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THE PATHOLOGY OF ARMANNI-EBSTEIN DIABETIC NEPHROPATHY *

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A distinctive glycogenic vacuolization of the cytoplasm of the epithelium of the renal tubules in diabetic patients has long been recognized as a lesion unique in its morphology and in its association with the diabetic state.[1,2] It is considered pathognomonic of diabetes[1,2] and appears related to the severity of the disease and to lack of adequate control.[3] Indeed, with the advent of insulin therapy the lesion has become rare and in the last 25 years has been the subject of but few studies.

Ever since the first descriptions of the lesion by Armanni[4] and Ebstein,[5] conflicting opinions have been expressed concerning the precise localization of the alteration within the affected nephrons and its relationship to renal function in the excretion of glucose. The epithelial vacuolization has been considered to affect principally the loop of Henle in its descending limb[5] or isthmic portion,[6] or both limbs.[7] Although Baehr,[8] in 1913, demonstrated conclusively that the terminal portion of the proximal tubules could also be affected, and Oliver,[8] in a brief reference to a single case, indicated that this was the principal localization of the change, this view has not gained wide currency.

The present study was therefore directed primarily at determining, by three-dimensional visualization of the lesions, their precise localization. On the basis of the findings presented below it now appears that the terminal straight portion of the proximal convoluted tubule is the principal focus of involvement, and that this portion of the nephron varies in its susceptibility to vacuolar change according to its location within the kidney. It seems possible also that the terminal proximal tubule may differ physiologically from those areas situated closer to

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the glomerular headwater, even though morphologic differences between the two zones of the proximal tubule are minor.

METHODS

Specimens of renal tissue from five diabetic patients were selected for study. Selection was based solely on the fact that the diagnosis of diabetes had been clearly established during life, and that routine microscopic sections of the kidneys at necropsy demonstrated the characteristic vacuolization of tubular epithelium in the innermost cortex and outer medulla. Blocks of renal tissue that were available for study had been fixed in either 10 per cent formalin or in Zenker's fluid. Paraffin sections were cut at 4 to 8 μ and stained with hematoxylin and eosin, HPS (hemalum, phloxine, and saffron), and by McManus' PAS technique.[9] In addition, some sections were prepared by the alcoholic PAS technique for the preservation of glycogen.[10] Confirmation of the presence of glycogen was done by PAS staining of sections initially treated with diastase and with Best's glycogen stain.[11] Additional stains included fat stains on frozen sections using oil red O with hematoxylin counterstain.

Microdissection studies were done in all cases by the hydrochloric acid maceration and dissection procedure as used by Oliver[12] and others.[13,14] Briefly, this method involves careful selection of a piece of formalin-fixed tissue (Zenker fixation renders the tissue too brittle for easy dissection) in such a way as to ensure continuity of cortical nephrons with medullary tubules. After the tissue had been suitably digested in concentrated hydrochloric acid, microdissection was carried out with fine needles under a stereoscopic binocular microscope. Isolated nephrons were picked up on glass slides and protected by coverslips mounted on vaseline runners. Specimens were examined either unstained using a green light source, or colored with Regaud's iron hematoxylin. During the study of individual kidneys several hundreds of nephrons would be observed in the dissecting dish. Many of these were selected for mounting and detailed microscopic study. It should be noted that after acid digestion, iron hematoxylin colors the cytoplasm but leaves nuclei unstained. Details of cell structure are poorly visualized but gross structural distortions are easily observed.

In the course of the investigation it was found that the time of maceration (normally extremely variable) could be accelerated to a few hours by placing the tissue in an incubator at 50° to 56° C. On the other hand, more precisely controlled and uniform maceration was best achieved by carrying out the digestion in the refrigerator at slightly higher than freezing temperatures. Under these conditions beautifully

uniform digestion could be achieved in 10 to 15 days. Permanent preservation of mounted nephrons was accomplished by replacing the aqueous mounting medium with Gelatinol (C). Photographs of mounted specimens were made by serial photomicrography using Ilford Pan F 35 mm. film. Subsequent trimming and montage of the prints allowed accurate reconstruction of the original specimen.

Summary of Cases

The important clinical features of the five cases are presented in Table I. The oldest patient was a woman of 36 years and three of the five patients were under 17 years. Four were females and all but one of the patients were known to have had diabetes prior to the terminal illness. In each, a complicating illness appeared prior to the terminal coma which developed in all. The complicating illness was an upper respiratory infection in two cases, bilateral otitis media in one, and dental extraction in two cases. In the latter two it appeared that the precipitation of coma was in part referable to infection, and in part to disturbance of normal dietary control resulting from loss of teeth. Impairment of urinary output was noted in one case and anuria in one. More or less

Table I

Clinical Resumé of Five Cases of Armanni-Ebstein Diabetic Nephropathy

Case no.	Age	Sex	Duration of diabetes	Terminal illness	Duration of terminal illness	Blood sugar		Urine output last 24 hrs.	Coma	Blood non-protein nitrogen
						Highest	Last 24 hrs.			
	yrs.					mg. %	mg. %			mg. %
1	16	F	7 days	Bilateral otitis media	7 days	945	945	0	+	240
2	8	F	5 yrs.	Upper respiratory infection	2 days	1,228	424	?	48 hrs.	65.8
3	30	F	15 mos.	Extraction of teeth	11–12 days	939	939	205 cc./15 hrs.	48 hrs.	
4	36	F	5 yrs.	Extraction of teeth	2 mos.	1,390		?	+	
5	5	M	3 yrs.	Upper respiratory infection	3 days	300		?	24 hrs.	

marked terminal uremia was noted in two patients. It is evident from the individual protocols that all patients suffered from the complex metabolic derangement that is characteristic of diabetic coma. That is to say that in addition to hyperglycemia and glycosuria, there were varying degrees of dehydration, shock, acidosis and, in case 1, distortion of the serum electrolyte pattern.

Observations

In all cases there was moderate to marked glycogenic vacuolization of tubular epithelium. In ordinary sections the lesion showed a striking and constant localization astride the corticomedullary junction; but mainly in the outer medulla (Fig. 1). Extension to the innermost cortex was usual, but lesions were never seen in the middle or outer cortex.

The affected epithelial cells were markedly swollen, bulging into and sometimes apparently occluding the lumen (Fig. 2). The cell borders were unusually sharp, perhaps accentuated by virtually total conversion of the cytoplasm into a single large vacuole, in the center of which lay a normal appearing nucleus. Cell borders were sometimes stretched and smooth, at others greatly wrinkled, as though there had been shrinkage of the distended cell (Fig. 2). In the portions of the tubule showing the change, all cells were affected in apparent continuity. Sections stained with either aqueous or alcoholic PAS technique showed high concentrations of globular PAS-positive material in the cytoplasm of the affected cells (Fig. 3). This reaction was prevented by preliminary treatment of the sections with diastase (Fig. 4). Best's reaction for glycogen was also positive in the one case in which alcohol-fixed tissue was available (case 1). Fat stains on kidneys from all five cases, using oil red O on frozen sections, showed no stainable lipid in the glycogen vacuolated epithelium. In cases 2 and 5, however, fine fat droplets were present in the basal portion of the cytoplasm of the non-vacuolated cells of the proximal convoluted tubules.

The affected tubules were usually more or less markedly dilated. There was no noticeable abnormality of the connective tissue or vessels in the vicinity of the lesions. The unaffected tubules did not appear abnormal, i.e., we did not observe gradations or transitions between severely affected and normal tubules. It appeared, therefore, on the basis of conventional microscopic sections that the lesion had probably a rather abrupt beginning and end within the nephron. On the basis of the location of the changes within the renal topography it might have been assumed, as others have done, that the portion of the nephron principally affected was Henle's loop. It was possible, however, in occasional instances where the tubule had been cut longitudinally, to

see toward the cortical aspect an abrupt transition from normal appearing proximal tubular epithelium and the clear distended cells of the lesion (Fig. 2). It was thus apparent that, whatever other areas of the nephron might be affected, the proximal tubule clearly was involved in at least some cases. Dissection studies in all five cases demonstrated this to be true, and showed further that the proximal tubule is invariably the principal site of change (Figs. 5, 6, 7, 8, and 9). More precisely, the area of involvement was the terminal straight portion of the proximal tubule, spilling over occasionally to affect the contiguous beginning portion of the thin limb of Henle's loop (Fig. 7). This terminal portion of the proximal tubule is the relatively straight segment of it that descends into the medulla, that is to say, the relatively nonconvoluted portion of the proximal convoluted tubule. While it might be argued that this indeed is part of Henle's loop, the cells of this zone are morphologically indistinguishable from those of the upper proximal tubule, and are normally considered to be proximal tubule when seen in conventional sections.

The linear portion of the tubule affected was extremely variable, even in nephrons from the same kidney. Sometimes only a very short segment was involved (Fig. 8); at others as much as 20 per cent of the length of the proximal tubule exhibited the alteration (Fig. 7). In the dissected specimens the lesion, in its most fully developed form, appeared as one, or as a series of irregular, fusiform dilatations of the tubule (Fig. 5). These were often eccentric with respect to the axis of the tubule. The lesions were readily visible in the dissecting dish, where, in addition to the obvious dilatation, it was apparent that the tubule was considerably paler and more translucent than the adjacent more normal proximal segments.

Microscopic examination of the dissected specimen usually showed the lesion to consist of a translucent open lacework pattern (Fig. 9), presumably created by the vacuolation and distention of the affected cells. The basement membranes usually appeared intact, although in case 1 where there were also changes of acute nephrosis, foci of necrosis and rupture were apparent (Fig. 10).

In cases where the involvement was discontinuous, the foci of damage formed sausage-like links connected with one another by short segments of normal caliber (Fig. 8). The epithelial cells even between the dilated segments showed vacuolation and pallor. In some instances, and this was true of occasional nephrons from each of the cases, dilatation of the tubule was inconspicuous and pallor of the epithelium was the principal sign of damage.

A curious observation was made in the course of the dissection of

tissue from case 3. Although conventional microscopic sections had shown lesions to be numerous, nephrons from the first block of tissue dissected showed no evidence of damage. In seeking a possible explanation for this discrepancy, it was realized that the nephrons that arise close to the capsule of the kidney have Henle's loops that often do not reach or penetrate the medulla. It was postulated that if these nephrons did develop Armanni-Ebstein lesions, such lesions should occasionally be seen in the central or outer cortex in conventional sections. Review of the sections from all five cases showed that in none of them were lesions present in these areas. From this it was reasoned that the exclusively cortical nephrons were probably either less susceptible to glycogenic vacuolization or perhaps entirely immune. Dissection of additional blocks of kidney from case 1 with special attention to this possibility revealed that lesions were numerous and extensive in nephrons that penetrated the medulla but rare in those that did not. Further confirmation could not be made in the remaining cases since no more tissue was available for dissection. On the basis of the distribution of lesions in sections from these cases, however, it is probable that the exclusively cortical nephrons in them, too, were relatively resistant to damage. It should be pointed out also that the "immune" nephrons, in addition to their cortical location, were usually ones whose loop of Henle was short, and in which the thin segment was either very short, or non-existent (Fig. 11).

Discussion

There are several other forms of vacuolar nephropathy from which it is not difficult to distinguish the Armanni-Ebstein lesion. In poisonings from diethylene glycol or dioxane the change affects the entire proximal tubule and is usually associated with hyaline droplet formation or necrosis of cells in patchy fashion.[2] The vacuolation in patients treated with hypertonic sucrose or dextran is much finer and again affects the entire proximal tubule.[15] The changes associated with potassium loss in severe diarrhea affect isolated cells in random fashion.[16]

The epithelial changes in cases of von Gierke's disease are similar to those in diabetic cases in their appearance[17] but differ in that the upper portion of the proximal tubule appears selectively affected. The evidence that in some cases of von Gierke's disease there is impairment of intracellular glycogenolysis[18] might, however, suggest a similar explanation in the kidneys of diabetic patients. Fatty vacuolization as is seen in potassium deficient rats[19] is generally basal, and of course the

fatty nature of the vacuoles is demonstrated by appropriate stains. Similar fatty change was found in some of our cases, but in all instances the upper proximal tubule was affected and not the portion involved by Armanni-Ebstein lesion.

The latter lesion is distinctive in its nephronal localization, and this is reflected in the distribution of the changes as seen in conventional sections, astride the corticomedullary junction.

The obvious association of the Armanni-Ebstein change with the state of uncontrolled diabetes suggests almost too readily an association between the renal alteration and the high level of blood sugar and of urinary glucose excretion. Robbins[20] related the appearance of the lesions in alloxan-diabetic rats to blood glucose levels of at least 350 mg. per cent for a duration of 5 days or more. In spite of this evidence, it is well to remember that the renal metabolic disturbance in human patients with this lesion is a complex one in which there is usually distortion of electrolyte and fluid distribution and that one or all of these solute changes may be responsible for the type of alteration observed, rather than glucose alone.

Whatever the contribution by the factors indicated above, it is clear that glycogen and glucose are fundamental in the development of the renal changes. The exact means by which glycogen accumulates in the renal epithelium remains unclear. It is possible that the affected portion of the tubule, normally exposed to little or no filtered glucose,[21] is less well equipped to handle this substance than are the more proximal areas. The cells in the affected segment of the nephron, though normally identical with their more proximal neighbors, may possess a greater capacity for glycogenesis than for glycogenolysis when faced with excessive concentrations of glucose substrate. Another possibility would be that excessive glycogenesis, or defective glycogenolysis, results in some way from the effects of acidosis or dehydration, rather than reflecting a simple response of a normal physiologic mechanism to uncomplicated substrate excess.

The most impressive finding in this study was the relative immunity to damage of those nephrons that did not penetrate the corticomedullary junction. This was in marked contrast to the almost general susceptibility of the penetrating nephrons. Although a number of possible explanations can be proposed for the different susceptibility of these two classes of nephrons, there is no direct evidence on this point in the material at hand. An obvious morphologic difference between the affected and uninvolved nephrons is that the latter have a short loop and a non-existent or rudimentary thin limb. On this basis it might be pro-

posed that the thin limb normally imposes a degree of stasis just above its commencement and that this plays a rôle in the pathogenesis of the lesion.

Resistance of the cortical nephrons to glycogenic change may, on the other hand, be related to the fact that their loops lie in a more richly vascular environment than do those of the penetrating nephrons. This could lead to improved glucose transfer with reduced liability to intracellular stasis in the form of glycogen. It would seem more probable that this is the case than that this minority of nephrons is functionally different from the rest, although the latter possibility cannot be excluded.

Whatever the reason for the resistance to glycogenic change demonstrated by the cortical nephrons, it would be of some interest to determine whether they are also resistant to other forms of damage, e.g., poisoning, acute nephrosis.[22]

Summary

The kidneys from five cases of Armanni-Ebstein diabetic nephropathy were studied by observation of conventional microscopic sections and by the technique of maceration and dissection of individual nephrons. The lesions in all cases consisted of marked glycogenic vacuolization of the epithelium of renal tubules in the outer medulla and innermost cortex. Tubules in the central and outer cortex were not affected.

Dissection of nephrons showed the lesions to be localized to the terminal straight segment of the proximal convoluted tubule, with occasional extension into the contiguous portion of the thin limb. It was established in one case, and inferred in the others, that the exclusively cortical nephrons, which have a short Henle's loop and which do not penetrate the medulla, are relatively resistant to glycogenic vacuolization. The possible reasons for this reduced susceptibility to damage are considered.

REFERENCES

1. Warren, S., and Le Compte, P. M. The Pathology of Diabetes Mellitus. Lea & Febiger, Philadelphia, 1952, ed. 3, 336 pp.
2. Allen, A. C. The Kidney. Grune & Stratton, New York, 1951, 583 pp.
3. Robbins, S. L. Renal lesions in diabetes mellitus. *Bull. New England M. Center*, 1948, 10, 78–88.
4. Armanni. Cited by Ebstein.[5]
5. Ebstein, W. Weiteres über Diabetes mellitus, insbesondere über die Complication desselben mit Typhus abdominalis. *Deutsches Arch. f. klin. Med.*, 1882, 30, 1–44.
6. Ehrlich, P. Ueber das Vorkommen von Glykogen im diabetischen und im normalen Organismus. *Ztschr. f. klin. Med.*, 1883, 6, 33–46.

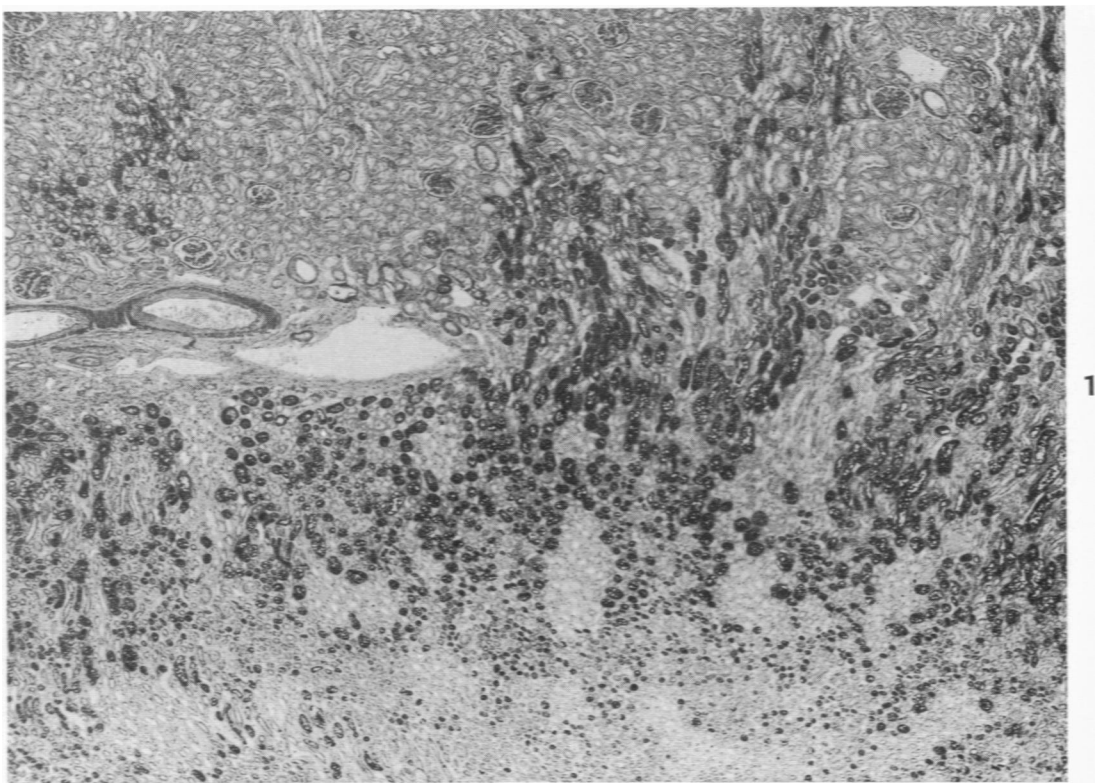
7. Straus, I. Contribution a l'étude des lésions histologique du rien dans le diabéte sucré. *Arch. de physiol. norm. et path.*, 1885, s. 3, **6**, 322–350.
8. Baehr, G. Über die Sekretion von Glykogen in Diabetikernieren. *Beitr. z. path. Anat. u. z. allg. Path.*, 1913, **56**, 1–12.
9. McManus, J. F. A. The periodic acid routine applied to the kidney. *Am. J. Path.*, 1948, **24**, 643–653.
10. Mowry, R. W., and Millican, R. C. A histochemical study of the distribution and fate of dextran in tissues of the mouse. *Am. J. Path.*, 1953, **29**, 523–545.
11. Lillie, R. D. Histopathologic Technic and Practical Histochemistry. The Blakiston Co., Inc., Philadelphia, 1954, pp. 276–277.
12. Oliver, J. Architecture of the Kidney in Chronic Bright's Disease. Paul B. Hoeber, Inc., New York, 1939, 256 pp.
13. Darmady, E. M., and Stranack, F. Examen histologique du néphron isolé. *Vie méd., Par.*, Special No., Dec., 1954, pp. 6–8. (Cited by Darmady, E. M.; Dempster, W. J., and Stranack, F. The evolution of interstitial and tubular changes in homotransplanted kidneys. *J. Path. & Bact.*, 1955, **70**, 225–231.)
14. Bialestock, D. The morphogenesis of renal cysts in the stillborn studied by micro-dissection technique. *J. Path. & Bact.*, 1956, **71**, 51–59.
15. Vickery, A. L. The fate of dextran in tissues of the acutely wounded: A study of the histologic localization of dextran in tissues of Korean battle casualties. *Am. J. Path.*, 1956, **32**, 161–183.
16. Kulka, J. P.; Pearson, C. M., and Robbins, S. L. A distinctive vacuolar nephropathy associated with intestinal disease. *Am. J. Path.*, 1950, **26**, 349–377.
17. von Gierke, E. Hepato-Nephromegalia glycogenica (Glycogenspeicherkrankheit der Leber und Nieren.) *Beitr. z. path. Anat. u. z. allg. Path.*, 1929, **82**, 497–513.
18. Recant, L. Recent developments in the field of glycogen metabolism and the diseases of glycogen storage. *Am. J. Med.*, 1955, **19**, 610–619.
19. Follis, R. H., Jr.; Orent-Keiles, E., and McCollum, E. V. The production of cardiac and renal lesions in rats by a diet extremely deficient in potassium. *Am. J. Path.*, 1942, **18**, 29–39.
20. Robbins, S. L. The reversibility of glycogen nephrosis in alloxan-treated diabetic rats. *Am. J. M. Sc.*, 1950, **219**, 376–381.
21. Oliver, J. When is a kidney not a kidney? *J. Urol.*, 1950, **63**, 373–402.
22. Waugh, D. Acute nephrosis: the kidney of acute renal failure. *Am. J. M. Sc.*, 1953, **226**, 310–325.

[*Illustrations follow*]

LEGENDS FOR FIGURES

FIG. 1. Case 3. Corticomedullary junction of kidney. Dark glycogen deposits are concentrated mainly in tubules of outer medulla with some irregular involvement of innermost zone of cortex. Periodic acid-Schiff (PAS) stain with hematoxylin counterstain. × 25.

FIG. 2. Case 1. Dilatation and vacuolization of tubules. Partial involvement of a proximal tubule is seen at the center of the photograph. Other tubules show involvement of all epithelial cells. Hematoxylin and eosin stain. × 200.



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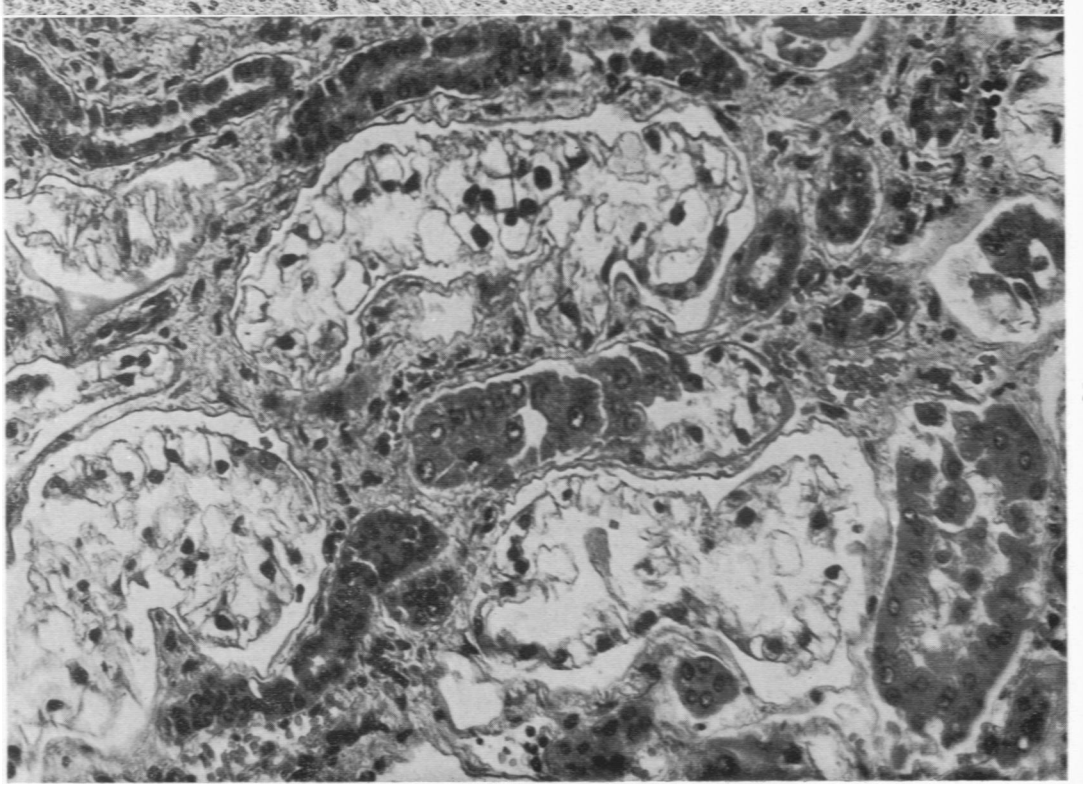

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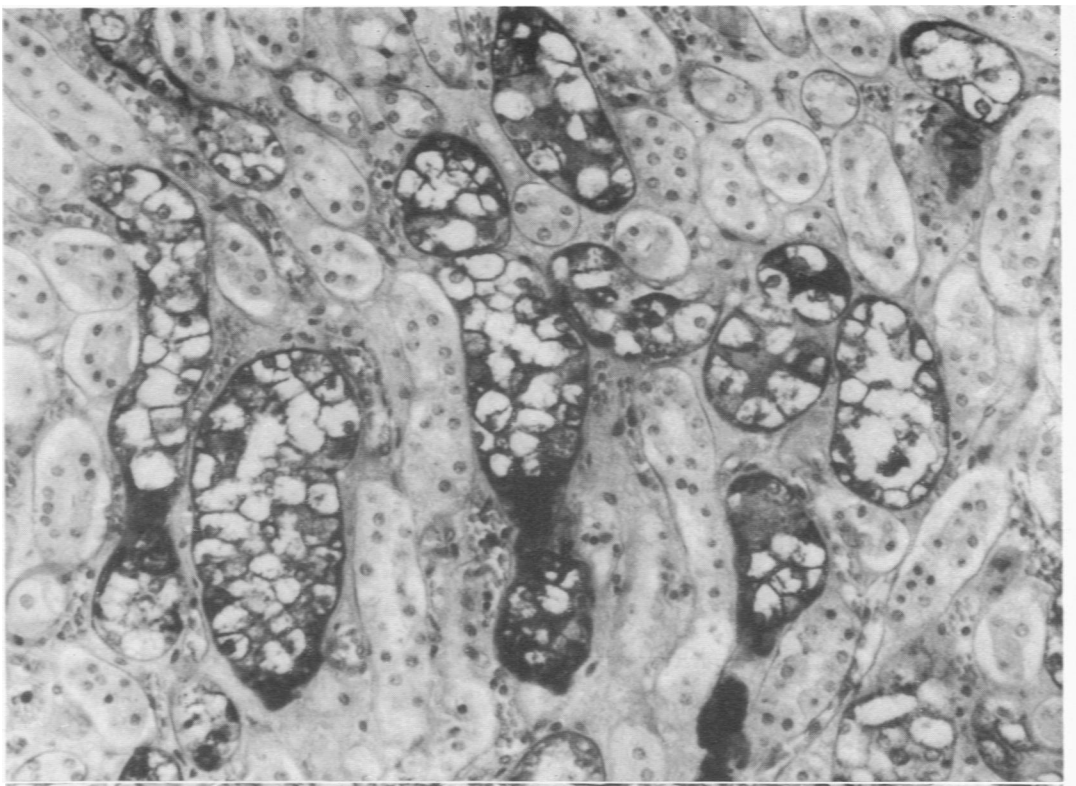
FIG. 3. Case 2. PAS-positive material in epithelial cytoplasm of involved tubules. PAS stain with hematoxylin counterstain. × 160.

FIG. 4. Case 2. PAS positivity removed from vacuolated cells by preliminary treatment with diastase. Diastase digestion followed by PAS reaction. × 200.



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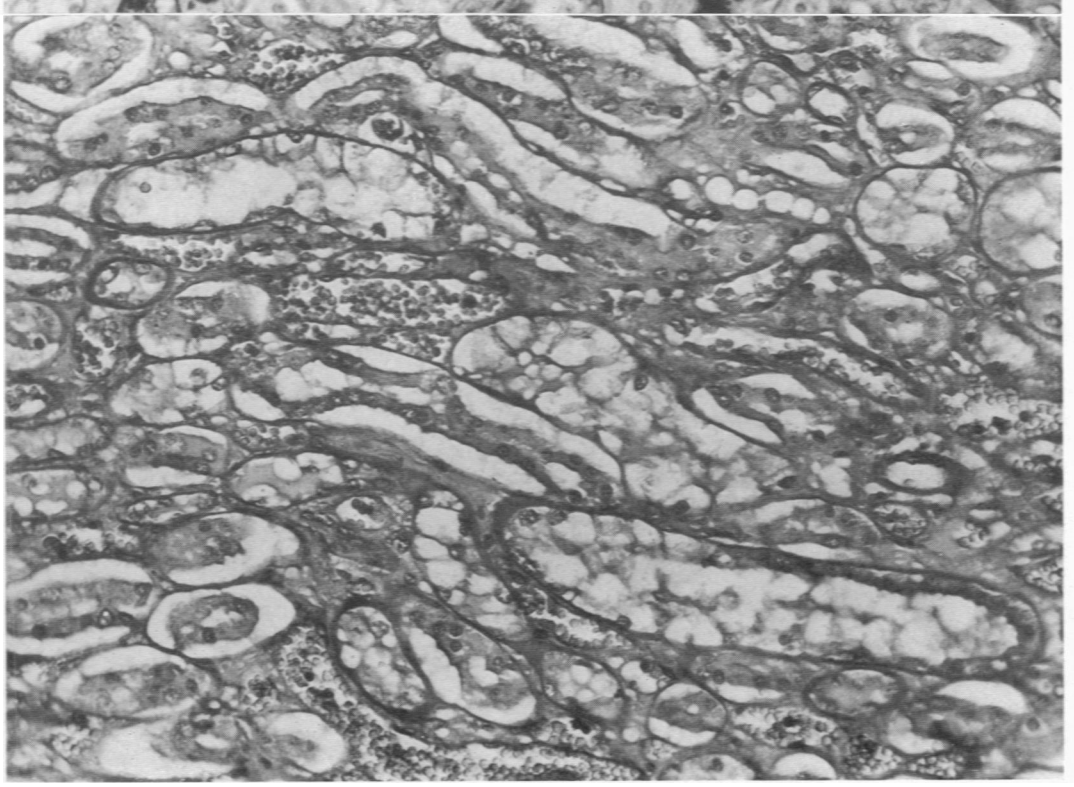

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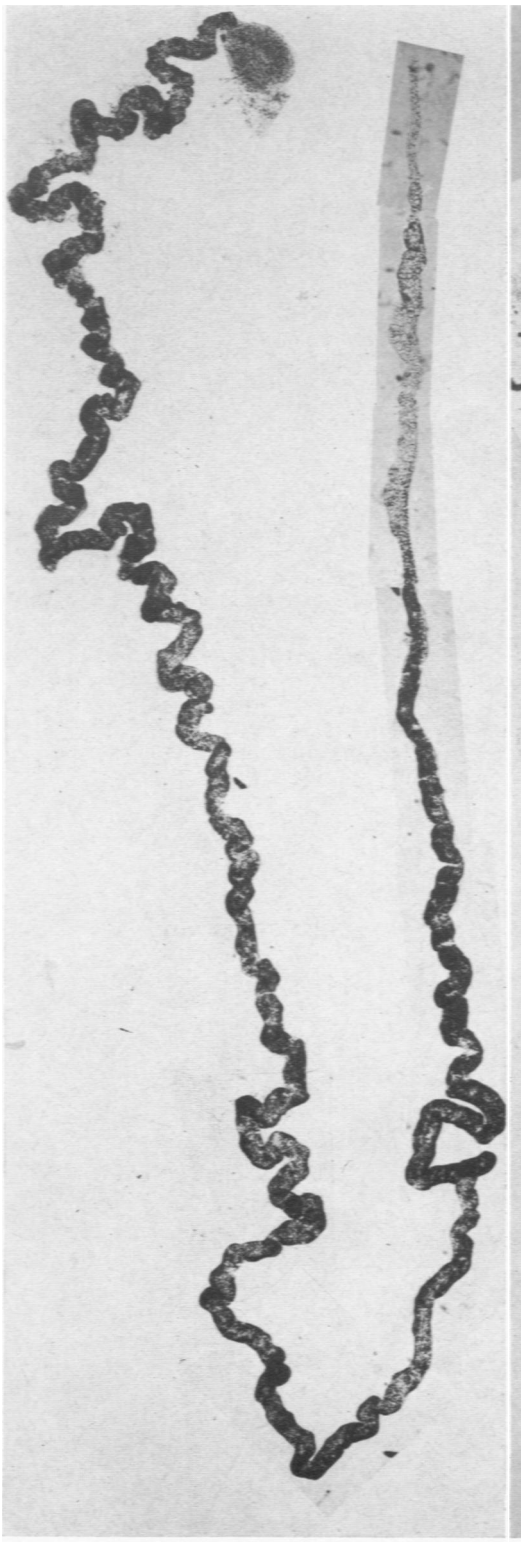
FIG. 5. Case 2. Photomontage reconstruction of isolated glomerulus, proximal tubule, and beginning of Henle's loop. The glomerulus and most of the proximal tubule are normal. At the distal end of the specimen there is irregular fusiform dilatation, pallor and translucency of the terminal proximal tubule, and possibly of the commencement of the thin limb. The remainder of the thin limb is normal in appearance. Unstained aqueous mount. × 40.

FIG. 6. Case 5. Isolated upper nephron showing epithelial pallor and dilatation of terminal proximal tubule. The mosaic appearance was produced by vacuolation of epithelium. Unstained aqueous mount. × 40.



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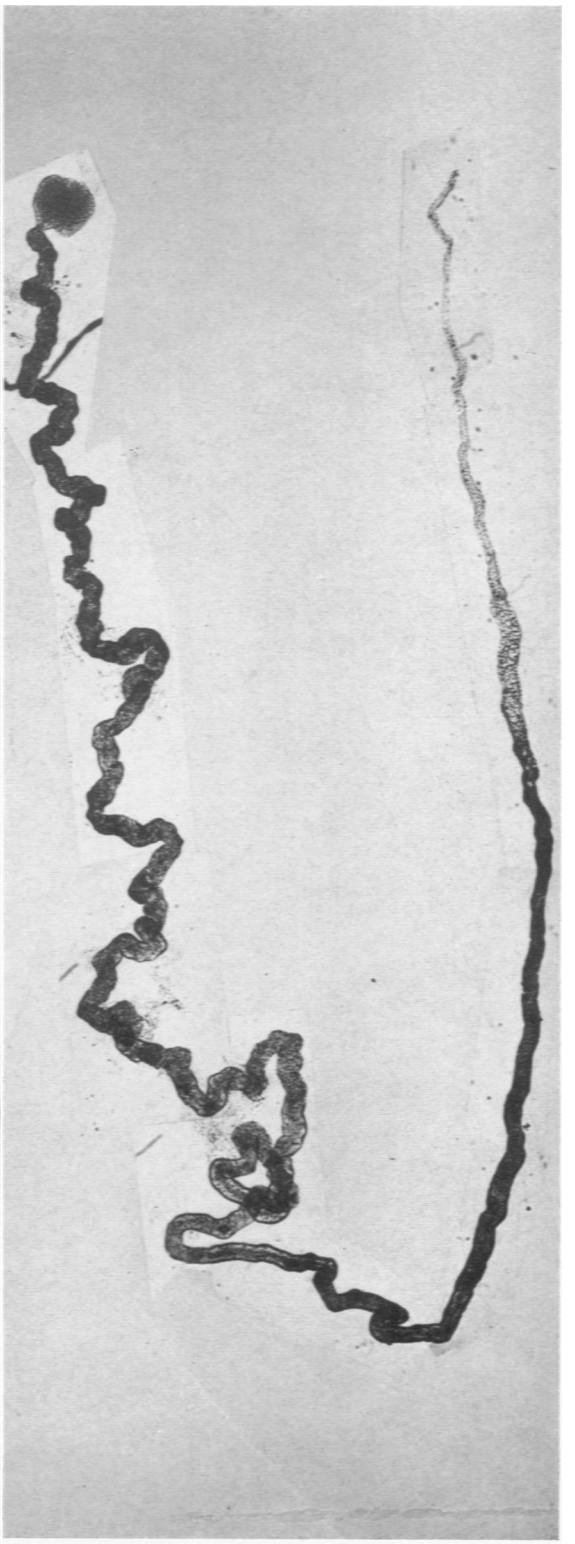

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FIG. 7. Case 3. Terminal portions of proximal tubule showing irregular saccular dilatation. Unstained aqueous mount, × 40; enlarged area, × 160.

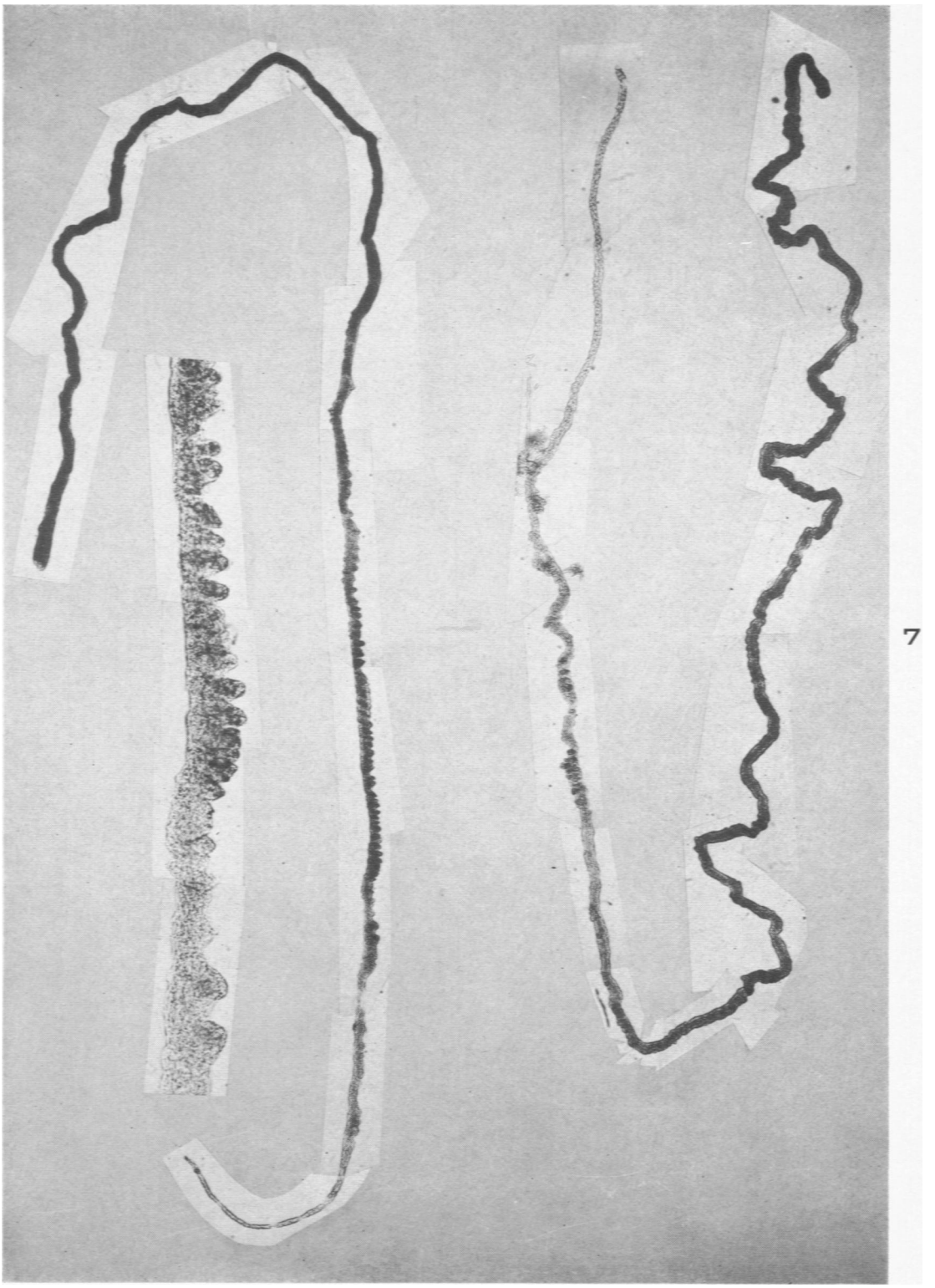

FIG. 8. Case 1. Small localized saccular lesions confined to the thin limb. Unstained aqueous mount. × 40.

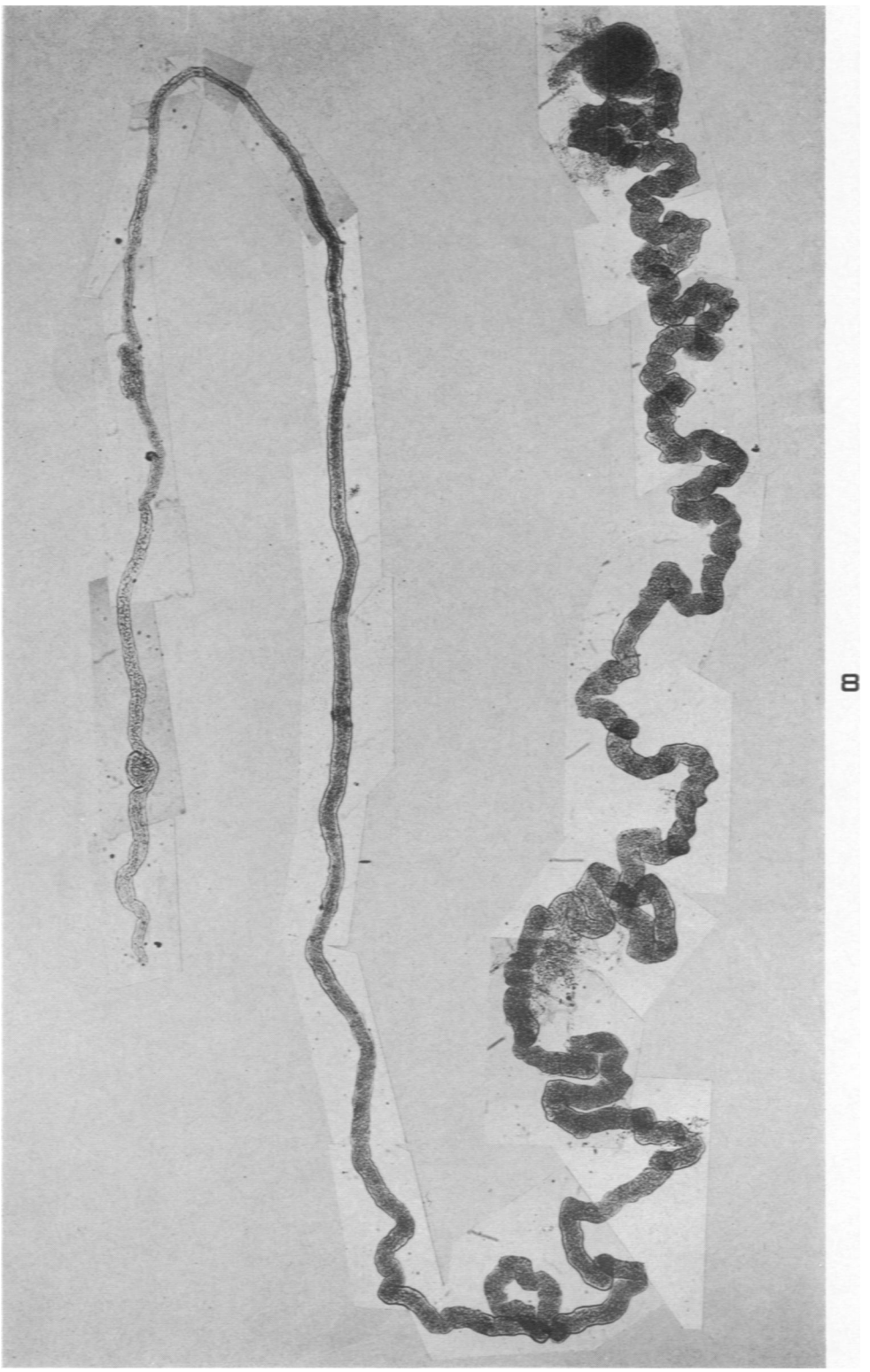

FIG. 9. Case 4. Diffuse Armanni-Ebstein lesion of terminal proximal tubule. High-power photograph shows complex refractile feltwork appearance produced by distended cells. Unstained aqueous mount. × 40; high power. × 165.

FIG. 10. Case 1. Two distal convoluted tubules and collecting tubule. Patient died in renal failure. Specimen shows irregular foci of necrosis of tubule. obstruction by casts. Adhesive strands of connective tissue are attached to the left tubule. Iron hematoxylin Gelatinol (C) mount. × 40; high power. × 160.

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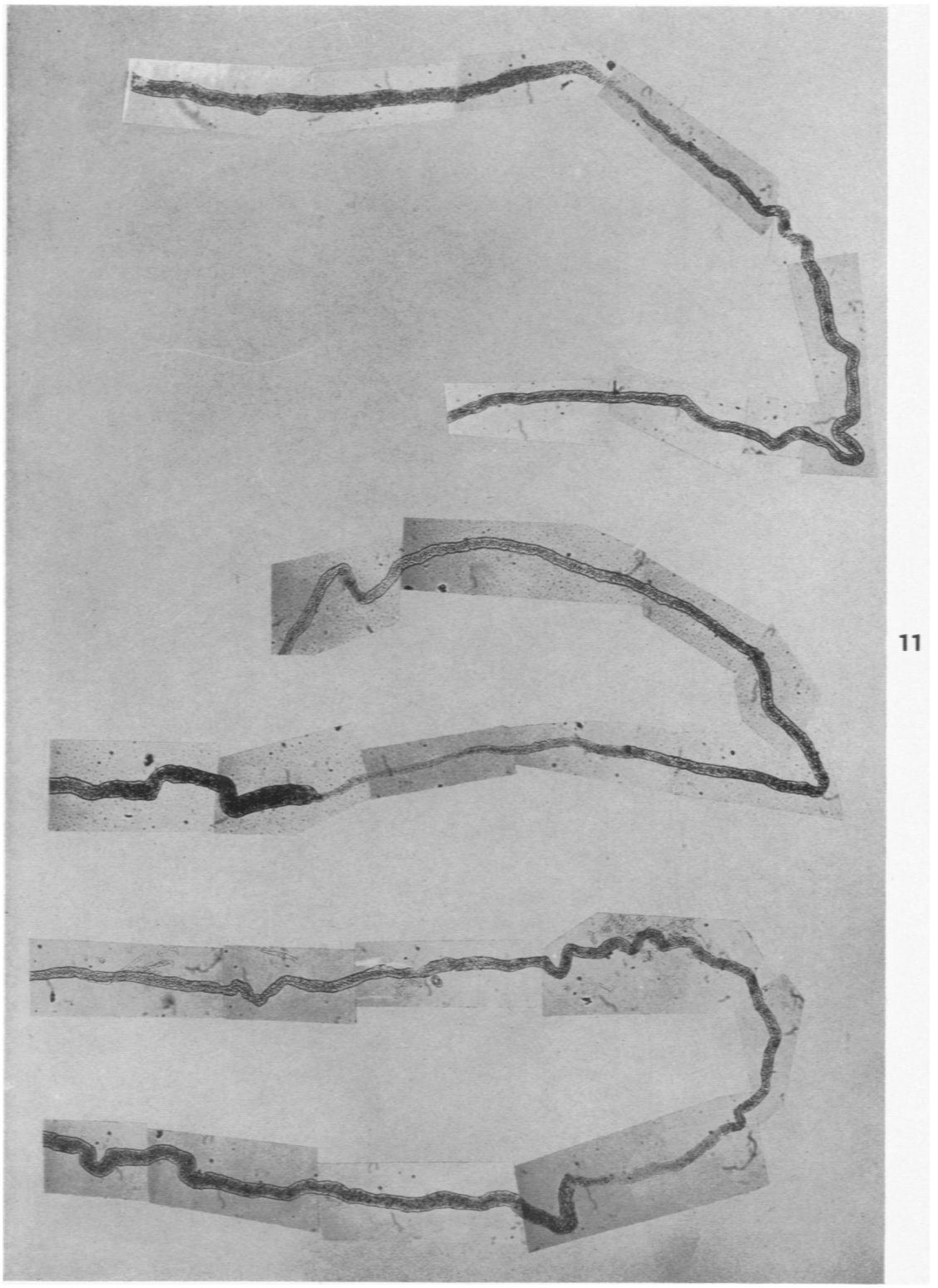
FIG. 11. Case 1. Three uninvolved loops from nephrons originating just beneath the renal capsule. A short thin limb is present in the center specimen only. Unstained aqueous mounts. × 40.



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