Ultrastructural Cytochemistry of the Ischemic (Endocrine) Kidney

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Partial ligation of the aorta between the renal arteries induces marked atrophy of the cortical tubules (including the macula densa) of the left (endocrine) kidney with a remarkable increase in the number and granularity of hypersecretory juxtaglomerular granulated cells (JGC) which are found not only at the glomerular pole of arterioles but also in the walls of arteries and arterioles far removed from the glomerulus. Staining of fine sections of Araldite-embedded endocrine kidneys according to the periodic acid–thiocarbohydrazide–silver proteinate technique of Thiery reveals abundant glycogen in the JGC and less in the blood vessels and tubules. Juxtaglomerular granules are argentaphobic, but their rim is positively stained when ultrathin sections of glutaraldehyde-fixed, glycol methacrylate–embedded kidneys are exposed to phosphotungstic acid at a low pH. A positive reaction is also shown by the cell coat and lysosomes of JGC as well as by the thickened basal lamina, cell coat, cytosomes, and cytosegresomes of the atrophic tubules. Atrophy is most pronounced in the proximal convoluted tubules, which lose their apical microvilli, their basal infoldings and the majority of their mitochondria and cytosomes. (Am J Pathol 82:527–548, 1976)

SELYE [1] FORMULATED the concept of the endocrine kidney by showing that partial constriction of the aorta between the renal arteries diminishes the arterial pressure of the left kidney to a level which is equal or inferior to the filtration pressure. The kidney then loses its excretory function and is transformed into an endocrine organ as evidenced by the rapid development of acute hypertensive disease.[2] Histologically, the endocrine kidney is characterized by a total absence of the nephrosclerotic lesions that typically affect the contralateral organ. The tubules lose their lumen, and the proximal convoluted tubules also lose their brush borders and eosinophilic granules. This is accompanied by a striking increase in the number and granularity of juxtaglomerular cells,[3,4] which ultrastructurally manifest all the signs of secretory hyperactivity.[3] Concomitantly, there is a marked rise in the renin content of these kidneys.[4,5]

The aim of the present investigation is to study, with ultrastructural

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cytochemical methods, the morphologic changes which occur in the renal vessels and tubules under such conditions of severe chronic ischemia.

Materials and Methods

Animals and Surgery

Surgery was performed on 50 female Sprague-Dawley rats (Fermes et Laboratoires Canadiens d'Elevage Ltée, St. Constant, Qué.) with a mean initial body weight of 200 g (range, 190 to 210 g) under ether anesthesia on the first day of the experiment. Surgery consisted of partial ligation of the aorta between the renal arteries using a silk thread and the style (diameter, 0.103 mm) of a No. 30 injection needle. Removal of the style, once the ligation was made, produced a severe but partial constriction of the aorta, inducing a rapid and marked atrophy of the left kidney.[3,6] The left ureter was also ligated in 10 rats to verify that urine formation from the atrophic kidney had ceased; the left ureter was not ligated in the remaining 40 animals, and those showing hydronephrosis or renal necrosis were eliminated (less than 10%). Ten other rats of the same breed and body weight were used as absolute controls (Table 1). All animals had free access to Purina Laboratory Chow and drinking water.

The 10 controls and 9 rats with endocrine kidneys were sacrificed on the tenth day. Their left kidneys were kept in 10% formalin for 24 hours and then weighed.

Preparation for Electron Microscopy

On the tenth day of the experiment, the left kidneys were perfused,[7] with the rats under ether anesthesia, first with 20 to 40 ml of Ringer-Locke solution and then for 10 minutes with 2% glutaraldehyde buffered with cacodylate-HCl (0.1 M at pH 7.1).[8] Small fragments of renal cortex were placed in the same fixative for 2 hours at 4 C. All specimens were washed for three 15-minute periods in cacodylate buffer to which 2% sucrose had been added. They were left in the buffer for 12 hours. Some renal fragments were post-fixed for 1 hour in 2% osmium tetroxide buffered with Veronal acetate. Only the specimens fixed in glutaraldehyde alone were embedded in glycol methacrylate (GMA), as already described.[8] The other fragments were embedded in Araldite. For routine electron microscopy, ultrathin sections of either Araldite-embedded or GMA-embedded kidneys were cut on a MT-1 ultramicrotome with glass knives, stained with uranyl acetate and lead citrate,[8] and examined under a Philips 201 electron microscope.

Table 1—Effect of Aortic Ligation on Final Body Weight and on the Weight of the Ischemic (Endocrine) Kidney (mean ± SE)

Group	Treatment	No. of animals	Final body weight (g)	Left kidney weight (mg/100 g body weight)
1	—	10	204.6 ± 2.46	467.7 ± 16.13
2	Partial ligation of aorta*	9	124.8 ± 3.83†	229.2 ± 17.47†

* Aortic ligation between renal arteries was performed on the first day of the experiment. The animals were killed on the tenth day.

† Significant ($P < 0.001$) in comparison with the respective controls.

Cytochemistry

Thiery's Method

The periodic acid–thiocarbohydrazide–silver proteinate technique of Thiery [9,10] was used for the demonstration of periodate-reactive vicinal glycols on tissues fixed either in glutaraldehyde alone or in combination with osmium tetroxide and embedded in Araldite.[8,11]

Low pH Phosphotungstic Acid (PTA)–Hydrochloric Acid Method

The technique [12-14] was employed for the visualization of hydroxyl groups in complex carbohydrates. Ultrathin (silver to gray) sections from glutaraldehyde-fixed, GMA-embedded tissues were floated on a 1% solution of PTA in 1 N HCl (pH 0.3) for 8 to 10 minutes, then rapidly washed in distilled water.[7,8,11]

Results

Clinical State

Table 1 shows a severe loss of body weight after partial ligation of the aorta between the renal arteries. The weight of the left ischemic (endocrine) kidney was less than half of the left kidney of control animals.

Juxtaglomerular Apparatus

Ultrastructure

Juxtaglomerular granulated cells (JGC) were very numerous in the preglomerular arterioles, while smooth muscle cells were extremely rare. Each of these cells was surrounded by a thick cell coat. The JGC of the arteriolar walls were ovoid or elongated; those of the external part of the wall frequently exhibited cytoplasmic projections. Their nuclei were similar to those of adjacent smooth muscle cells. Micropinocytotic vesicles were present at the periphery of both types of cells. Two kinds of granules were encountered in the JGC. Specific granules (JGG), by far the most abundant, were round, oval or irregularly-shaped and were surrounded by a unit membrane. Their content was often dense and finely granular, rarely amorphous. Some of these granules were much more electron lucent but were still granular. Several of them showed a mixed content with dense portions adjacent to more electron-lucent ones. Granules in the Golgi area were frequently in the process of fusion. Nonspecific granules corresponding to residual bodies were more rare, of lesser diameter, with irregular contours and a heterogeneous content; they were generally very

osmiophilic and made up of small granules and membrane fragments. The mitochondria were relatively few and were of the cylindrical type with short cristae. The rough endoplasmic reticulum was often dilated and filled with a flocculent, electron-lucent material. The Golgi apparatus was frequently prominent; it usually occupied a large paranuclear area, and consisted mostly of small, extremely numerous vesicles.

Lacis cells appeared to be undisturbed by renal ischemia. They were small, elongated, separated by a thick cell coat, and contained a few dense lysosomes.

Macula densa cells were often separated from one another by large intercellular spaces extending to the basement lamina. No basilar interdigitating processes were visible. The cytoplasm was extremely simplified, with only a few mitochondria and lysosomes, but the frequent residual bodies were filled with membrane whorls. Their lumina most often had entirely collapsed. Contrary to what was found in normal macula densa (i.e., light and dark cells),[15,16] only one type of atrophic cell could be identified.

Ultrastructural Cytochemistry

All the JGG, regardless of the method of fixation (with or without osmium tetroxide), were argentaphobic when stained according to Thiery's technique, even after 5 days of exposure to thiocarbohydrazide (Figures 1 and 2). They showed a pale, uniform matrix and reacted positively to PTA (Figures 3 and 4). The stained area was confined to a narrow zone along the limiting membrane. The smaller, nonspecific granules displayed irregular deposits of silver grains when stained according to Thiery's method, even without periodic acid oxidation. They reacted intensely to PTA.

The *surface* or *cell coat* (glycocalyx) was barely visible after Araldite-embedded tissues were stained with uranyl acetate and lead citrate, nor could it be seen convincingly with the Thiery technique, even after 5 days of exposure to thiocarbohydrazide. After staining with PTA, the cell coat appeared as a dark, thick, irregular line separating each cell from its neighbors and from endothelial cells.

All Golgi structures were silver-negative when stained by Thiery's technique, even in sections exposed to thiocarbohydrazide for 5 days. No structures corresponding to the Golgi complex could be recognized convincingly with PTA.

Glycogen (monoparticulate, β-type) could be demonstrated after only 30 minutes of exposure to thiocarbohydrazide. The granules were spherical and were distributed singly or in clumps between the organelles.

They were more abundant in JGC than in smooth muscle cells, particularly around the JGG. They were not stained when the pêriodic acid or thiocarbohydrazide steps were omitted but reacted with the same intensity whether or not the tissues were fixed in osmium tetroxide. Glycogen was not demonstrated by PTA.

The lacis cells, after staining with Thiery's technique, showed only a few small lysosomes covered focally by silver precipitates. Beta particles of glycogen were scarce. PTA demonstrated a thick surface coat and a few small lysosomes.

Macula densa cell components were generally not stained by Thiery's technique except for a few focally reactive lysosomes and residual bodies and relatively abundant glycogen granules (Figure 1). These lysosomes and a portion of the material contained in the residual bodies were stained by PTA which, in addition, revealed part of the cell coat (Figure 3).

Renal Cortical Blood Vessels and Glomeruli

Ultrastructure

All the arteries and arterioles were remarkably well preserved and showed no evidence of injury. In practically all the arterioles examined, even in those far away from the glomeruli and in several arteries, otherwise normal smooth muscle cells—easily recognized by their filamentous cytoplasm—contained granules which had the same shape, density, and reactivity as the JGG (Figure 5). The Golgi complex was much more developed in these cells than in ordinary smooth muscle cells, and the rough endoplasmic reticulum was comprised of several saccules filled with a flocculent, electron-lucent material. The glomerular epithelial, endothelial, and mesangial cells mostly seemed to be normal. A few basement membranes in some glomeruli were focally thickened.

Ultrastructural Cytochemistry

Thiery's technique revealed β-glycogen in arterial and arteriolar smooth muscle cells but not in endothelial cells. Silver grains were also located in a few lysosomes and residual bodies of smooth muscle and endothelial cells. PTA stained the smooth muscle cell coat as well as the JGG rim (Figure 5). No glycogen was detected in the epithelial, endothelial, or mesangial cells of the glomeruli. A few lysosomes and residual bodies were focally stained, even upon omission of the periodic acid oxidation or thiocarbohydrazide steps. PTA delineated the glomeruli basement membranes as well as the endothelial, epithelial, and mesangial cell surfaces. The lysosomes in these cells were also stained.

Renal Cortical Tubules

Ultrastructure

The architecture of the tubules was the same whether or not the ureter had been ligated. The lumina had collapsed in all the tubules examined. In the proximal convoluted tubules, there were frequent interdigitations of the few remaining microvilli in cells facing each other (Figure 6). Apical vesicles and tubules, Golgi complexes, and basilar interdigitating processes could not be visualized. Lateral cell processes were often evident, and the intercellular spaces were usually markedly enlarged. The basement laminae were considerably thickened. The number and size of mitochondria had decreased, and those that remained were distributed haphazardly in the cytoplasm. The nuclei were unaltered and even showed nucleoli. Cytosomes were absent in the majority of cells, but there were abundant cytosegresomes containing membrane fragments and other cellular debris, including lipids. In some cells, lipid vacuoles occupied more than half of the remaining cytoplasm (Figure 7). Atrophy was similarly apparent in distal convoluted tubules and in collecting ducts, but it was generally less severe. Basilar interdigitating processes were missing whereas lateral cell processes persisted in some cases. The basement lamina had thickened. The mitochondria had diminished in size and number, and cytosegresomes were prominent.

Ultrastructural Cytochemistry

Although it is virtually absent from renal cortical tubules in adult rats,[7] glycogen was present in relatively large amounts in the form of β-particles (Figure 1). The few remaining cytosomes as well as the more prevalent cytosegresomes were focally covered with silver precipitates, even without exposure to periodic acid or thiocarbohydrazide. PTA strongly stained the thickened basement lamina and parts of the cell coat (Figure 9). A few cytosomes showed a characteristic reaction to PTA,[7] i.e., at their rim. Also stained was a part of the content of structures probably corresponding to cytosegresomes.

Discussion

Electron microscopic studies of JGC in the endocrine kidney reveal abundant rough endoplasmic reticulum, prominent Golgi complexes, and numerous JGG of varied shape and electron density. According to observations on other types of secretory cells,[17] it may be concluded that these JGC are hypersecretory. Such ultrastructural signs of hyperactivity

are accompanied by an extremely high juxtaglomerular granulation index,[3,4] by an elevated renal renin content,[4,5] by the presence of typical JGG in the smooth muscle cells of renal arteries and arterioles and—judging from the intense catabolism, high blood pressure, and rapid development of arterial and arteriolar lesions—by the secretion of a large quantity of renin into the circulation.

The presence of typical JGC in renal cortical arteries and arterioles, which possess numerous characteristics of smooth muscle cells (such as filaments and attachment bodies [3]) raises questions about the origin of these cells. By light microscopy, no mitosis can be visualized in the walls of these arteries and arterioles [3] and, during the present study, we observed only a few binucleated, granulated cells whose cytoplasm sometimes contained numerous microtubules in a paranuclear position. Although it is possible that the remarkable and rapid increase of JGC noted in the endocrine kidney is due to division of JGC already present in the arterial and arteriolar walls, it seems more likely that there is metaplasia of smooth muscle cells. This hypothesis is strengthened by the presence not only of JGG but also of the much more prominent rough endoplasmic reticulum and Golgi complex in otherwise typical smooth muscle cells. If such is the case, the question of why ischemia in other tissues does not elicit the same phenomenon, since renal arterial and arteriolar smooth muscle cells are ultrastructurally similar to vascular smooth muscle cells located elsewhere, then arises.

In striking contrast to the juxtaglomerular apparatus and blood vessels, all the renal cortical tubules of the endocrine kidney are atrophic. The various changes observed in these atrophic cells are not artifactual, since the same fixation technique reveals a classic renal tubular ultrastructure in normal animals.[7,18] Furthermore, the tubular alterations were not due to hydronephrosis, for they were similar in rats whose ureter had not been ligated. These changes of renal tubular cells are very different from those of acute ischemia.[19,20] In several aspects, as revealed by light microscopy,[2] they are similar to the ischemic tubular atrophy characteristic of human hypertension.[21-23] In these cases, crowded intact glomeruli are surrounded by small but viable tubules lined with cuboidal cells that usually have a clear cytoplasm. The changes are also analogous to the tubular atrophy induced by sclerosing glomerulonephritis or other obliterative glomerular disease.[24] The presence of an identical degree of tubular atrophy seen in all the endocrine kidneys examined as well as the absence of arterial and arteriolar lesions indicate that the present technique produces more uniform results than those previously described following renal artery ligation in the rabbit.[25] Earlier studies [24,26] have shown that thickening of the

basement membranes precedes tubular cell atrophy. The basal infoldings diminish in height and often disappear in the early stages of the process. At the apical pole, the microvilli become less numerous and lose their uniformity. The cells themselves diminish in size and contain fewer organelles, except for cytosegresomes, which increase in number and are transformed into large, highly osmiophilic masses surrounded by a membrane. Lipid vacuoles become numerous. Their origin has been ascribed either to a decreased utilization or to an anomalous intracellular transport.[24] Glycogen has already been noted in atrophic tubular cells [27] and might be attributed to the lower metabolic requirements of these cells. That the process of ischemic atrophy can be reversed by restoration of an adequate blood supply [28] shows the viability of the tubular cells. The picture of tubular atrophy and cellular simplification, as observed in the present experiment, is analogous to the regression stage of mesonephros in the chicken embryo [29] and the immature kidney of newborn mice.[30]

Although proteins seem to be the major constituent of JGG,[31] it has long been known that they are PAS positive [32-37] and that they become PAS negative when oxidation is followed by acetylation, which might indicate that they contain neutral glycoproteins instead of sulfated polysaccharides.[38,39] The PAS positivity of JGG in man might be due in part to the presence of abundant lipofuscin granules,[32] but such is not the case in the rat where most of the granules in the JGC are specific and where nonspecific granules are rare. Our results confirmed these findings inasmuch as the JGG were positively stained by PTA at a low pH. Direct staining of hydroxyl groups in complex carbohydrates has been achieved, with a high degree of specificity, with PTA (at a low pH) in GMA-embedded sections of glutaraldehyde-fixed tissues.[13,14,40] Complete agreement between the PTA and PAS methods has already been reported.[8-11,41-44] However, other studies [45-47] have cast doubt on the validity of the PTA technique for the detection of complex carbohydrates and have indicated that PTA is an anionic stain for certain positive groups of proteins. The fact that some polysaccharides (viz, glycogen) do not show up in PTA-stained sections would be an argument against the specificity of this stain. Whatever the mechanism of the staining reaction, no one—to our knowledge—has disproved that, in fine sections of glutaraldehyde-fixed, GMA-embedded tissues, or in fine sections obtained by ultracryotomy, PTA at a low pH reveals carbohydrates, which are PAS positive when seen light microscopically. Glycogen is intensely reactive in fine sections obtained by ultracryotomy [48,49] and, under appropriate conditions, is also stained in GMA-embedded tissues.[50] Although the Golgi complex of JGC is quite extensive in routine electron microsopy, we have

been unable to localize it in PTA-stained sections. It is possible that, as in atrial cells,[11,42] only a small part of the Golgi complex is stained under these conditions. The PAS and PTA positivity of JGG may be due, at least in part, to the acid phosphatase,[51] β-glucuronidase,[52] and renin [53] present in these granules. Although acid phosphatase has not been purified from JGG, it may be assumed from other studies [54,55] to be a type of glycoprotein. In the same manner, β-glucuronidase is known to contain 3 to 6% carbohydrate and is considered to be a glycoprotein.[56] Purified renin is also positively stained by the Hotchkiss method.[36]

The results obtained with Thiery's technique have not been systematically compared with the PAS and PTA stains. According to Thiery,[9,10] these results depend upon the duration of exposure to thiocarbohydrazide: 30 to 40 minutes are sufficient for glycogen, 24 to 48 hours are adequate for mucopolysaccharides, and 48 to 72 hours are suitable for glycoproteins. This technique, however, appears to be much less sensitive than the PTA method;[8] nevertheless, it reveals a much greater amount of glycogen in JGC than in smooth muscle cells.

The staining of the cell coat and of the nonspecific granules of JGC by PTA is similar to what has already been observed in a large number of cell types.[7,11,41,57] Like JGG, these structures are well known to be PAS positive.[58,59]

The same remarks apply to the cell coat, cytosomes, and cytosegresomes of tubular cells. The simplification of these cells in the endocrine kidney is strikingly evident after staining with PTA. The basilar interdigitating processes, apical tubular invaginations, apical dense tubules, apical vesicles and apical vacuoles are well stained in normal proximal convoluted tubule cells, but are not apparent in the endocrine kidney. The apical surfaces of remaining microvilli and the cell coat are generally much less intensely stained than in normal tubular cells.

As revealed by the present study, the ultrastructure of the endocrine kidney raises some questions regarding the role of the macula densa in the function of the juxtaglomerular complex. Several investigations have shown that the activity of glucose-6-phosphate dehydrogenase and of 6-phosphogluconate dehydrogenase increases under conditions of hyperactivity of the juxtaglomerular complex (ischemic kidney, sodium deficiency, adrenalectomy) and decreases under inverse circumstances (contralateral, nonischemic kidney, overdosage with desoxycorticosterone and sodium). It has been postulated that the macula densa may act as a sodium sensor, transmitting information to the JGC for renin release. Opinions differ, however, on whether the sensor-triggering signal is a high [60] or low [61] sodium concentration of the tubular fluid. It has also been

hypothesized that the macula densa cells might detect changes in their own rate of sodium uptake and in the intracellular sodium concentration rather than in the sodium content of the intraluminal fluid.[61,62] These hypotheses evidently do not apply to the endocrine kidney. Not only is there no urine flowing in the tubules, but their lumens are closed and the macula densa cells are so atrophic that it is difficult to think of them as being actively engaged in any meaningful monitoring. Furthermore, most of the JGC are located in arterioles and arteries far removed from the macula densa. A dissociation between the juxtaglomerular index, renin release, and the enzymatic activity of the macula densa has also been observed in magnesium deficiency,[63] in acute diuresis,[64] in congestive heart failure,[65] and in aminonucleoside nephrosis.[66] Analogous results have been obtained from a physiologic viewpoint: in nonfiltering, denervated kidneys of adrenalectomized dogs, Davis [67] found an increase in renin secretion in response to both hemorrhage and suprarenal aortic constriction. This acute experiment, like the more chronic present study, effectively eliminates both sodium and the macula densa as the originator of renin release.

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[*Illustrations follow*]

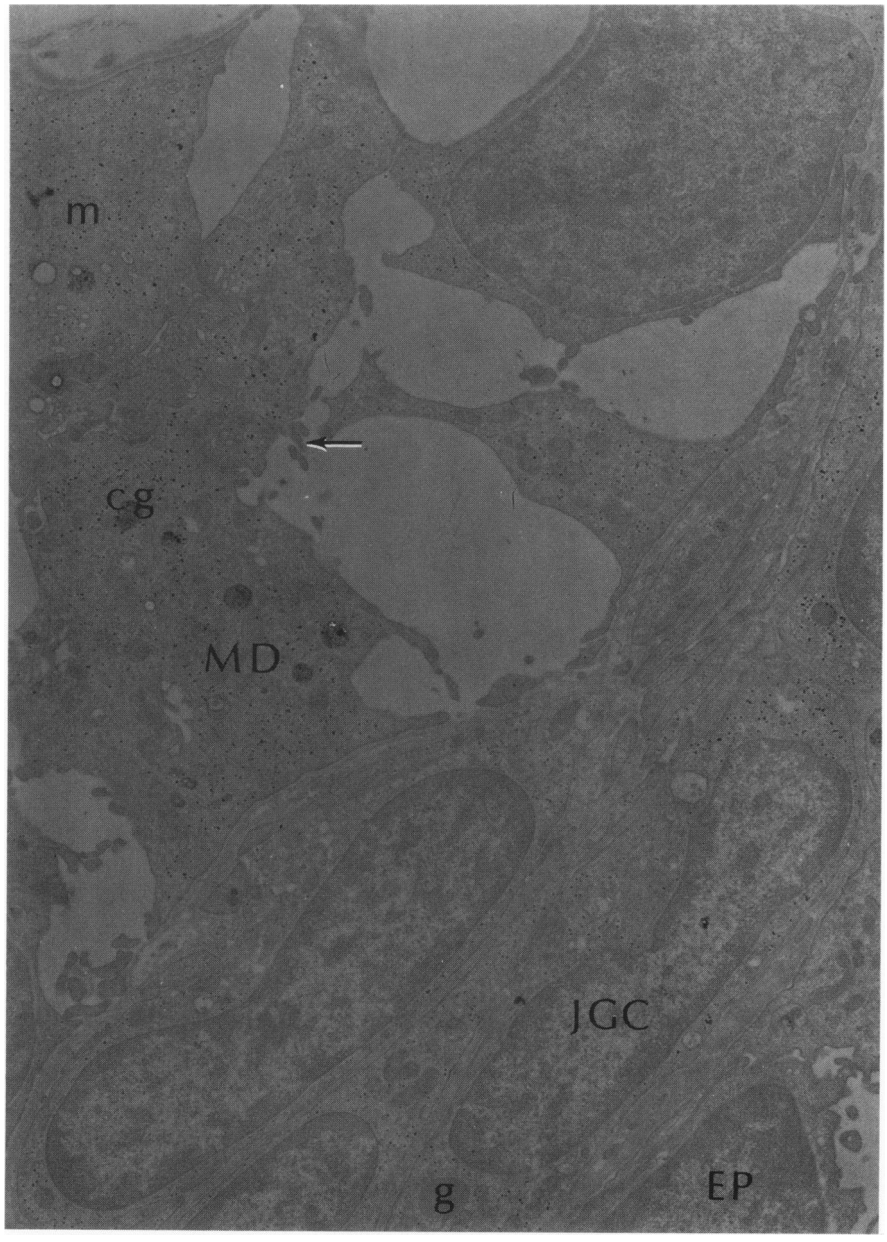

Figure 1—Juxtaglomerular complex stained according to Thiery's technique (floated on thiocarbohydrazide for 24 hours), showing from bottom to top: glomerular parietal cells of Bowman's capsule (*EP*), juxtaglomerular cells (*JGC*) with specific granules (*g*) and fine, densely stained β-particles of glycogen, as well as macula densa (*MD*). The cells of the macula densa are separated by large intercellular spaces containing a few microvilli (*arrow*). They are comprised of glycogen and several cytosegresomes (*cg*) with an argentophilic content. These cells are also markedly atrophic and show only a few mitochondria (*m*). ($\times$ 14,500)

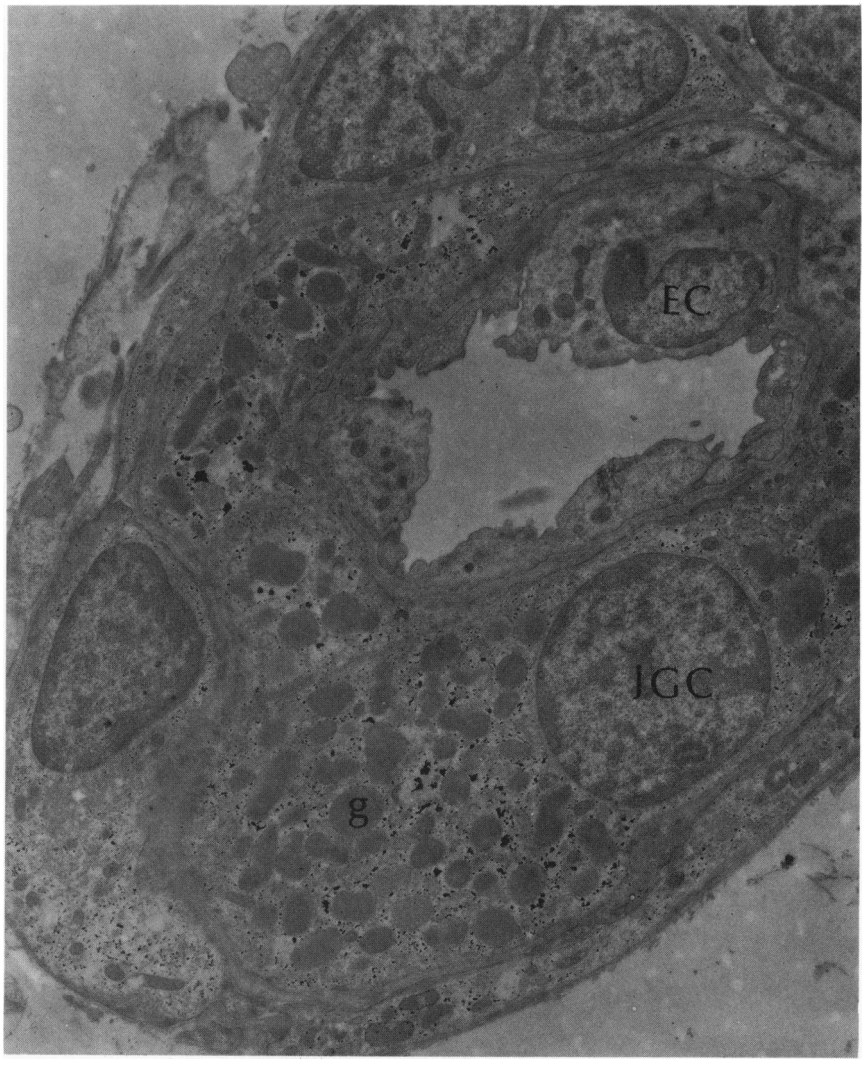

Figure 2—Renal arteriole (located near a glomerulus) stained as in Figure 1. The lumen is lined with endothelial cells (*EC*) devoid of glycogen. Several juxtaglomerular cells (*JGC*) filled with numerous specific granules (*g*) contain a large quantity of β-particles of glycogen forming either single particles or clumps. ($\times$ 13,400)

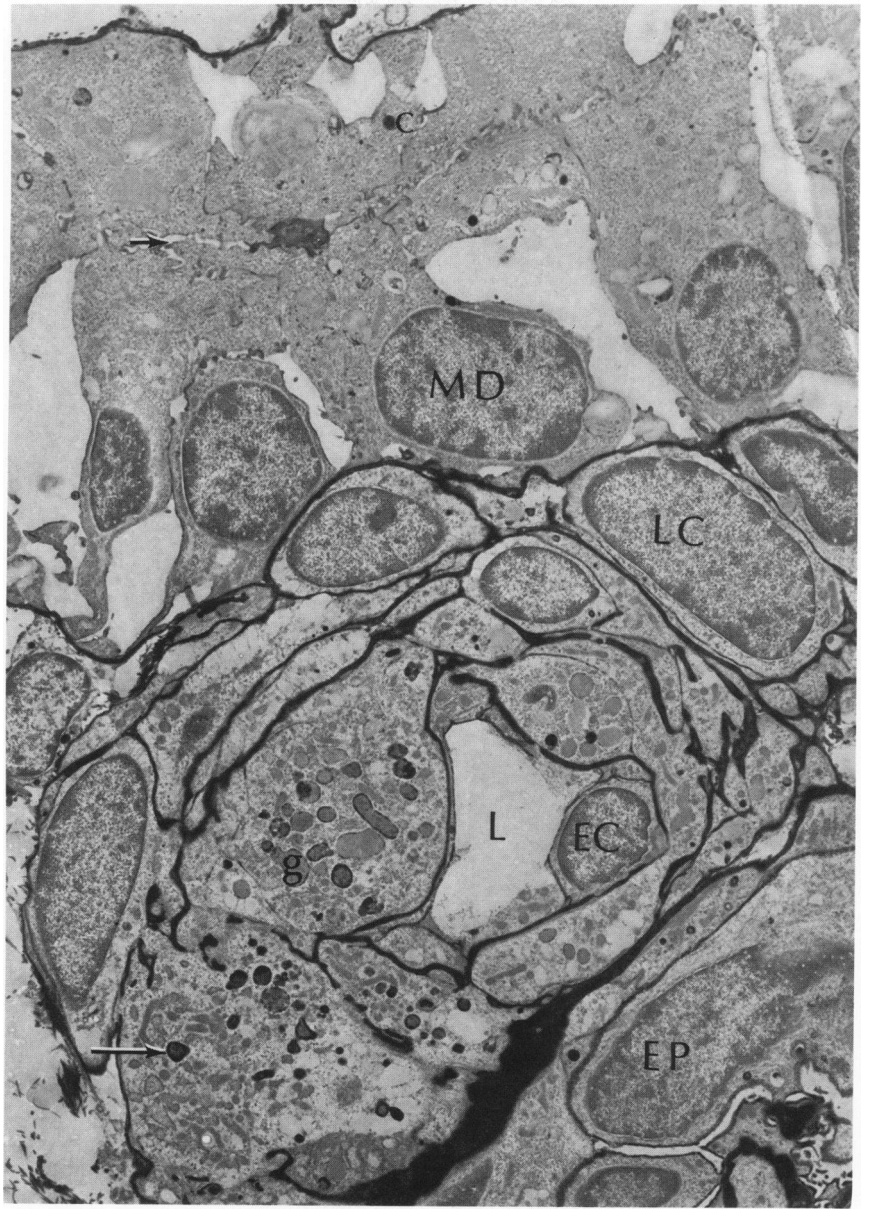

Figure 3—Survey picture of the juxtaglomerular complex of an endocrine kidney embedded in glycol methacrylate and stained with PTA-HCl. The arteriole with its lumen (*L*) is located between a glomerulus showing parietal cells of the Bowman's capsule (*EP*) and the macula densa (*MD*) the lumen of which (*arrow*) is collapsed. The lumen of the arteriole is lined with endothelial cells (*EC*). Its wall contains several juxtaglomerular cells with numerous specific granules (*g*) showing a positively stained rim. Nonspecific granules or lysosomes (*arrow*) are generally smaller, possess irregular contours, and are intensely reactive. Each cell is surrounded by a thick, very reactive cell coat. The arteriole is separated from the macula densa by lacis cells (*LC*) with very little cytoplasm and a thick cell coat. The macula densa cells are often separated by large intercellular spaces and show a poorly stained cell coat with a few lysosomes (*C*). (× 3,300)

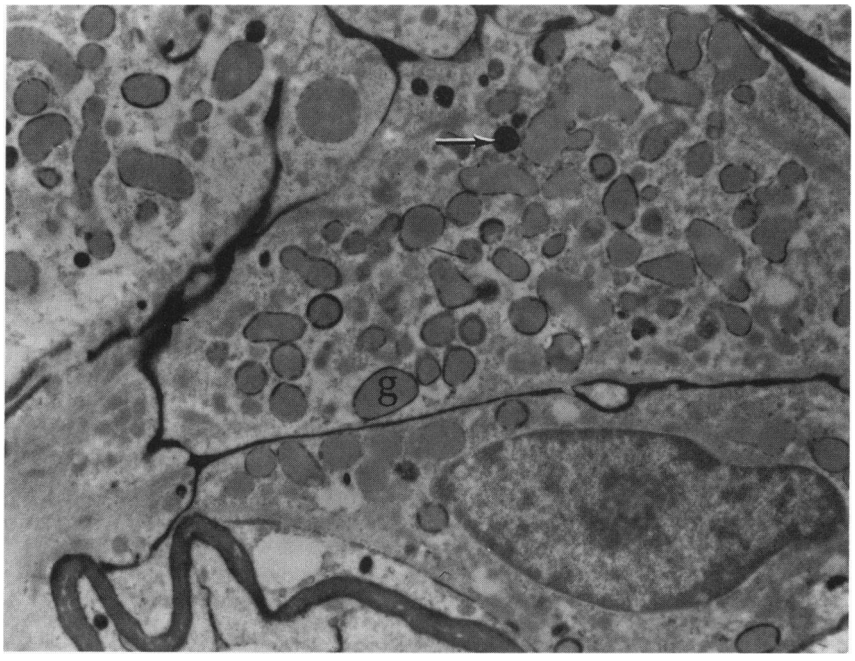

Figure 4—Higher magnification of juxtaglomerular cells processed as in Figure 3. Note the intensely reactive cell coats and the numerous specific granules (*g*) with a positive rim. Smaller, markedly-stained lysosomes (arrow) are evident. (× 9500)

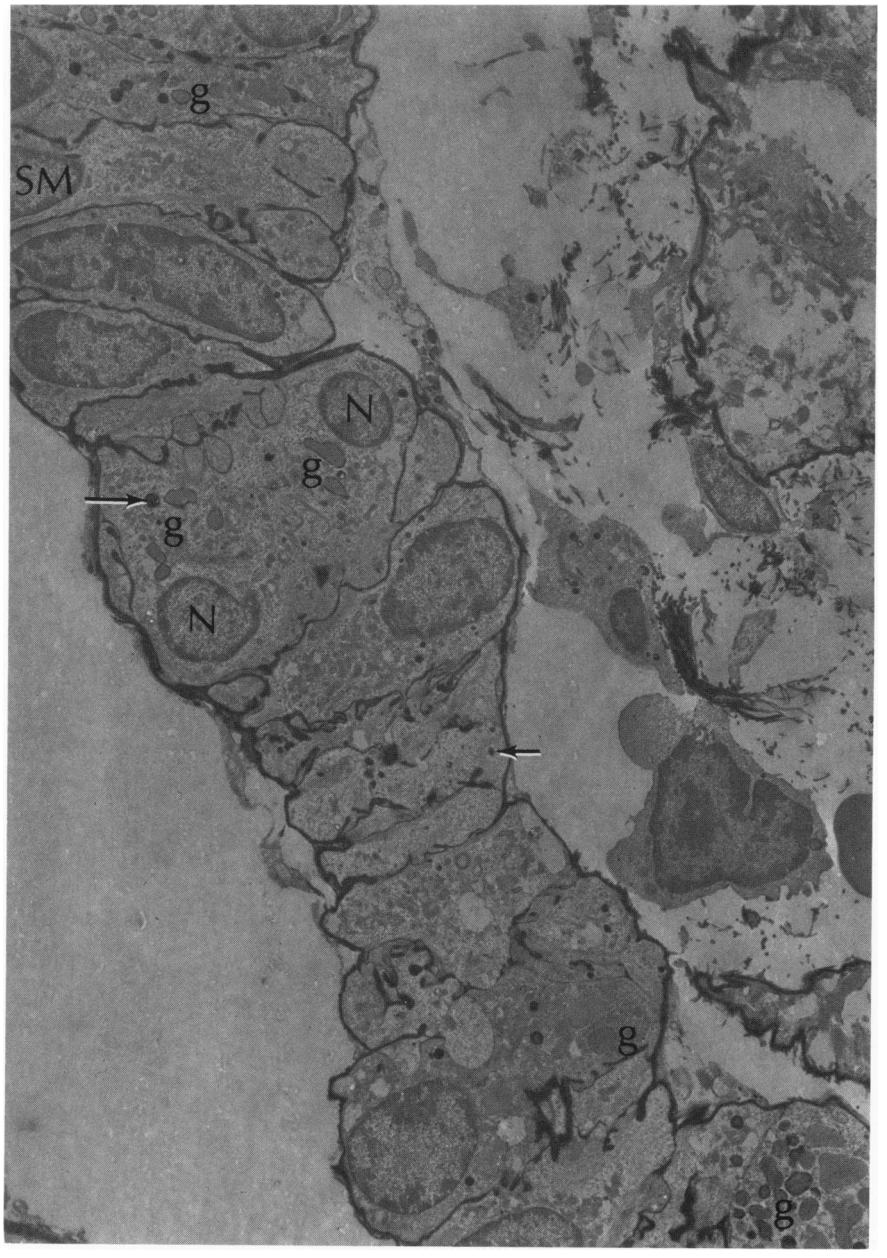

Figure 5—Wall of an arteriole (located far from the glomerulus) processed as in Figure 3. The lumen is on the left. The arteriole wall shows several typical smooth muscle cells (*SM*); the other cells contain specific granules (*g*). One granulated cell appears to be binucleated (*N*). Densely stained, small lysosomes (*arrow*) are evident in both cell types. The latter are enclosed within an intensely reactive cell coat. (× 5200)

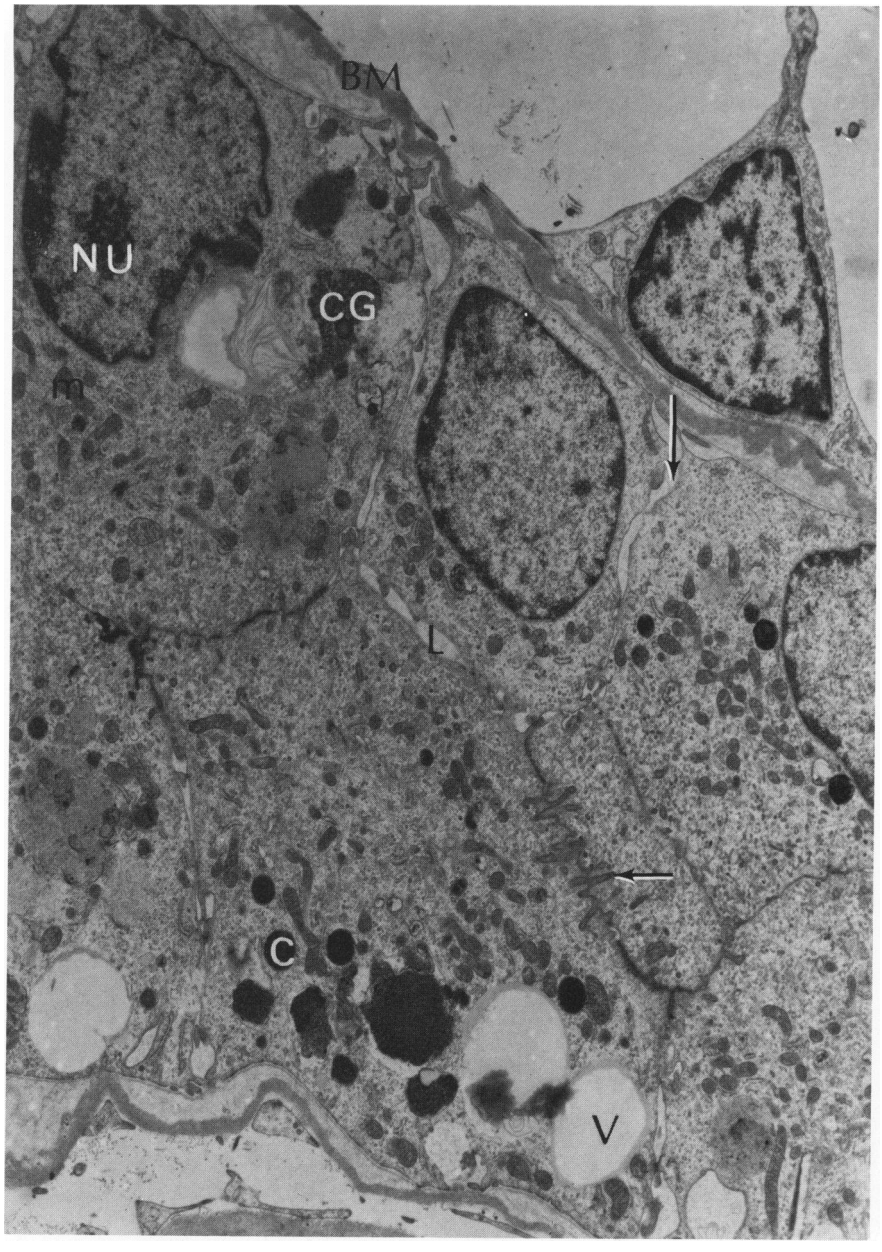

Figure 6—Survey picture of an atrophic proximal convoluted tubule embedded in Araldite and stained with uranyl acetate and lead citrate. The lumen (*L*) is virtually collapsed. A few interlocking apical microvilli are still visible (*horizontal arrow*). The basement membrane (*BM*) is thickened. Some of the intercellular spaces (*vertical arrow*) are still present. The hyaloplasm contains small mitochondria (*m*), several cytosegresomes (*CG*), and a few cytosomes (*C*). Basilar interdigitating processes are not visible but some basal vacuoles (*V*) are evident. *NU* = nucleolus. (× 7700)

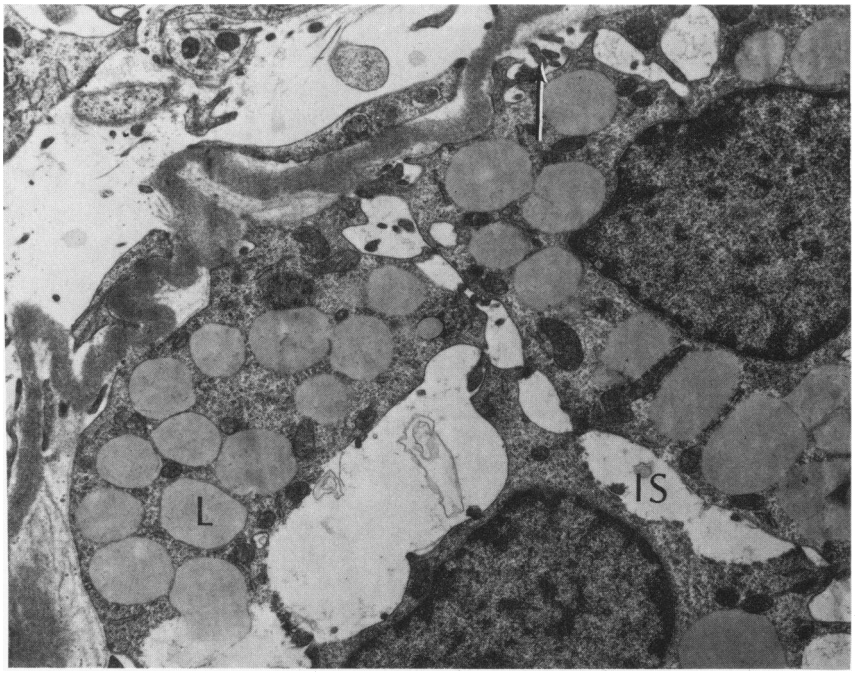

Figure 7—Atrophic proximal convoluted cells embedded in Araldite and stained with uranyl acetate and lead citrate. The basement membrane is very thick. The cytoplasm contains only a few mitochondria and numerous lipid droplets (*L*). Note the large intercellular spaces (*IS*). A few basilar interdigitating processes (*arrow*) are still present. (× 9500)

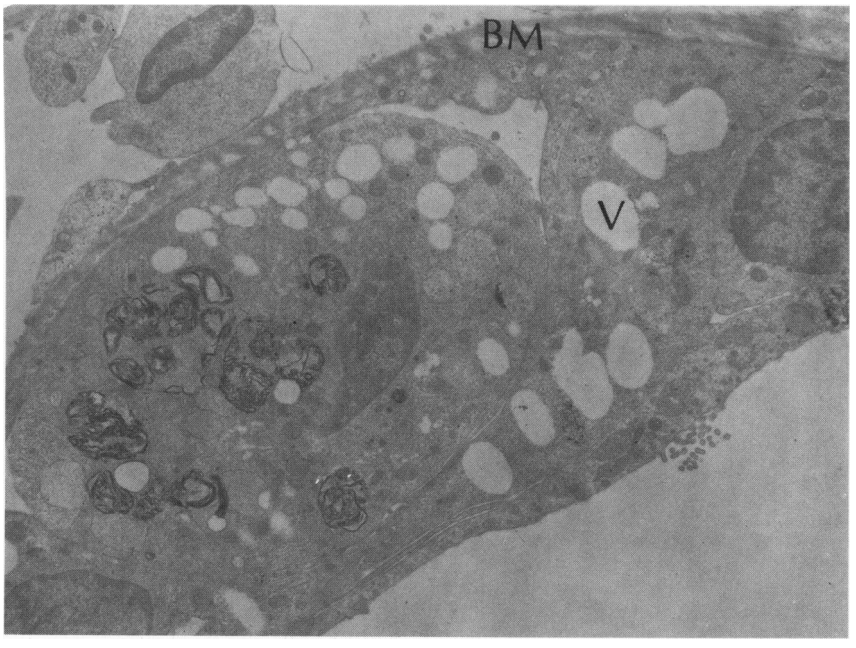

Figure 8—Portion of atrophic cortical collecting duct stained according to Thiery's technique (floated on thiocarbohydrazide for 24 hours). The basement membrane (*BM*) is thickened. The cytoplasm contains numerous vacuoles (*V*) and several cytosegresomes filled with positively stained membrane whorls. Note the absence of glycogen. (× 6200)

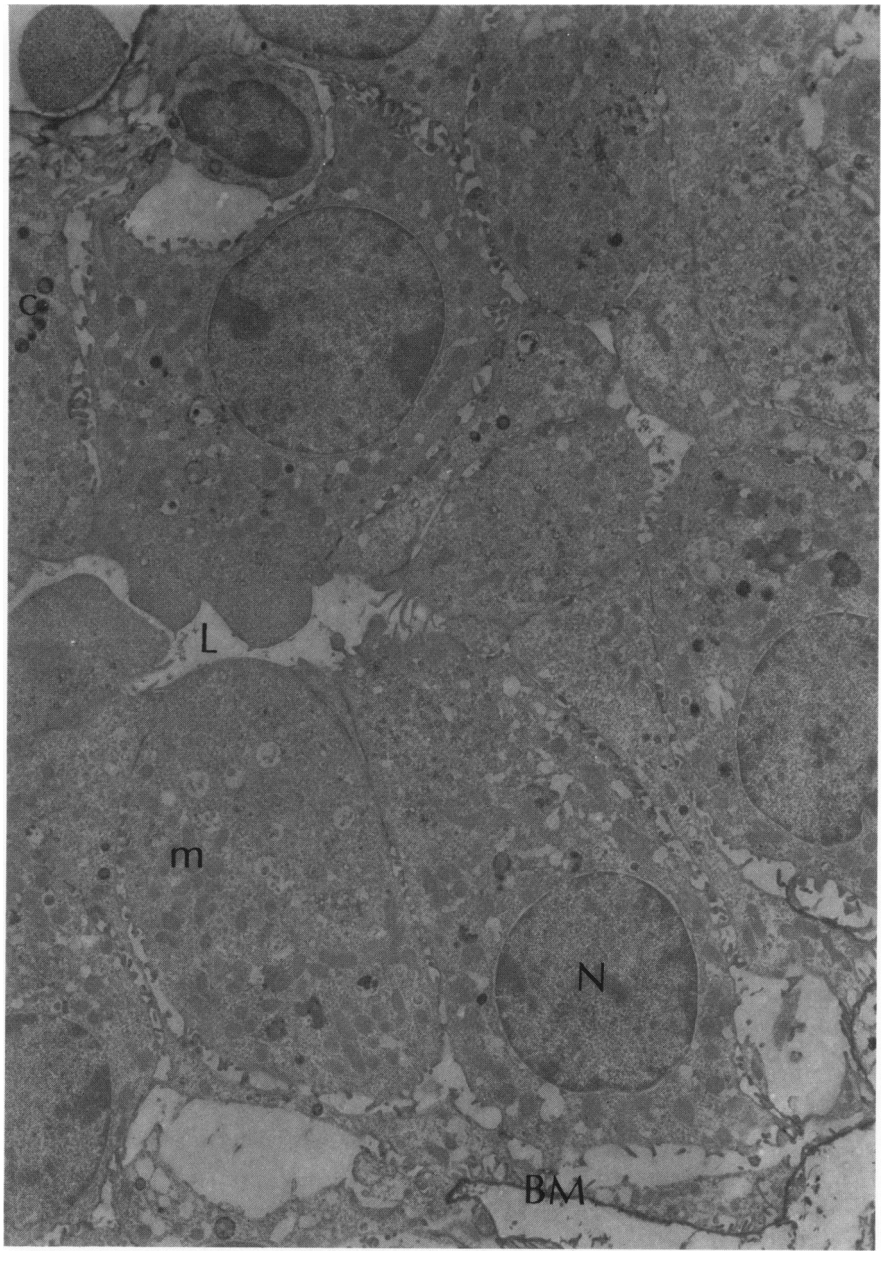

Figure 9—Survey picture of an atrophic proximal convoluted tubule embedded in glycol methacrylate and stained with PTA-HCl, showing lumen (*L*) (at left center) and a few apical microvilli. Note the absence of basilar interdigitating processes, apical tubular invaginations, apical dense tubules, apical vesicles, and apical vacuoles. The lateral cell spaces contain several interdigitating processes. The basement membrane (*BM*) here is not thickened. A few cytosomes (*c*) are present. *M* = mitochondria, *N* = nuclei. (× 6500)