THE RENAL LESIONS OF ELECTROLYTE IMBALANCE*

II. The Combined Effect on Renal Architecture of Phosphate Loading and Potassium Depletion

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In 1935 MacKay and Oliver (1) observed that the ingestion by rats of a diet containing an excess of inorganic phosphate in the form of orthophosphoric acid, acid, basic or neutral sodium, or potassium phosphate resulted in severe alterations in renal architecture; later studies by means of microdissection (2) showed that the lesion originated as a necrosis of the epithelium of the terminal medullary portion of the proximal convolutions and of isolated segments of tubule in the ascending limbs of Henle loop; calcification was frequently associated with the cellular damage. The phosphate ingested in these experiments was of the order of 4 to 5 mM per weanling rat per day; the changes occurred independently of the cation given with the phosphate or the pH of the salt used. It was later noted by Holliday and Schultz (3) that in potassium deficiency with acute alkalosis a much more severe renal architectural disarrangement occurred with phosphate loading than in its absence.

Because of these findings an experiment was designed to determine in what manner phosphate loading and potassium deficiency might mutually influence each other in the production of renal alterations.

Methods

Four groups of 10 male Sprague-Dawley rats weighing between 300 and 370 gm. were placed on a basal diet previously described (4) which was deficient in sodium, potassium, and chloride and low in phosphorus, for a period of 30 days, each group receiving up to 50 cc. per day of a supplemental drinking solution containing the electrolytes indicated in Table I.

At the end of 30 days the animals were deprived of drinking water for 24 hours, anesthetized

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with sodium hexobarbital, exsanguinated from the abdominal aorta, and blood and thigh muscle obtained for analysis. The kidneys were weighed and tissues placed in 10 per cent neutral formalin and in Zenkers' solution for fixation. Histological sections were prepared and stained with hematoxylin and eosin; microdissection and staining of individual nephrons followed the usual procedure (5). The chemical methods used in serum and tissue examinations have been described previously (4).

RESULTS

Biochemical Alterations

In Table II are seen two contrasting patterns of serum and muscle constitution. The first, comprising groups B and C, shows the typical alterations of potassium depletion, *i.e.*, alkalosis, hypokalemia, and a reduction in muscle potassium comparable to that described in many previous studies (6, 7); in the second (groups A and D), these alterations are absent. In group A all values are normal and it thus serves as a general control for the experiment;

TABLE I

Electrolyte Composition of the Supplemental Drinking Solution

Group	Na	K	Cl	HCO_3	PO_4
	mM/liter	*mM/liter*	*mM/liter*	*mM/liter*	*mM/liter*
A. Control	30	30	30	25	5
B. K-deficient	135	—	—	135	—
C. K-deficient phosphate-loaded	135	—	—	—	75*
D. Phosphate-loaded	135	30	30	—	75*

* Na_2HPO_4—NaH_2PO_4 buffered to pH 7.4.

in group D, which also shows no evidence of potassium depletion, all serum and muscle constituents are essentially unchanged, although the animals had received the same amount of phosphate as group C, *i.e.*, 3.75 mM per day for 300 to 370 gm. rats; in relation to their body weight this was much less than was used in the MacKay-Oliver experiments. It produced no change in the weight of the kidney in contrast to the moderate effect noted in the potassium-depleted group B and the much greater increase in the potassium-depleted, phosphate loaded group C.

In summary, the phosphate load of the experiment produced in itself little or no significant alteration of serum and tissue constituents or in the size of the kidney; judging from the blood urea nitrogen (BUN) renal function was not greatly affected. Potassium depletion resulted in typical disturbances of serum and tissue constituents with some increase in the size of the kidney and a moderate elevation of BUN; when the two electrolyte disturbances were combined the kidneys were greatly enlarged and a greater elevation of BUN was observed.

The question thus presents itself, did a relatively innocuous load of phosphate produce an aggravation of the renal lesions that are known to accompany potassium depletion? Or is the converse true and may the graver renal damage observed in group C be the result of an intensification of the phosphate lesion described by MacKay and Oliver? Or are we dealing with simple additive lesions of potassium depletion and phosphate load?

The last question seems answered by the fact that the dosage of phosphate which was used in group D produced no effect by itself; the former should

TABLE II

Chemical Analysis of Serum and Tissues

Group		Na	Cl	K	CO_2	BUN‡	Phosphate	Muscle K FDS§	Kidney weight per 100 gm. rat
		m.eq./liter	*m.eq./liter*	*m.eq./liter*	*m.M/liter*	*mg./per cent*	*mg./per cent*	*m.eq./100 gm.*	
A. Control (10)	Mean	135.9	103.6	4.13	26.1	19.2	6.48	45.8	0.747
	S.E.	±0.8	±1.7	±0.22	±0.7	±1.3	±0.16	±0.5	±0.013
B. K-deficient (10)	Mean	129.5	83.4	2.90	40.3	30.6	5.34	36.8	0.913
	S.E.	±2.2	±2.1	±0.19	±1.8	±4.2	±0.66	±1.0	±0.027
C. K-deficient (8) Phosphate load	Mean	134.9	85.3	2.76	39.1	41.5	5.21	36.0	1.586
	S.E.	±2.0	±3.7	±0.12	±1.9	±5.9	±0.22	±1.6	±0.175
D. Phosphate load (10)	Mean	135.0	94.7	3.83	25.4	21.2	6.50	44.8	0.704
	S.E.	±2.2	±3.3	±0.24	±0.9	±3.1	±0.35	±0.8	±0.018

Nos. in parentheses indicate number of rats in each group.

* Total CO_2.

‡ Blood urea nitrogen.

§ Fat free substance.

yield to an examination of the nature of the renal lesion in group C since the structural changes caused by the two factors, potassium depletion and phosphate loading, have been shown to differ both in their character and their localization in the nephrons. In potassium depletion the collecting tubules show sharply localized and specific structural alterations (8), hyperplasia, and proliferation of intercalated cells in the outer zone of the medulla and intracellular accumulation of material in the form of colloid droplets (9) in the inner zone. In the nephrons proper the tubules show little structural changes other than a moderate swelling and vacuolization in the mid-portion of an occasional proximal convolution. The lesion of phosphate loading is quite different (2), consisting of an epithelial necrosis in the terminal medullary segment of the

proximal convolution and in isolated segments of tubule scattered along the course of the ascending limb of Henle's loop; the necrosis is commonly associated with calcification; the collecting tubules are not affected.

Structural Changes

Gross inspection of the kidneys of the four groups showed definite abnormalities only in group C. These large kidneys had a pale, brownish-yellow color and mottled granular surfaces. When cut in their long axis a dark red stripe was noted at the cortico-medullary junction and, contiguously, on its papillary side, a grey-white line composed of parallel radial streaks.

Histological examination showed normal renal structure in the control group A. In the potassium depleted group (B) the typical lesions of this condition were present in all animals; colloid droplets were seen in the epithelial cells of the collecting tubules in the inner zone and intercalated cell hyperplasia in the outer zone of the medulla. The cortical structures were well preserved, showing only an occasional dilated tubule and even more rarely a group of cross-sections of a proximal convolution in which swelling and vacuolization were present.

In the kidneys of the potassium-depleted, phosphate-loaded group C, extensive architectural changes were present in histological section. Throughout the cortex were greatly dilated, cystic tubules (Fig. 1), while below, in the subcortical region, the outer stripe of the outer zone was completely transformed by alterations in the tubules (Fig. 2). The lesion consisted in epithelial necrosis, the lumens of the distorted tubules being filled with masses of debris,—which in many places stained the deep blue of calcification,—and a marked tissue reaction which took the form of both atypical regeneration of the epithelial walls of the tubules and proliferation of fibroblastic tissues between them; in some places the effect of a centrally placed necrotic tubule with its surrounding connective tissue was to give the appearance of a "granulomatous" lesion (Fig. 3). So pronounced were these changes that it was impossible to identify the type of tubules involved in the broad area of architectural alteration (Fig. 2). Occasionally, however, in a cross-section of collecting tubule in the less involved areas there could be seen the hyperplastic proliferation, with an excessive number of intercalated cells, of the typical lesion of potassium depletion. The inner zone of the medulla was free of structural change except for the presence of colloid droplets in the collecting tubules and the occurrence of an occasional isolated, completely necrotic, narrow tubule (Fig. 4).

In dissected specimens the distribution of the lesions in the nephron, the identity of the affected tubules and the relation of the alterations in the subcortical zone to the cystic dilatation in the cortex were revealed. In Figs. 5 to 8 are seen the necrosis, calcification, and regenerative proliferation of atypical epithelium that transform the terminal medullary segments of proximal convolutions which lie in the severely altered subcortical zone.

The incipient form of the alteration is shown in Fig. 5; the first half of the convolution is essentially unchanged, while the terminal half is swollen, presenting a number of areas (arrows) where the normal cellular pattern is disturbed and where

desquamation of necrotic cells forms clumps of debris in the tubule lumen (Fig. 6 A). In Figs. 7 and 8 is seen a more severely affected example. Swelling of the entire convolution is evident and the areas of necrosis are more numerous; the marked swelling of the individual cells of the tubular epithelium and the transition to necrosis is particularly evident in Figure 8 A (arrow). The convolution ends with a complete destruction of its normally tapering tip, which is now represented by a mass of cellular debris surrounded by proliferating, intertubular, fibrous tissue so firmly adherent to the tubule wall that it could not be removed from the dissected specimen; within the debris bluish-black stained areas of calcification are evident (Fig. 8 B). The destroyed tubule and surrounding fibrous proliferation thus show the structure of what in the histological section appears to be a "granulomatous" lesion (Fig. 3).

In Figs. 6 B, C, and D are shown the curiously isolated segment-like areas of necrosis which are scattered along the course of the broad ascending limb of Henle's loop. There may be one or several in a single limb; in the more severe examples there is calcification of the necrotic debris and surrounding interstitial fibrous proliferation.

Contrasting to the lesions so far described, all of which have appearance of "primary" effects of some deleterious action, are changes involving all portions of the nephron; these, though producing severe architectural alterations in the kidney, seem to be definitely the "secondary" consequences of a disturbance of the continuity of the nephrons. The more obvious alteration (Fig. 1), a pronounced feature of the lesion as it appears in the histological section, is dilatation of tubules; in Fig. 9 a greatly dilated proximal convolution is shown, an effect that might be anticipated as a result of destruction of the tubule lumen at some lower point in its course. Conversely, other nephrons show an irregular atrophy of various portions of their tubule (Fig. 10), presumably a counterpressure effect of their greatly dilated neighbors. It is the combination of these two types of tubular distortion that accounts for the general alteration of cortical architecture.

In contrast to the severe architectural alterations of group C, the sections of kidneys from the equally phosphate-loaded group D, which was not potassium-depleted showed little abnormality. The architecture of cortex and medulla were essentially normal; there was no general dilatation of tubules in the cortex. A considerable search was needed to reveal an occasional necrotic, terminal segment of a proximal convolution, and there was no calcification of the scant cellular debris.

DISCUSSION

The changes in serum electrolyte and muscle potassium require brief comment. The fact that the phosphorus load in the control group D had no demonstrable effect on electrolyte composition, or on real architecture, does not conflict with the earlier findings of MacKay and Oliver (1) because of the difference in the load in relation to weight and age in the two experiments. The development of classical findings of potassium deficiency was to be expected in both groups B and C. Previous workers, using potassium-deficient diets, have employed both a high and low phosphorus intake without demonstrably different results in serum or tissue electrolyte composition.

The elevated urea nitrogen in groups B and C may reflect a reduction in

glomerular filtration rate, greater in the phosphorus-loaded group. Although no glomerular alterations were noted histologically in either group, obstructive tubular lesions and cystic dilatation of proximal convolutions were present in both, and greatest in the group C with the highest urea nitrogen concentration.

Hypophosphatemia of comparable degree was noted in both potassium-deficient groups, despite the fact that group C had a high phosphorus intake. However, the higher phosphorus intake resulted in greater alterations in tubular structures, so that the possible effects on phosphorus excretion and reabsorption are obscured. Hypophosphatemia and phosphaturia have been reported to coincide with potassium deficiency and to be corrected with its repair in a patient with potassium-losing renal disease (10). Hypophosphatemia is frequently associated with aminoaciduria and glycosuria in a variety of conditions that have other effects on the proximal convolution (11). However, amino acid excretion was not increased in the patient just mentioned and was not measured in these studies. At this point, therefore, the finding of hypophosphatemia may simply be noted as an interesting finding.

The structural lesions in the kidneys of the potassium-depleted rats which had also received phosphate (group C) were predominantly those previously described as an effect of a simple phosphate load (1); they were in fact as severe as those noted in the earlier experiments of MacKay and Oliver, in spite of the lesser phosphate load and hence obscured the specific alteration of potassium depletion.

The occurrence of damage to the ascending limb of Henle's loop in phosphate-loaded rats merits particular mention. This portion of the nephron, though commonly involved secondarily in general disturbances of circulation or interstitial fibrosis, which may cause indiscriminate damage to all parts of the nephron, rarely shows primary specific localized alteration; moreover, the pattern of the damage in the phosphate lesion is unusual and distinctive, segmented areas of necrosis alternating with stretches of intact tubule. There is therefore a striking contrast with what commonly occurs in the proximal convolution where primary localized damage is frequent and presumably related to the reabsorptive function of this segment of the tubule. The significance of these contrasts in behavior is not at present clear but they may be reflections of differences in the functional capacities of the two segments of the nephron.

In a recent report Selye and Bajusz (12) have described the interrelations of the ill effects of various combinations of ion deficiency and excess. In their evaluation of lesions in the kidney they make no qualitative distinction between different sorts of renal damage; the general term "nephrocalcinosis" is used to describe both the changes resulting from potassium deficiency and phosphate loading. From this premise that the renal lesions are similar and the observation that the specific cardiac lesions of potassium depletion are increased by phosphate loading they conclude that the latter has exaggerated generally the ill-

effects of potassium deficiency. In our experiments a separation of the renal lesion into its structurally specific component elements indicates rather that in this organ it is the potassium depletion which has aggravated the effect of the phosphate load, since the alterations produced by the combination of potassium depletion and phosphate load were predominantly those peculiar to the latter.

This interpretation in no way prejudices but rather confirms and amplifies the main conclusion of Selye and Bajusz as to the complexity of the interrelations in the influence of various ions in the syndrome which by oversimplification is usually ascribed to "potassium deficiency." For whereas in their experiments phosphate loading increased the *cardiac* damage due to potassium deficiency, in ours potassium depletion clearly augmented the renal lesions of phosphate loading. Converse ill effects of electrolyte imbalance (potassium depletion-phosphate load) are therefore to be seen in the two organs, a paradox which may be explicable by the action of the kidney in modifying the relations of the various electrolytes within its tissues.

SUMMARY

Potassium depletion in rats augments the specific *renal* lesions of phosphate loading.

Since it has been shown that phosphate loading increases the specific *cardiac* lesions of potassium deficiency (12), it is concluded that the similar types of electrolyte imbalance (potassium depletion-phosphate load) act conversely in the two organs.

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EXPLANATION OF PLATES

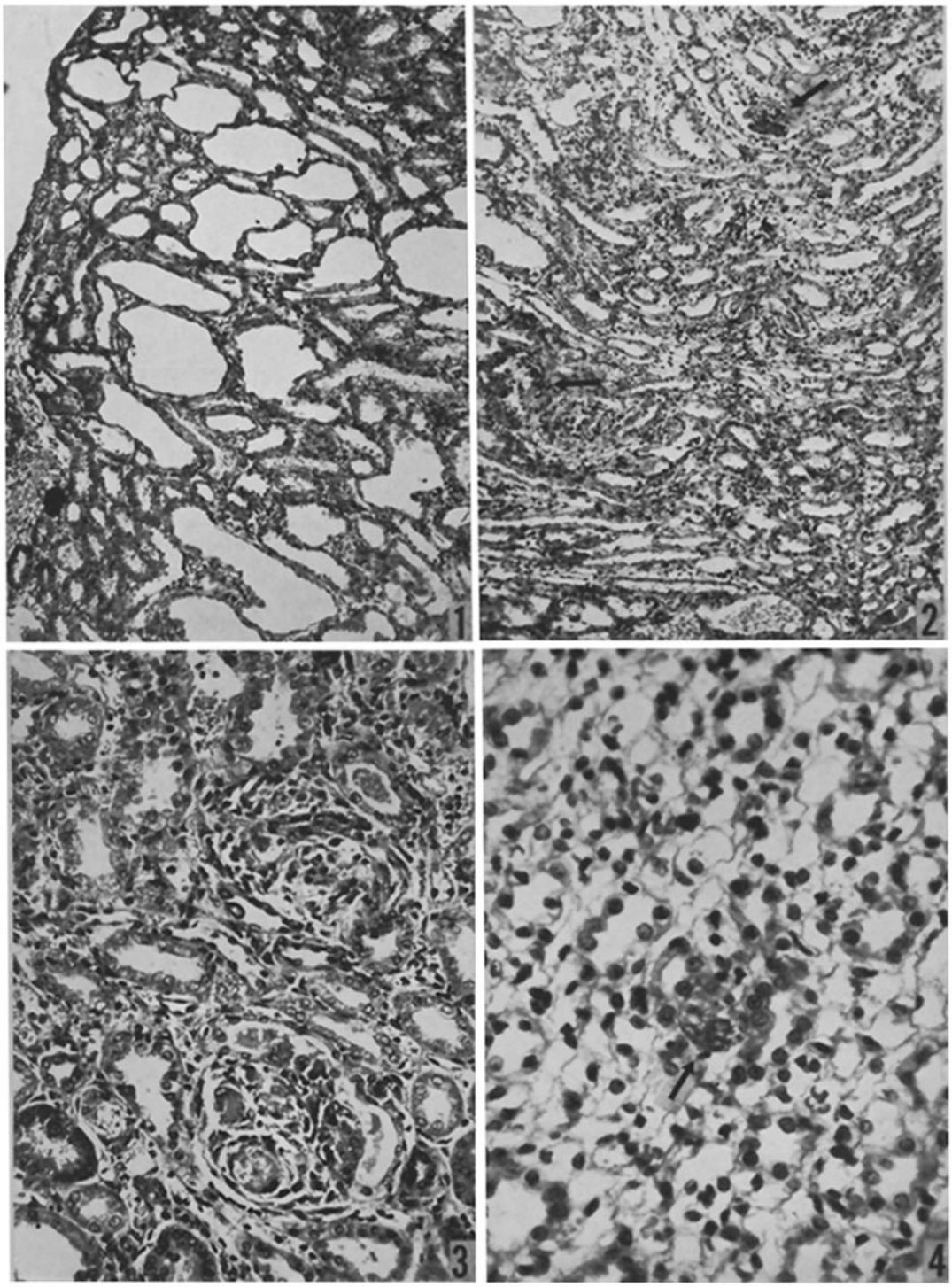
PLATE 13

FIG. 1. Architectural transformation in the renal cortex of a potassium-depleted, phosphate-loaded rat: note irregular cystic dilatation of some tubules and atrophy of others. *Cf.* Figs. 9 and 10 for these changes in the dissected specimens. Zenkers' fixation. × *ca.* 80.

FIG. 2. Alterations in the subcortical zone of the same kidney. Marked distortion of all tubules with both dilatation of lumens as well as atrophic compression. There is an extensive proliferation of intertubular connective tissue and scattered areas of calcification (arrows) of necrotic tubules. *Cf.* Figs. 1 in reference 8 for comparison of the extreme architectural alterations noted in this and the preceding figure with the relatively slight changes that result from simple potassium depletion. Zenkers' fixation. × *ca.* 80.

FIG. 3. Two "granulomatous" lesions in the subcortical zone of a similarly treated rat. Complete destruction of tubule; atypical regenerated epithelial cells have formed "giant-cell"-like complexes. *Cf*, Fig. 8 B for the appearance in a dissected specimen which shows the "granuloma" to be the terminal portion of a proximal convolution. Zenkers' fixation. × *ca.* 150.

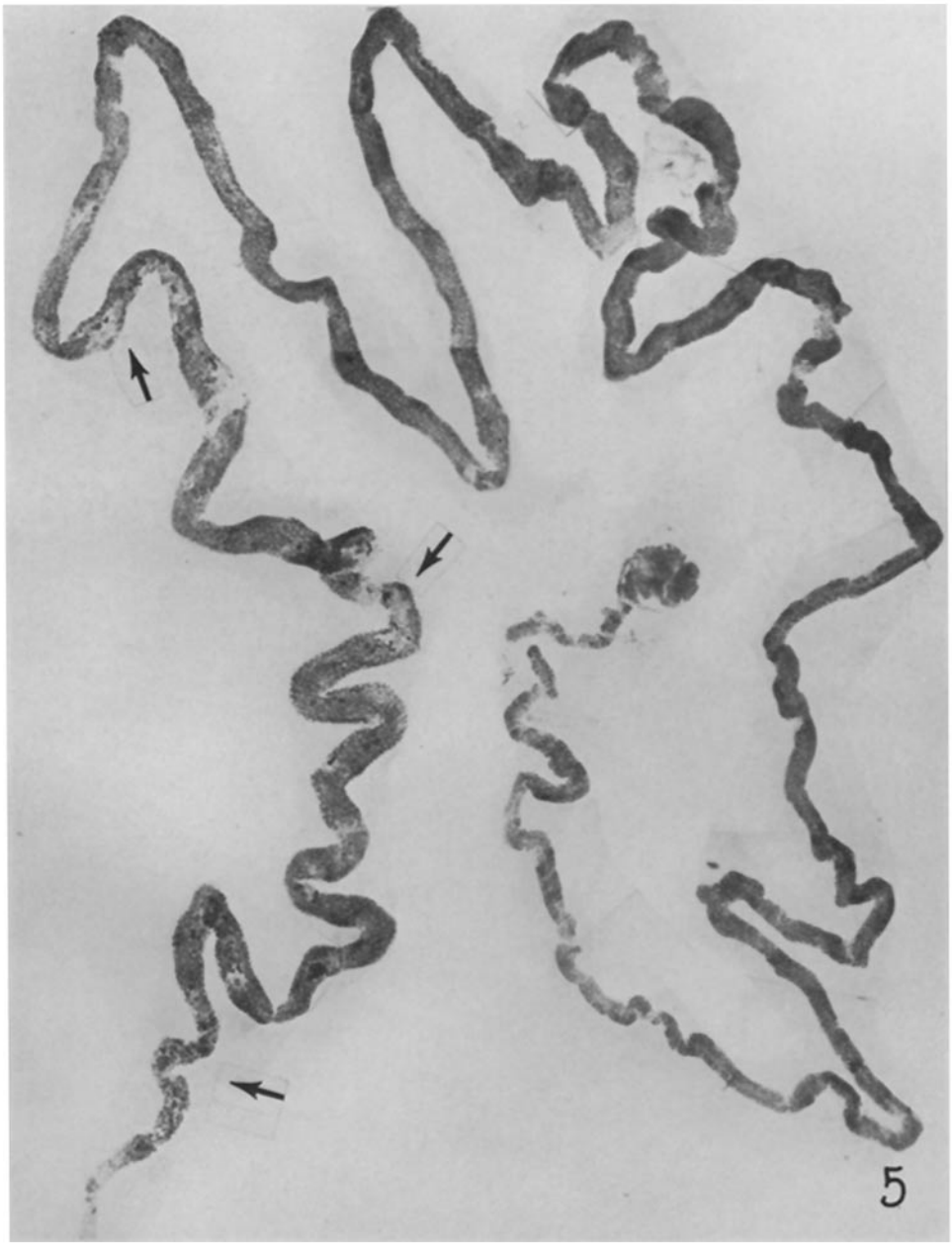
FIG. 4. Cross-section of a single tubule lying deep in the inner zone of the medulla; it is completely necrotic, partially calcified and surrounded by tissue reaction; the sections of all the tubules about it are normal. *Cf.* Figs. 6 B, C, and D which show the section to have passed through one of the isolated areas of damage in the ascending limb of Henle's loop. Zenkers' fixation. × *ca.* 150.



(Holliday *et al.*: Renal lesions of electrolyte imbalance. II)

PLATE 14

FIG. 5. Glomerulus and complete proximal convolution from the kidney of a rat of group C (potassium depletion with phosphate load). The first third of the convolution is normal; note its diameter; the remainder is swollen, and at the arrows is seen cellular damage which is most pronounced in the tip of the convolution. Formalin fixation. × *ca.* 55.



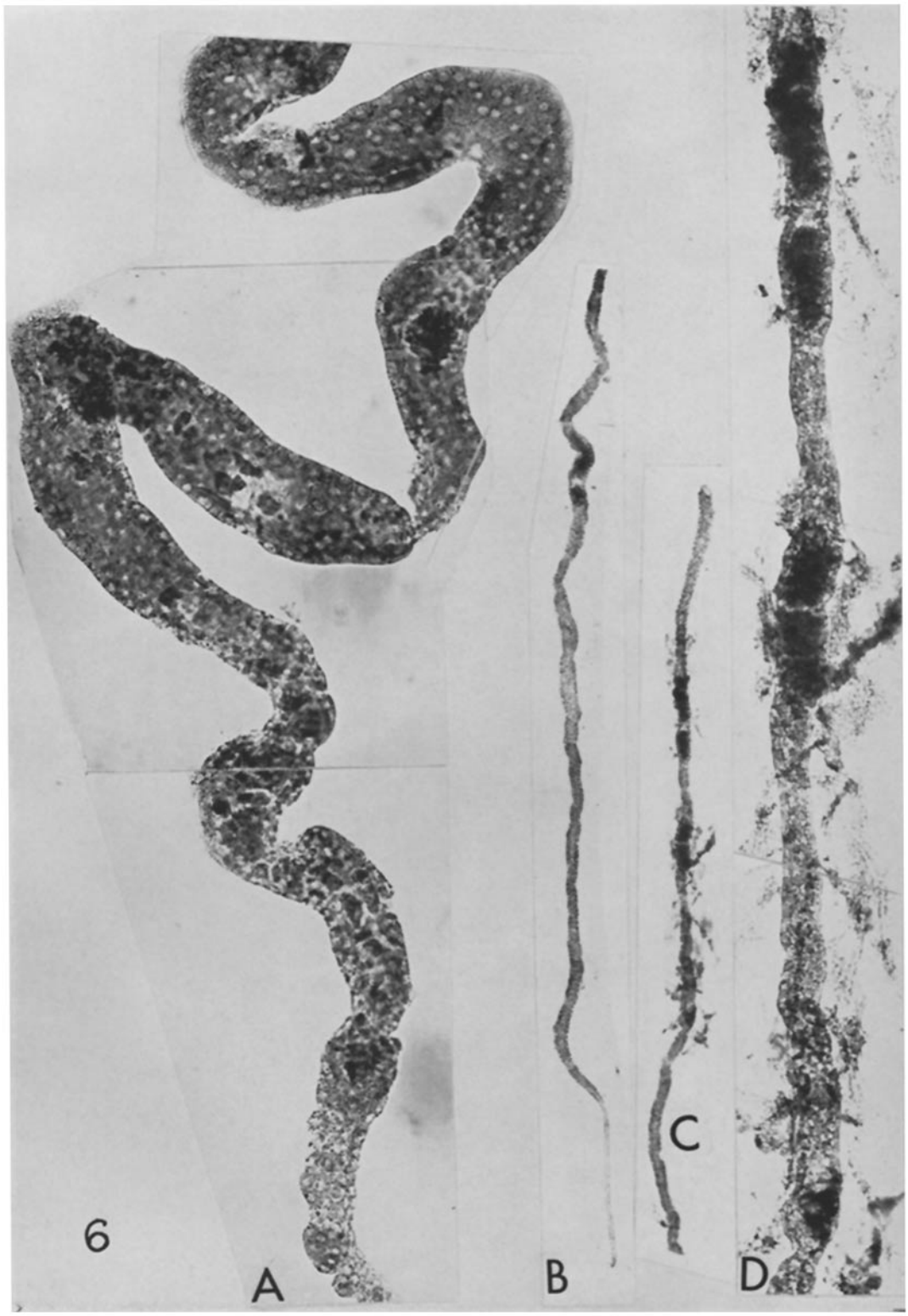
(Holliday *et al.*: Renal lesions of electrolyte imbalance. II)

PLATE 15

FIG. 6 A. High power of the lesion in the tip of the convolution shown in the preceding figure. On a background of normal epithelium (unstained nuclei and dark cytoplasm) are seen scattered areas of cellular necrosis forming irregular masses of deeply stained debris. The tip of the convolution is almost completely necrotic. Formalin fixation. × *ca.* 190.

FIGS. 6 B, C. Ascending limbs from the same kidney showing varying numbers of segmental lesions where the lumen of the tubule is filled with necrotic debris; between them the tubular epithelium is intact. Formalin fixation. × *ca.* 55.

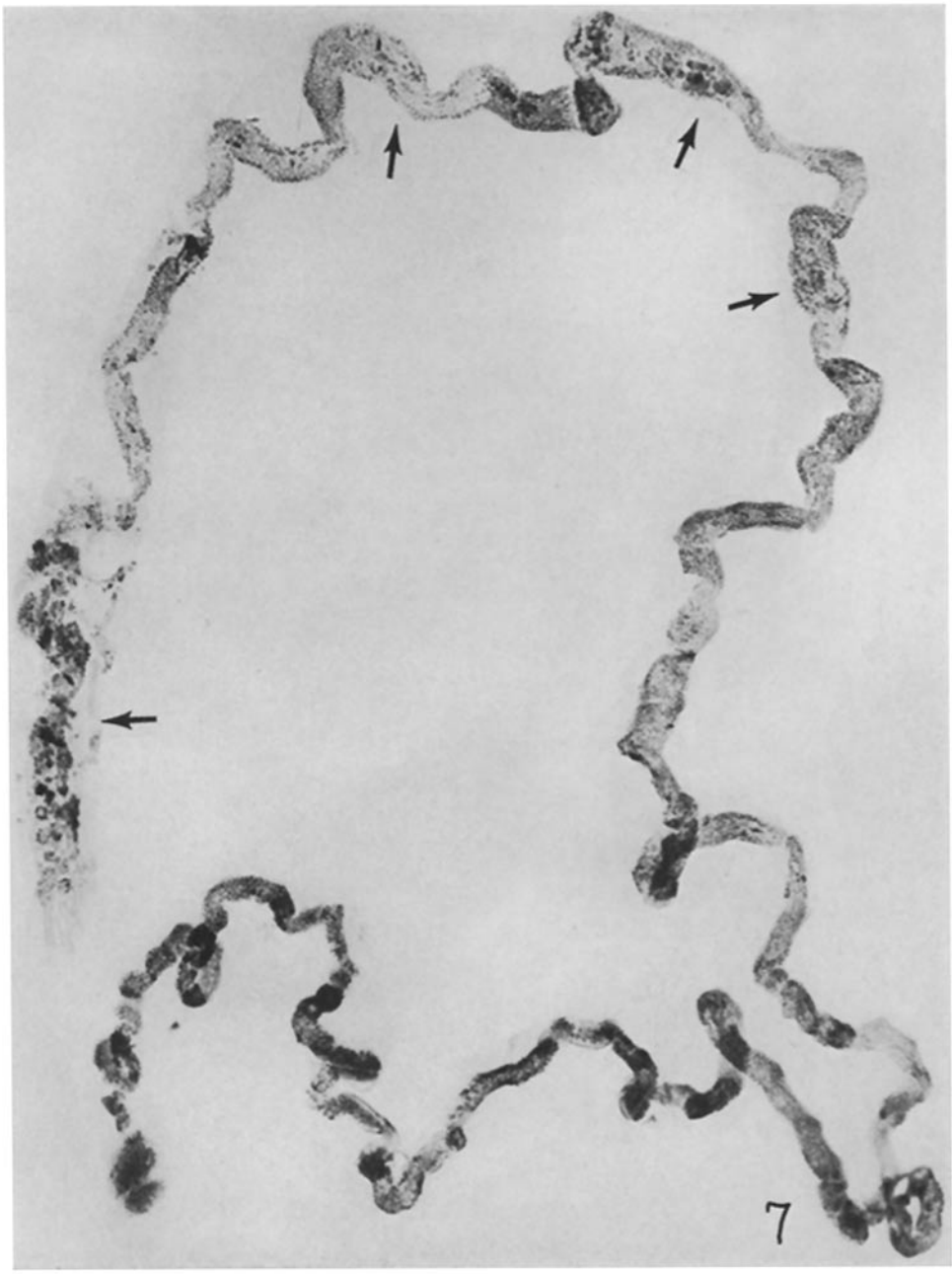
FIG. 6 D. High power of tubule in Fig. 6 C; the necrosis and obliteration of the lumen are evident; surrounding the tubule are adherent strands of dense connective tissue which could not be removed by dissection. Formalin fixation. × *ca.* 190.



(Holliday *et al.*: Renal lesions of electrolyte imbalance. II)

PLATE 16

FIG. 7. A complete proximal convolution from the same kidney showing extreme lesions. Note the general swelling of the tubule as compared with that seen in the lesser involvement of Fig. 5. There are scattered areas of cellular necrosis (arrows) and the end of the convolution is entirely destroyed. Formalin fixation. × *ca.* 55.

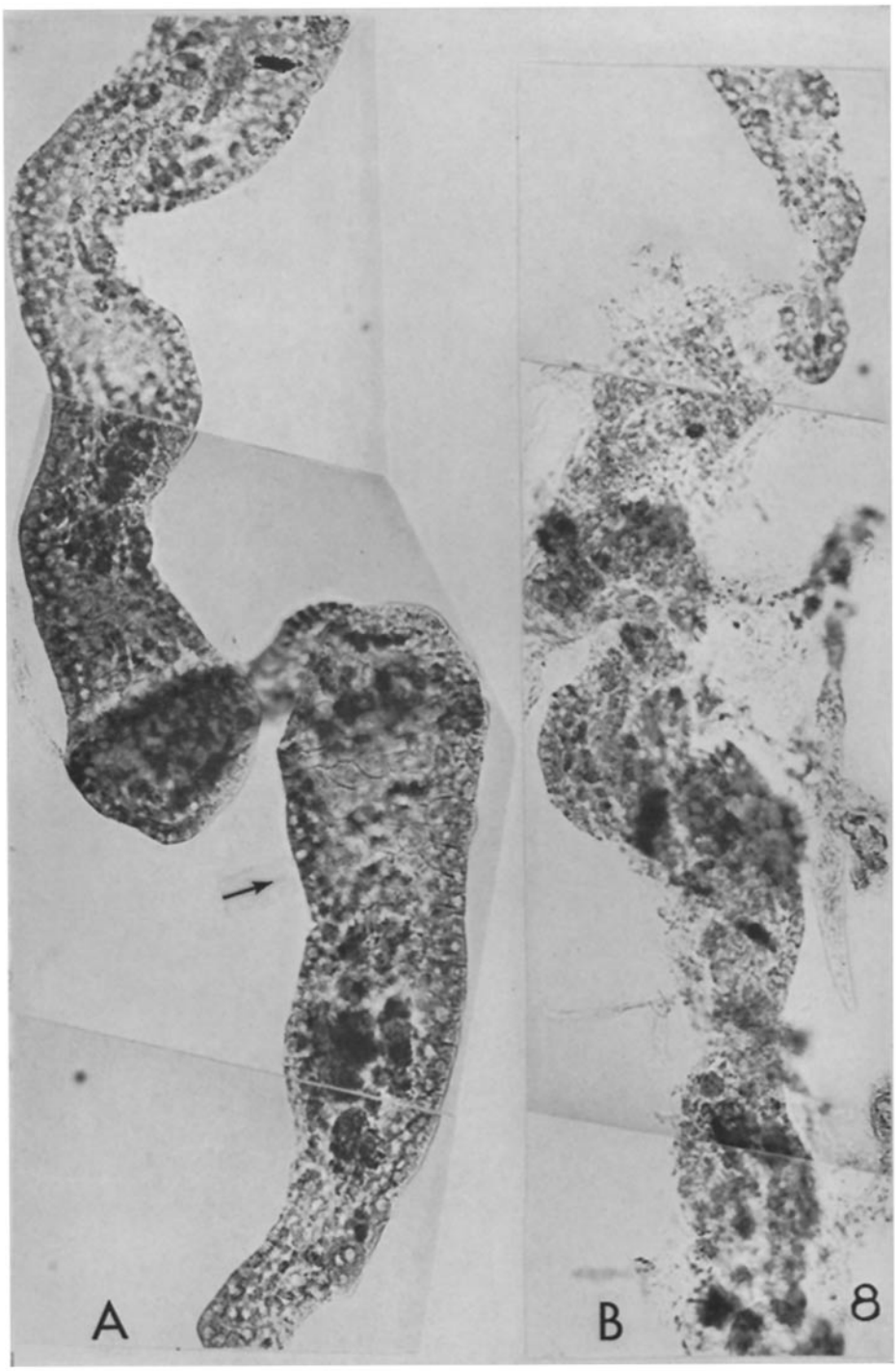


(Holliday *et al.*: Renal lesions of electrolyte imbalance. II)

PLATE 17

FIG. 8 A. High power of the lesion at top of Fig. 7 showing (arrow) the progress of the epithelial lesion from marked swelling of individual cells to necrosis and the formation of cellular debris which gives the deep black staining reaction of calcification. Formalin fixation. × *ca.* 190.

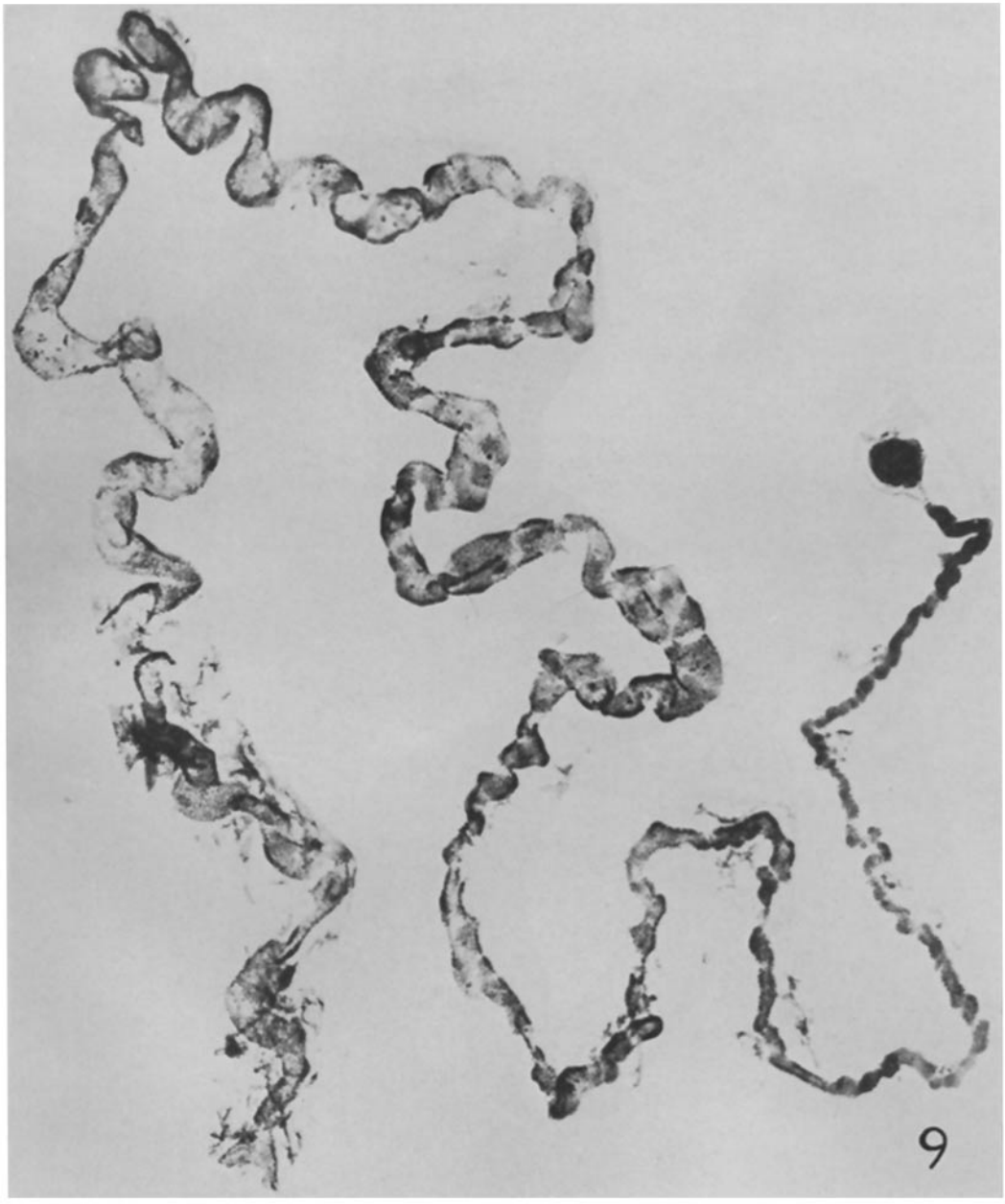
FIG. 8 B. Detail of the complete destruction and calcification of the end of the convolution shown in Fig. 7. The tubule is surrounded by adherent fibrous tissue. It is histological cross-sections of such disintegrated tubules that give the appearance of the so-called "granulomatous" lesion (*cf.* Fig. 3). Formalin fixation. × *ca.* 190.



(Holliday *et al.*: Renal lesions of electrolyte imbalance. II)

PLATE 18

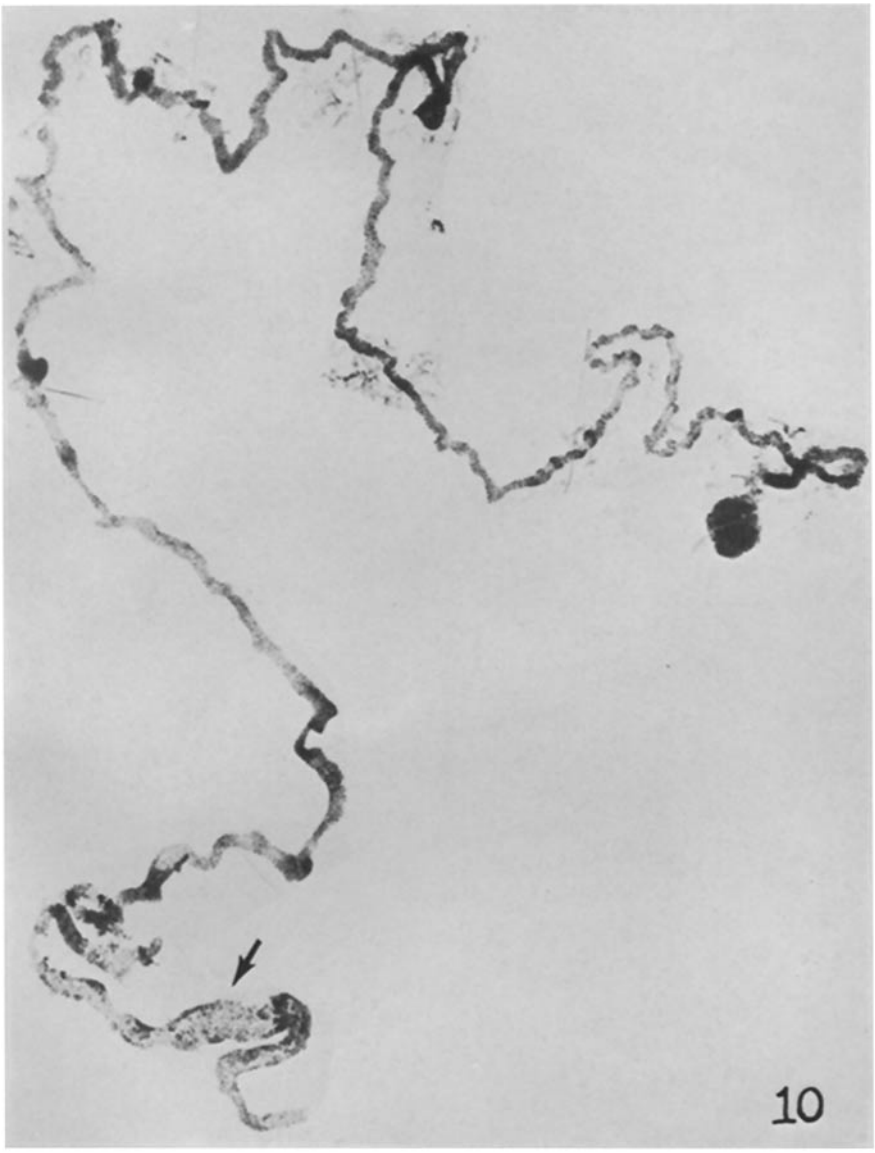
FIG. 9. A complete proximal convolution from a similar kidney showing irregular cystic dilatation of its lower portion. *Cf.* Fig. 5 for contrast between normal and swollen tubular dimensions. There is little if any evidence of actual epithelial destruction, the distention apparently being the result of occlusion from tubular damage, or compression in the subcortical zone. Formalin fixation. × *ca.* 55.



(Holliday *et al.*: Renal lesions of electrolyte imbalance. II)

PLATE 19

FIG. 10. A complete proximal convolution from the same kidney showing irregular atrophy of its tubule. There is a typical necrotic lesion near its tip (arrow). It is the combination of such tubular distortions, occlusive dilatation, and pressure atrophy, that produce the architectural alterations in the cortex that are shown in Figs. 1 and 2. Formalin fixation. × *ca.* 55.



(Holliday *et al.*: Renal lesions of electrolyte imbalance. II)