cellular alteration. In the least affected the cells were simply swollen and the nuclei more prominent (Fig. 1); others more severely involved showed a definite

disintegration of their protoplasm associated with the accumulation of eosinophilic material in the lumens of the tubules (Fig. 2). Many of the nuclei in these tubules were swollen and fragmented while others were intact and greatly enlarged and hyperchromatic. The latter were interpreted as premitotic evidences of cellular proliferation, since scattered among them were many mitotic figures; the intensity of the process was evident in the pluripolar configuration of many of these figures (Figs. 2 to 5).

The location of the changes described was determined by microdissection

TABLE II
Serum and Muscle Composition 3 Days After Dialysis

Group		Serum			Muscle	Kidney lesion
		CO ₂	Cl	Urea N	K	
1	Na and Cl-depleted	24.7 (±2.5)	99.3 (±6.2)	46.2 (±10.1)	44.5 (±2.28)	9/10
	Controls	22.8 (±2.4)	98.5 (±3.1)	26.5 (±6.2)	44.3 (±2.13)	0/6
2	Cl-depleted alkalosis	41.2 (±6.3)	74.5 (±3.9)	50.8 (±10.5)	32.5 (±2.31)	7/8
	Controls	36.2 (±5.4)	91.0 (±6.0)	28.0 (±5.5)	36.1 (±2.50)	0/4
3	Cl-depleted alkalosis K added	40.7 (±3.5)	78.4 (±7.7)	37.9 (±7.9)	38.9 (±1.31)	8/10

* m.eq. per 100 gm. of dried fat-free muscle solids.

and was found to be confined to the middle half of the proximal convolutions (Fig. 6); no other portions of the nephron or the collecting tubules showed significant lesions except for an occasional cast in their lumens; these were evidently derived from the cellular destruction which had occurred in the proximal convolution.

The sections were scrambled and graded blindly on the basis of extent and severity of the lesion. Group 2 with severe potassium deficiency had the most severe structural changes. Both group 1, depleted of sodium chloride with no alkalosis, and group 3 with alkalosis, but given potassium, had less severe changes.

In a separate experiment a group of six animals was depleted of chloride and

made alkalotic in a manner similar to group 2 except that the dialysis contained 0.15 M NaHCO₃, not 0.3 M NaHCO₃. At the end of the 2nd day water was withheld, pitressin given, and urine collected over the next 16 hours. A control group of six animals, dialyzed and not depleted of chloride, was similarly tested. Maximal urine osmolality for the chloride-depleted group was 1910 ± 150

TABLE III

Effect of Phosphate Intake and Bicarbonate Intake in Animals with Chloride Depletion and in Control Animals

The alkalosis is less severe than that noted in Table I because 0.15 M NaHCO₃ was injected intraperitoneally whereas 0.3 M NaHCO₃ was injected in the first study.

Group		Solution	Serum			Muscle	Kidney lesion	
			CO ₂	Cl	Phos- phate	K	Cortical	Phos- phate†
			<i>mM/liter</i>	<i>m.eq./liter</i>	<i>mg. per cent</i>	<i>m.eq./100 gm. FFDS*</i>	<i>No. affected /total No.</i>	
1	Control‡	0.03 M NaHCO ₃	19.3	104.0	7.3	45.4	0/6	0/6
	Na ₂ HPO ₄ given	0.11 M NaCl 2 per cent glucose	(±2.7)	(±1.0)	(±.3)	(±1.6)		
2	Cl-depleted‡	0.15 M NaHCO ₃	33.2	87.2	6.2	36.9	6/6	6/6
	Na ₂ HPO ₄ given	2 per cent glucose	(±1.7)	(±4.0)	(±.9)	(±3.4)		
3	Cl-depleted	0.15 M NaHCO ₃	33.1	81.1	7.0	36.6	5/6	0/6
	NaHCO ₃ given	2 per cent glucose	(±2.4)	(±2.8)	(±.4)	(±1.9)		

* m.eq. per 100 gm. of dried fat-free muscle solids.

† Lesion seen in phosphate loading (7).

‡ Groups 1 and 2 given 0.15 M NaHPO₄ as drinking solution, 40 ml. per day.

|| Group 3 given NaHCO₃ as drinking solution, 40 ml. per day.

mOsm/liter and for the control group was 2170 ± 190 mOsm/liter. Although both were significantly lower than normal (2720 ± 301) the decrease in concentrating capacity of the chloride-depleted group relative to its control was of uncertain significance ($p = 0.05 - 0.01$ by t test). Muscle potassium of the experimental group averaged 36.6 m.eq. per 100 gm. fat-free dry solids (FFDS) and of the control group 44.3 m.eq. per 100 gm. FFDS.

Another study was done to observe the effects of modest phosphate ingestion in acute chloride depletion and alkalosis. Three groups of animals were pre-

pared. A control group was dialyzed with an isotonic sodium bicarbonate-chloride solution and no chloride removed. The animals received 40 ml. per day of 0.15 M Na_2HPO_4 for 3 days until killed. The second group was dialyzed using 0.15 M NaHCO_3 and thereby depleted of chloride. They also received 40 ml. per day of 0.15 M Na_2HPO_4 . A third group was dialyzed with 0.15 M NaHCO_3 and received 40 ml. per day of 0.15 M NaHCO_3 instead of Na_2HPO_4 . They served as a control group with chloride depletion. Results of changes in serum and muscle composition are indicated in Table III. Both the groups depleted of chloride developed a comparable alkalosis and a definite potassium deficiency.

On histological examination of the kidney no changes were noted in the kidneys of the group given phosphate but not depleted of chloride. Both the chloride-depleted groups had the changes in the proximal convolution already described. In the group depleted of chloride and given phosphate there occurred calcification and hyperplasia in the terminal half of the proximal convolution similar to that noted to occur with massive phosphate ingestion (2, 7). No amino-aciduria was noted by chromatographic analyses of 24 hour urine samples (10).

DISCUSSION

These studies identify acute loss of chloride as a cause of cellular damage and subsequent hyperplasia occurring in the mid-portion of the proximal convolution. This change is not dependent on alkalosis nor on potassium deficiency but there is evidence that potassium deficiency does increase its severity.

In analyzing the situation which may be responsible for the renal lesions it must be noted that factors other than disturbances of the electrolyte pattern *per se* may be involved; for example, the technique used for effecting chloride depletion also results in a reduced volume of extracellular fluid in all the experiments. In the sodium chloride-depleted group this is accompanied by a transient reduction in the concentration of chloride in the serum, whereas in the chloride-depleted group made alkalotic the reduction in concentration of chloride persists.

The reduction in extracellular fluid volume is certainly accompanied by a reduced plasma volume and a reduced renal blood flow; aldosterone excretion is very likely increased in these circumstances. Whether aldosterone excretion alone could effect the changes noted or whether a reduction in renal blood flow is also necessary remains to be determined.

The mechanism whereby potassium deficiency enhances the renal lesion is obscure. It has been noted (6) that chronic potassium deficiency alone may lower glomerular filtration rate. However this effect is not likely to be present during the 3 days for which the present experiment lasted.

It is interesting to note that there were no lesions in the collecting tubule in

any experimental group even though potassium deficiency as shown by muscle analysis was severe in group 2. This accords with previous observations that the collecting lesions develop more as a function of duration than of degree of potassium deficiency (6). It is also interesting that in the absence of the structural lesion in the collecting tubules little if any defect in urine-concentrating capacity due to chloride depletion could be elicited even though potassium deficiency coexisted.

The relation of the lesion of chloride depletion, as we have described it, to the lesions noted in the proximal convolutions of potassium-deficient rats raises the question whether they may have the same origin and significance. Not only is the location in the nephron the same but the characteristic cellular alterations—swelling, disintegration, and regenerative hyperplasia—are similar (see reference 1, Figs. 11 to 13). The lesion is pronounced in chloride-depleted rats, but variable and often absent in potassium-deficient rats.

As previously stated in the paper, the experimental design of our earlier work included a diet low in chloride content. In many of the studies in the literature in which proximal tubule damage is reported a coexistence of varying degrees of chloride depletion seems to have been likely. In the light of the observation that potassium deficiency exaggerated the lesion of chloride depletion, we conclude therefore that the lesion in the proximal convolution is more likely to be causally related to a deficit in chloride rather than in potassium.

The effect of phosphate ingestion further complicates the problem of the significance of the lesion in the proximal convolution, for in potassium deficiency and in acute chloride depletion with alkalosis, the striking changes characteristic of phosphate excess were noted to occur with intakes of phosphate well tolerated by normal animals. What part phosphate may be playing in the general insult to the proximal convolution in the "typical" experiment of electrolyte imbalance is obscure, though in our experience its occurrence can be recognized by the presence of calcification in the tubular lesion. It is clear, however, that knowledge of the *complete* chemical pattern of the electrolyte imbalance will be necessary for interpretation of the origin of the proximal lesion in any given circumstance.

Discussion of the lesions in the kidney tubules in states of experimental electrolyte imbalance in rats opens the question as to the relation of these lesions to those described as occurring in the tubules of humans who were assumed on the basis of clinical evidence to be suffering from a "potassium deficiency"; the clinical origin of such states is widely varied and includes aldosterone-producing tumors.

Our experience with human material has been limited but in general confirms the wider experience of others. We have examined 6 cases of clinical "kaliopenic nephropathy" in which the concentration of potassium in serum was noted over a range of 2.5 to 3.4 m.eq./liter and in which autopsy material

permitted the examination of the entire range of architectural change from the surface of the kidney to the papillary tip, an essential requirement which is seldom if ever found in biopsied specimens. The clean-cut vacuoles in the proximal convolution which have been described as "typical" of potassium deficiency (11) were present in 2 cases, a diffuse swelling and indefinite "vacuolization" in 3, and in 1 the proximal convolutions appeared normal. None of the cases showed the hyperplastic lesions in the outer zone of the medulla and 1 showed severe droplet formation in the terminal collecting ducts and in the epithelium covering the papilla.

Evaluation of the findings in man is subject to many qualifications such as: (a) the unknown duration of the hypokalemic state; (b) the possible effects of therapy, for in the experimental animals the lesions are known to disappear in 24 hours on correction of the electrolyte imbalance; and (c) the presence of a complicating renal fibrosis ("pyelonephritis") in most of the kidneys we have examined which may have obscured or modified the lesions in the medullary tubules. Since we have never observed the clean-cut vacuoles in the proximal convolutions of several hundred examples of experimental potassium deficiency in the rat, it would seem that their presence in man constitutes a species peculiarity and that the lesions in the proximal convolutions of the two species are analogous but not identical.

The droplets seen in the collecting tubules in chronic potassium deficiency have been seen in 1 of the human cases as noted above. We have also seen the droplets in a monkey on a potassium-deficient diet for 29 days (muscle potassium 33.9 vs. 42.0 m.eq. per 100 gm. FFDS in the control) (12). This kidney, in addition to the droplets, had the generalized hyperplasia of the collecting tubular epithelium and the specific hyperplasia of the intercalated cells also noted in potassium-deficient rats. There were no changes in the cells of the proximal convolution.

The studies to date show that in rats various specific structural renal changes are associated with correspondingly specific patterns of electrolyte imbalance; *i.e.*, potassium deficiency (1), phosphate ingestion (7), and chloride depletion (with or without sodium). There are interdependent relations of these states; *e.g.*, potassium deficiency results in the lesion associated with excess phosphate ingestion appearing more readily (2), and it appears to increase the severity of change noted in acute chloride depletion. The total pattern in any one instance thus may be analyzed into its characteristic elements. This procedure can be applied in analyzing the effects of other variables that affect renal architecture directly or indirectly. Aldosterone excess is one such example. Does it exert its effects on renal architecture and function directly or through the electrolyte imbalances usually associated with it?

CONCLUSIONS

Acute chloride depletion in rats is associated with the occurrence of an extensive cell damage in the mid-portion of the proximal convolutions which is followed by an excessive hyperplastic reaction of the renal epithelium; no other significant lesions were found by microdissection in either the tubules of the nephrons or the collecting system.

Potassium deficiency is not essential to the development of this lesion but does increase the severity of the reaction.

As in the case of potassium deficiency, chloride depletion predisposes to or exaggerates the structural alterations that accompany excess phosphate intake.

The relations of the different structural changes in renal architecture that occur in various states of electrolyte imbalance are discussed as well as the relation of the lesions seen experimentally in the rat and monkey and clinically in man.

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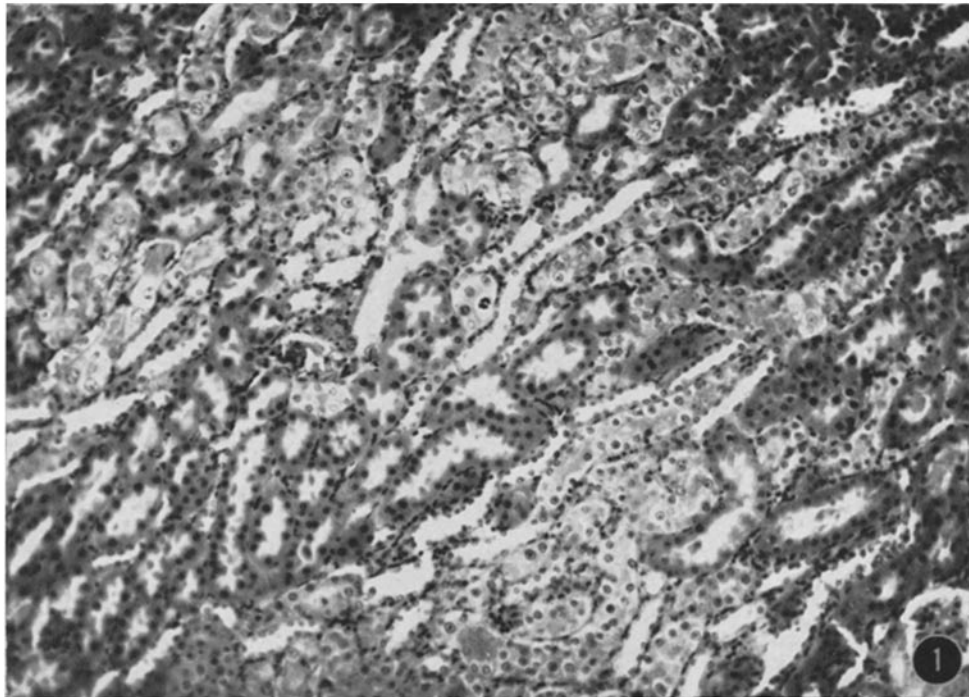
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EXPLANATION OF PLATES

FIGS. 1 to 5. Fixation in Zenker's solution with hematoxylin and eosin stain.

PLATE 98

FIG. 1. The lesion in mid-cortex; clusters of greatly swollen, light staining tubules are seen on a background of dark staining, normal proximal convolutions. Magnification $\times ca.$ 100.



(Holliday *et al.*: Renal lesions)

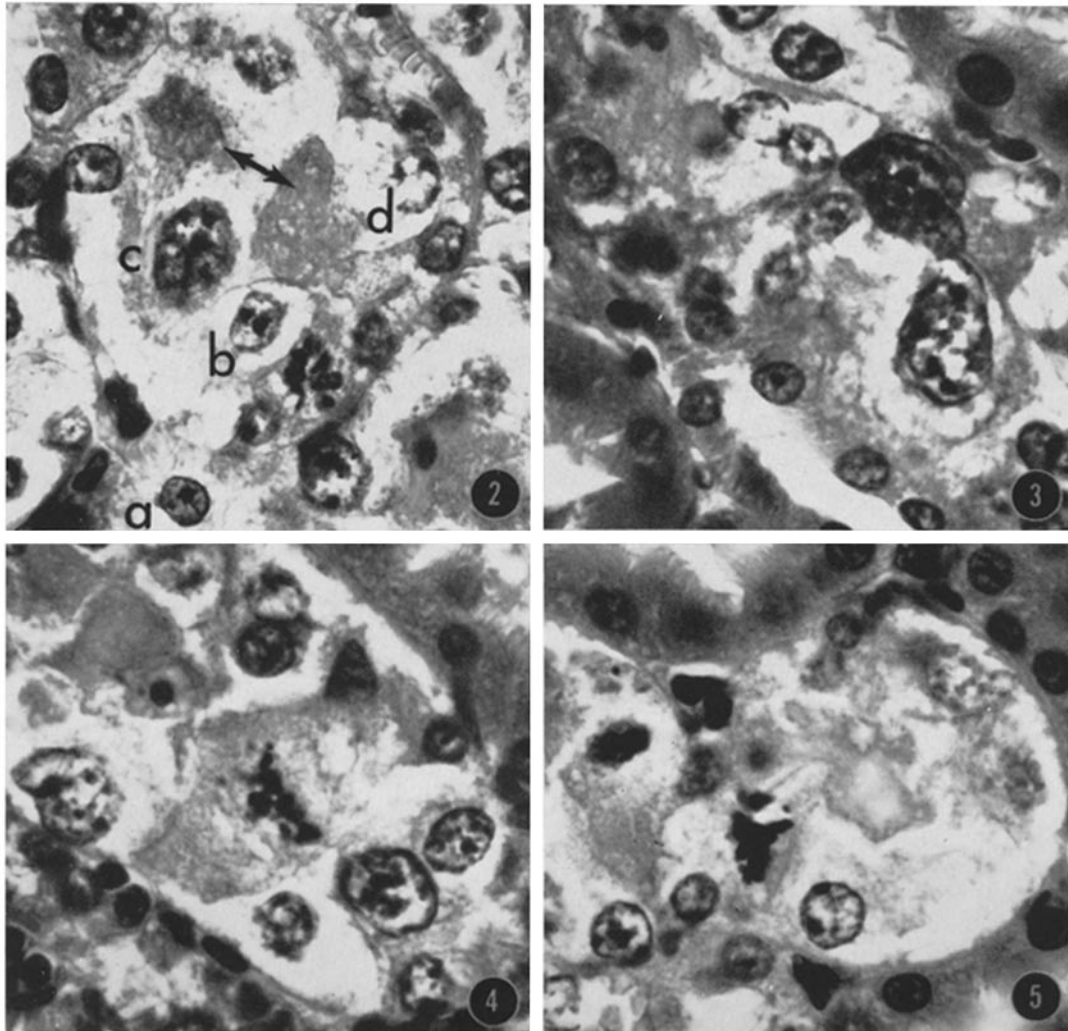
PLATE 99

FIG. 2. Cross-section of a swollen proximal convolution showing the detail of the cellular lesion. At the lower edge (*a*) is a somewhat swollen epithelial cell of the neighboring tubule, the nucleus of which is relatively normal in size and chromatic configuration. The cells of the more severely affected tubule above show various degrees of swelling and cytoplasmic disintegration. The nuclei present the same essential type of lesion, *i.e.*, swelling to the point of disintegration, $b < c < d$ and the huge equatorial plate of a mitotic figure is visible. At \updownarrow masses of eosinophilic material are seen. Magnification $\times ca.$ 1000.

FIG. 3. Two huge "megalokaryocytic" epithelial nuclei are seen among the variously damaged tubular cells. Magnification $\times ca.$ 1000.

FIG. 4. A gigantic epithelial cell with a huge hyperchromatic equatorial plate. Magnification $\times ca.$ 1000.

FIG. 5. Two hyperchromatic mitotic figures; that to the right is tri-polar. Magnification $\times ca.$ 1000.



(Holliday *et al.*: Renal lesions)

PLATE 100

FIG. 6. A typical proximal convolution stained with iron hematoxylin. The normal pattern would show an even gradient of darkening due to deep-stained mitochondria, decreasing from the glomerulus downwards. Scattered along the mid-portion of this convolution are moth-eaten patches of tubular swelling and epithelium disintegration. Magnification $\times ca.$ 60. Fixation in 10 per cent formalin.



(Holliday *et al.*: Renal lesions)