Metaplasia of Smooth Muscle Cells Into Juxtaglomerular Cells in the Juxtaglomerular Apparatus, Arteries, and Arterioles of the Ischemic (Endocrine) Kidney

An Ultrastructual-Cytochemical and Autoradiographic Study

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Partial ligation of the aorta between the renal arteries induces marked atrophy of the cortical tubules of the left (endocrine) kidney with a remarkable increase in the number and granularity of hypersecretory juxtaglomerular 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. Typical vascular smooth muscle cells (SMC), in which secretory granules appear, show a concomitant development of their Golgi complex and rough endoplasmic reticulum, with a gradual decrease in the number of their filaments. Microtubules also appear in the Golgi area. Thiery's periodic acid–thiocarbohydrazide–silver proteinate technique demonstrates that in these "intermediate" cells, as in mature JGC, the amount of glycogen is greater than in SMC. The newly-developed secretory granules of intermediate cells are stained by phosphotungstic acid at a low pH, as are the mature granules of JGC, an indication that both types contain glycoproteins. Light and electron microscopic autoradiography reveal that both JGC and "intermediate" cells of the vascular wall do not incorporate radioactive thymidine (injected during the 10-day observation period). Thus, they develop by metaplasia of preexistent SMC. In control kidneys, radioactive thymidine is practically never incorporated into the nuclei of SMC but is found in a few glomerular and tubular cells of all zones except the papilla. The endocrine kidney shows virtually no reactive nuclei in vascular SMC, glomeruli, or tubular cells of the outer cortex. Thymidine is incorporated into practically all nuclei of the straight portion of proximal tubules and into about half the nuclei of all medulllary tubular cells including the papilla. (Am J Pathol 87:581–602, 1977)

ALTHOUGH MITOTIC FIGURES have been noted in juxtaglomerular cells (JGC),[1-3] several authors have long suspected that these cells originate from metaplasia of smooth muscle cells (SMC)[4-9] because of their close anatomic relationship and the frequent appearance of filaments and attachment bodies typical of SMC in the cytoplasm of JGC.

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The aim of the present investigation was to study—by ultrastructural-cytochemical methods and by light and electron microscopic autoradiography with radioactive thymidine—the occurrence of JGC in the vasculature of the ischemic (endocrine) kidney produced by partial ligation of the aorta between the renal arteries.[10] This experimental model is ideally suited to such a study because of the extremely rapid increase in the number of JGC, not only in the juxtaglomerular apparatus (JGA) itself but also in the walls of arteries and arterioles far removed from the glomerulus.[11,12] The uptake of radioactive thymidine by the nuclei of the normal JGA and by the nuclei of cells of the glomeruli, tubules, and vascular smooth muscle of normal and ischemic kidneys was also studied for control and comparative purposes.

Materials and Methods

Animals and Surgery

Surgery was performed on 25 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. It 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 ligation was complete, produced a severe but partial constriction of the aorta, inducing a rapid and marked atrophy of the left kidney.[11,13] Fifteen other rats of the same initial body weight were used as controls. All animals had free access to Purina Laboratory Chow and drinking water.

Administration of Radioactive Thymidine

Methyl-^{3}H-thymidine (specific activity, 20 Ci/mM) was given intraperitoneally at a dose level of 1 μ Ci/g body weight in 0.2 ml of 0.9% NaCl, twice daily (9 AM and 5 PM), to 15 rats with ischemic kidneys and to 5 controls, from the first to the tenth day of the experiment.

Determination of the Juxtaglomerular Granulation Index

Ten rats with ischemic kidneys and 10 control animals not injected with radioactive thymidine were sacrificed on the tenth day with chloroform. Their left kidneys were fixed in Helly's fluid, embedded in paraffin, cut longitudinally at 4 μ, and stained according to the Bowie technique as modified by Hartroft and Hartroft.[14] The juxtaglomerular granulation index (JGI) was determined by assessing the number and granularity of JGC at a magnification of ×400 according to the method of Hartroft and Hartroft;[14] the counts obtained from two sections of each kidney were averaged in all cases[15] (Table 1).

Electron Microscopy

On the tenth day of the experiment, the left ischemic kidney of 10 animals injected with radioactive thymidine was perfused,[16] 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).[17] Small fragments of renal cortex were placed in the same fixative for 2 hours at 4 C. All specimens were then washed for three 15-minute periods in cacodylate buffer to which

Table 1—Effect of Aortic Ligation on the Juxtaglomerular Granulation Index

Group	Treatment	No. of animals	JGI (mean ± SE)
1	—	10	29.8 ± 1.52
2	Partial ligation of aorta*	10	106.4 ± 10.53†

* Aortic ligation between the renal arteries was performed on the first day of the experiment. The animals were killed on the tenth day.
† Significant ($P < 0.0001$) in comparison to the respective controls.

2% sucrose had been added. They were left in the buffer for 12 hours. Some renal fragments were postfixed 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.[17] All 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 or an LKB ultramicrotome with glass or diamond knives, stained with uranyl acetate and lead citrate,[17] and examined with a Philips 201 electron microscope.

Cytochemistry

Thiery's Method

The periodic acid–thiocarbohydrazide–silver proteinate technique of Thiery[18,19] was used for the demonstration of periodate-reactive vicinal glycols on tissues that were fixed either in glutaraldehyde alone or in combination with osmium tetroxide and embedded in Araldite.[17,20]

Low pH Phosphotungstic Acid–Hydrochloric Acid Method

This technique[21-23] 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 phosphotungstic acid (PTA) in 1 N HCl (pH, 0.3) for 8 to 10 minutes, then rapidly washed in distilled water.[17]

Electron Microscopic Autoradiography

Ultrathin (silver) sections were placed on collodion-coated glass slides, stained with uranyl acetate and lead citrate, and covered with evaporated carbon.[24] The slides were dipped in dilute Ilford L4 emulsion according to the method of Whur *et al.*[25] The autoradiographs were developed in Microdol X after exposure for 1 month.[26]

Light Microscopic Autoradiography

Paraffin-Embedded Sections

On the tenth day of the experiment, 5 rats bearing endocrine kidneys and 5 controls injected with radioactive thymidine were sacrificed with chloroform. Their left kidneys were fixed in Bouin-Hollande fluid for 24 hours, embedded in paraffin, and cut transversally at 5 μ. The sections were deparaffinized, covered with dilute Ilford K5 emulsion, and exposed at 4 C for 15 days. The autoradiographs were developed and stained according to the PAS technique or with hematoxylin-phloxine-saffron.

Semifine Sections

Sections 1 μ thick were cut from Araldite-embedded ischemic kidneys with glass knives, placed on glass slides, and treated exactly as described above except that the time of exposure was 30 days.

Results

Juxtaglomerular Granulation Index

As can be seen in Table 1, the JGI of rats with ischemic kidneys was more than three times that of untreated controls. This was due to a tremendous increase in the number and granularity of JGC, not only in the JGA itself but also all along the walls of afferent arterioles and in the walls of arterioles and several arteries far from the glomeruli. In the walls of arterioles near or far from the glomeruli, the impression was often gained that practically all the SMC had been replaced by JGC. Only a few JGC could be seen in the arteries, mostly in zones of bifurcation.

Ultrastructure of Arterioles and Arteries in the Endocrine Kidney

As already noted,[11] all the arteries and arterioles were remarkably well preserved and showed no evidence of injury. The endothelial cells appeared to be normal. The SMC contained a great number of filaments, often seemingly interrupted by dense attachment bodies (Figure 1). Mitochondria were relatively frequent, while Golgi complexes were small and poorly developed. The rough endoplasmic reticulum was limited to rare small saccules studded with ribosomes. In virtually all the arterioles examined (even in those far away from the glomeruli) and in several arteries, otherwise normal SMC (easily recognized by their filamentous cytoplasm) contained specific granules which had the same shape, density, and reactivity as juxtaglomerular granules (Figures 2–4). The Golgi complex in these cells was much more developed than in ordinary SMC, being composed of saccules and innumerable small vesicles that extended from one side of the nucleus, often almost up to the plasmalemma on the same side (Figure 4). The rough endoplasmic reticulum showed multiple dispersed cisternae (Figures 3 and 4), often filled with a flocculent, electron-lucent material. Pinocytotic vesicles were extremely numerous all along the inner aspect of the plasmalemma and sometimes extended so deeply within the cells that they mingled with, and became indistinguishable from, the small Golgi vesicles.

Ultrastructural Cytochemistry of Arterioles and Arteries in the Endocrine Kidney

Use of Thiery's technique revealed B-glycogen in arterial and arteriolar SMC (Figure 5) but not in endothelial cells. The number of glycogen

particles was augmented in SMC showing typical granules (Figure 6) or an increase in the size of the Golgi complex and rough endoplasmic reticulum. Silver grains were also located in a few lysosomes and residual bodies of SMC and endothelial cells. Phosphotungstic acid stained equally well the cell coat of ordinary SMC and that of SMC showing granules or an increase in the size of the Golgi complex and rough endoplasmic reticulum. The granules' rims were almost always stained by PTA. Small lysosomes were intensely reactive (Figure 7).

Light Microscopic Autoradiography (Paraffin-Embedded and Semifine Sections)

Control Kidney

Arteries and Arterioles. Silver grain accumulations were occasionally found over the nuclei of endothelial cells but very rarely over those of SMC in arteries and arterioles.

Juxtaglomerular Apparatuses. No silver grains were seen over the JGC, which could be identified by the weak PAS positivity of their granules.[16] Two nuclei covered by silver grains were found in the macula densa, identifiable by the typical crowding of its nuclei. None could be observed in the lacis cells.

Glomeruli. Several nuclei covered by silver grains were detected in the glomeruli in all sections examined.

Tubules. Nuclei densely covered by silver grains were rare in all zones of the cortex and the medulla and especially in the inner medullary region (the papilla) (Figure 10). Accumulations of silver grains in the outer cortex were found over proximal and distal convoluted tubular cells whereas, in the inner cortex, they were localized over nuclei in mostly all the straight portions of the proximal convoluted tubules (Figure 8) and, to a lesser extent, in the ascending limbs of the loops of Henle and the collecting tubules. Marked nuclei were not as frequent in the outer medulla; they were identified in the cells of the ascending limbs of Henle, the thin loops, and the collecting ducts. Individual silver grain–covered nuclei were found most often, but some were grouped in the same portion of the tubule. There was a slight difference between the various parts of the proximal tubules, the convoluted portion containing less reactive nuclei than the straight portion.

Endocrine Kidney

Arteries and Arterioles. Silver grains were encountered over the nuclei of some endothelial cells but very rarely over the nuclei of SMC in the

walls of arteries and arterioles. The PAS stain did not reveal any reactive nuclei in JGC located in the walls of arteries and arterioles far from the glomeruli.

Juxtaglomerular Apparatuses. Marked nuclei could not be found in the JGC of arterioles near glomeruli, in cells of the macula densa, or in the lacis cells.

Glomeruli. An occasional group of silver grains was noted in a few glomeruli.

Tubules. Reactive nuclei were almost completely absent from the outer cortex. Practically all the nuclei of the straight portion of the proximal tubules were covered by silver grains (Figure 9), forming a sharply delimited band corresponding to the inner cortex. Silver grains also covered approximately half the nuclei of the tubules in all medullary regions, including the papilla (Figure 11). As in the inner cortex, these grains were more dispersed and generally less numerous than in control tubules.

Electron Microscopic Autoradiography of the Endocrine Kidney

Arteries and Arterioles. These structures revealed only a few reactive nuclei in endothelial cells. None were found in either SMC or JGC.

Juxtaglomerular Apparatus. Silver grains were not evident over the nuclei of JGC, macula densa, or lacis cells.

Cortical Tubules. It was not possible to determine whether the atrophic proximal tubules examined were from the convoluted or straight portions. Some nonradioactive proximal tubules were encountered and in many others all the nuclei were covered by silver grains (Figure 12). A few distal tubular cells (Figure 13) and some collecting duct cells (Figure 14) had reactive nuclei.

Discussion

It is evident from determination of the JGI and from electron microscopic examination that renal ischemia due to partial ligation of the aorta produces a tremendous increase in the number of JGC, not only in the JGA itself but also in the walls of afferent and efferent arterioles and in renal arterioles and arteries far from the glomeruli. The JGC population in the JGA is augmented, and these cells become hypersecretory, as judged by the enhanced size of the Golgi complex, the abundant rough endoplasmic reticulum, and the variations in size, shape, and internal structure of the juxtaglomerular granules.[11,27] The same picture is presented by JGC in the walls of arterioles and arteries far from the JGA, but generally to a

lesser degree. Some JGC in arteries and arterioles, which have only a few granules, contain more Golgi bodies and rough endoplasmic reticulum than do SMC but ordinarily less than JGC from the JGA. As soon as they acquire granules, the JGC from arterioles and arteries seem to contain more glycogen than SMC. The granules themselves are sometimes sparingly filled with flocculent, electron-lucent material. They show the same positivity to PTA as the granules of JGC in the JGA, i.e., at their rim, which means that they contain a certain amount of glycoproteins.[11,16]

Various manifestations, interpreted as signs of secretory hyperactivity, have been noted in SMC of the walls of arteries and arterioles in the rat and rabbit ischemic kidney.[28,29] In the beginning, the cells are swollen, the rough endoplasmic reticulum and Golgi complex become more prominent, and the mitochondria multiply. Later on, the signs of secretory hyperactivity disappear but the cells may remain clear, having lost most of their filaments. These manifestations have been observed in both "minor" and "major" renal ischemia.[30] We could not confirm several of the changes in the rat ischemic kidney: the SMC did not swell, the mitochondria did not multiply, and lost filaments were replaced by granules which are, by any standard, the only yardstick of endocrine-like secretory activity in such cells. The above-mentioned changes were possibly due to the unequal degree of ischemia induced in various regions of the rabbit renal cortex by partial obstruction of the renal artery.[30] If this is true, then these changes would be on the borderline of pathology.

The complete absence of silver grains over the nuclei of JGC in the JGA and the walls of afferent arterioles or other arterioles and arteries indicates that, in JGC, mitoses must be very rare events. The dose of radioactive thymidine that we injected is generally considered adequate to label every nucleus in the S phase. An intraperitoneal injection is believed to be equal to a pulse label of less than 1 hour.[31] Since the S phase of most nuclei varies between 6 and 9 hours[32,33] (in extreme cases, between 1.5 to 30 hours), our chances of labeling the nuclei of cells in the renal vasculature, which could have entered into the S phase, would have been quite high. We must then conclude that JGC originate from metaplasia of SMC, not only in renal arteries and arterioles but also in the JGA itself where, allegedly, mitosis is extremely rare. This paucity of mitotic activity in JGC is also common to normal SMC of the vascular walls.[34]

As in other tissues,[35-39] the SMC and JGC of renovascular walls would possess a certain latent population of G_2 cells which could enter mitosis immediately upon stimulation without incorporating radioactive thymidine. The same could apply to divisions of tubular cells.[40] These G_2

populations in the kidney are ordinarily small. Their presence could not account for intermediate stages between SMC and JGC in the walls of renal arteries and arterioles.

The occurrence of DNA synthesis in the nuclei of glomerular cells and in nuclei of tubules in the normal kidney is in agreement with previous studies.[41-44] It is already known that normal cortical tubular cells constitute a fairly permanent but slowly expanding population in which DNA synthesis does take place even in adult animals. According to Heine and Stöcker,[45] less than 3% of the cells divide at any time in the kidney, and this figure is even lower in the medulla. In the endocrine kidney, however, DNA synthesis is rare in arteries and arterioles, practically absent in the outer cortex, extremely high in the nuclei of cells in the inner cortex (i.e., in cells of the straight portion of proximal tubules), and to a lesser extent, also present in medullary tubular cells. Our ultrastructural study[11] has shown that these cells are severely atrophic and, at least on the tenth day after partial ligation of the aorta, they do not divide. Mitosis has, however, been noted in the inner cortex of the endocrine kidney, under the influence of renotrophic steroids or renotrophic pituitary extracts.[10] It is well known that compensatory hypertrophy and hyperplasia of the remaining kidney occur following unilateral nephrectomy in most mammals. The mechanism(s) underlying this phenomenon is, however, unknown. On the one hand, stimulating factors have been sought: Lyons *et al.*[46] suggested the existence of such a factor which would modify uridine incorporation into RNA, and Preuss *et al.*[47-49] found evidence that this factor might be renin activity. The correlation between renin and DNA synthesis has not, however, been confirmed.[50,51] On the other hand, a negative feedback mechanism could also explain the growth arrest seen in normal adults; the cell proliferation following a reduction of the renal mass could be caused by the suppression of some inhibitory factors, as suggested by various authors.[52-60] Since the endocrine kidney is the functional but not the anatomic equivalent of unilateral nephrectomy and since an increase in DNA synthesis is observed in the ipsilateral kidney, it is almost impossible to extrapolate the results of previous investigations to the present case. That the increase in DNA synthesis noted in the present experiment might have been initially due to ischemia itself is possible, since renal ischemia of a short duration,[61] like hydronephrosis[44,62,63] and ligation of the renal artery,[64] has been found to cause a proliferative response in the tubules of the affected kidney.[65] Why such a stimulus should particularly affect the inner cortex and, to a lesser extent, the medulla may be related to the degree of ischemia inflicted in various regions of the kidney by the present surgical technique.

The results of our investigation indicate that JGC develop in the ischemic kidney and probably also in the normal kidney by metaplasia of SMC. It is possible that a marked decrease in stretch, such as may undoubtedly occur in the renal vasculature of the ischemic kidney, is the signal for the metaplastic process to start in the renovascular smooth muscle. Recent investigations have revealed relationships between the composition of the arterial wall and the degree of medial stress.[66-68] In cultures, SMC respond to cyclic stretching by an increase in the rate of collagen, hyaluronidase, and chondroitin-6-sulfate synthesis but not in DNA synthesis.[69]

Why this type of metaplasia should be restricted to renovascular SMC, which are structurally identical to SMC of other vascular beds, remains to be determined.

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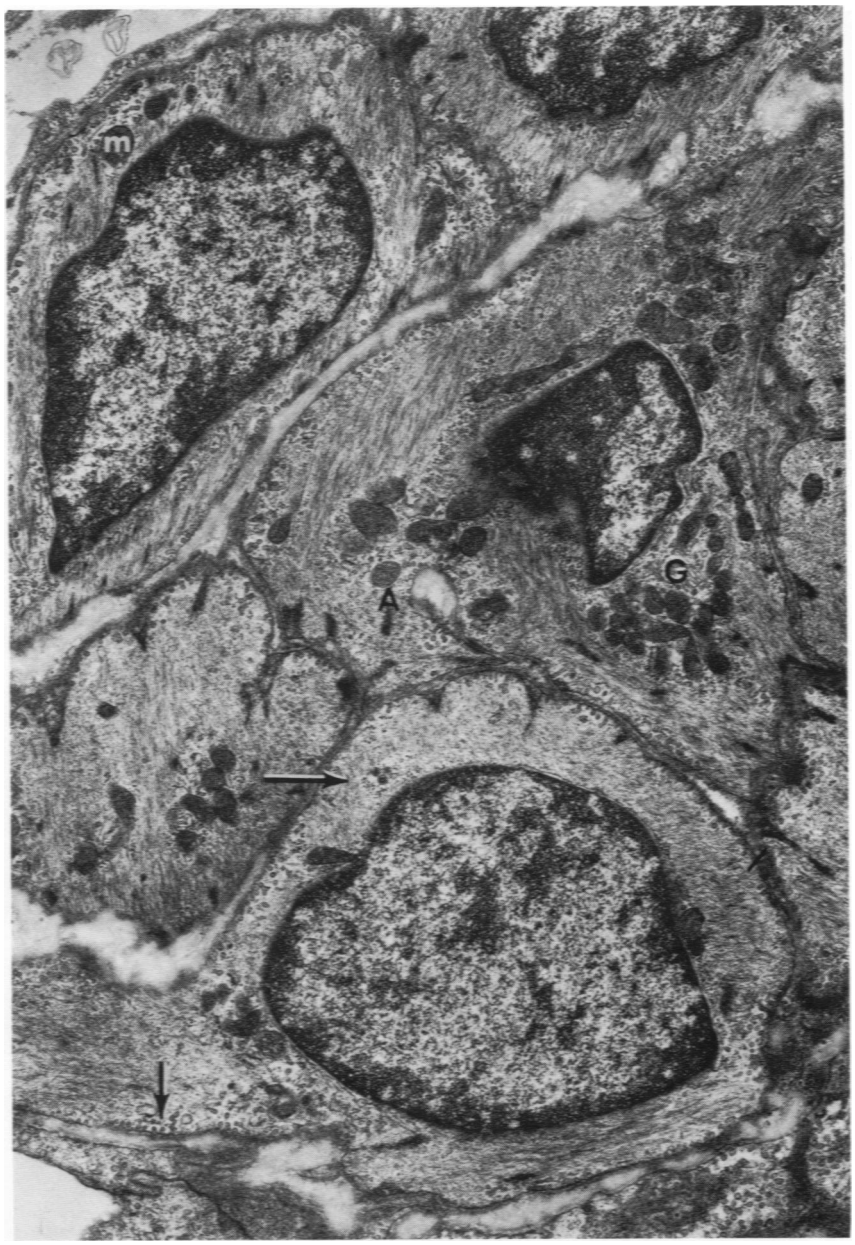


Figure 1—Smooth muscle cells in an arterial wall of an endocrine kidney containing numerous filaments with attachment bodies (*A*), mitochondria (*m*), a few ribosomal aggregates (*horizontal arrow*), a poorly-developed Golgi complex (*G*), and abundant pinocytotic vesicles (*vertical arrow*) all along the inner aspect of the plasmalemma (× 19,000).

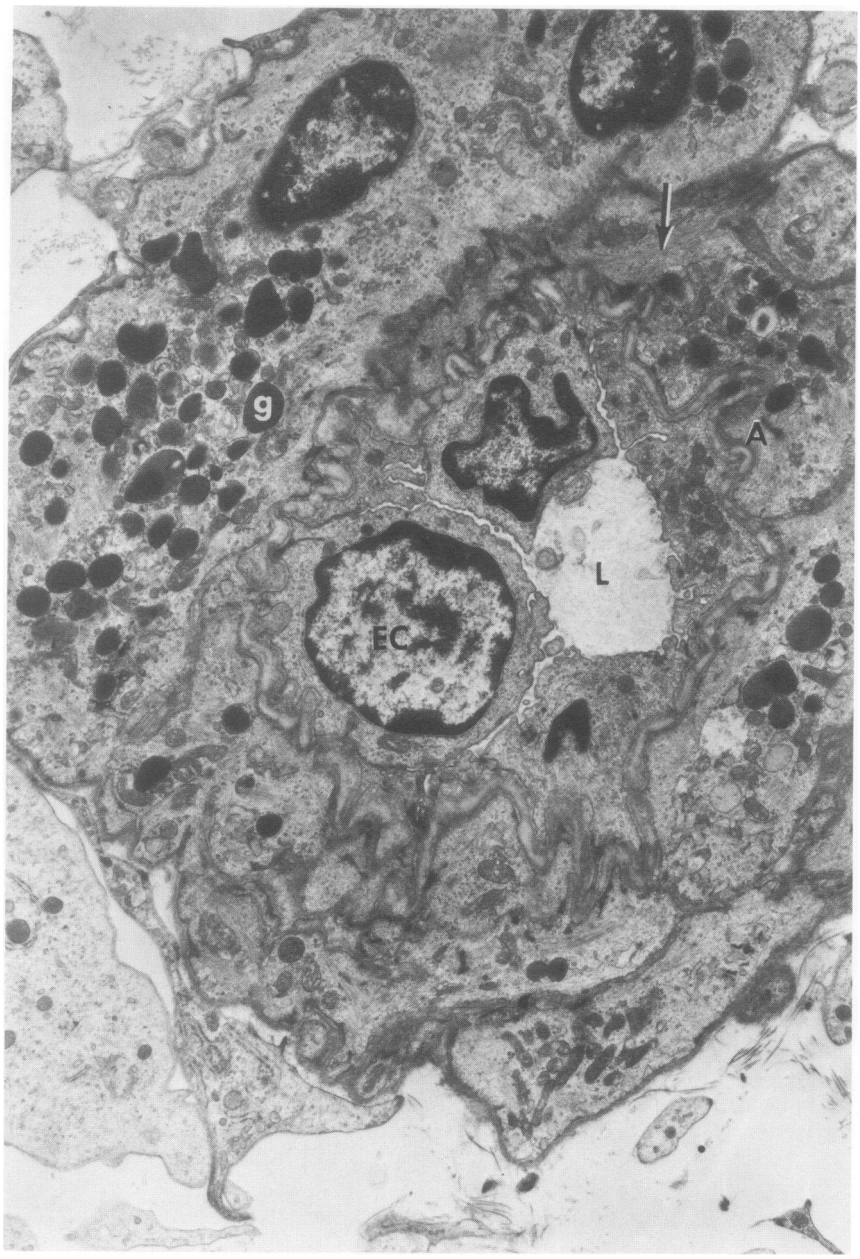


Figure 2—Transverse section of an arteriole far from a glomerulus in an endocrine kidney with lumen (*L*), endothelial cells (*EC*), and several cells containing specific granules (*g*), numerous filaments (*arrow*), and attachment bodies (*A*) typical of smooth muscle cells (× 9500).

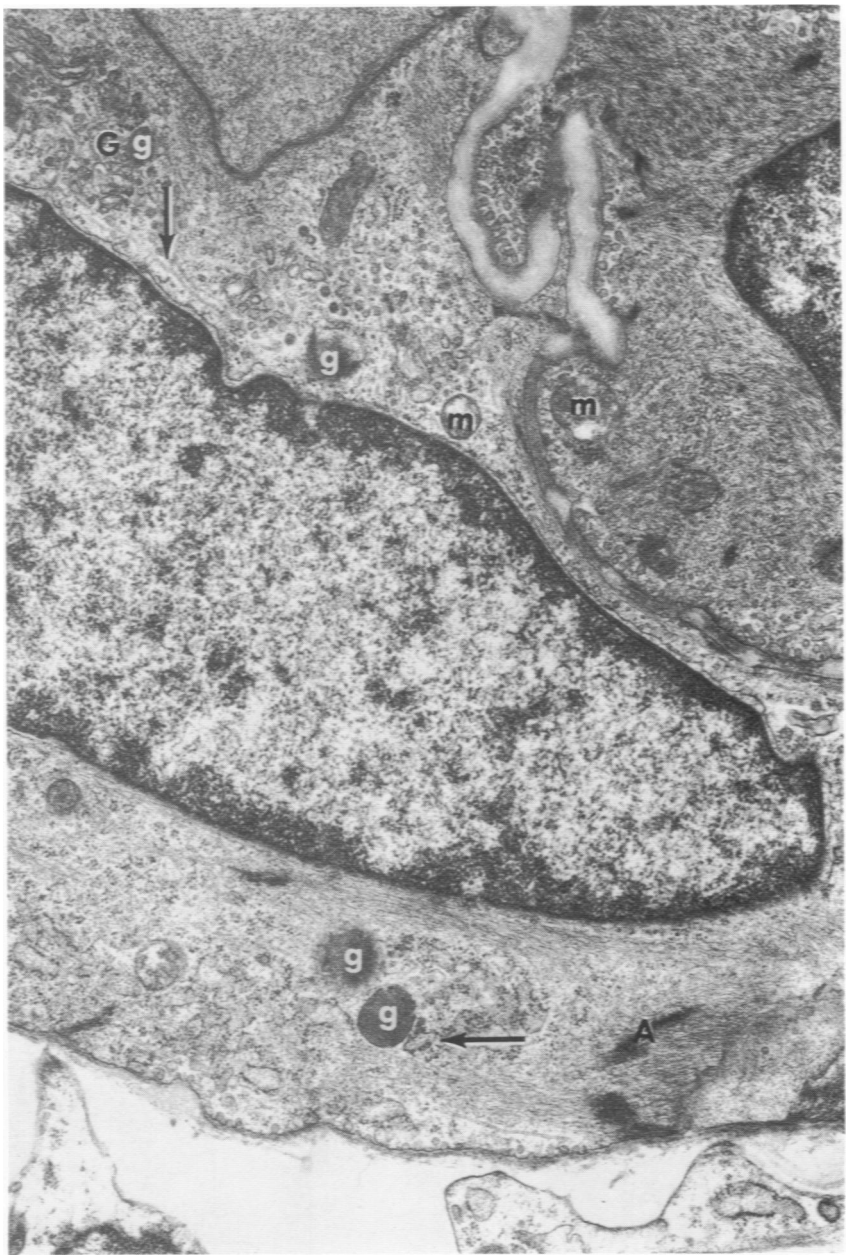


Figure 3—Part of the wall of an arteriole far from a glomerulus in an endocrine kidney. Numerous filaments and attachment bodies (*A*) are present throughout the cells. Four specific granules (*g*) are visible in one cell as are a large Golgi complex (*G*), with saccules and vesicles, and portions of rough endoplasmic reticulum (*horizontal arrow*). Microtubules (*vertical arrow*) and mitochondria (*m*) are seen. (× 21,000)

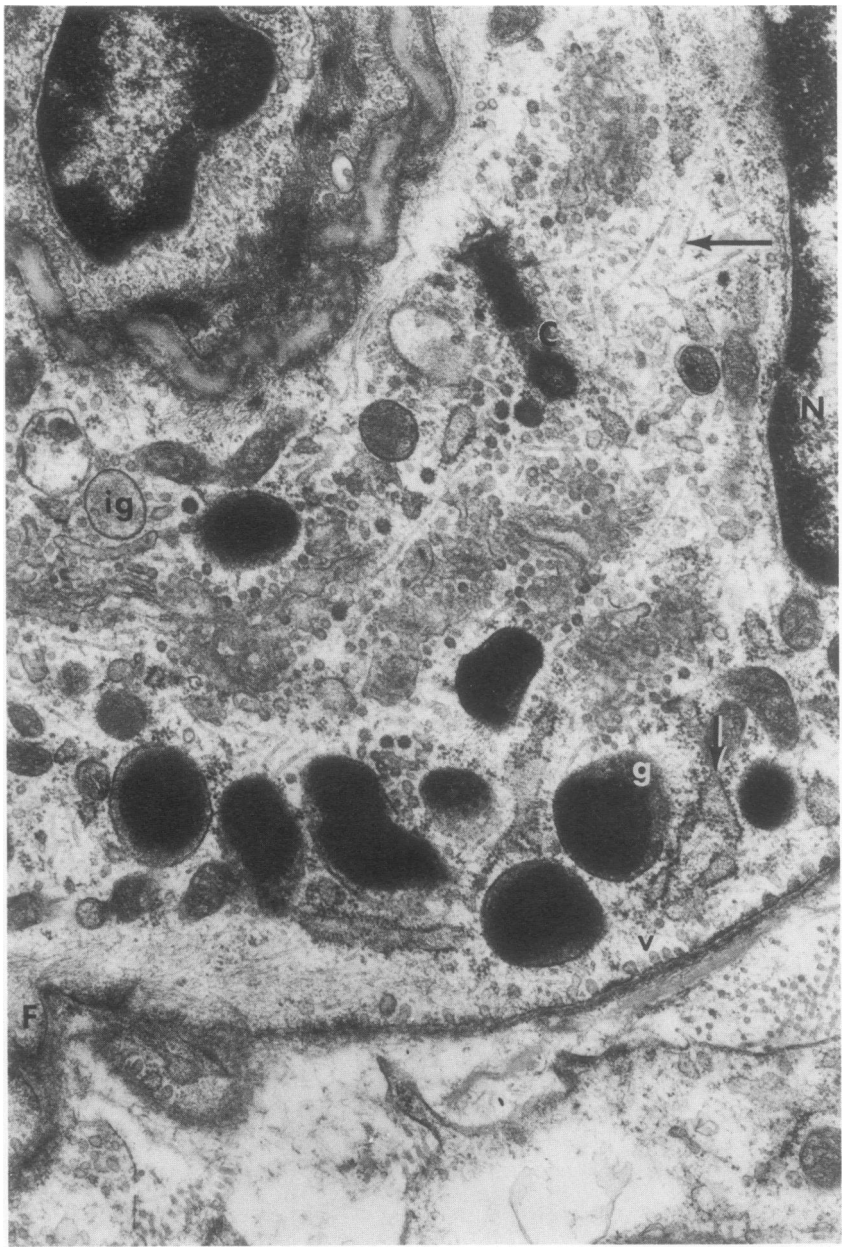
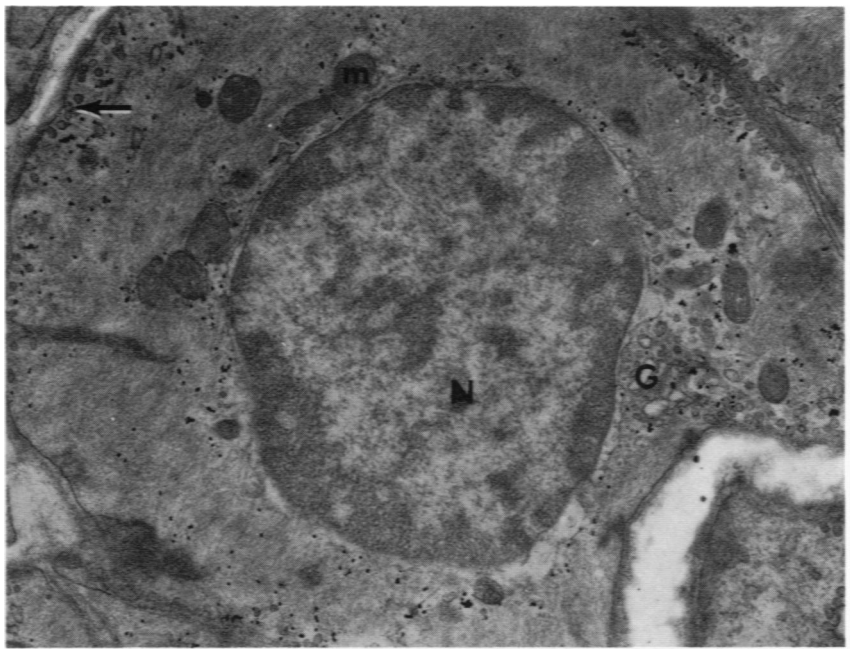


Figure 4—Part of the wall of an arteriole far from a glomerulus in an endocrine kidney. One large cell contains filaments (*F*), several specific granules (*g*), an extremely large Golgi complex made up of numerous vesicles and a few saccules, and rough endoplasmic reticulum (*vertical arrow*). Note immature granules (*ig*) near the Golgi complex. Microtubules (*horizontal arrow*), pinocytotic vesicles (*v*), centrioles (*C*), and nucleus (*N*) are seen. (× 42,200).



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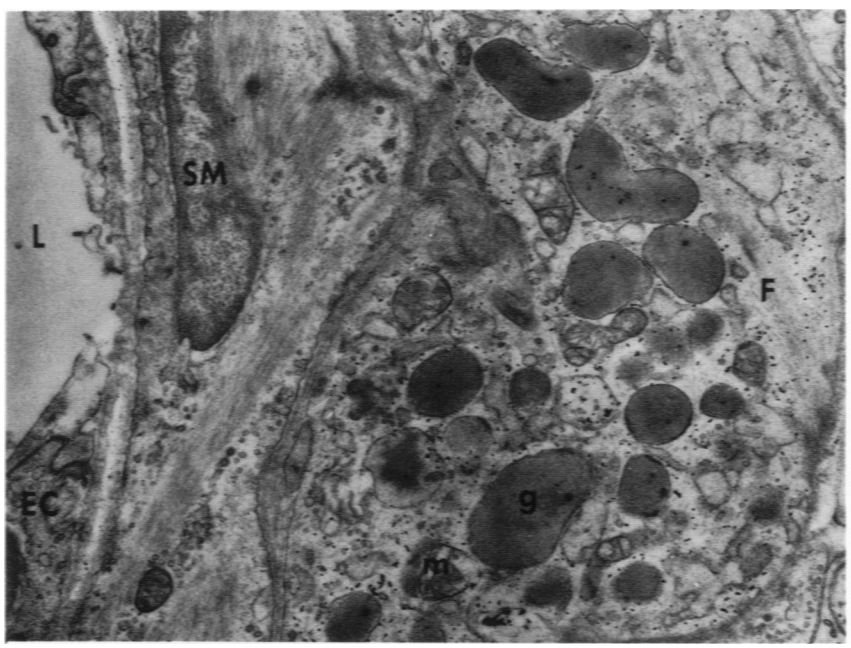

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Figure 5—Smooth muscle cells in the wall of an arteriole far from a glomerulus in an endocrine kidney, stained according to Thiery's technique (floated on thiocarbohydrazide for 24 hours), showing nucleus (*N*), a few dark, single granules of B-glycogen, a poorly-developed Golgi complex (*G*), a few mitochondria (*m*), and pinocytotic vesicles (*arrow*) (× 31,600). **Figure 6**—Part of an arteriole far from a glomerulus in an endocrine kidney, stained as in Figure 5, with lumen (*L*), endothelial cells (*EC*), a smooth muscle cell (*SM*) devoid of glycogen and a cell with filaments (*F*), numerous specific granules (*g*), a few mitochondria (*m*), and abundant dark glycogen particles (× 17,100).

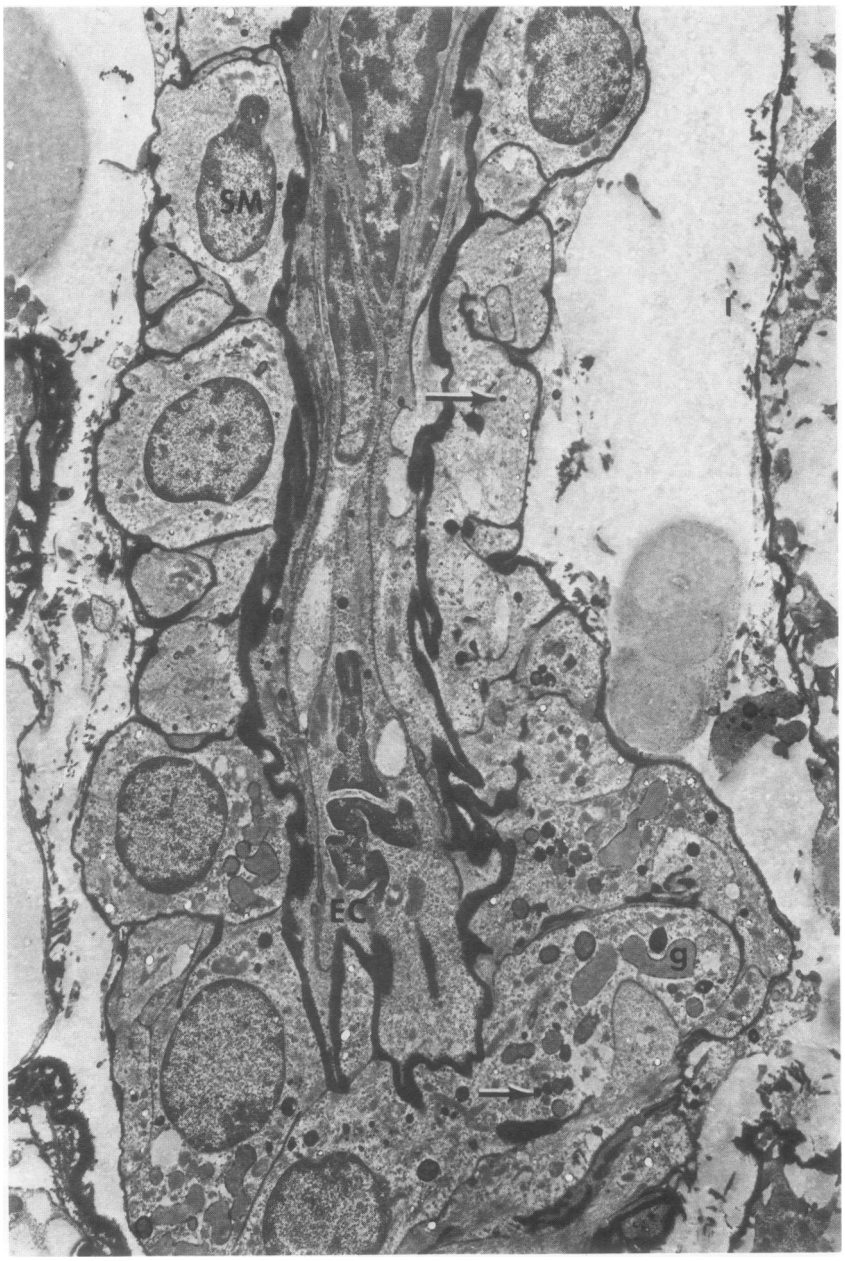
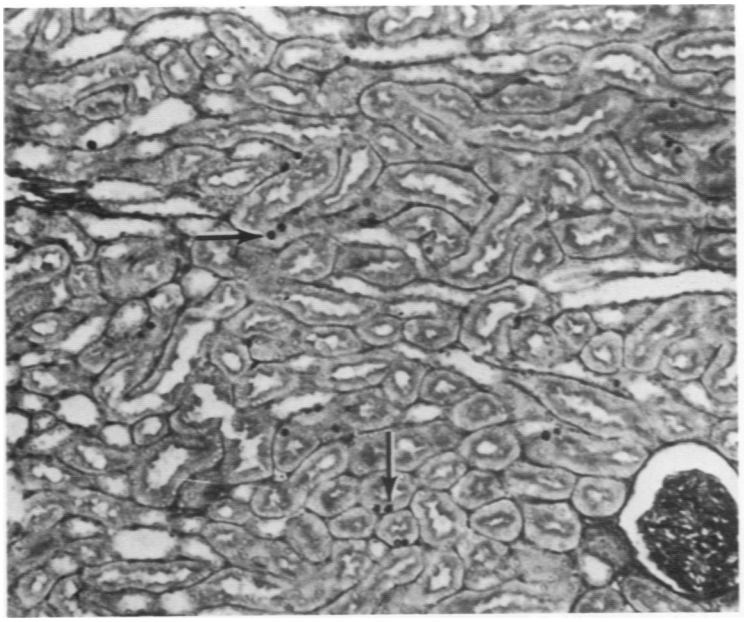


Figure 7—Longitudinal section of an arteriole far from a glomerulus in an endocrine kidney embedded in glycol methacrylate and stained with phosphotungstic acid at a low pH. The endothelial cells (*EC*) are separated from the media by a thick, extremely reactive basement membrane. The wall contains typical smooth muscle cells (*SM*) as well as cells with specific granules (*g*). Small, dark lysosomes (*arrows*) are visible in both cell types. (× 6200).



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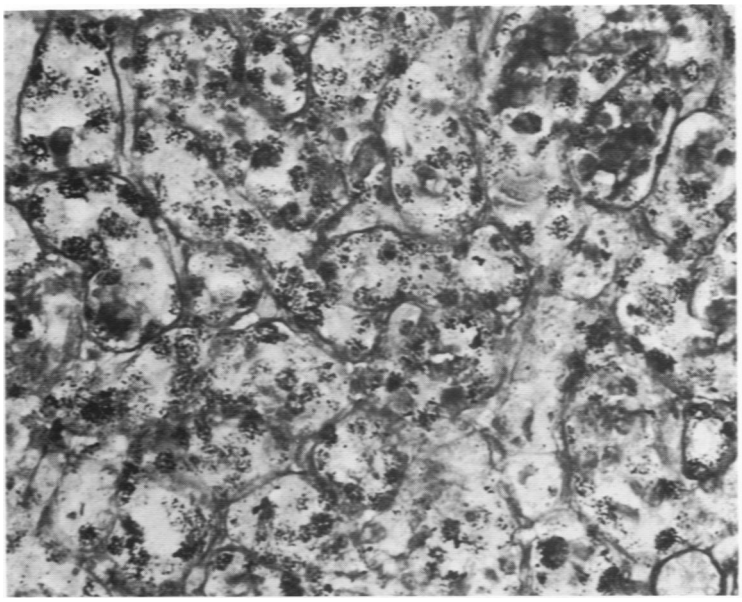

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Figure 8—Part of the inner cortex of a control rat. (The limit of the outer cortex is located at the level of the glomerulus, to the right of the photograph.) Light microscopic autoradiography shows reactive nuclei overlaid by densely-packed silver grains (*arrows*) mostly localized in the straight portion of the proximal tubular cells. (PAS, × 100) **Figure 9**—Part of the atrophic inner cortex of an endocrine kidney processed as in Figure 8. Most of the atrophic cells of the straight portion of proximal tubules contain reactive nuclei overlaid by more dispersed and less numerous silver grains. (PAS, × 250)

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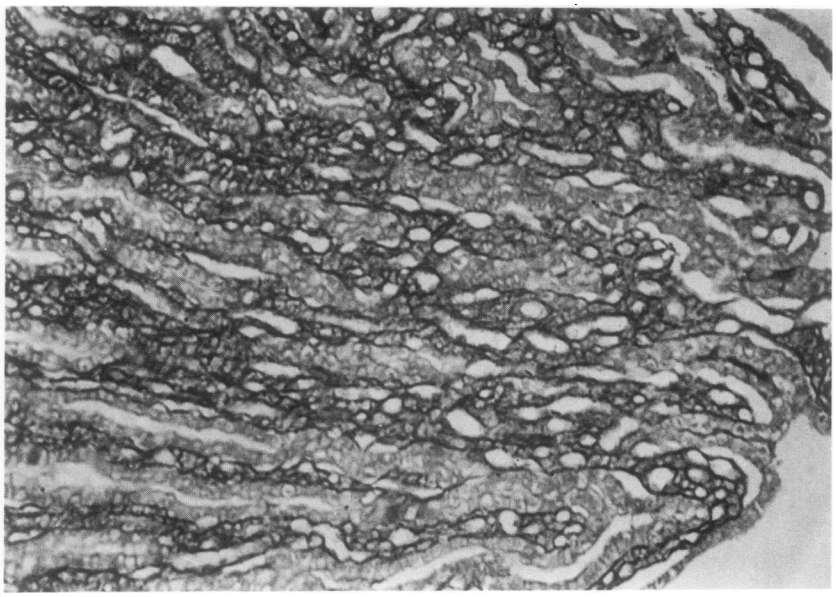

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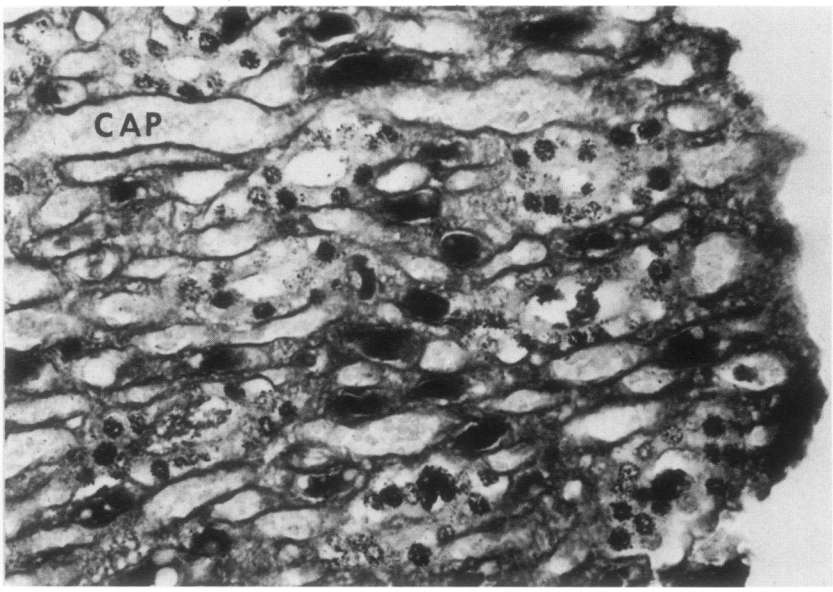
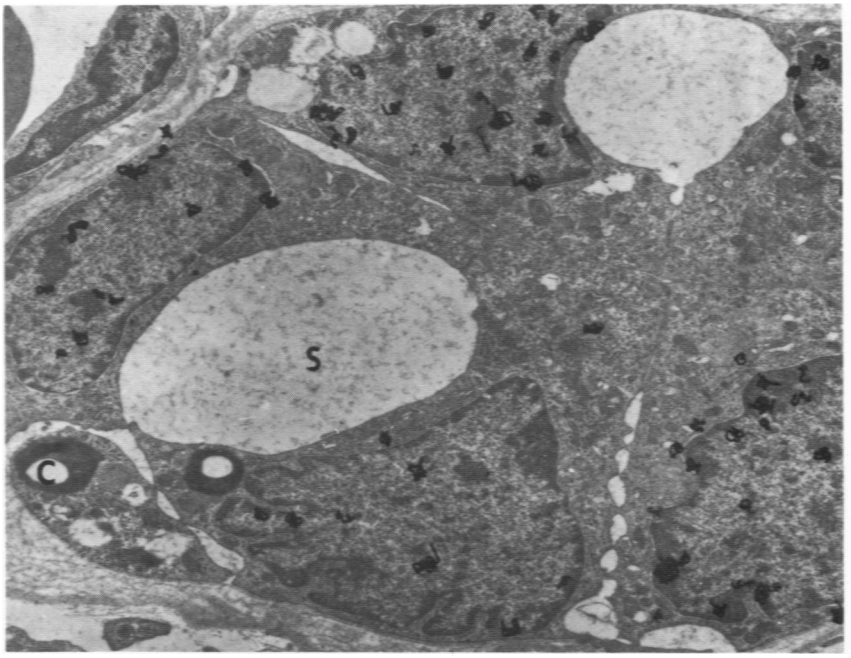


Figure 10—Papillary tip of a control kidney processed as in Figure 8. Note the complete absence of silver grains over the nuclei of tubular cells. (PAS, × 100) **Figure 11**—Papillary tip of an endocrine kidney processed as in Figure 8. Abundant silver grains are present over a great number of nuclei in the tubular cells. *CAP* = capillaries. (PAS, × 250)



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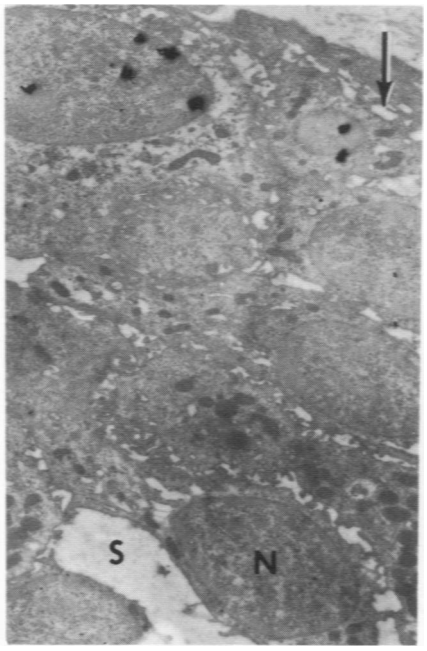

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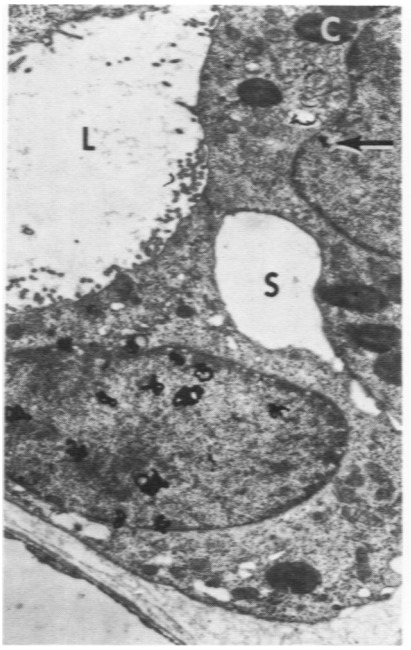

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Figure 12—Extremely atrophic proximal convoluted tubule of an endocrine kidney processed for autoradiography. Numerous silver grains are visible over all nuclei. Note the absence of lumen, large intercellular spaces (*S*), and cytosegresomes (*c*). (× 4800) **Figure 13**—Part of an atrophic distal convoluted tubule in an endocrine kidney processed as in Figure 8. Two nuclei are overlaid by a few silver grains, while the others (*N*) are not. Large intercellular spaces (*S*) as well as remnants of basilar interdigitating processes (*arrow*) are evident. (× 10,400) **Figure 14**—Part of a cortical collecting duct with lumen (*L*) into which a few microvilli protrude. One nucleus is overlaid by several silver grains while the other shows only a single grain (*arrow*). Large intercellular spaces (*S*) and cytosegresomes (*C*) are visible. (× 9000)

[*End of Article*]