

FINE STRUCTURE OF NORMAL HUMAN JUXTAGLOMERULAR CELLS

II. SPECIFIC AND NONSPECIFIC CYTOPLASMIC GRANULES

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In an earlier report, the cytoarchitecture and general cytologic features of the normal human juxtaglomerular apparatus (JGA) were described.¹ Three main types of juxtaglomerular cells (JC) were distinguished on the basis of their nuclear characteristics, and these were correlated with the degrees of development of major cytoplasmic organelles such as the Golgi apparatus and ergastoplasm. All cells appeared to consist of modified arteriolar smooth muscle cells (SMC). Transitional forms between the latter and JC were commonly observed. Not included in the earlier description were findings relative to cytoplasmic granules of JC, of which there appear to be two main types. One type of granule is considered specific for JC as it is not found in other renal vascular or tubular cells. The second type consists of nonspecific lipofuscin-like granules which, in human kidneys, are particularly prominent in both juxtaglomerular and vascular SMC.² These granules represent a potential source of confusion when estimating the degrees of "granularity" of JC by light microscopy.

It is the object of the present report to describe specific juxtaglomerular granules in detail and to contrast their fine structure and cellular distribution with those of nonspecific granules. The findings will be correlated with the results obtained by light microscopy with different staining procedures demonstrating juxtaglomerular granules.

MATERIAL AND METHODS

Seven renal biopsy specimens were processed for light and electron microscopy and juxtaglomerular apparatuses examined in serial sections as described in the first part of this study.¹ For the particular purposes of the work reported here, paraffin sections were stained with two groups of procedures. The first group included Mason's trichrome stain with ponceau fuchsin as used by McManus,³ Bowie's stain as used by Pitcock and Hartroft,⁴ and a fluorochrome stain with thioflavin-T as recommended by Janigan.⁵ To date, these have been the procedures principally recom-

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mended for the demonstration of cytoplasmic granules in JC. The second group of procedures included Sudan black, Ziehl-Nielsen, periodic acid-Schiff (PAS) and performic acid-Schiff (PFAS) stains as outlined by Lillie⁶; Jones' periodic acid-silver methenamine stain (PASM)⁷; Halmi's aldehyde fuchsin stain⁸ following oxidation with performic acid (PFAF); and Pearse's phthalocyanin method for phospholipids.⁹ Several of these procedures have been previously employed to demonstrate nonspecific granules in renal vascular SMC² and are known to stain lipofuscins in a variety of cells.^{6,8-10} Deparaffinized sections were also mounted in Apáthy's syrup or glycerin and examined with ultraviolet light for autofluorescence. The light source was a high-pressure mercury bulb HBO 200W in a Zeiss housing. BG-12 or UG-5 exciter filters with various barrier filters were used as recommended by Janigan,⁵ since the same sets of filters were used for examining the sections after staining with thioflavin-T. A dark-field condenser was used throughout.

For comparison purposes, the above stains were also applied to normal kidneys from 4 rats and 4 mice, as in these species, juxtaglomerular granules are more numerous and more readily visualized than in humans. Sections from these specimens were also used as controls whenever the Bowie's and thioflavin-T stains were applied to human kidneys. In view of the well known usefulness of the Bowie's and related stains for the demonstration of zymogen granules,^{11,12} sections of normal human pancreatic tissue were used as additional controls for the Bowie's stain. The tissue was obtained from a partial pancreatectomy specimen removed because of early carcinoma of the ampulla of Vater. Zymogen granules in this specimen were numerous and prominently stained with the Bowie's technique.

RESULTS

Electron Microscopic Observations

Ultrastructure of Nonspecific Cytoplasmic Granules. In thin sections (0.2 to 0.5 μ) of Epon-embedded blocks, nonspecific granules in JC stood out most prominently when examined by phase contrast microscopy, with or without toluidine blue staining. They appeared as dense cytoplasmic clumps, varying in size from the limits of visibility of the light microscope to several μ in diameter (Fig. 1). Larger granules were composed of clusters of smaller round clumps and frequently showed small clear vacuoles. In adjacent ultrathin sections examined by electron microscopy (Fig. 2), they could be readily correlated with electron-dense, markedly osmiophilic bodies scattered throughout the cytoplasm of JC, but most often located in perinuclear regions. Dense bodies with similar appearances were found in SMC throughout the renal vascular tree. As described more in detail in another report,² the bodies were composed of a coarsely granular matrix with particles 100 to 120 Å in diameter which, in larger bodies, were focally arranged in regular hexagonal lattices to form crystalline inclusions. The latter varied greatly in number, size and shape and, at high magnification, exhibited dot patterns or line patterns depending on the angle of sectioning (Fig. 3). The bodies contained, in addition, several fat droplets which were usually extracted during dehydration of the tissues with ethyl alcohol and appeared as round clear spaces.

Studies of serially sectioned JGA showed that nonspecific granules were present in most of the type 1 and 2 cells comprising the bulk of juxtaglomerular bodies.¹ Type 3 epithelioid cells, on the other hand, only occasionally contained such granules. The latter, when present, were few and small (Fig. 10).

Ultrastructure of Specific Cytoplasmic Granules. Specific granules occurred mostly in epithelioid JC. These cells consistently showed a preferential localization in limited portions of JGA, namely, in short eccentric segments in the walls of afferent arterioles at the points where these vessels first approached the macula densa and gave origin to juxtaglomerular bodies (Figs. 4 and 5). Usually no more than 4 to 8 epithelioid JC were located in these regions. A few additional cells of this type were inconstantly found scattered along the macula densa in deeper portions of juxtaglomerular bodies.

Infrequently, a few specific granules were found in type 2 JC, mostly in elements adjacent to epithelioid cells. No granules were present in type 1 cells. They were likewise lacking in renal vascular SMC outside of the JGA.

The structural characteristics of specific granules differed somewhat from one granule to another. Three main forms could be distinguished. There were, however, all structural gradations between these forms, indicating that they most likely reflected different stages in the development and maturation of the granules. The first, and possibly earliest, structural form was particularly characteristic in that it was comprised of granules shaped as narrow, elongated rhomboids, presenting sharply angulated, diamond-shaped outlines in cross sections (Figs. 6 to 10). The granules were most often seen within, or in the neighborhood of, Golgi cisternae (Figs. 7, 8 and 10). The Golgi apparatus was particularly well developed and was frequently composed of multiple segments extending from the perinuclear regions to the peripheral cytoplasm of epithelioid cells (Figs. 6 and 7). The granules were surrounded by single agranular membranes, presumably derived from Golgi cisternae, and contained a poorly osmiophilic, but densely packed, material which stained lightly with lead hydroxide and more intensely with uranyl acetate alone or in combination with the lead stain. When examined at relatively low magnification, this material appeared homogeneous. At higher magnifications it appeared to be composed of tightly packed filamentous units, less than 50 Å in diameter and predominantly orientated in directions parallel to the long axes of the granules (Figs. 8 and 9).

A second structural form was comprised of larger granules, found at the periphery of Golgi regions and measuring 500 to 800 m μ in diameter. The granules consisted of spherical membranous sacs containing

several rhomboidal clumps similar, in every respect, to the rhomboid granules described above (Fig. 6). Some of these clumps were sharply outlined while others tended to disaggregate and distribute their material more diffusely within the membranous sacs. These granules were believed to represent compound forms resulting from fusion of several rhomboid granules. A third, and apparently more mature, structural form of specific granules consisted of membranous sacs uniformly filled with finely filamentous material (Figs. 6, 7 and 10). The majority of granules in epithelioid cells were of this type. They were distributed predominantly at the periphery of the cytoplasm and showed relatively uniform sizes, shapes and structural appearances in contrast to the polymorphism exhibited by nonspecific granules. They were usually ovoid or cylindrical in shape, measuring 0.8 to 1.2 μ in diameter and up to 2.0 μ in length (Fig. 10). Their outer limiting membranes were closely applied to their component material without the interposition of electron-lucent spaces. Their content was poorly osmiophilic, finely filamentous and compact. In larger granules, however, the filamentous components tended to become coarser (about 70 Å in diameter) and more widely spread (Fig. 8). In some of these granules, focal accumulations of denser osmiophilic materials could be noted, as well as the presence of various structures such as fragments of membranes, glycogen particles and, occasionally, small crystalloids (Fig. 11). These components, however, were inconstant and most probably represented cytoplasmic constituents accidentally trapped in some granules, possibly during their growth, by fusion with other granules. Some of the larger granules near the cellular membranes showed variable degrees of rarefaction and loss of material from their periphery while compact cores remained in their central portions (Fig. 11). At the same time, their limiting membranes became less distinct and, on occasion, appeared missing. Clear-cut images of fusion between the membranes of specific granules and the cell membranes, with extrusion of the granule contents outside JC were not observed; nor were granules found free in extracellular spaces.

Light Microscopic Observations

The staining reactions observed in human and animal kidneys with the two groups of staining procedures employed are recorded in Table I. It can readily be appreciated that both groups of procedures demonstrated juxtaglomerular granules in human kidneys, while in animal kidneys, only the procedures in the first group gave positive results. In general, the procedures in the second group reflected the presence of mucopolysaccharides or bound lipids, and stained granules in JC in a positive manner (Figs. 12 and 13) as well as in arteriolar SMC (Figs. 14 and 15).

In addition, they stained a variety of hyaline and pigmented or non-pigmented lipid droplets in tubular cells. In the JGA, the strongest color reactions were obtained with the PASM and PFAF procedures. Positively stained granules were sparsely distributed, a few being present in

TABLE I
STAINING REACTIONS OF JUXTAGLOMERULAR GRANULES IN HUMAN AND ANIMAL KIDNEYS

Procedures	Human JGA granules		Rat and Mouse JGA granules
	Nonspecific	Specific	
<i>Group I</i>			
Masson's trichrome (fuchsinophilic)	++	+++	+++
Bowie's stain	++	+++	+++
Thioflavin-T	?	?	+++
<i>Group II</i>			
Periodic acid-silver methenamine	++++	—	—
Performic acid-aldehyde fuchsin	++++	—	—
Periodic acid-Schiff	++	—	—
Performic acid-Schiff	++	—	—
Ziehl-Nielsen	+	—	—
Sudan black	+	—	—
Luxol fast blue (phthalocyanin)	+	—	—
Autofluorescence	+++	—	—

most JC. Although quite variable in size and shape, they were usually coarse and lobulated. At times they were seen within, or at the margins of, juxtannuclear vacuoles. When examined under ultraviolet light, they showed a bright yellow autofluorescence with the OG-12 exciter filter and a more subdued, greenish-blue autofluorescence with the UG-5 filter. These granules corresponded closely in morphologic appearances and topographic distribution, to the polymorphic osmiophilic bodies seen in electron micrographs of JC and SMC and are to be considered as non-specific in nature.

With the first group of staining procedures, fuchsinophilic and Bowie-positive granules were readily demonstrated in the JC of rat and mouse kidneys. Characteristically, the granules were uniformly round and small and tended to crowd the cytoplasm of the cells. After staining with thioflavin-T, the granules showed a bright yellow fluorescence with the OG-12 exciter filter and a subdued bluish fluorescence with the UG-5 filter as reported by Janigan.⁵ In human kidneys, similar fuchsinophilic, Bowie-positive granules, not apparent in sections stained with the second group of procedures, were seen crowded in a few large JC located within the walls of afferent arterioles near the macula densa (Fig. 17). These granules corresponded in appearance and topographic distribution to the specific granules seen in electron micrographs of epithelioid JC.

Throughout the juxtaglomerular bodies, however, several of the coarser and more sparsely distributed nonspecific granules were also stained with the Masson's trichrome and Bowie's stains (Fig. 18), so that outside of the frankly epithelioid JC, a clear distinction between specific and nonspecific granules could not be achieved. In addition, Bowie-positive granules were observed in vascular SMC and in tubular cells (Fig. 16). With thioflavin-T the results were inconclusive since the autofluorescence of the more numerous nonspecific granules was quite similar in color and intensity to the fluorescence of positively stained specific granules, as seen in animal kidneys. The amount of tissue examined, however, was limited and a more exhaustive re-evaluation of this stain in human kidneys seems indicated.

DISCUSSION

It is clear from the results that in human JC there are at least two types of cytoplasmic granules distinct in staining properties, ultrastructure, topographic distribution and specificity. A comparable admixture of specific and nonspecific cytoplasmic granules has been described recently in other types of secretory cells.^{13,14} With regard to nonspecific granules, the findings reported here confirm earlier observations focused on similar granules in renal vascular SMC.² Their staining reactions and autofluorescence indicate that they consist, in general, of complex glycolipoproteins and that they are equivalent to lipofuscin-containing granules seen in other cell types.^{6,8-10} Their main function, as suggested by the accumulation of increasing amounts of osmiophilic materials during their evolution, may be the segregation, by adsorption to a glycoprotein matrix, of lipid products derived from the cellular metabolism and turnover of cytoplasmic constituents.

Specific granules, in contrast to nonspecific ones, are present in only a small minority of JC. Most of these are large, epithelioid, type 3 cells localized in segments of the afferent arteriolar walls nearest to the macula densa. The granules would seem to contain mostly proteins, as judged by their lack of reactivity with stains for lipids and carbohydrates, and by the fact that they stain deeply with the Bowie's and related stains¹⁵ used in other cells for the demonstration of zymogen granules known to be composed predominantly of enzyme proteins. Mature specific granules in human JC resemble, in size and structure, the granules found in epithelioid JC of the rat¹⁶⁻¹⁸ and the cat.¹⁹ Their 50- to 70-Å-thick filamentous units, however, are one-third to one-half the size reported for the component units of specific granules in the rat.²⁰ More strikingly characteristic of human JC are the angulated rhomboidal shapes and compactness of the smaller specific granules which, like in the rat,¹⁸ seem to arise

within, and separate from, the Golgi apparatus. These structural appearances strongly suggest that, in man, the content of early specific granules is concentrated in crystalline or coacervate form. The lack of such crystalline-like appearances, together with the coarser size of the structural units in rat granules, point to the existence of species differences in the molecular configurations of the specific products synthesized by JC. The nature of these products is not known with certainty, but there is considerable evidence that they represent secretory material, probably renin.²⁰ Maturation of the granules is accompanied by loss of their crystalline-like packing and by a looser arrangement and coarser structure of their constituent units, probably reflecting some degrees of hydration and polymerization of their material. The structural disorganization in some of the larger granules at the periphery of the cytoplasm suggests the possibility that their material may diffuse into the surrounding cytoplasm and be released from the cells.

Of particular interest in this study was the consistent distribution of specific granules in cells localized in limited regions of the juxtaglomerular apparatus. It is possible, therefore, that differentiation of these cells occurs in response to specific stimuli prevalent in such zones, rather than as a random phenomenon of cell maturation. In particular, the localization of epithelioid cells on the sides of the arteriolar walls facing the macula densa, and their consistent absence from the opposite sides, suggests that the responsible stimuli originate from macula densa cells and are most effectively exerted on cells within, or near, the vascular walls. The information presently available strongly suggests that sodium concentration gradients between the urinary fluid reabsorbed by macula densa cells and the plasma filtering through arteriolar walls may be the most important stimuli.²¹

The morphologic characterization of two distinct types of juxtaglomerular granules in human JC, as has emerged from the results of the present study, has some of its most important implications in relation to the problem of the "granularity" of the human JGA as visualized in conventional light microscopic preparations. This problem arises basically from the fact that, while in animal kidneys, atypical granules, presumably equivalent to those described here as non-specific granules, are insufficient in number and size to interfere with the identification of specific granules.^{17,18} The converse is true in human kidneys. This fact seems to explain the peculiarities which, in the literature, have been frequently noted to distinguish human from animal juxtaglomerular granules. In Goormaghtigh's classic study,²² fuchsinophilic granules in human JC were observed to be coarser, less numerous, and more sparsely distributed than in animals, and to stain less brightly. Most significantly,

Goormaghtigh also recorded the presence of lipid granules in JC, seemingly with the same distribution as fuchsinophilic granules. In a later study, McManus²³ observed that human juxtaglomerular granules were strongly stained by a method employing Sudan black in paraffin sections. This suggested a high content of masked lipids in the granules.

Since the original description of fuchsinophilic granules in human JC by Oberling,²⁴ the Masson's trichrome stain with ponceau fuchsin has remained in prevalent use for the demonstration of granules in JC until the work of Wilson.¹⁵ Searching for a more selective and reliable stain, this author found that, in the rat, neutral stains usually employed for the demonstration of zymogen granules in various cells could be successfully applied to juxtaglomerular granules. He obtained the best results with a modification of the Bowie's ethyl violet-Biebrich scarlet stain for zymogen granules. Hartroft and Hartroft²⁵ successfully used the Wilson's and Bowie's stains in their studies on the granularity of the JGA in rats. Since their work, the Bowie's stain has been widely adopted as the method of choice for the demonstration of juxtaglomerular granules. When applied to human kidneys, however, the results obtained have been less consistent and more difficult to interpret. Bowie stain-positive granules in human JC were noted to be fewer than in animals and, as reported earlier by McManus,²⁶ to react in positive manner with the PAS technique.⁴ Bowie stain-positive granules were also found in tubular epithelium.²⁷ For these reasons, increasing reliance has been placed on the cellularity rather than on the granularity of human JGA as an index of juxtaglomerular activity.^{27,28} More recently, Janigan⁵ has introduced a fluorescent stain with thioflavin-T which, in the kidney of various animal species, appears to be highly specific for juxtaglomerular granules. Again, however, the evaluation of this stain in human kidneys was complicated by the fact that, in this tissue, the granules had strikingly different distributions and were unexpectedly found to be autofluorescent. Almost simultaneously, we reported the autofluorescence of most human juxtaglomerular granules and showed that these granules corresponded ultrastructurally to nonspecific osmiophilic bodies commonly occurring in JC and arterial SMC.²

When the data reviewed above are considered in the context of the light and electron microscopic findings reported here, it becomes evident that the peculiar difficulties encountered in appraising the "granularity" of human JC are, in the main, attributable to the following: nonspecific granules in the human JGA are sufficiently prominent in number and size to overshadow the relatively scarce specific granules; the opposite is true in the JGA of most laboratory animal species; the Masson's and Bowie's stains do not discriminate between specific and nonspecific granules. This lack of selectivity, while relatively inconsequential in animals, is seri-

ously misleading in man, due to differences in the predominant types of juxtaglomerular granules encountered in the JC of human and animal kidneys.

It is hoped that with more systematic histochemical investigations on human JC, better means will be found to differentially stain specific and nonspecific granules in the same tissue sections. For the present, the results obtained with the limited number of procedures used in the present study indicate that:

1. The Bowie's stain has been, and remains, a most useful stain for demonstrating specific granules in JC, although its selectivity is more limited than formerly believed.

2. In human kidneys, this stain should be used in conjunction with one of the procedures which stain nonspecific granules most intensely and selectively, such as the PASM or PFAF.

3. The term "granular" cells, if used, should be reserved for JC containing specific granules. In normal kidneys, such cells consist almost exclusively of epithelioid cells.

SUMMARY

The cytoplasmic granules in normal human juxtaglomerular cells (JC) were studied by electron microscopy and the findings correlated with the results obtained with various staining procedures by light microscopy. Two basic types of granules were distinguished; these differed in ultrastructural patterns, staining reactions and cellular distribution. One type of granule was nonspecific in nature and was related to lipofuscins. The second type was specific for JC, and probably secretory in nature. While nonspecific granules were sparsely distributed throughout juxtaglomerular bodies and were most numerous in non-epithelioid JC, specific granules were almost exclusively localized in epithelioid cells. The latter constituted a small minority of JC and were mostly eccentrically distributed in short segments of the afferent arteriolar walls on the sides of the macula densa.

To help the correlation between the fine structure and the staining reactions of cytoplasmic granules in human JC, advantage was taken of the fact that in rat and mouse JC nonspecific granules are insufficient in number and size to be readily visualized by light microscopy. The results of various staining procedures were, therefore, compared with those obtained in the kidneys of these animal species. It was found that nonspecific granules were selectively stained by a group of procedures, among which the periodic acid-silver methenamine and the performic acid-aldehyde fuchsin stains gave the best results. On the other hand, procedures used to demonstrate specific granules, particularly the

Bowie's stain, stained nonspecific granules as well. It was concluded that a combined use of both types of staining procedure is advisable in order to determine by light microscopy the contributions made by each type of cytoplasmic granule to the overall "granularity" of the juxtaglomerular apparatus in human kidneys.

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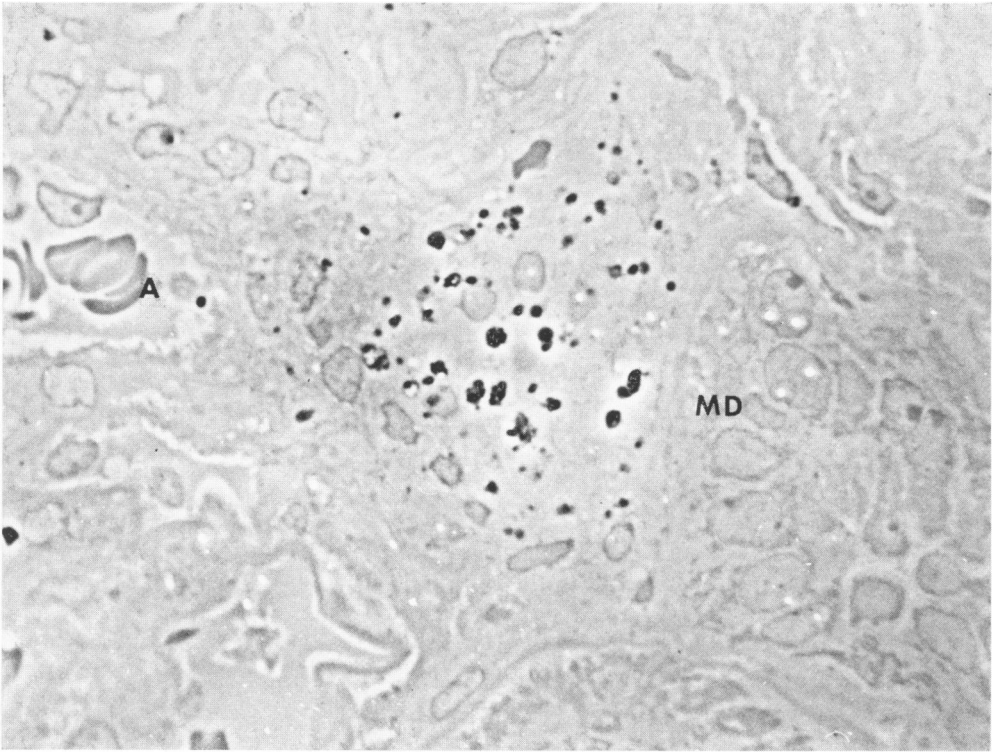
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[*Illustrations follow*]

LEGENDS FOR FIGURES

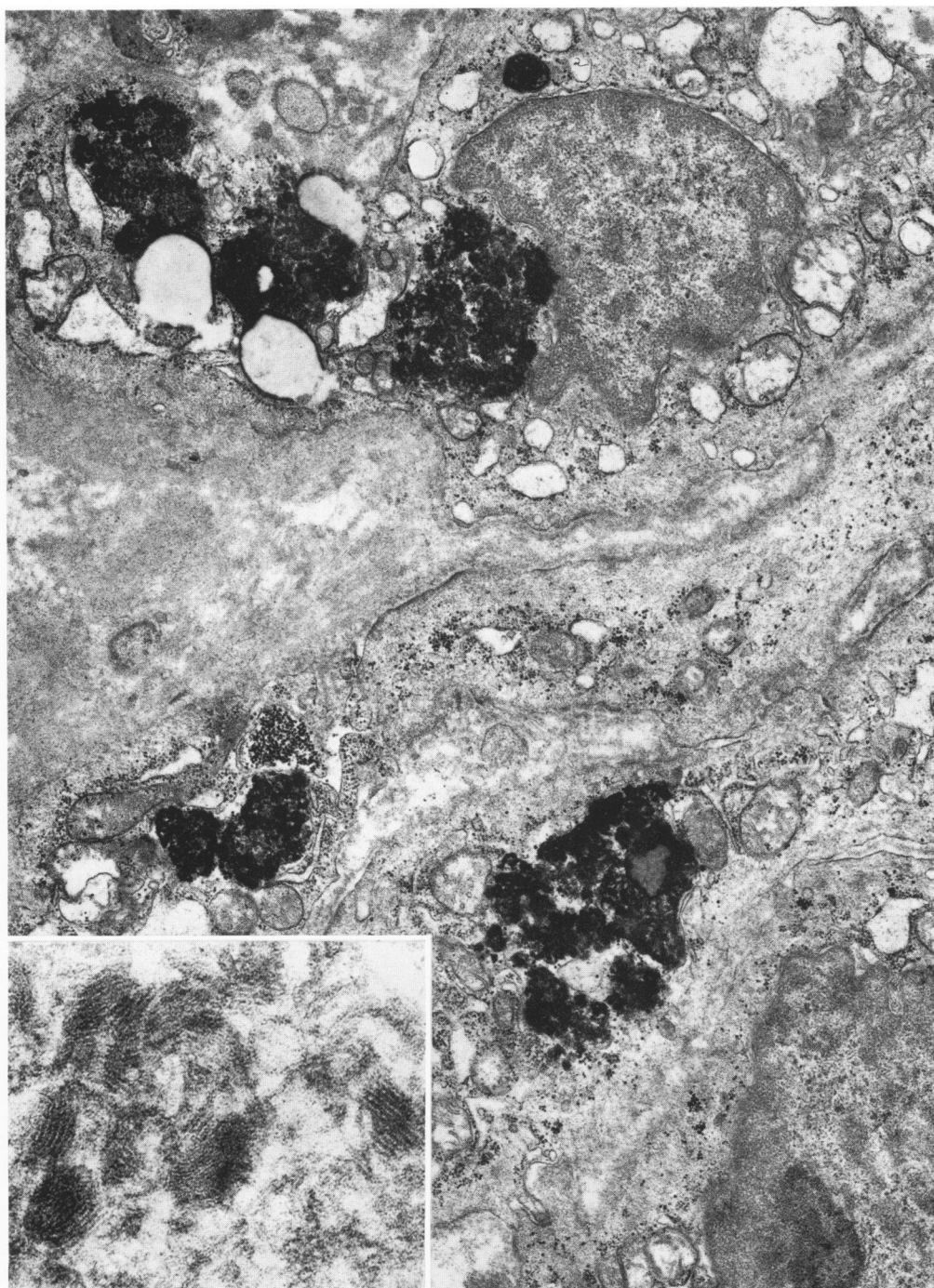
FIG. 1. Phase contrast; JGA in a 0.2- μ -thick unstained section of Epon-embedded tissue. Nonspecific cytoplasmic granules in JC appear as dense cytoplasmic masses which, in spite of the thinness of the section, stand out prominently while the outlines of other tissue components are only faintly made out. The granules are coarse, often lobulated, and sparsely distributed throughout the juxtaglomerular body. Afferent arteriole (A); macula densa (MD). $\times 1,100$.



1

FIG. 2. A representative field from an ultrathin section of the juxtaglomerular body illustrated in Figure 1. Nonspecific granules in JC are seen as polymorphic dense bodies containing a coarsely granular matrix, osmiophilic inclusions and fat droplets. Lead hydroxide stain. $\times 16,000$.

FIG. 3. Detail of osmiophilic inclusions in a large nonspecific granule. The inclusions exhibit line-patterns resembling myelin figures. Lead hydroxide stain. $\times 124,000$.

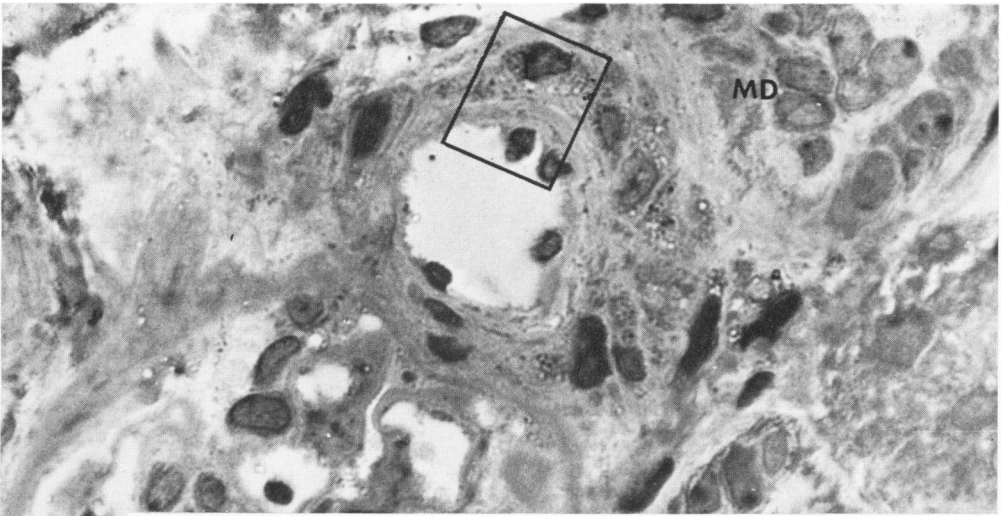


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FIG. 4. The JGA is illustrated at the level where cells containing specific granules are predominantly localized. The afferent arteriole is sectioned at some distance proximally to its entrance into the glomerulus. At this level, the juxtaglomerular body has only begun to emerge and consists simply of a moderate, eccentric thickening of the arteriolar wall on the side of the macula densa (MD). This thickened segment contains several epithelioid JC, the cytoplasm of which is crowded with uniformly round small granules. The field enclosed corresponds to that shown in the electron micrograph in Figure 5 taken in an adjacent ultrathin section. Toluidine blue stain. $\times 1,000$.

FIG. 5. Composite of two electron micrographs illustrating, in mirror image, the field enclosed in Figure 4. The epithelioid cell in the media of the afferent arteriole contains a large Golgi region (G), dilated endoplasmic reticulum, several specific granules (SG) and a few accumulations of particulate glycogen (g). Nonspecific granules are lacking. Lead hydroxide stain. $\times 6,400$.



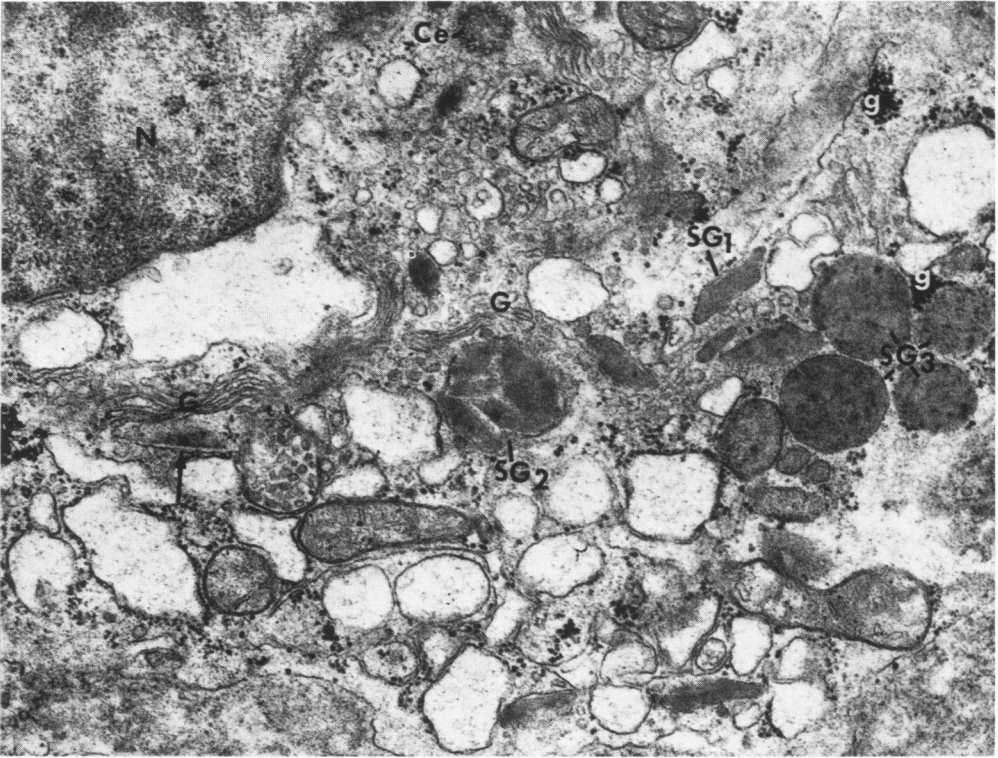
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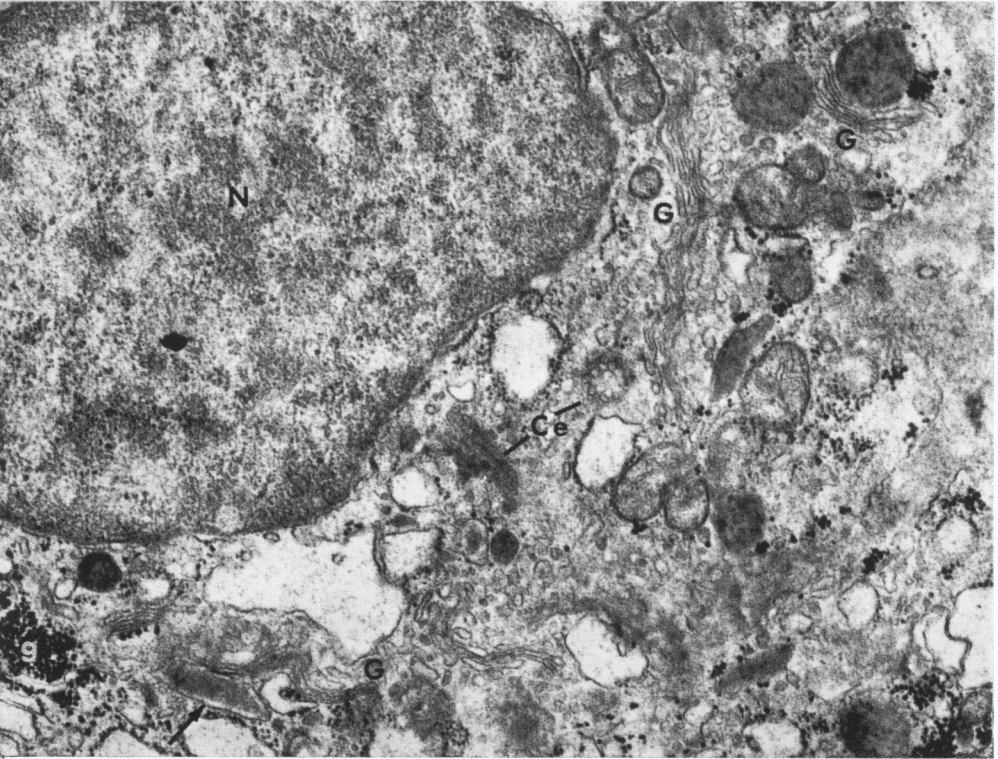
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FIG. 6. Detail of the epithelioid cell shown in Figure 5. The Golgi apparatus is particularly well developed and composed of multiple segments (G). Several specific granules (SG) are scattered between the Golgi region and the periphery of the cell. The granules are surrounded by a single membrane and contain a material apparently homogeneous at this magnification and of fairly high electron density with the uranyl acetate-lead hydroxide stain used on this section. One form of specific granules (SG₁) characteristically shows elongated rhomboidal configurations. A second form (SG₂) is round and contains material subdivided into several rhomboidal masses. A third form (SG₃) consists of round granules containing uniformly distributed materials. A tangentially sectioned rhomboid granule (arrow) is present in a Golgi cisterna. Cisternae of granular or partly degranulated endoplasmic reticulum show close spatial relationships with the Golgi apparatus. Nucleus (N); centriole (Ce); glycogen (g). $\times 26,000$.

FIG. 7. The same epithelioid cell as that shown in figure 6 is sectioned at a deeper (about 0.3μ) level. The specific granule (arrow) in the Golgi cisterna has emerged in full view. Additional segments of the Golgi apparatus (G) associated with different forms of specific granules (SG) have appeared and extend from the nucleus (N) to the outer upper region of the cell. A second centriole has emerged in the centrosphere region (Ce). Glycogen (g). Uranyl acetate-lead hydroxide stain. $\times 26,000$.

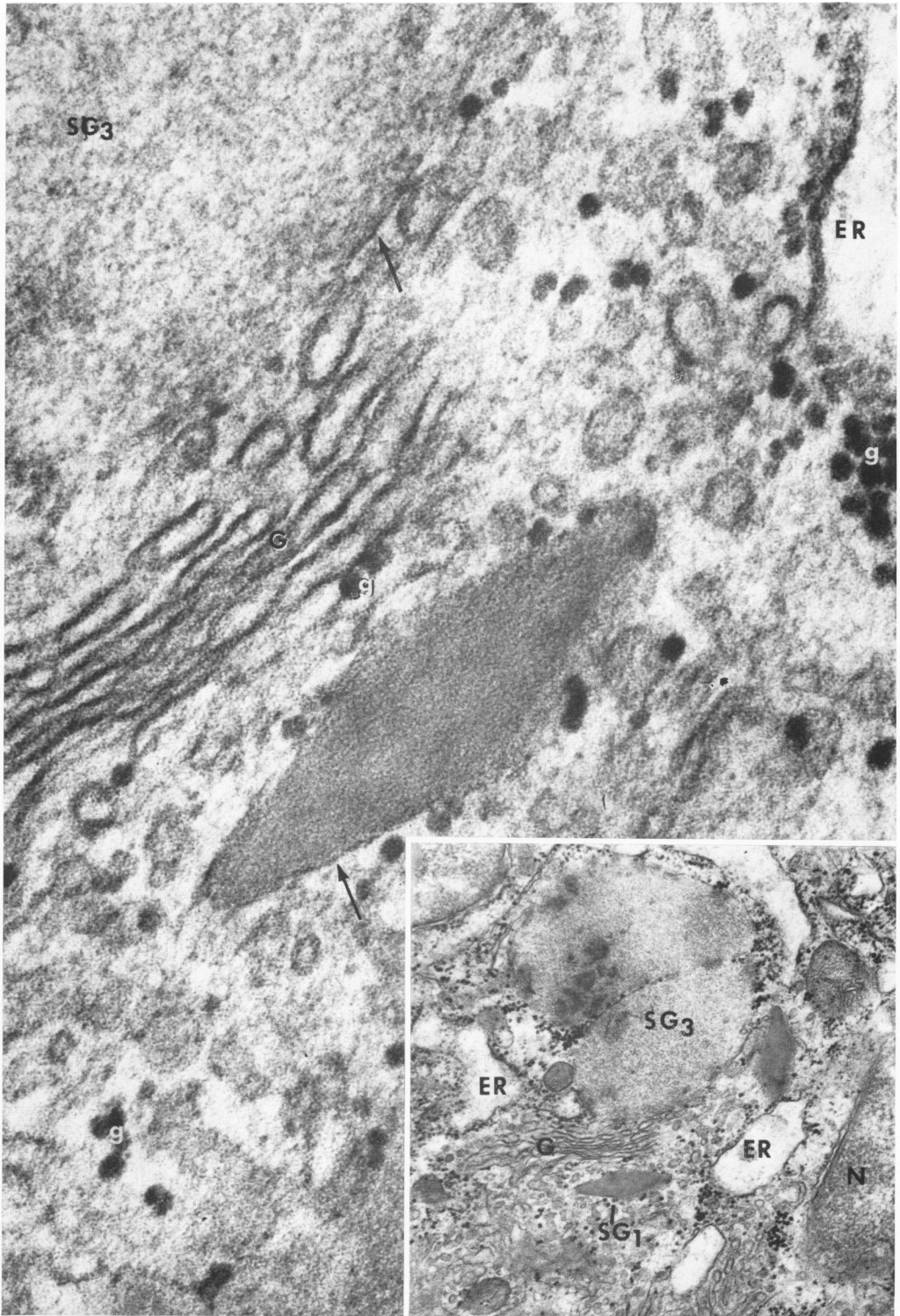


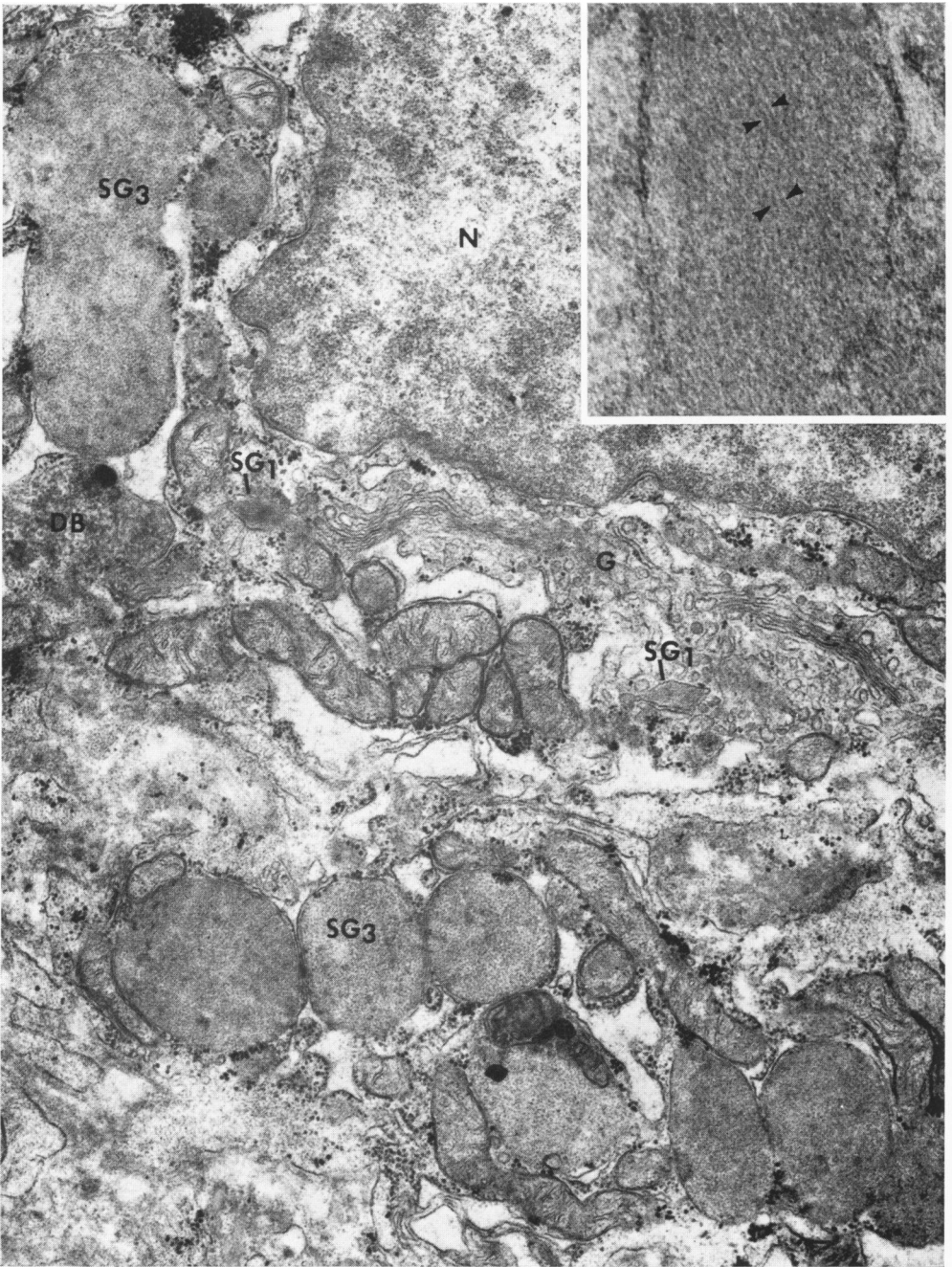
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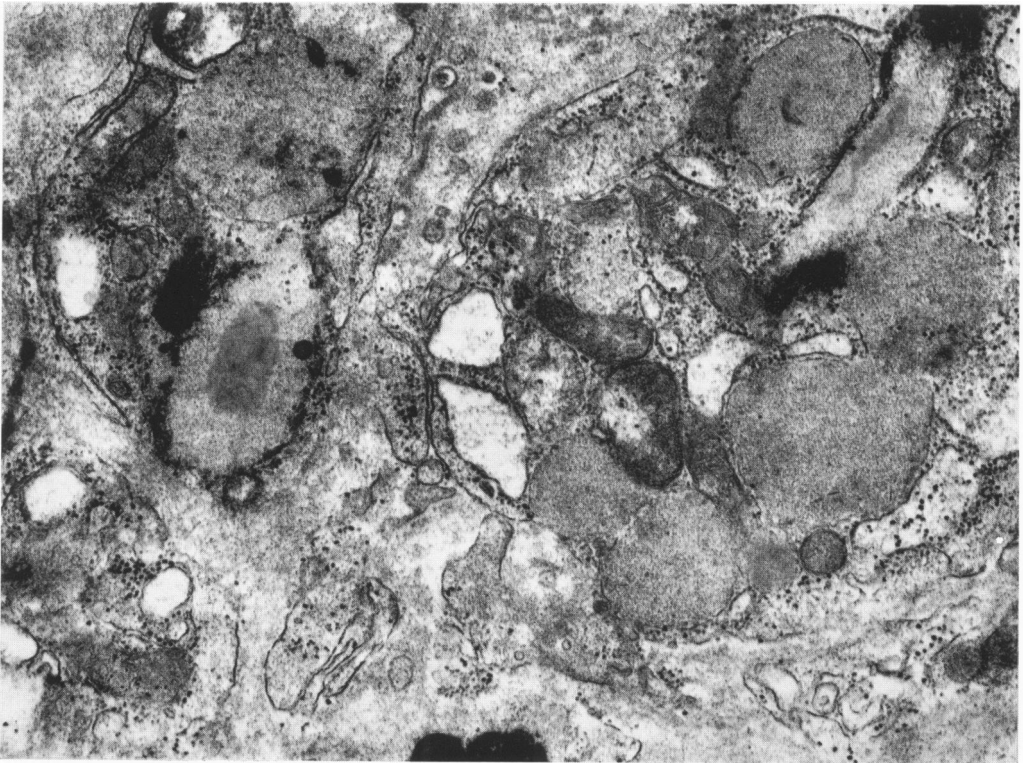
FIG. 8. Detail of a rhomboid granule (SG_1) and of portions of a large mature specific granule (SG_3). The granules are near the Golgi apparatus (G) in the perinuclear cytoplasm of an epithelioid cell as shown at lower magnification in the insert. The rhomboid granule, cut in cross section, exhibits a characteristic diamond-shaped configuration. Its material is composed of tightly packed filamentous units seen mostly in cross section and appearing as fine dots less than 50 Å in diameter. A single outer membrane (arrow) is so closely applied to this material that it can be made out only with difficulty. The mature granule is composed of coarser filamentous units more loosely and randomly distributed. Its outer limiting membrane (arrow) is indistinct and closely applied to its content. Cisternae of granular endoplasmic reticulum (ER) extend to the immediate vicinity of the Golgi apparatus. Glycogen particles (g) are scattered diffusely in the ground cytoplasm. Nucleus (N). Lead hydroxide stain. $\times 150,000$. *Inset*, $\times 22,000$.





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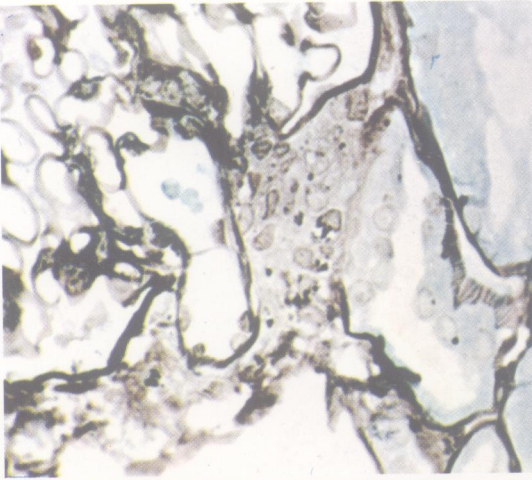
FIG. 9. Detail of a rhomboid specific granule shows several longitudinally orientated filamentous components about 40 Å in diameter (arrow points). Lead hydroxide stain. $\times 150,000$.

FIG. 10. Large mature specific granules (SG_3) are distributed at the periphery of an epithelioid JC. The granules have round, ovoid or cylindrical shapes. They are surrounded by single membranes without the interposition of electron-lucent spaces, and contain tightly packed finely filamentous material. Two rhomboid specific granules (SG_1) are separating from the well developed Golgi apparatus (G) near the nucleus (N). A dense body (DB) corresponding to a small non-specific granule is also present in the adjacent cytoplasm. Lead hydroxide stain $\times 98,000$.

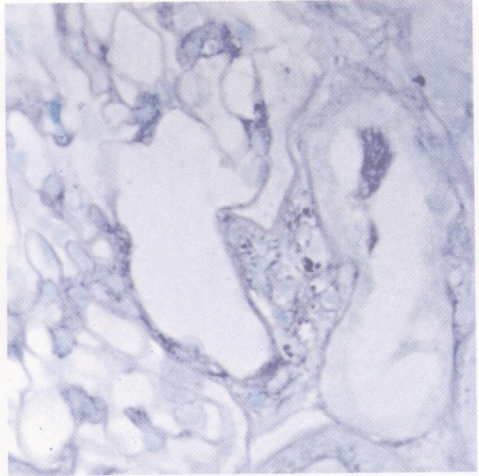
FIG. 11. Specific granules in the peripheral cytoplasm of two epithelioid cells. The material in the granules is loosely aggregated. A denser core and small osmiophilic inclusions are seen in the granules on the left. These granules also show poorly defined outlines and limiting membranes. Lead hydroxide stain. $\times 26,000$.

- FIG. 12. A juxtaglomerular body extends eccentrically from the afferent arteriolar wall. Nonspecific granules (black) are sparsely distributed and variable in size and shape. PASM stain, light green counterstain. $\times 500$.
- FIG. 13. A juxtaglomerular body extends eccentrically from the afferent arteriolar wall. Nonspecific granules (deep blue) stand out prominently in the lightly counterstained tissue. They are sparsely distributed and show considerable variation in size and shape. PFAF stain, light green counterstain. $\times 500$.
- FIG. 14. Nonspecific granules in arteriolar SMC. PASM stain, light green counterstain. $\times 500$.
- FIG. 15. Nonspecific granules in arteriolar SMC. PFAF stain, light green counterstain. $\times 500$.
- FIG. 16. Bowie stain-positive nonspecific granules (red purple) are shown in SMC of interlobular arteriole and in tubular cells. Bowie's stain, no counterstain. $\times 500$.
- FIG. 17. Bowie stain-positive specific granules (red purple) are evident in two epithelioid JC. The cells are typically localized in the wall of the afferent arteriole near the macula densa, almost outside of the JGA. The granules are exceedingly small, uniformly round, and crowd the cytoplasm of the cells. Bowie's stain. $\times 500$.
- FIG. 18. Bowie stain-positive granules appear in the main cellular mass of a juxtaglomerular body. The granules are sparsely distributed and, on the average, coarser in size than specific granules in epithelioid cells. While the larger granules can be inferred to be nonspecific, the smaller ones could be either specific or nonspecific, the latter possibility being most probable. Bowie's stain, no counterstain. $\times 500$.

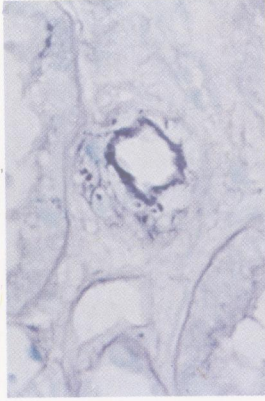
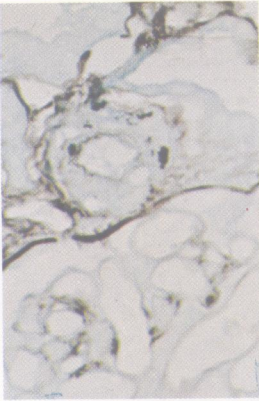
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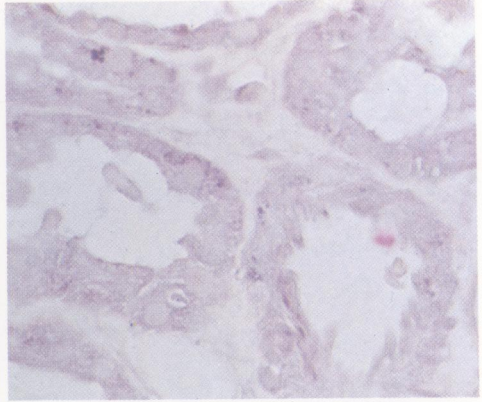
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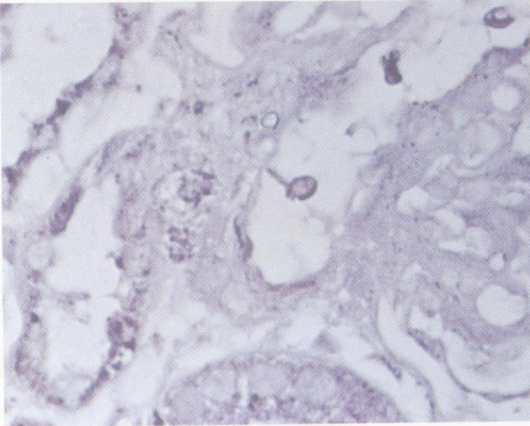
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