THE RENAL LESIONS OF ELECTROLYTE IMBALANCE*

I. The Structural Alterations in Potassium-Depleted Rats

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Plates 50 to 59

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Since the demonstration by Schrader, Prickett, and Salmon (1) of structural alterations in the tubules of the kidney of rats fed a diet deficient in K a series of investigations (2–12) has described renal lesions associated with K depletion both in experimental animals and in clinical states in which electrolyte balance has been disturbed by such incidents as prolonged diarrhea or vomiting.

All the investigators agree that changes occur in the tubular epithelium of the nephron; there is no unanimity of opinion, however, on their nature or location. Fatty, vacuolar, and hydropic degeneration and even a general tubular necrosis, which in its severity is compared to the "necrotizing nephrosis" of heavy metal poisoning, have been noted along with the progressive changes of epithelial hyperplasia and cystic dilatation of tubules.

Regarding the location of the lesions there is also disagreement: they are described by different investigators as in the proximal convolutions, the ascending limbs of the loops of Henle, the distal convolutions and the collecting tubules, either limited to certain of these segments or in various combinations.

The more recent studies have emphasized the predominance of alterations in the collecting tubules. Spargo (10) describes increase in the size of the lumen of the "distal" and collecting tubules, along with an accumulation of hyaline or "colloid" droplets in the cells of the latter. There was an increase in both size and number of the cells in the tubular wall and mitotic figures were frequent. The droplets were PAS (periodic acid–Schiff reaction) positive and especially prominent near the papillae. Milne, Muehrcke, and Heard (11) report a similar finding of droplets, stressing not only their frequency in the ducts near the papilla but their occurrence in the epithelial

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covering of the pelvis. Craig and Schwartz (12) note the droplets in the collecting tubules and also an increased PAS reaction in the interstitial tissue of the papillae related to the basement membranes of the tubules; in the inner medulla the PAS-positive material was contained in proliferating swollen cells, described as macrophages, lying between the tubules.

There are two aspects of the problem which may account for these divergences of opinion; the experimental procedures by which K depletion was produced were not identical; nor is "K depletion" an entity in the sense that this ion alone is deficient but rather is part of a general disturbance of electrolyte balance which may assume a varied pattern. Among the alterations that may be associated in varying degree with K deficiency are the effects of increases in serum bicarbonate concentration, of increases in muscle sodium, of phosphate loading, of administered sodium, and of desoxycorticosterone. The lesions therefore may have differed as a result of these variations.

The second factor concerns the location of the lesions in the course of the nephron, and here the reason for uncertainty may be largely technical. It is obviously impossible to recognize the characteristics of the distinctive epithelium of its segments when these characteristics have been completely altered by pathological change and so no longer exist; nor can continuity of change in the long and tortuous tubule be followed in histological sections. It would seem therefore that the localizations that have been made by the latter technique need reexamination by a more adequate method.

A considerable amount of anatomical material has accumulated from experiments in which disturbances of electrolyte and water balance, including K deficiency, were produced by dietary modification or by the ionic exchanges that result from peritoneal dialysis with solutions containing differing electrolyte content. Although the structural lesions that have been observed have shown more consistency than previous reports might suggest, their pattern is extremely varied, doubtless owing to the first of the difficulties noted above. This has required experimental analysis of various forms of electrolyte imbalance having K deficiency as a common denominator; the structural alterations have then been compared to provide some evidence concerning the lesion which appears to depend specifically on K deficiency. The second technical uncertainty has been met by the use of microdissection and the examination of the lesions in the continuity of entire nephrons.

This first report describes the structural changes in what may be considered the basic experiment of a series. K depletion was produced by feeding a diet deficient in this ion but abundant in Na; the data on the chemical and functional disturbances have been given in full elsewhere (13). In this study it was found that with increasing degrees of potassium depletion, as estimated from the concentration of potassium in fat-free skeletal muscle, there occurred a progressive decrease in the maximum urinary concentration that could be achieved. This defect appeared to be better correlated with the degree than with the duration of the potassium depletion; it was shown that this impairment of the renal concentrating mechanism was not due to the load of sodium bicarbonate *per se*, since concentrating power was normal in animals receiving large amounts of

this salt when they were simultaneously protected from potassium deficiency by the addition of adequate quantities of potassium in their dietary regimen. It was not the result of diminished food intake; it was related to the total concentration of solutes in the urine rather than to that of one particular solute; and it could be demonstrated either by the use of exogenous vasopressin or by water deprivation.

TABLE I

Experiment	No. of rats per group	Duration	Body weight				Kidney weight*		Serum CO		Muscle K mm.		
			Initial		Final								
			Control	K-deficient	Control	K-deficient	Control	K-deficient	Control	K-deficient	Control	K-deficient	
		wks.	*gm.*	*gm.*	*gm.*	*gm.*	*gm./100 gm. body wt.*	*gm./100 gm. body wt.*	*mM/L*	*mM/L*	*mM/100 gm. f.f.s.‡*	*mM/100 gm. f.f.s.‡*	
A (control)	11	1	394 ±25		−4 ±23		0.781		26.0 ±2.7		47.7 ±1.89		Fig. 8 G
B	5	3	334 ±31	328 ±29	+65 ±34	+18 ±16	0.759	0.933	26.0 ±2.7	31.4 ±4.39	47.7 ±1.89	41.7 ±2.26	Fig. 2, 3
B^1	5	4	342 ±26	321 ±17	+70 ±18	+52 ±18	0.660	0.909	26.0 ±2.7	41.9 ±6.02	47.7 ±1.89	34.0 ±5.13	Figs. 8, 10
C	8	4	273	270	+77	+20			25.5 ±1.49	31.2 ±3.17	47.6 ±2.05	28.3 ±2.45	Figs. 1, 4, 5, 7, 9, 11–13

* One kidney, wet wt.

‡ Fat-free solids.

The following description of the structural lesions in these experiments is limited to a definite time period, 1 to 4 weeks, for, as will be reported later, complications of fibrosis and architectural change develop at a later time.

Methods

The studies were done on male, Sprague-Dawley rats weighing 300 to 400 gm., housed individually, with free access to a basal diet which was deficient in potassium, sodium, phosphate, and chloride. A feeding of supplementary electrolytes was administered to group A in 5 ml. of water once each day by gavage, the control group thus receiving normal amounts of potassium chloride and of a neutral mixture of monosodium and disodium phosphate. The potassium-deficient groups, B and B_1, received sodium bicarbonate and the same mixture of sodium phosphate. Group C received the same basal diet, with free access to a 0.15 M solution of NaCl in place of drinking water. The ingested sodium exceeded the sodium provided in the other experiments because of the developing polyuria. Rats were sacrificed by exsanguination from the abdominal aorta after anesthetization with intraperitoneally administered hexobarbital sodium (evipal) after 1, 2, 3, and 4 weeks on the experimental regimen. The kidneys

were removed promptly, weighed, and placed in 10 per cent formalin and Zenker's solution. Histological sections were prepared from paraffin blocks and stained with hematoxylin and eosin and Heidenhain azan stains. The nephrons were isolated by microdissection after maceration in HCl and stained with iron hematoxylin by the procedures previously described (14). Certain data including the degree of K depletion and related acidosis are shown in Table I. Additional details, particularly with respect to composition of the diet and quantities of electrolytes administered are reported elsewhere (13).

The Structural Alterations in the Nephrons

Since the structural changes in the kidneys that follow dietary K restriction depend on both the intensity and the duration of the resulting K depletion and the former may be modified by experimental procedures a certain variability was noted in the development of the lesion. In general, it can be stated that while the characteristic structural changes were definitely established by the end of the first week of K restriction their full development was best seen at 2 weeks; in the following 2 weeks there was some exaggeration of the tissue alterations and a beginning was noted of changes in the architecture of the kidney which appear to be a secondary effect of the earlier primary lesion.

At 2 weeks the kidneys were essentially normal to gross examination; they were of normal size and colour; at 3 and 4 weeks they appeared swollen and their surfaces somewhat roughened. The general localization in the kidney of the tubular alterations at 2 to 4 weeks is shown in low magnification of a histological section passing through the papilla in Fig. 1. Extending in a broad band beneath the cortex, the outer zone of the medulla was clearly outlined by the dilatation and enlargement of the collecting tubules. Some of these tubules extended out into the cortex, but the demarcation from the inner zone of the medulla was quite sharp; the larger collecting ducts of the latter appeared essentially unchanged at this magnification.

At higher magnification structural alterations of two distinct types were noted in the outer and inner zones of the medulla. Most pronounced in the inner stripe of the outer zone, the clear epithelium of the collecting tubules was greatly swollen, so that some cells protruded into the tubule lumen. In many instances these cells had ruptured, resulting in a frayed appearance of their apices (Figs. 2 and 4). There were no droplets in their finely granular, distended protoplasm. Not only was there an increase in the size of the clear cells, but also an increase in their number; the original single layer of cells was replaced by masses of irregular cells which encroached on the lumen. Mitotic figures were frequent. Since there was no excess of intracellular lipides to account for the vacuolar swelling of the cells, the appearance of the lesion was therefore that of an excessive hydration of the cell protoplasm which had increased to the point of cellular damage and which was followed by hyperplastic proliferation.

A more remarkable evidence of this hyperplasia was observed in the intercalated cells of the tubules in the involved area. In normal collecting tubules these cells are so few and insignificant in appearance, as they lie compressed between the predominant, clear cells, that they are seldom noted by the histologist;[1] in the hyperplastic collecting

[1] As these cells are seldom mentioned in histological texts the reader is referred to von Möllendorf's Handbuch (15) for a historical review of the older literature concerning them and to a more recent description of their appearance as it may be observed in dissected specimens (16).

tubules of the K-depleted animals they stood out plainly in great numbers as deeply stained cells crowded either between the clear cells or protruding in long bulbous projections into the lumen of the tubule (Fig. 2). In many instances these protrusions, cut by the plane of the section, appeared as rounded masses free in the lumen. An occasional fortunate section showed the characteristic crab-like shape of the entire cell with its cellular projections and the presence of twin nuclei (Fig. 4). Tangential sections passing through the base of the epithelium (Figs. 3 and 5) showed an alternation of intercalated cells with the swollen clear cells, the long processes of the former passing between the latter and reaching to the thickened basement membrane.

The collecting tubules in the inner zone of the medulla showed, in contrast to the marked alterations in the outer zone an essentially normal configuration; their lumens were not dilated nor their epithelium hyperplastic. Intercalated cells are not normally present in the collecting tubules of this region nor were they seen in the K-depleted kidneys. Beginning at the junction with the outer zone and increasing in number as the papilla was approached, the normally shaped epithelial cells were filled with fine eosinophilic granules or droplets; these were present in even greater numbers in the papillary epithelium of the renal pelvis.

Although the two lesions described, cellular hyperplasia and granule-droplet formation, had considerably altered the original histological appearance of the collecting tubules, the identification of these structures in histological section was possible from their general morphological characteristics of contour, size, and position and by the presence of the intercalated cells. Since the two lesions differ, however, in their distribution in a tubular system which extends from the papilla through medulla and cortex to the surface of the kidney, the exact localization and in particular the continuity of the structural changes was therefore examined by microdissection.

Fig. 6 shows a camera lucida tracing of the outlines of a considerable portion of a dissected collecting system. Superimposed on this specimen are tracings of other dissections of various portions of nephrons oriented to the collecting ducts as they were observed in the kidney during dissection; the figure therefore presents a reconstruction of actual structures. Microphotographs of the individual specimens are shown in separate figures.

During the progress of the dissection it became apparent that some change had occurred in the intertubular connective tissues at the junction of the outer and inner zone, for it was impossible to strip the collecting tubules in this region from the peculiarly adhesive substance which coated them (Figs. 7 B and 8 A). No great increase in collagen had been noted in the sections stained with either eosin or a modified Mallory method, though the individual intertubular fibers and basement membranes appeared swollen and diffusely thickened.

In regard to the exact site and distribution of alterations in the collecting system, it was found that the tubules in the inner zone from the ducts of Bellini to the junction with the outer zone showed no alteration in their external configuration; they were easily freed from the surrounding interstitial tissue (Fig. 7 A). There was no proliferation of their clear epithelial cells nor, as in normal animals, were intercalated cells present. When the epithelium was stripped from the papilla and stained *in toto*, its cells were seen to be filled with great numbers of fine granule-droplets (Fig. 14). The intensity of this change outside the tubular system could therefore be more accurately appreciated than from its appearance in the histological section which shows only a

single layer of cells. The epithelium of the ducts of Bellini which is continuous with the papillary epithelium was also filled with similar granules and these gradually decreased in prominence as one departed from the papilla so that they were rarely present in the tubule cells at the junction with the outer zone. The granules stained feebly with iron hematoxylin and blue, as does collagenous material, with the Heidenhain azan procedure.

At the junction of the inner and outer zone swelling and proliferation of the epithelium of the collecting tubules abruptly began (Figs. 7 and 8) and from this point extended in decreasing intensity toward the cortex. The result was a greatly thickened but not markedly distorted segment of tubule in which the increase in size and number of cells, both clear and intercalated, encroached upon and at times almost obliterated the lumen. Since the entire thickness of the tubule is visible in the dissected specimen, those stained with iron hematoxylin showed great numbers of intercalated cells which appeared as a sprinkling of dark objects on the lighter background of the clear epithelium (Fig. 8 A). From the middle of the outer stripe of the outer zone upward into the lower cortex the proliferation and swelling decreased, the excess of intercalated cells diminished, and the lumens of the tubules were widely patent and in many instances definitely dilated (Fig. 8 C and 8 E); the dilatation was regularly associated with the proliferative cellular occlusion situated below it (Figs. 8 D and 8 F).

This effect of the lower obstructive lesion fades in mid-cortex; the great majority of the connecting tubules and the distal convolutions were not dilated and their cellular components were normal (Figs. 8 E and 9 A and 9 B). An exceptional distal convolution, however, which lay at the surface of the kidney, where its coils were not surrounded by other structures which maintained a supporting counter pressure, was greatly dilated (Fig. 10). It is noteworthy that in these convolutions wherever a lesser degree of distention allowed observation of the cellular pattern (Fig. 10 (arrows)), the cells appeared intact. Such occasional dilated distal convolutions may be found in histological sections as clusters of cross-sections of distended tubule protruding beneath the capsule; their epithelium, though flattened, shows no evidence of cytoplasmic damage.

The broad ascending limbs of Henle's loop were entirely normal (Fig. 8 E, 9 C) except in the rare example where dilatation had extended from a distended distal convolution (Fig. 10). There were no changes in caliber or in the cells of the thin portion of the loop.

In summary, the "distal tubule," as the term is commonly used to designate some region in the nephron beyond the proximal convolution, had undergone no change in these experiments except that of a passive distention. This distention is explicable on the basis of a demonstrable structural impedance to the flow of tubular fluid which results from cellular hyperplasia (Figs. 8 B, 8 D and 8 F) located in the region of the junction of outer and inner zones of the medulla. As will be described in a subsequent report dealing with the effect of long continued electrolyte disturbances on the architecture of the kidney, these secondary alterations become in the end so pronounced that all portions of the tubules are eventually affected by the renal lesion.

Due to the difficulty of accurately identifying abnormal tubules in histological section, particularly in the subcortical outer stripe of the outer zone of the medulla, where the lesion in the collecting tubule lies interspersed between medullary terminal proximal convolutions and broad ascending limbs of Henle's loop, the structural changes in the nephron proper, as contrasted to the collecting system, will first be described in dissected specimens in which structures can be identified with certainty; the cytological detail visible in sections will follow.

Frank epithelial lesions in the nephron proper, *i.e.* from glomerulus to the collecting tubule, were found only in the proximal convolution. Rarely present in the earlier periods, they became progressively more frequent in the experiments of longer duration and more severe K depletion. It is noteworthy, however, that in none of the experiments were the lesions present in the proximal convolutions of all kidneys; in many they appeared normal. Moreover, varying degrees of the lesion, from its most slight to severest manifestation, were present in different nephrons of the same kidney.

The least and apparently earliest change in the cells of the proximal tubule consisted in a swelling with no great change in cell configuration; there resulted a consequent increase in the thickness of the tubule with no widening of its lumen. This swelling was not general throughout the convolution but involved only a limited segment of solid appearing tubule with normal round nuclei that lay in the middle third of the convolution. The other coils of the convolution which originally lay entwined with it, appeared thinned as if compressed by the increased volume of the thickened segment (Fig. 11).

Other proximal convolutions in the same kidney showed more advanced change. The progression of the lesion along the tubule is shown in Fig. 12 in which successive stages of the cellular alteration are visible. At the lower left the first change of swelling of the epithelial cells and consequent thickening of the tubule with some alteration in nuclear shape, size, and number is apparent in comparison to the normal portion at the lower right of the illustration; at (*a*) the mitochondrial background is blurred though nuclei are still present and from (*b*) to (*c*) absence of nuclear staining and complete mitochondrial disintegration and clumping indicate frank cell damage. From (*c*) to (*d*) regenerative hyperplasia is evident in the irregular pattern of large oval nuclei which are arranged in clusters on the sparse mitochondrial background that is characteristic of a new formed renal epithelium. By this interpretation, which is only possible by observation of the continuity of change in the dissected tubule, the lesion is incipient from (*a*) to (*b*), fully developed from (*b*) to (*c*), and oldest from (*c*) to (*d*) and is therefore extending down the convolution.

The alterations thus evident in a dissected specimen of proximal convolution can be seen in histological section though here completely disoriented in the plane which cuts at random through its coils so that no relation exists between contiguous cross-sections.

The general appearance of the cortex in such a preparation even in the 4th week of the dietary restriction, may not be greatly altered (Fig. 1); among generally well

preserved cross-sections of proximal convolution is found an occasional single example which is swollen, the cytoplasm of its cell show some protoplasmic disturbance and its nuclei are few or absent. As this cross-section is completely surrounded by others of the same convolution which are entirely normal it is difficult to appreciate that a lesion, extending through an indeterminate and at times considerable length of tubule (*cf.* Fig. 12), has completely interrupted the structural integrity of an entire nephron.

Besides the solitary lesions described, and apparently completely disassociated from any orderly spatial relation to other renal structures, are seen clusters of a few cross-sections of atypical tubule which are lined with an irregular layer of proliferating epithelium containing masses of large vesicular nuclei; mitotic figures are frequent among them. A search through serial sections may at times show their identity. In Fig. 13 cross-sections *a*, *b*, and *c* are completely transformed into unidentifiable structures; *d*, *e*, and *f*, however, can be recognized as altered proximal convolution, for though the character of their epithelium is greatly changed, faint remnants of their brush borders are visible. In them the origin of the cellular proliferation can be seen at one point in the tubule wall (arrows); evidently the section has passed through the tubule at the very edge of the lesion, somewhat as indicated by the heavy line in Fig. 12.

Neither in sections nor dissected specimens did the glomeruli and vessels from any of the experimental animals show significant alterations.

DISCUSSION

It is to be noted that the description of structural changes has been limited to the first phases of their development in the belief that, being less complicated by secondary alterations, they are more likely to be specific effects of the ionic imbalance. One secondary complication had already appeared as the result of luminal obstruction by hyperplastic proliferation in the collecting tubules in the lower level of the outer zone of the medulla, namely a dilatation of upper cortical collecting tubules which in certain instances extended into distal convolutions and even reached the ascending limb of Henle's loop. As will be reported later, these secondary changes become progressively more prominent with passage of time and, being associated with alterations in the supporting fibrous tissues, result in the ultimate development of marked architectural change in the kidney. Under these circumstances all tubular elements, both of the nephron and the entire collecting system, become involved.

A remarkable feature of the two lesions in the collecting system, *i.e.* cellular hyperplasia with relative increase in intercalated cells and intracellular granule-droplet formation, that is visible in the dissected specimens is the sharp localization of the former to the outer zone of the medulla and of the latter to the inner zone. Moreover, a definite and conversely directed gradient of intensity of both lesions can be observed; beginning sharply at the line of demarcation between inner and outer zone the cellular hyperplasia and intercalated cell predominance decrease as the cortex is approached; conversely, droplet accumulation is barely perceptible at the line of junction of the two zones and increases

towards and is at its maximum in the pelvic epithelium. In analogous fashion, the lesion in the proximal convolution can be seen in the isolated specimens to begin in the middle of this segment and to progress by extension towards and into its terminal segment.

The structural changes in the kidneys of the potassium-depleted rats were accompanied by a functional alteration characterized by an inability to concentrate the urine properly (13). There are many reasons why it would appear unlikely that this disturbed function can be causally related to the lesion in the proximal convolutions. From the structural standpoint, the change in this region was widely variable in degree and was absent in many, often the majority, of the nephrons; the collecting tubule lesion, in contrast, was constant and all the ducts were involved. Although it is theoretically possible for a structural alteration of the proximal convolution to result in an apparently impaired renal concentrating mechanism by increasing the rate at which solutes are delivered to more distal tubular sites, this is not likely to be the explanation of the renal concentrating defect in the current studies since not only were few proximals structurally altered but the rate of excretion of solutes was not increased (13).

In contrast, there are many reasons for considering the lesion in the collecting tubule as the structural correlate of the functional defect in the concentrating mechanism. It has been established by direct observation that the water reabsorption which occurs in the proximal convolution results in a tubule fluid isosmotic with the filtrate from which it is derived (17, 18); concentration of the tubule fluid must therefore occur distally to this level. From the observation that animals which avidly conserve water have remarkably long papillae and extensive collecting systems, it has been suggested (19) that the concentrating mechanism may lie in this region, a conjecture supported more explicitly by the direct cryoscopic evidence of Wirz, Hargitay, and Kuhn (20), and by Wirz's (21) more recent findings by micropuncture that in antidiuresis with a highly concentrated bladder urine the tubule fluid is still isotonic when it leaves the distal convolution.

In view of these data with respect to the over-all processes involved in the renal absorption of water, the constancy of the specific structural alteration in the collecting tubules of the kidneys of rats with potassium depletion and their inability to concentrate the urine normally, would seem reasonable evidence that these anatomical and functional lesions are causally related. From the presently available data it would be premature to speculate on the nature of the cellular mechanisms by which the lesion in the intercalated segment might interfere with the renal concentrating mechanism and what might be the significance of the remarkable increase in the normally obscure intercalated cells.

The suggestion that the final operation in the formation of urine is in the collecting tubule might seem to place this last event beyond the influence of nephron activity. It should be remembered, however, that the intercalated segment lies in the indeterminate region where the embryonic mesenchymous

nephronic tubule joins the outgrowing ducts of hypoblastic origin that form the collecting system proper. The distinguishing element of this segment, the intercalated cell, has its origin from or is genetically related to the granular epithelium of the distal convolution (16). Thus the activity of the nephron may extend beyond the strict limitations of its topographical confines.

Further studies are in progress concerning the reversibility of the functional defect and the relation of this restitution to the structural alterations of repair and these may provide further insight with respect to these problems.

SUMMARY

Renal tubular lesions during the early phases of progressive potassium depletion in rats were found in nephrons isolated by microdissection in two locations, the collecting tubules and the proximal convolutions. All other portions of the nephron, in particular the "distal tubule," *i.e.* ascending limbs of Henle's loop and distal convolutions, showed no structural alterations except the passive effects of dilatation and cellular compression which developed as a result of primary disturbances lower in the tubular system.

The alterations affected all the collecting tubules uniformly and took two forms; the more severe, a swelling and hyperplasia of the tubular epithelium and the lesser, an intracellular accumulation of granule droplets. The former was limited to the outer zone of the medulla, the latter to its inner zone. In the proximal convolution the structural alteration began in its middle third and extended downward towards the medulla; only occasional nephrons were affected.

The essential nature of the more severe epithelial lesion was similar in both collecting tubule and proximal convolution, beginning as a swelling of cell bodies, increasing to protoplasmic disturbances with disintegration of the mitochondrial pattern, followed by rupture of cells and nuclear disappearance. These retrogressive alterations were followed by prolific regenerative hyperplasia. In the collecting tubules of the outer zone these epithelial alterations were present in both the clear and the intercalcated cells; in the latter the swelling of the cells was not prominent, but the hyperplastic proliferative increase in their number was the predominating feature of the lesion when the dissected tubules were viewed intact in the continuity of their topographical relations.

The cellular alterations in the tubules are associated with an inability to concentrate the urine; reasons are given for considering this functional disturbance a correlate of the structural lesion in the collecting tubules.

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EXPLANATION OF PLATES

PLATE 50

FIG. 1. Low power showing cortex and medulla of a rat after 4 weeks of K depletion. The general appearance of the cortex is not greatly altered though at the arrow an indefinite area of thickened tubules is visible (*cf.* FIG. 13 for detail). The striking change is in the outer zone of the medulla where the collecting tubules are large and dilated. The inner zone shows no marked alterations at the magnification of Hematoxylin and eosin stain. × *ca.* 20.

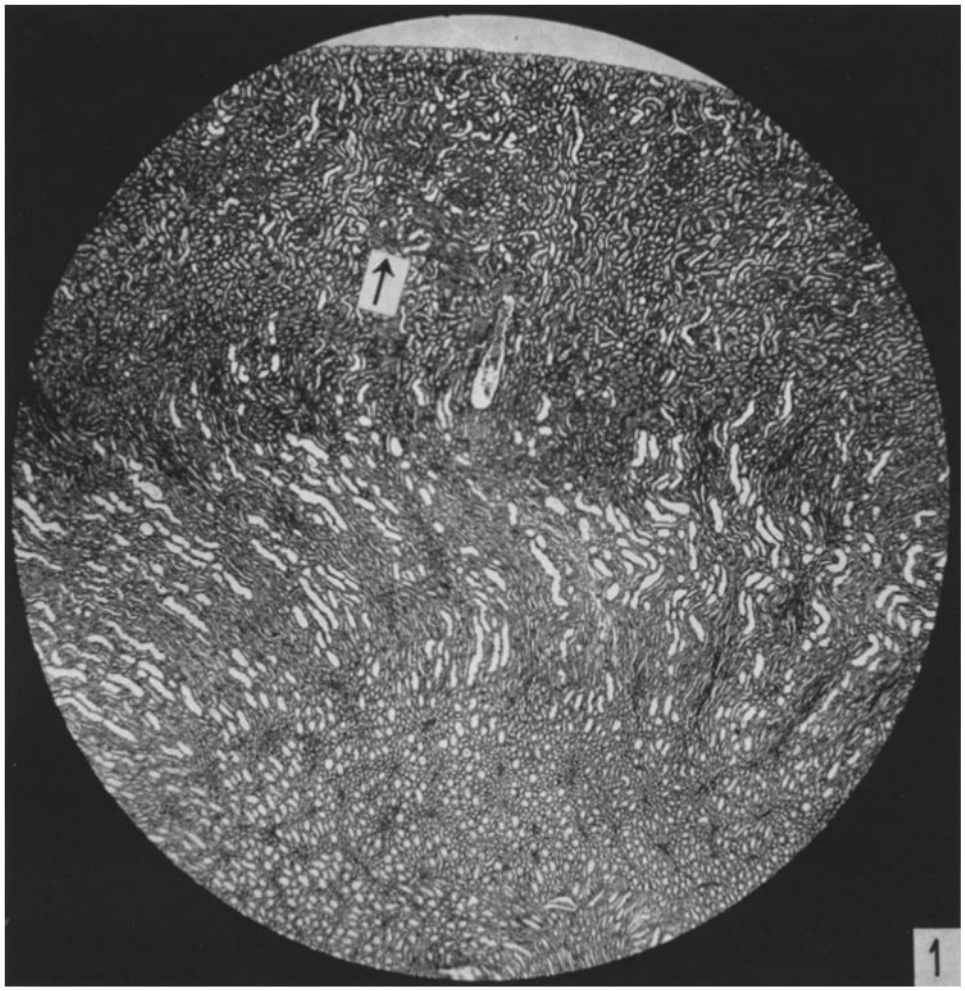

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PLATE 51

FIG. 2. High power of collecting tubules in the inner stripe of the outer zone of the medulla of a kidney from a similar experiment. The clear cells are greatly swollen and their protoplasm vacuolar. Scattered irregularly between them are the darkly stained intercalated cells, their bulbous apices protruding into the lumen of the tubule. × *ca.* 750. Heidenhain azan stain.

FIG. 3. Two collecting tubules from the same kidney are cut tangentially. The long processes of the intercalated cells are shown passing between the clear cells and reaching to the basement membrane which is diffusely thickened. Azan stain. × *ca.* 750.

FIG. 4. Cellular detail in a collecting tubule in a kidney from an experiment similar to that of Fig. 1, showing the swelling, vacuolization, and disintegration of the clear cells. There are five darkly stained intercalated cells; above to the left, the bulbous extension into the lumen; above to the right, the double nuclei which are common in these cells; below to the right the irregular shape of the protoplasmic body and the origin of processes. Hematoxylin and eosin stain. × *ca.* 900.

FIG. 5. A tangential section passing through the base of the epithelium from the same section shows the anastomosing processes of the dark stellate intercalated cells lying between the swollen clear cells. Hematoxylin and eosin. × *ca.* 1200.

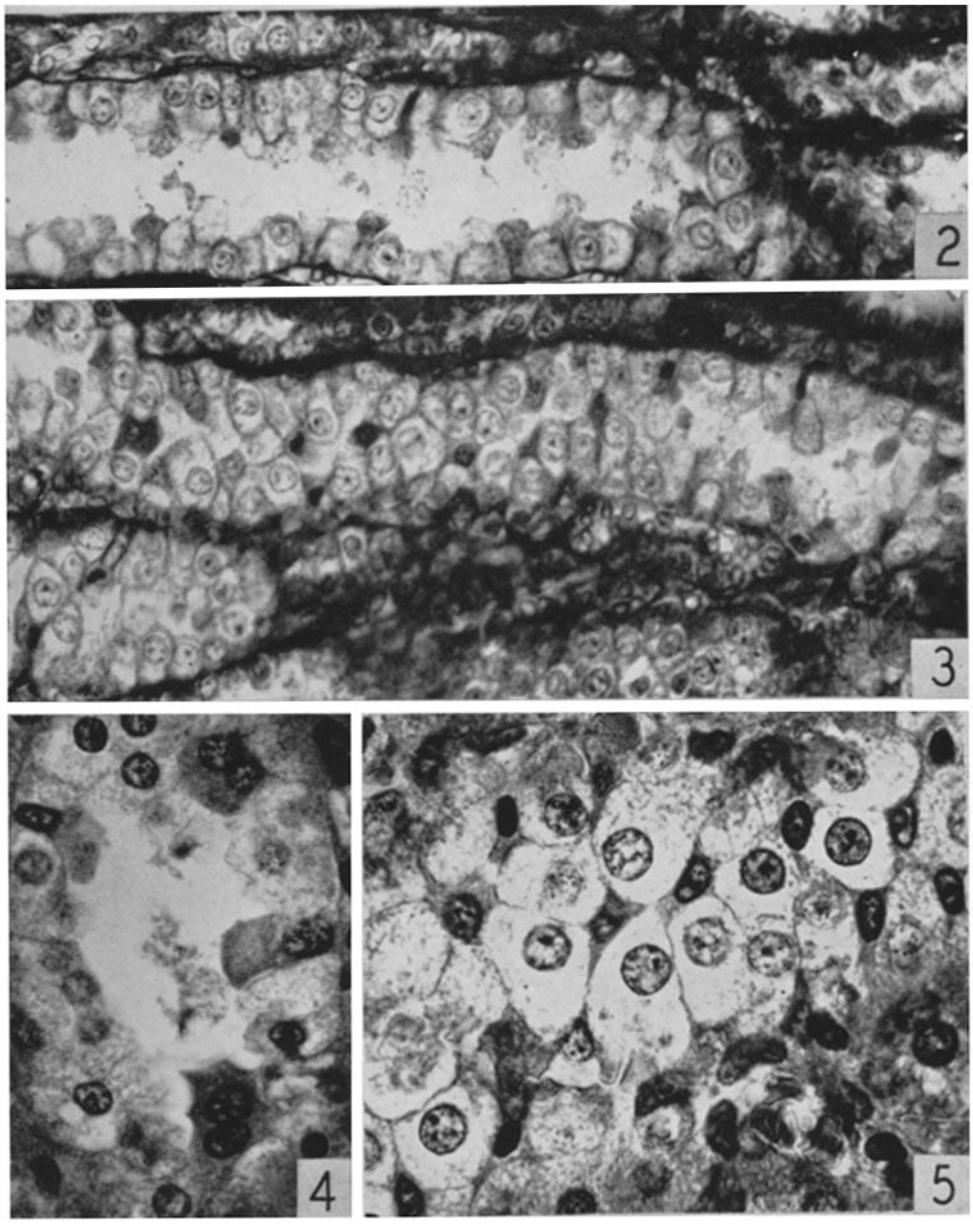

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PLATE 52

FIG. 6. Camera lucida tracings of dissected nephrons and a portion of the collecting system arranged as they were observed during the dissection. The individual specimens are illustrated in the following figures. × *ca.* 15.

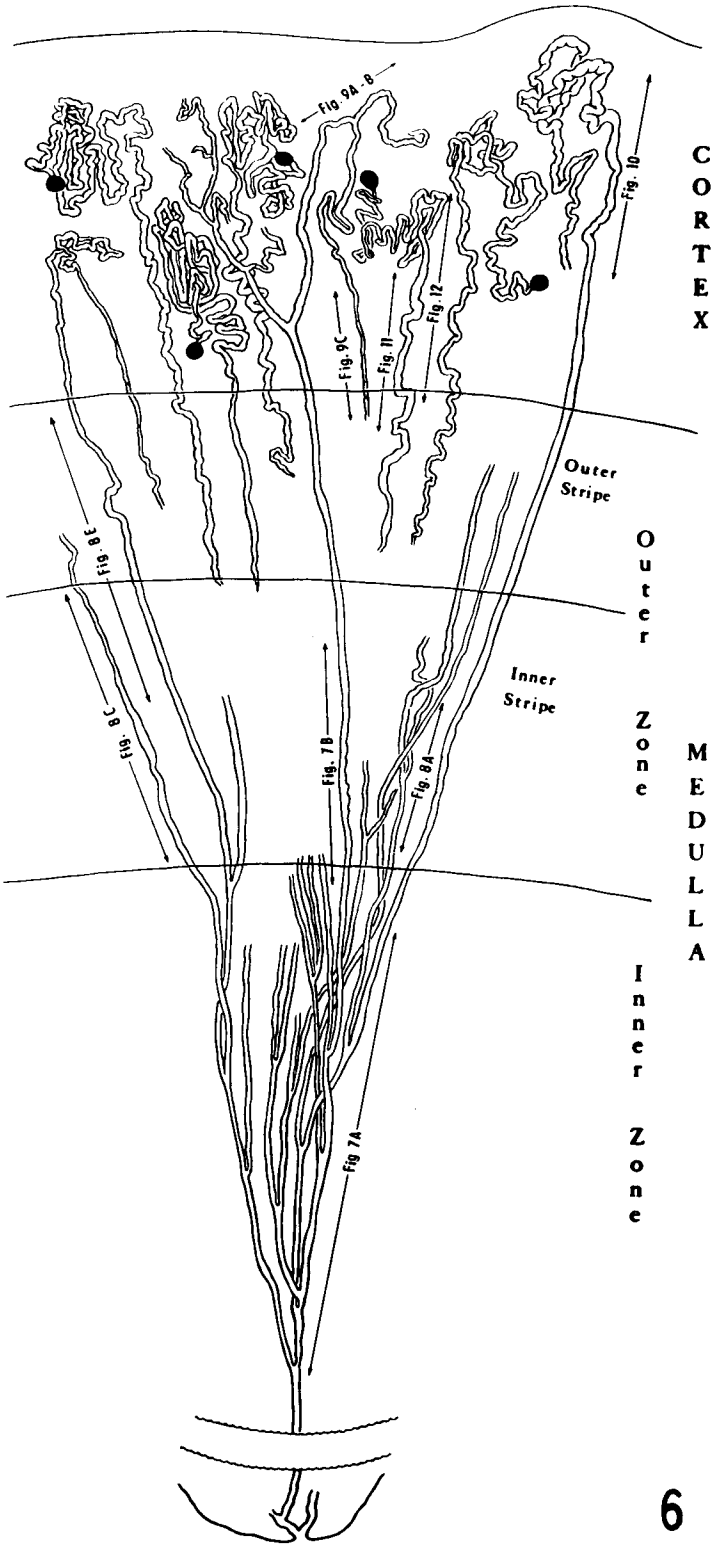

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PLATE 53

FIG. 7. A. Collecting tubules from the inner zone of the medulla after 4 weeks of K deficiency diet. Their external contours and epithelial pattern are normal; note the preservation of the original "tuning-fork" junctions. At this magnification the fine granules within their cells are invisible.

FIG. 7 B. Uninterrupted continuation of the same dissected specimen into the inner stripe of the outer zone (*a*, *b*, *c*, *d*, *e*, *f*). In the region of transition they could not be cleaned of the surrounding connective tissue which adhered to their walls. The tubules appear dark from the presence of many intercalated cells (upper right for detail at higher magnification); these cells decrease in number as the tubule proceeds into the outer stripe where it is considerably dilated. × *ca.* 40. and × *ca.* 70.

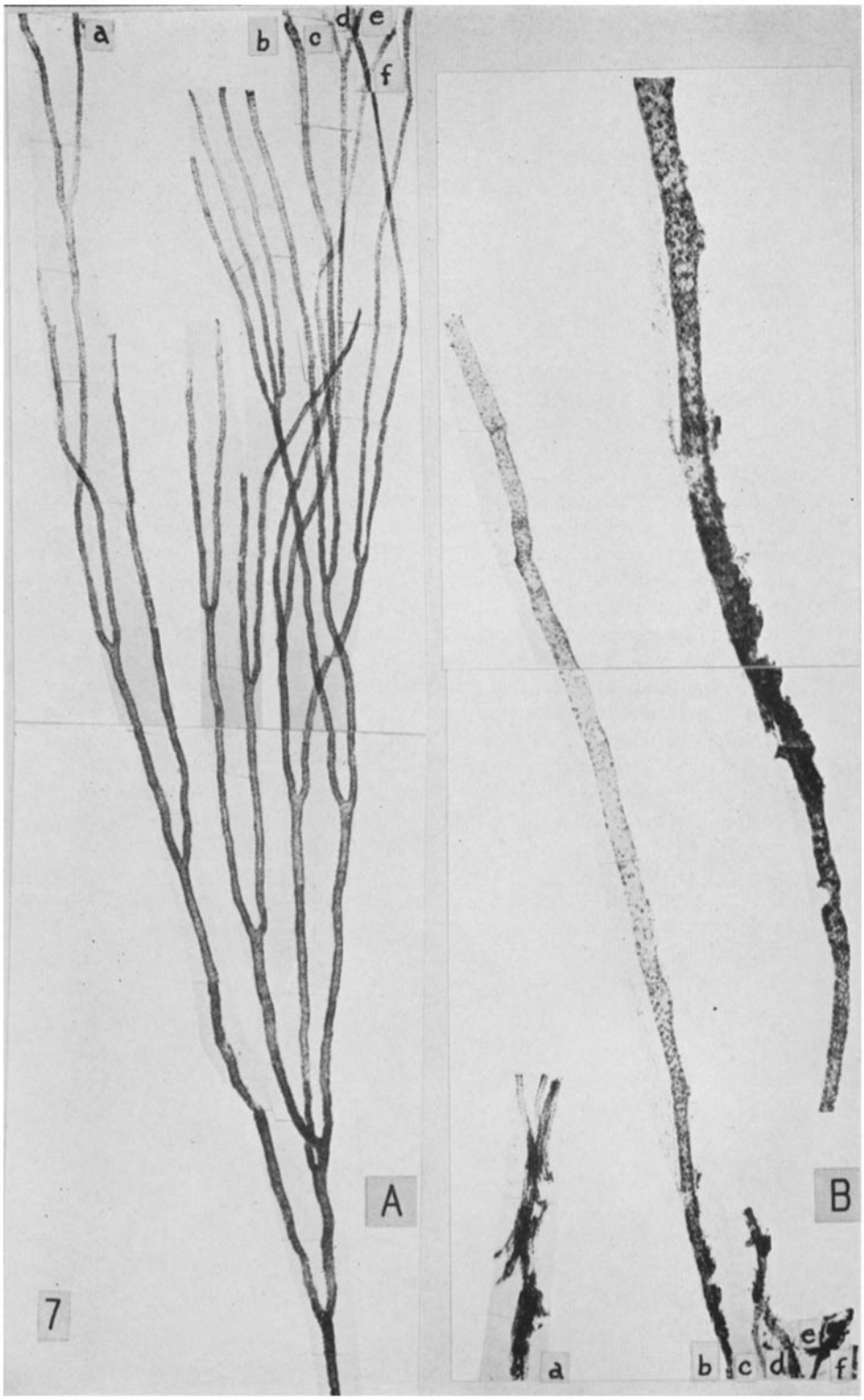

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PLATE 54

FIG. 8. Collecting tubules from three different animals of a set which had been on K restriction for 4 weeks. Fig. 8 A. Three tubules in the outer zone of the medulla at its juncture with the inner zone. Normally they should appear as lightly stained delicate tubules (*G*); they are thickened and darkened by myriads of intercalated cells which fill their epithelium. Fig. 8 B. Detail at higher magnification showing the proliferation of the epithelium and the predominance of intercalated cells. Fig. 8 C. A collecting tubule extending from the inner stripe of the outer zone of medulla to the cortex. Below at the junction with the inner zone a similar epithelial proliferation with intercalated cells which decreases as the tubule nears the cortex above. Fig 8 D. At higher magnification the marked irregularity of the proliferation is apparent. Fig. 8 E. A collecting tubule from the juncture of inner and outer zone extending into the cortex with an attached distal convolution and its ascending limb of Henle's loop. Below the proliferative lesion; in Fig. 8 F, irregular masses of epithelial cells encroach upon the lumen. Throughout the outer zone the tubule is moderately dilated and shows only a scattering of intercalated cells. As in the great majority of nephrons in this and all other kidneys of the experiments, the distal convolution and the broad ascending limb of Henle's loop is of normal appearance and is not dilated. The black staining of the former is due to its normal rich mitochondrial content. Fig. 8 G. The intensity and degree of structural alteration in Figs. 8 B, 8 D, and 8 F can be appreciated by comparison with the delicately built walls of a normal tubule from the same region in which the infrequent intercalated cells are randomly scattered in an epithelium composed in greater part of clear cells. × *ca.* 50 and × *ca.* 150.

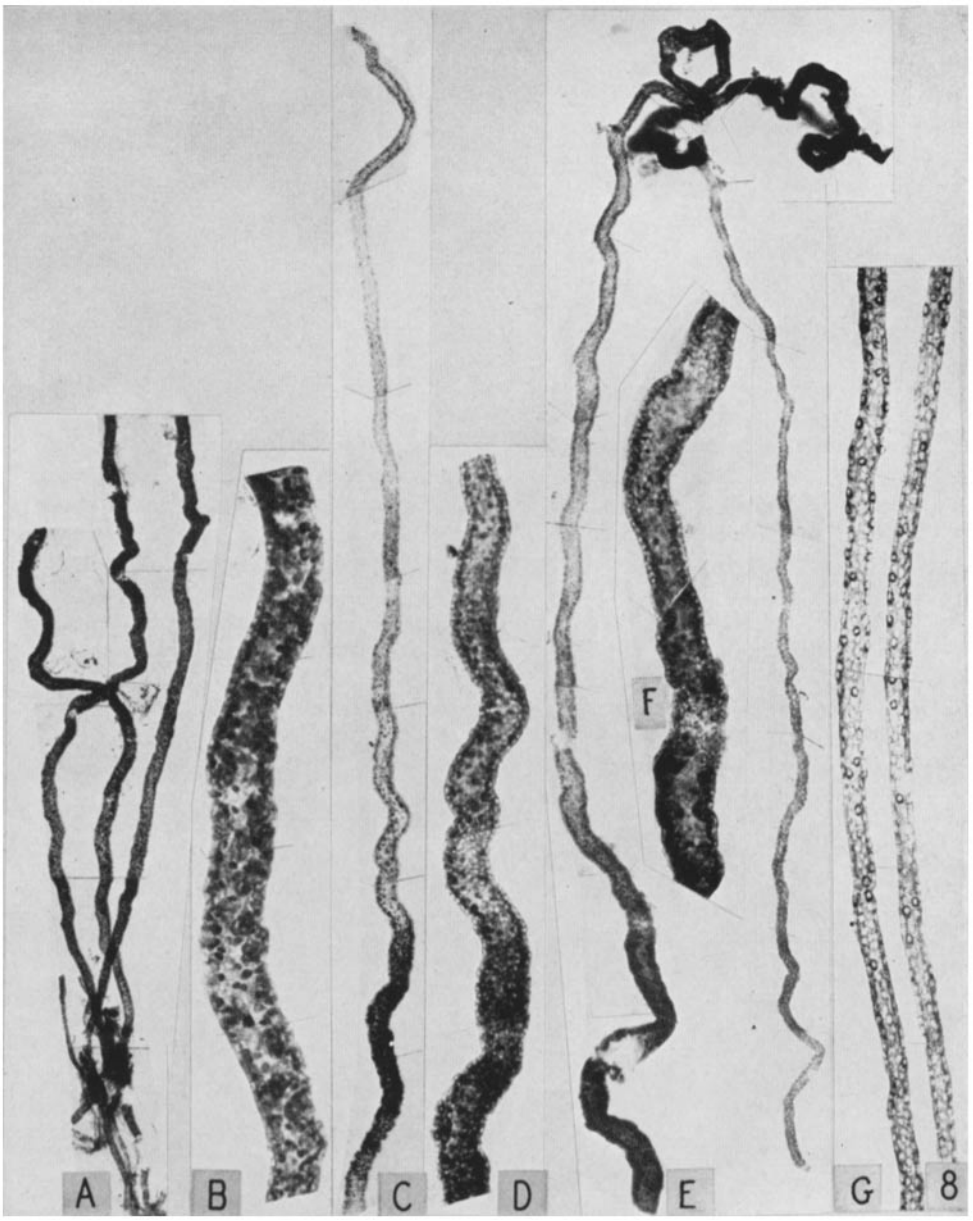

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PLATE 55

FIG. 9 A. A terminal collecting tubule, from a similar experiment of 4 weeks K depletion, entering at the lower left corner, subdivides and, at the upper right, ends in two connecting tubules. One passes to a distal convolution and its ascending limb of Henle's loop. Their contours are normal. × *ca.* 30. Fig. 9 B. Detail of the distal convolution and connecting tubule. The nuclei and mitochondrial pattern of the former are normal. Extending along the connecting tubule, twisted at one point during dissection (arrow), is a scattering of dark cells resembling in their granular component the cells of the distal convolution; in the collecting tubule above, similar granular cells are definitely intercalated cells, a sequence which suggests some genetic relation and the possible origin of the latter. Fig. 9 C. The broad ascending limb of Henle's loop, its contours and cellular components are normal. × *ca.* 40. and × *ca.* 110.

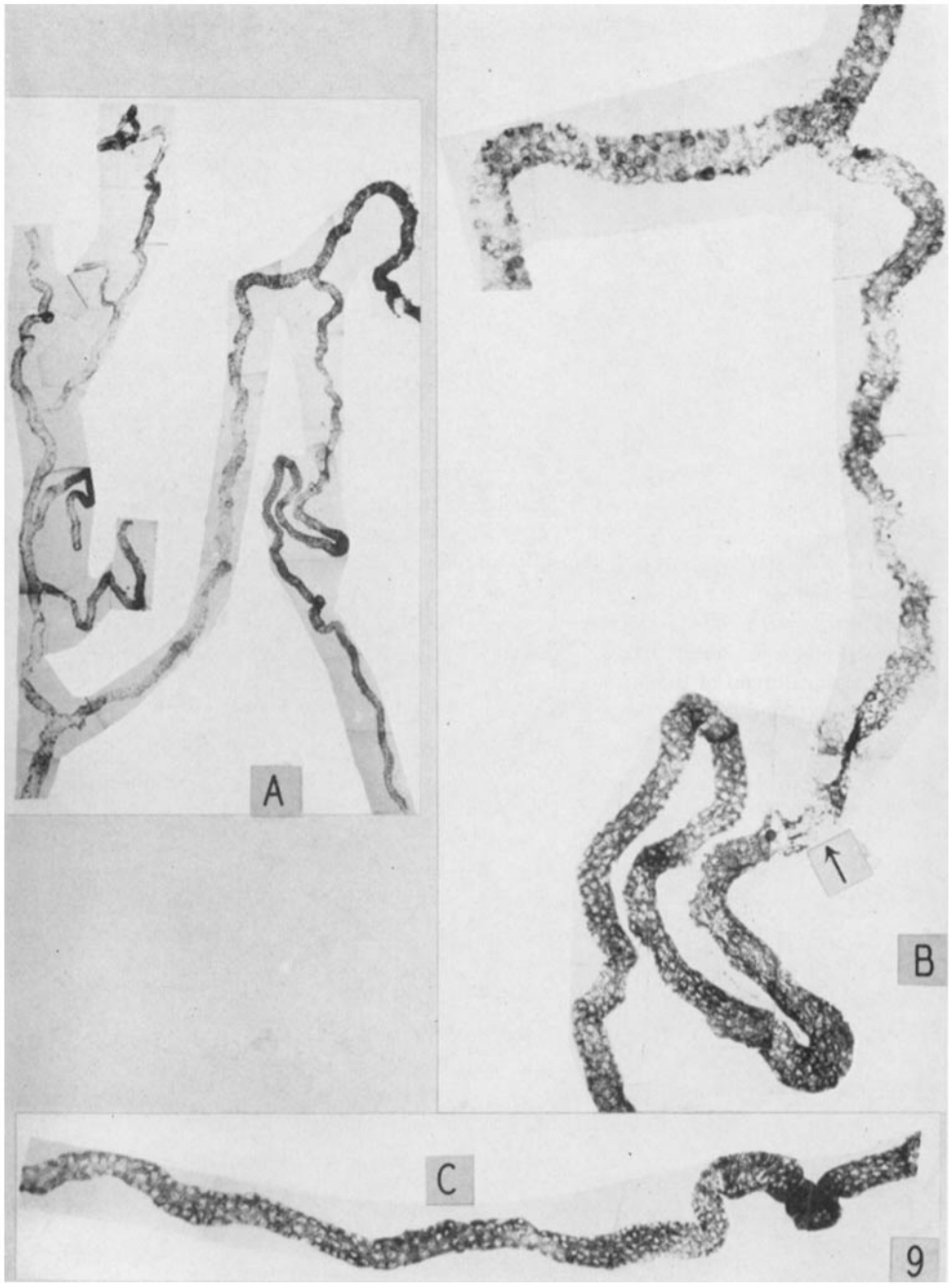

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PLATE 56

FIG. 10. An exceptional distal convolution from the same kidney which lay at the surface of the kidney and which is greatly and irregularly dilated; on the left an equally distended ascending limb of Henle's loop. In areas of lesser distension where the epithelium pattern is still visible (arrows), the cells are intact and show no alteration save thinning. × *ca.* 50.

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PLATE 57

FIG. 11 A. A complete proximal convolution from the kidney of a rat after 4 weeks of K restriction. At the end of its middle third the tubule is considerably thickened. As the coils of the convolutions originally lay in the kidney closely pressed in a knotted mass, there is the corresponding effect of compression in the first part of the tubule. This portion shows in Fig. 11 B a normal cellular and mitochondrial pattern. In Fig. 11 C the thickening is seen to be due to swelling and a hyperplastic increase of the epithelium which otherwise shows no abnormality. This is the condition of the majority of the proximal convolutions through the first weeks of K deficiency. × *ca.* 37 and 100.

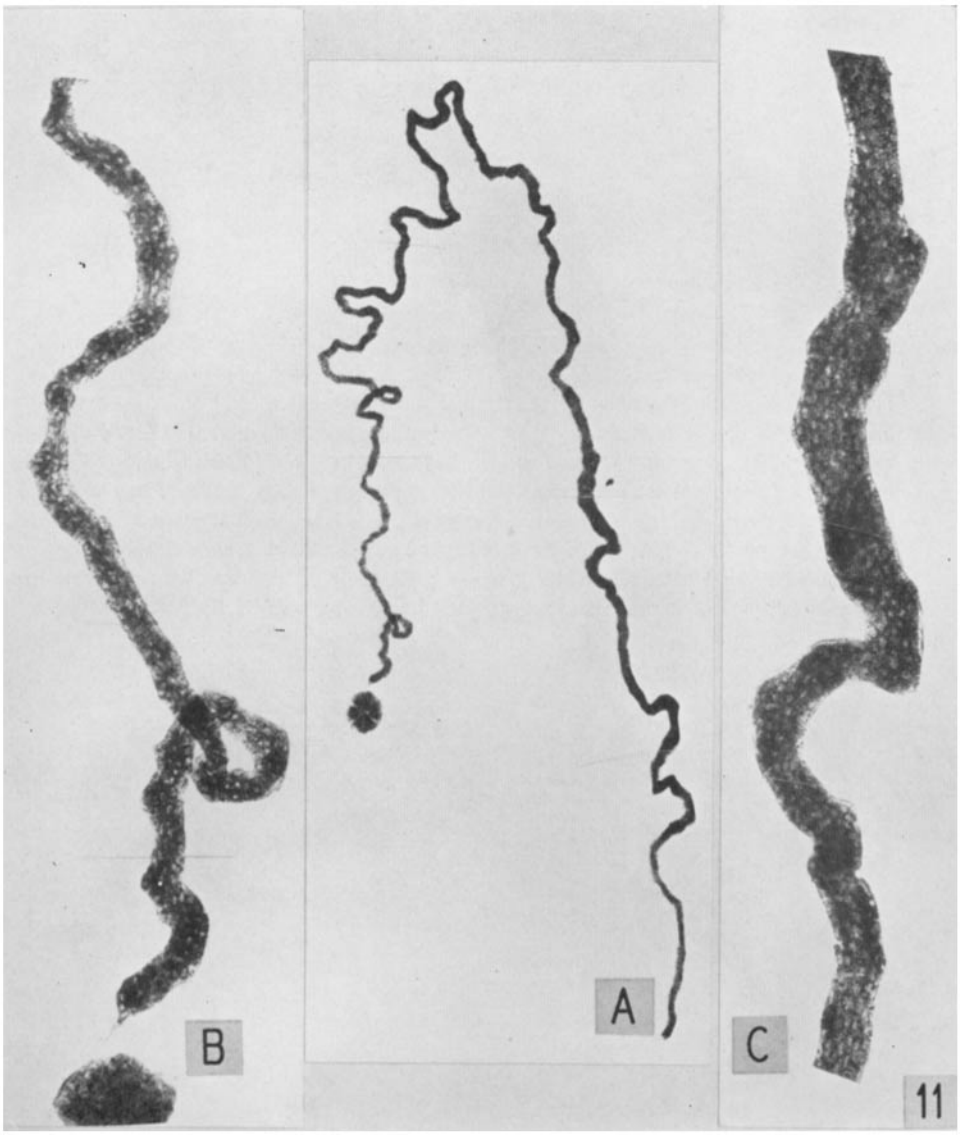

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PLATE 58

FIG. 12. An example from a similar experiment of the exceptional proximal convolutions which were scattered through the cortex in increasing frequency during the later development of the renal lesion. Epithelial and mitochondrial pattern are normal except in the middle third (*a* to *d*). At *a*, the tubule is swollen and the clarity of the mitochondrial background blurred though the nuclei are still visible; from *b* to *c*, the mitochondria are irregularly diffused and in places clumped and no nuclei are visible; from *c* to *d*, large, oval, clear nuclei are scattered irregularly, often in clusters, throughout the regenerated epithelium. From *d* downward to the glomerulus the tubule is normal. The lesion is therefore oldest where the hyperplastic reparative proliferation is prominent (*d*) and is progressing down the convolution. × *ca.* 130.

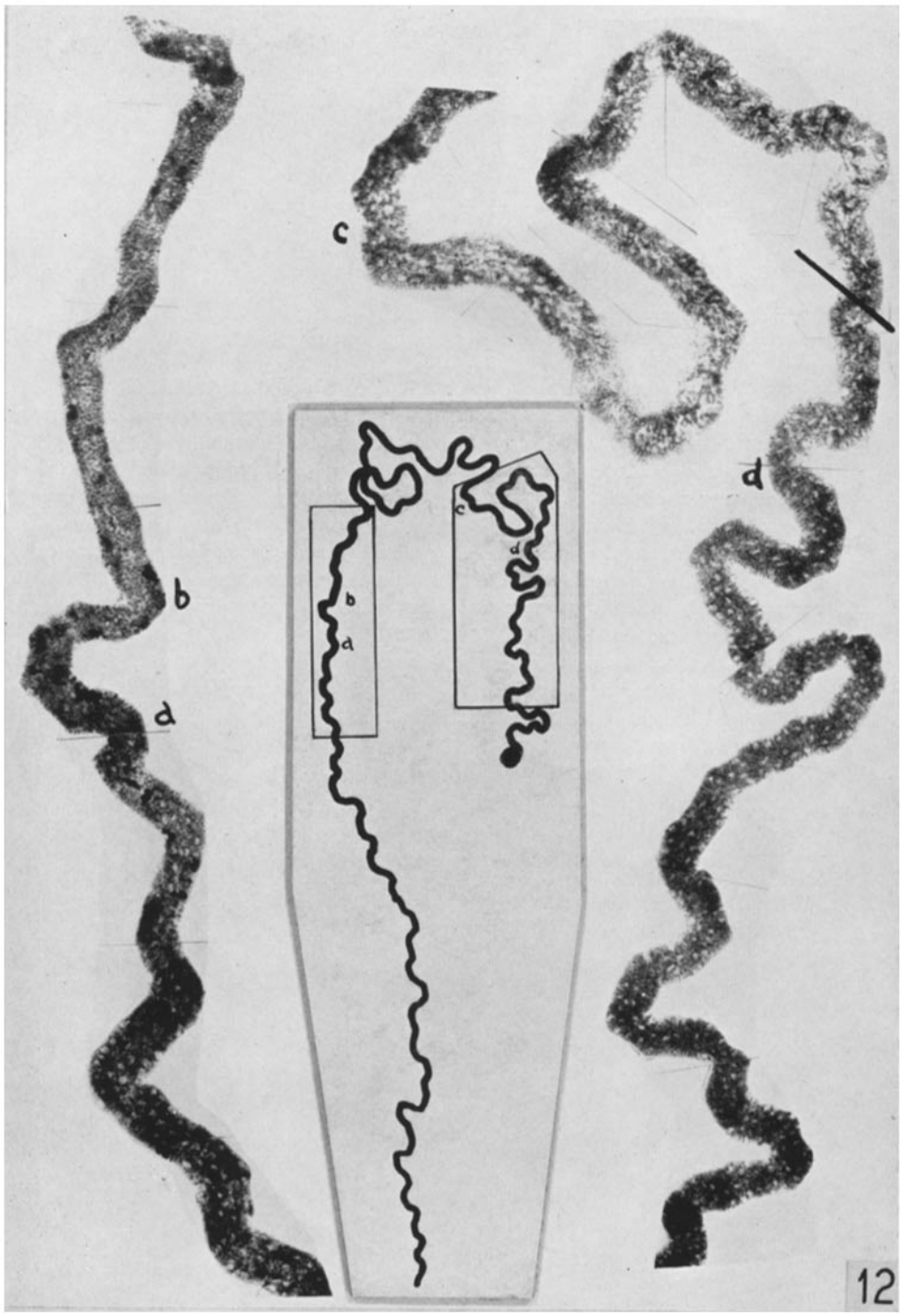

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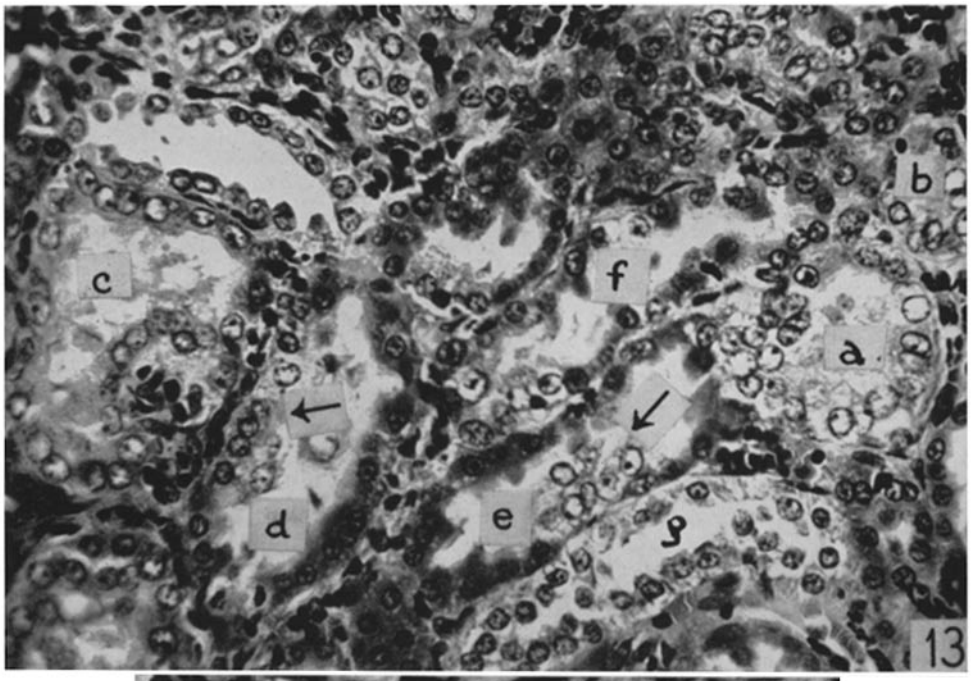
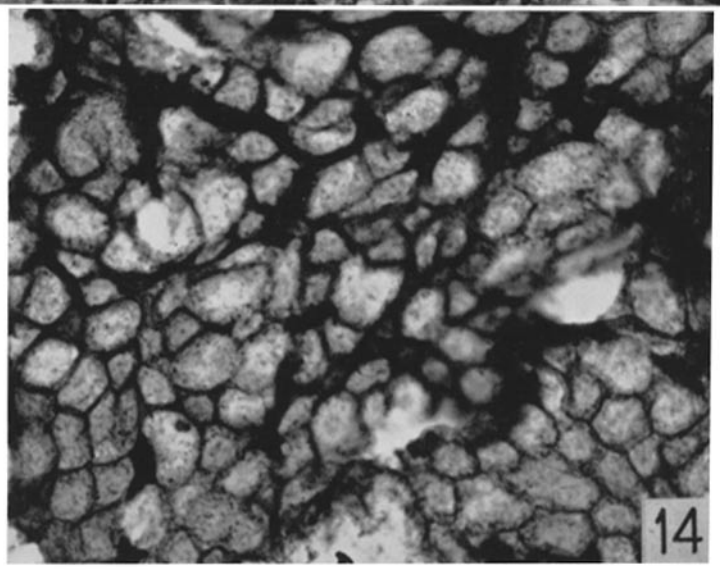
PLATE 59

FIG. 13. In a histological preparation of the cortex of the same kidney groups of sections through tubules *a*, *b*, and *c* are seen which are so altered as to be unidentifiable. In others, *d*, *e*, and *f*, the deeply stained epithelium is distorted (the "peaking" of earlier observers) but in places remnants of lighter brush border identifies them as proximal convolution. In each the point of origin of the hyperplastic alteration can be seen in heaped clusters of large vesicular nuclei (arrows). The tubule at the extreme right (*a*) has apparently been completely transformed; at *g* is what appears to be a not greatly altered cortical collecting tubule. Apparently the section has passed through the transition from regenerative hyperplastia to normal unaffected tubule as is indicated by the line in Fig. 12. × *ca.* 500.

FIG. 14. A sheet of epithelium stripped from the papilla near the entrance of ducts of Bellini from an experiment of 4 weeks' duration. The cells are filled with fine granule-droplets which extend into the collecting ducts and gradually decrease in number to disappear in the inner zone of the medulla. Iron hematoxylin stain. × *ca.* 375.





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