# Electron Microscopy of Renal Juxtaglomerular Cells[1]

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The secretory nature of renal juxtaglomerular (JG) cells, specialized cells in the media of the afferent glomerular arteriole, has been assumed since Goormaghtigh ('39) first studied them in renal ischemia. Indirect evidence includes the presence of stainable granules within these cells and the fact that changes in granule-content can be produced by certain experimental conditions, such as renal ischemia (Goormaghtigh, '39), variations in salt intake (Hartroft and Hartroft, '53), adrenalectomy (Dunihue, '49) and changes in blood pressure (Hartroft, '57; Tobian et al., '58). If JG cells are indeed secretory or endocrine in nature, electron microscopy should reveal ultrafine structure similar to that of other secretory cells.

Braunsteiner (Braunsteiner et al., '56) reported an electron microscopic study of three JG cell fields in rats in which he described the presence of canilicular structures as indicative of secretory activity. Unfortunately his observations seem invalid. The cells he described and illustrated were outside the afferent arteriole in interstitial spaces, contained no granules, and closely resembled plasma cells. It must be emphasized that JG cells are specific granulated cells having an epithelial-like appearance and are located *in the media* of the afferent arteriole (Hartroft and Hartroft, '53; Hartroft, '57).

The purpose of this investigation was to determine whether JG cells were functioning and healthy rather than degenerating or aging cells; to distinguish the granules observed by light microscopy from other cytoplasmic organelles and inclusions, such as mitochondria and lipid material; and to correlate features of JG cells as seen by the electron microscope with those seen by both conventional and phase light microscopy.

## METHODS

*Animals.* Three species were chosen for this investigation: rat, dog, and cat. Kidneys were selected from several animals from each species, most of whom had been adrenalectomized or maintained on a sodium-deficient diet for several months to produce hypergranulation and hyperplasia of their JG cells (Hartroft et al., '58). These animals were used in order to take advantage of greater number and size of JG cell fields that would be encountered in the small, thin sections necessary for electron microscopy.

*Techniques—electron microscopy.* With a razor blade, small blocks of tissue, about $1 \times 1 \times 0.5$ mm, were cut from the superficial renal cortex along a mid-sagittal plane, while animals were under anesthesia. Tissue was fixed for 30–90 minutes in either Dalton's osmium tetroxide solution at pH 7.6 or Palade's sucrose fixative. After rinsing, dehydrating with alcohol, and immersion in a mixture of butyl and methyl methacrylate, tissue blocks were carefully imbedded in methacrylate (polymerized at 50° C overnight) so that the tissue would be cut in the sagittal renal plane. Thin sections (400–700 A) were cut with a Porter-Blum microtome using disposable glass knives. RCA EMU3B and 2B electron microscopes were used for viewing and photographing.

*Techniques—phase sections.* Each time several thin sections were cut for electron microscopy, a thicker section (0.5–1.0 μ) was cut with the same glass knife for phase microscopy and placed on a glass slide. These slides were immersed in toluene to dissolve the plastic, hydrated through alcohol to water and mounted with Paragon, a water-soluble medium.

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Before electron microscopic examination was carried out, desirable fields were first located and studied in these phase sections. JG cells were easily identified with phase contrast, due to the presence of osmophilic granules (fig. 1). Ultra-thin sections were then examined with the electron microscope and the corresponding fields located and photographed at low magnification (fig. 2) for further orientation at higher magnifications (fig. 3). In this way, JG cells could be identified with certainty in electron micrographs.

## RESULTS

### *Cellular components of JG cells*

*JG granules.* JG granules were ordinarily observed as individual homogeneous osmophilic inclusions of the cytoplasm. In all three species, their shape was ovoid to spherical (figs. 3, 6). Although a distinct membrane has not been demonstrated, the smooth contour of these granules suggests that they have a limiting membrane.

In a sodium-deficient dog with hyperplastic JG cells, deviation from homogeneous appearing granules was observed. JG granules contained smaller particles, or "granules within granules" (figs. 9, 10, 11). The possibility that this appearance was due to compression artefact is unlikely, because compression of surrounding structures was not seen. In two instances, such granules were found abutting an indentation of the nucleus and in close association with a Golgi apparatus (fig. 13).

*Nuclei.* Nuclei of JG cells, as previously shown in light microscopy, were large, ovoid in shape, and sometimes indented. In favorable planes of section, prominent nucleoli were seen (fig. 5).

*Mitochondria.* Within the cytoplasm of JG cells, many characteristic mitochondria were found. They were ovoid, had definite cristae (figs. 6, 7), and were pale in contrast to denser mitochondria of renal tubular cells (fig. 3).

*Endoplasmic reticulum.* An outstanding feature of JG cells was the presence of extensive endoplasmic reticulum, or ergastoplasm, with abundant RNA granules (fig. 6). When JG granules were not packed too closely together, the endoplasmic reticulum could be seen in the form of sacs lined by RNA granules (figs. 3, 7).

*Golgi apparatus.* The presence of a Golgi apparatus in JG cells has been described with special stains using light microscopy (Harada, '54). In the present electron microscopic study, Golgi elements were observed in hyperplastic JG cells. This structure was found near the nucleus and consisted of a small spheroid vacuole partly surrounded by parallel lamellae and microvesicles, without associated RNA granules (figs. 13, 14).

*Plasma membrane.* The plasma membrane of JG cells was frequently observed to be involuted (figs. 6, 11). Its folds penetrated the interior of the cell and in some cases appeared continuous with endoplasmic reticulum.

### *Cells adjacent to JG cells*

*Macula densa.* The macula densa, that portion of the distal tubule situated in the angle between the two glomerular arterioles, is in close anatomical association with JG cells. With electron microscopy, suitable planes of section also demonstrate this close relationship. Distinct separations between JG and macula densa cells in some places were difficult to find and may have been absent (figs. 3, 13) in that infoldings of plasma membrane of both cell types often appeared continuous. Golgi apparatus (more typical than that in JG cells) in macula densa cells was observed in several fields (figs. 3, 13, 15).

*Smooth muscle cells.* Smooth muscle cells, adjacent to JG cells in the arteriolar media, differ from JG cells in that they have fewer mitochondria, a pale fibrillar cytoplasm and scant endoplasmic reticulum (fig. 5). They do not differ significantly from smooth muscle cells in other arterioles of comparable size.

*Intima of afferent arteriole.* Because of their position in the arteriolar media, JG cells also abut on the intimal coat. Typical endothelial cells line the lumen of the arteriole in which JG cells are found, but the internal elastica at this site is not as distinct as in arterioles distant from glomeruli.

## DISCUSSION

As mentioned above, previous studies using light microscopy have provided indirect evidence that JG cells are secretory in nature (Goormaghtigh, '39; Hartroft and Hartroft, '53; Dunihue, '49; Hartroft, '57; Tobian et al., '58). Goormaghtigh described a glandular cycle for JG cells in ischemic kidneys of experimental animals and proposed that these cells might elaborate the pressor substance, renin (Goormaghtigh, '39). Support for Goormaghtigh's theory has been obtained recently in this laboratory when it was found that renin-content of kidneys from sodium deficient animals was increased in proportion to degree of granulation of JG cells (Pitcock et al., '59). Tobian et al., ('59) have reported similar findings in hypertensive rats. This physiomorphologic evidence for the secretory nature of JG cells is further corroborated by observations from the present electron microscopic study.

The electron microscopic characteristics of JG cells, described above, suggest that they are metabolically active, rather than aging or degenerating cells, and are compatible with features of other secretory cells.

The osmophilic secretory granules of JG cells that were apparent in both phase and electron microscope sections closely resemble zymogen granules. By electron microscopy, JG granules usually had a homogeneous appearance. However, in hyperplastic canine JG cells, denser "granules within granules," were observed. In two instances such granules were found in cytoplasm that was partially surrounded by indentations of the nucleus and in close association with Golgi apparatus. This unusual finding is suggestive of an active stage of formation or release of these secretory granules but confirmation of this possibility must await further study.

Additional evidence of cellular activity of JG cells was provided by the presence of numerous mitochondria and rich endoplasmic reticulum with abundant RNA granules. The presence of Golgi elements, especially in hyperplastic cells, is consistent with secretory activity.

In describing the proximity of JG cells and macula densa cells, McManus ('47) suggested the possible passage of substances from one cell type to the other. Electron microscopic observations support his suggestion, and indicate possible cytoplasmic bridges between these cells.

Further studies with the electron microscope, including comparisons of depressed and active JG cells and stages between these two extremes, should shed more light on formation and release of secretory products in JG cells.

## SUMMARY

Observations on electron microscopy of renal juxtaglomerular (JG) cells have been made in rats, dogs and cats. Phase microscopy was used for orientation of fields seen with the electron microscope to assure correct identification of JG cells. Ultra fine structure of JG cells described in the present paper is indicative of metabolically active cells with secretory function and supports results of physiomorphologic experiments reported previously.

## LITERATURE CITED

Braunsteiner, H., K. Fellinger and F. Pakesch 1956 Elektronenmikroskopische Untersuchungen an juxtaglomularen Zellen. Klin. Wschr., *34:* 375.

Dunihue, F. W. 1949 The effect of adrenal insufficiency and of desoxycorticosterone acetate on the juxtaglomerular apparatus. Anat. Rec., *103:* 442.

Goormaghtigh, N. 1939 Existence of an endocrine gland in the media of the renal arterioles. Proc. Soc. Exp. Biol. Med., *42:* 688.

Harada, K. 1954 Histochemical studies of the juxtaglomerular apparatus. Rev. Belge. Path. Med. Exp., *26:* 311–320.

Hartroft, P. M. 1957 Studies on renal juxtaglomerular cells. III. The effects of experimental renal disease and hypertension in the rat. J. Exp. Med., *105:* 501–508.

Hartroft, P. M., and W. S. Hartroft 1953 Studies on renal juxtaglomerular cells. I. Variations produced by sodium chloride and desoxycorticosterone acetate. Ibid., *97:* 415–428.

Hartroft, P. M., L. Newmark and J. A. Pitcock 1958 Further observations on the effects of dietary sodium deficiency on renal juxtaglomerular cells and adrenal cortex (rat, dog, cat). Am. J. Path., *34:* 602.

McManus, J. F. A. 1947 Further observations on the glomerular root of the vertebrate kidney. Quart. J. Micr. Sci., *88:* 39–44.

Pitcock, J. A., P. M. Hartroft and L. N. Newmark 1959 Increased renal pressor activity (renin) in sodium deficient rats and correlation with juxtaglomerular cell granulation. Proc. Soc. Exp. Biol. Med., *100:* 868–869.

Tobian, L., J. Janecek and A. Tomboulian 1959 Correlation between granulation of juxtaglomerular cells and extractable renin in rats with experimental hypertension. Proc. Soc. Exp. Biol. Med., *100:* 94–96.

Tobian, L., J. Thompson, R. Twedt and J. Janecek 1958 The granulation of juxtaglomerular cells in renal hypertension, desoxycorticosterone and post-desoxycorticosterone hypertension, adrenal regeneration hypertension, and adrenal insufficiency. J. Clin. Invest., *37:* 660–671.

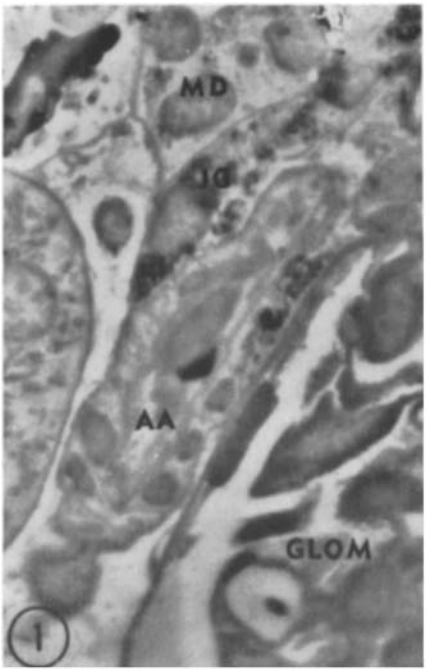
## PLATE 1

### EXPLANATION OF FIGURES

1 Section cut from methacrylate-imbedded tissue photographed with phase microscope showing juxtaglomerular (JG) cells in wall of afferent arteriole (AA). Macula densa cell (MD) at upper left; glomerulus (GLOM) to the right. Note osmophilic granules in JG cells. Normal rat. Dalton's fixative. × 2400.

2 Low power electron micrograph (× 5000) showing same field as in figure 1 in an adjacent thin section. Details as above. Squared area is shown at higher magnification in figure 3 (rotated 180° clockwise).

3 Electron micrograph at higher magnification showing a portion of the same field as above illustrating proximity of JG cell (bottom) and macula densa cell (MD) above. Note osmophilic JG granules (GR), mitochondria (M), and endoplasmic reticulum with RNA granules between JG granules. A Golgi (GO) apparatus can be seen close to the nucleus in the macula densa cell. × 14,000.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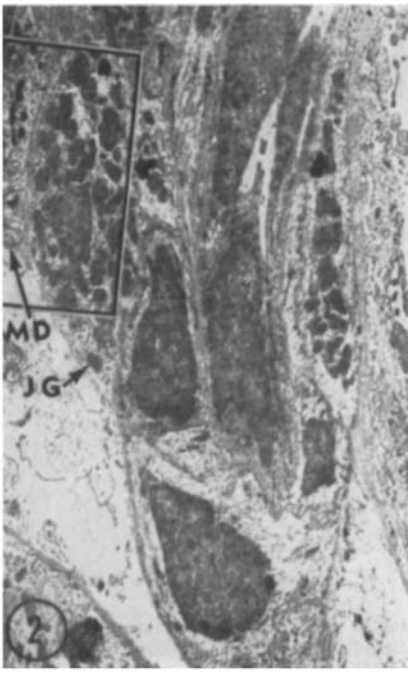
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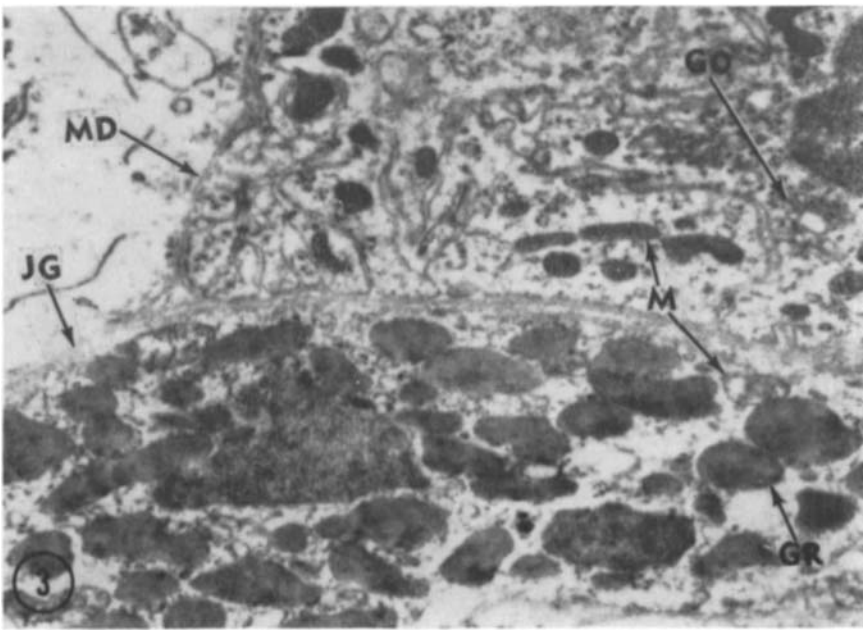
MD
JG
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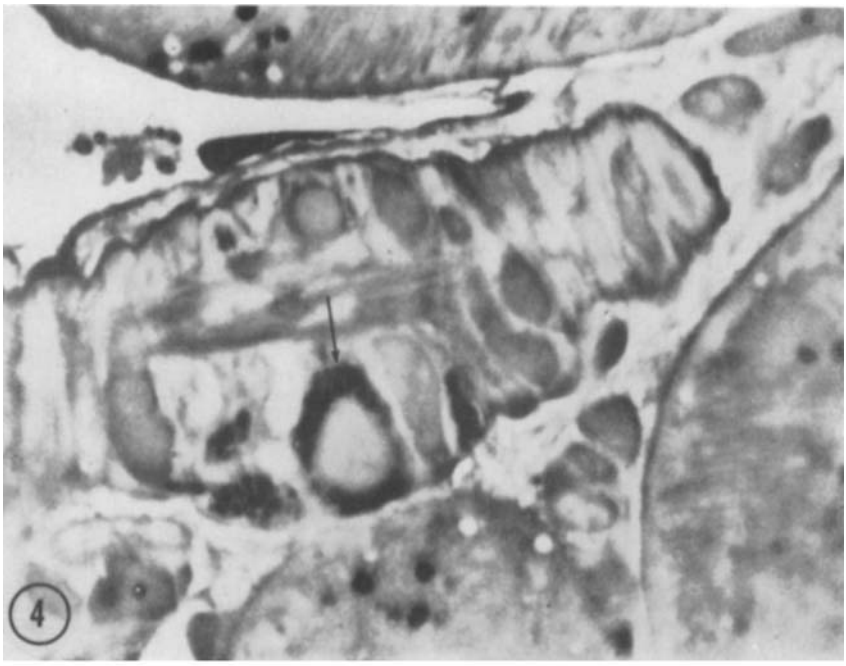
3

## PLATE 2

EXPLANATION OF FIGURES

4 Phase photomicrograph showing renal arteriole cut longitudinally (glomerulus out of field) with several JG cells in its wall (dark granules). Adrenalectomized rat. Dalton's fixative. × 3000.

5 Low power electron micrograph (× 5000) from same field as figure 4 in an adjacent thin section. For orientation, arrow points to same JG cell as above. Squared areas (labeled 6 and 7) are shown at higher magnifications in figures 6 and 7.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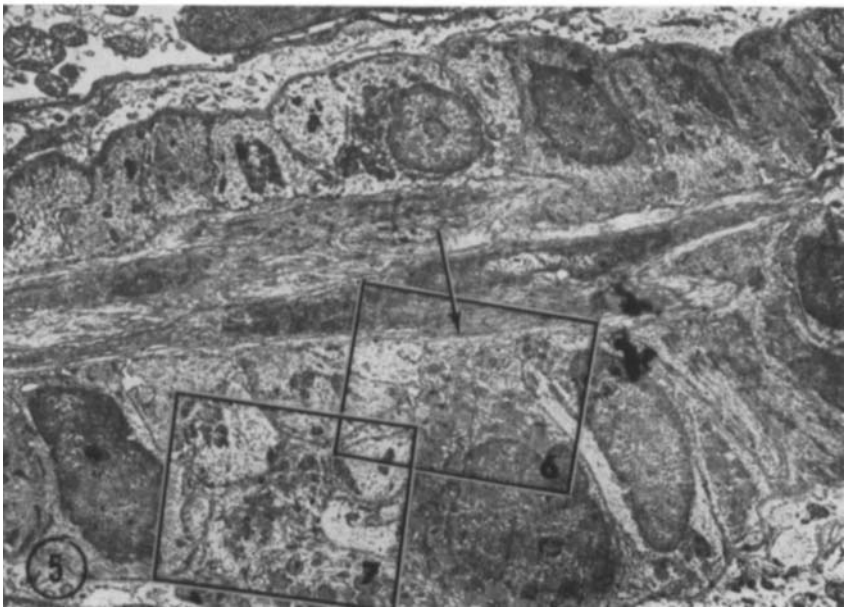
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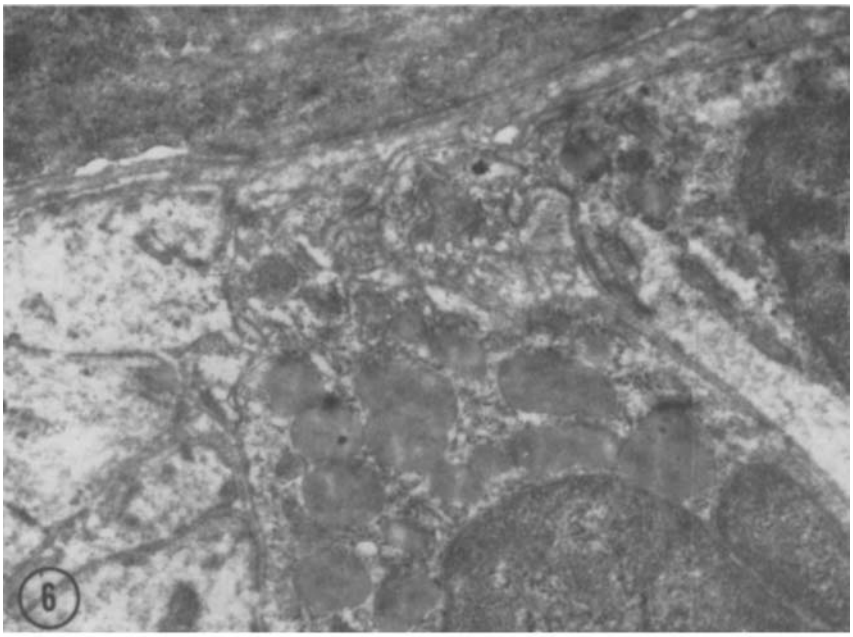
## PLATE 3

EXPLANATION OF FIGURES

6 Portion of a JG cell abutting on endothelium of arteriole (upper left) from same field as figure 5. Note infoldings of plasma membrane and numerous mitochondria at apex of cell. Large JG granules occupy most of the cytoplasm. × 20,000.

7 From same field as figure 5. Portions of several JG cells are shown. Note sacs of endoplasmic reticulum lined by RNA granules between JG granules in cell at lower right. × 18,000.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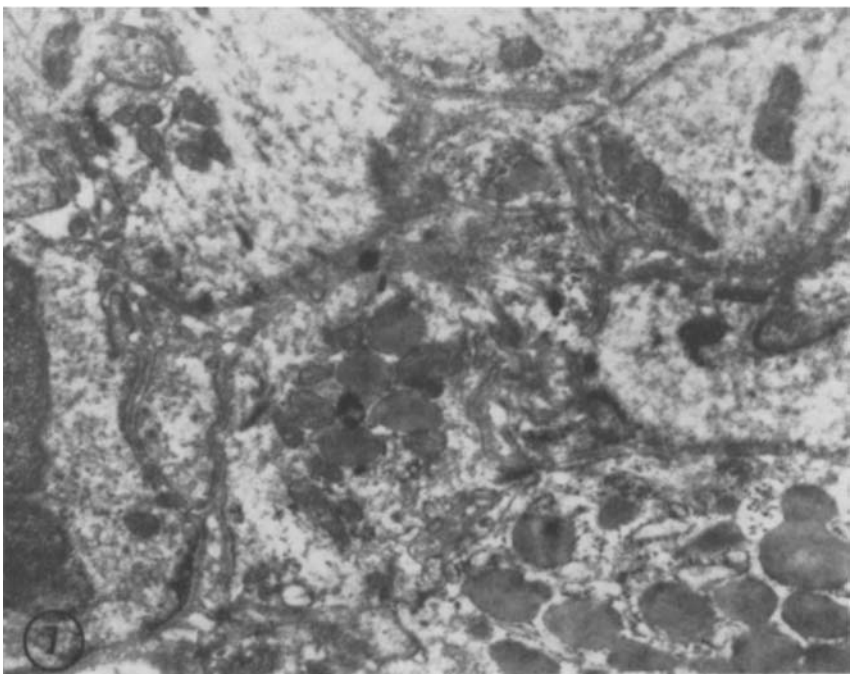
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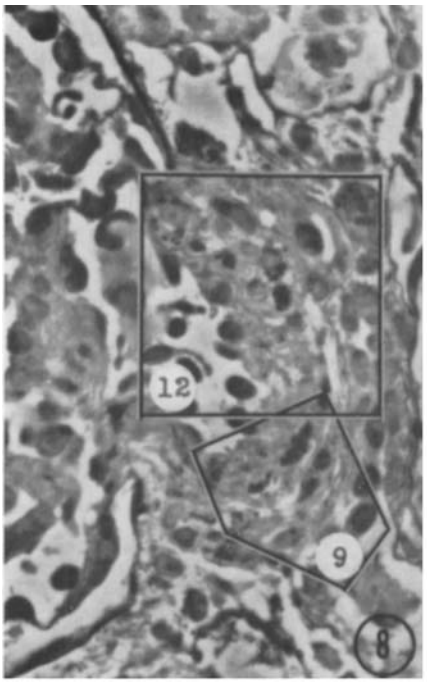
## PLATE 4

EXPLANATION OF FIGURES

8 JG cells from a sodium-deficient dog photographed with phase microscope. Glomerulus is to the left and macula densa at extreme right. Lumen of afferent arteriole is seen where it enters the glomerulus. Wall of the arteriole consists of hyperplastic JG cells. Squared areas refer to figures 9 and 12. × 1000.

9 Low power electron micrograph (× 3700) from same field as figure 8 in an adjacent thin section. AA: lumen of afferent arteriole; MD: lumen of distal tubule with wall consisting of macula densa. Squared area refers to figure 10, below.

10 Portion of same field as figure 9 at a higher magnification, showing sparsely granulated JG cells. Usually JG granules are clumped together in a perinuclear position but in hyperplastic fields they are often scattered, as seen here. Note dense particles within JG granules (GR) and numerous infoldings of plasma membrane. × 24,000.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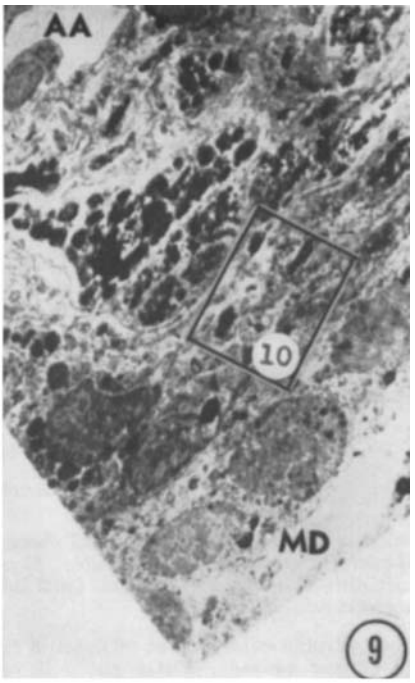
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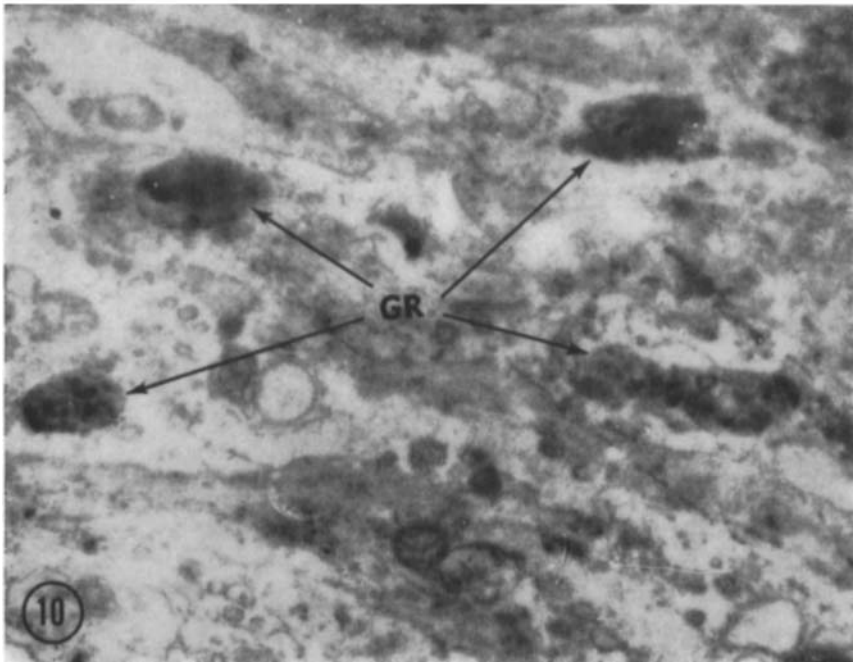

GR
10

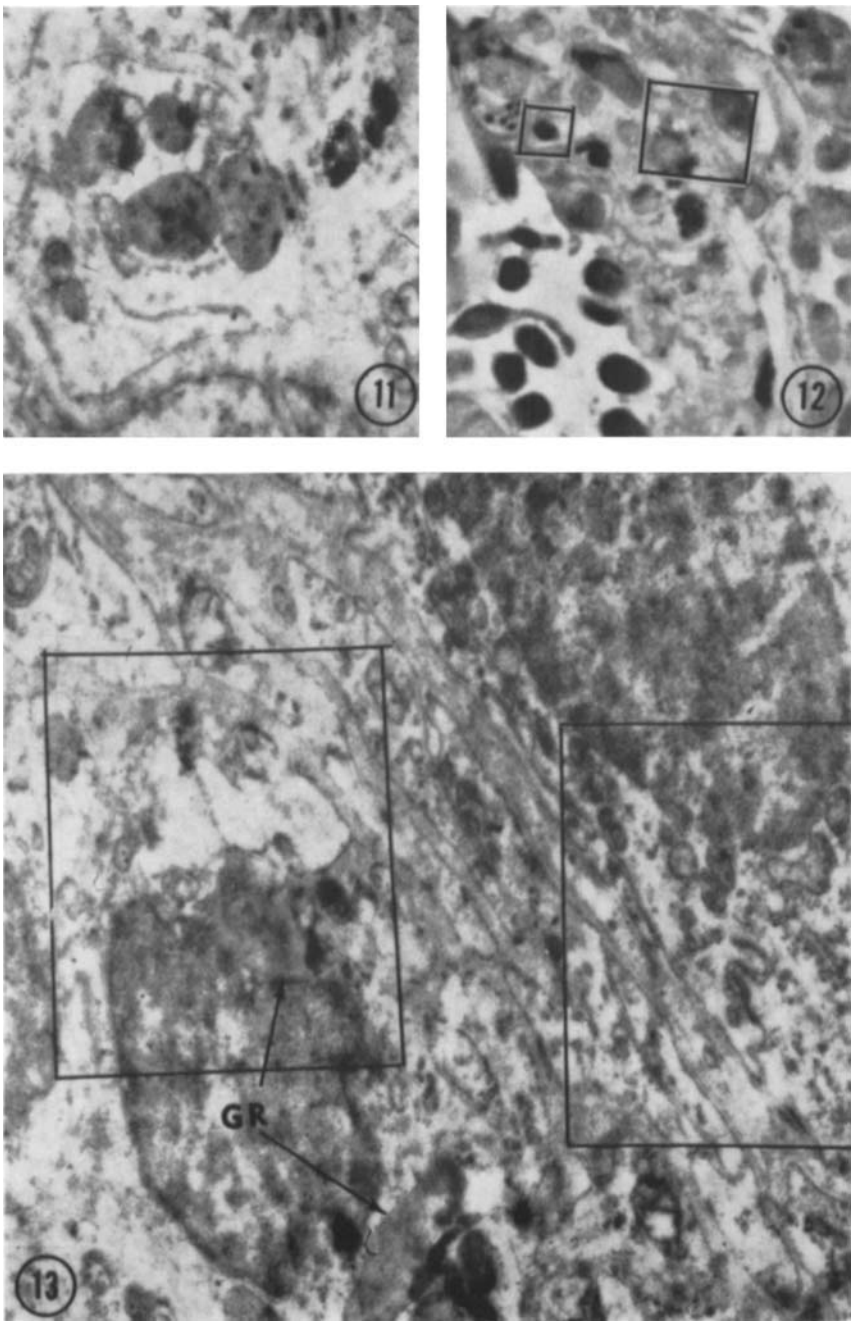
## PLATE 5

### EXPLANATION OF FIGURES

11 Another portion of the same field shown in figure 8 (refer to figure 12 for orientation), showing a group of 4 JG granules. Note dense particles, or "granules within granules," infolding of plasma membrane (just beneath granules) and intercellular spaces (above and below). × 20,000.

12 Photographic enlargement of figure 8 for orientation of figure 11 (smaller square) and 13 (larger square). Phase photomicrograph. × 2000.

13 Electron micrograph showing a macula densa cell on the right (nucleus at upper right) and a JG cell on the left (refer to figure 12). In the JG cell, a cluster of granules is present close to the nucleus (middle, bottom) and one granule is closely applied to the opposite pole of the nucleus next to the Golgi apparatus (within the squared area on the left). The latter structure is seen at higher magnification in figure 14. A Golgi aparatus is also present in the macula densa cell in the squared area on the right (see figure 15). Note numerous involutions of plasma membrane of both the JG and macula densa cell with intercellular spaces forming continuous interlacing channels between the two cell types. × 24,000.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GR
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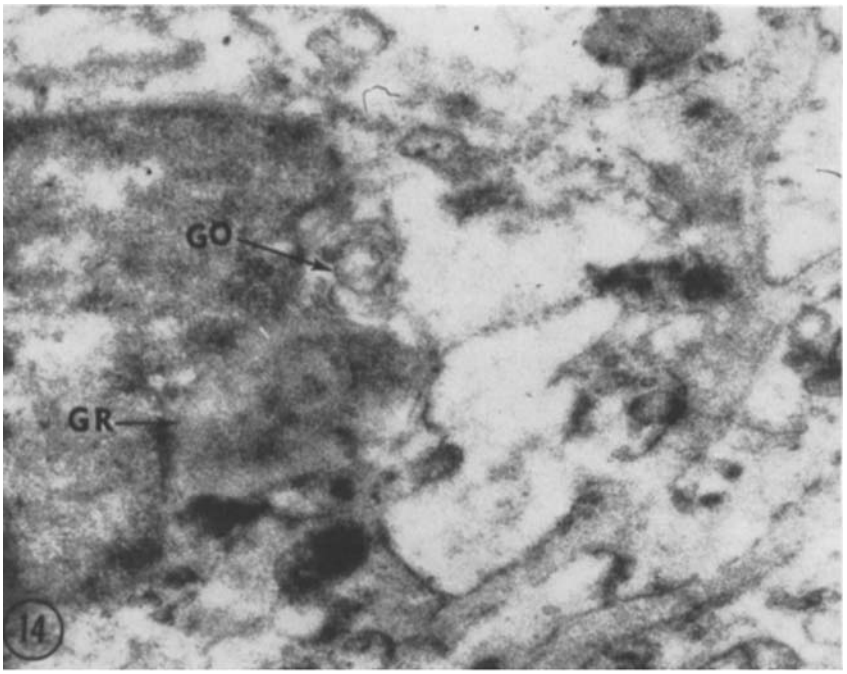
## PLATE 6

### EXPLANATION OF FIGURES

14 Photographic enlargement of a portion of the JG cell shown in figure 13. Nucleus is to the left. GO, Golgi apparatus; GR, JG granule. Note the Golgi apparatus close to the nucleus just above a large JG granule. Although not as typical as that in other cells, e.g., in the macula densa cell in figure 15, the position and appearance of this structure fulfills the criteria of Golgi apparatus. × 48,000.

15 Photographic enlargement of the macula densa cell shown in figure 13. GO, Golgi apparatus. × 48,000.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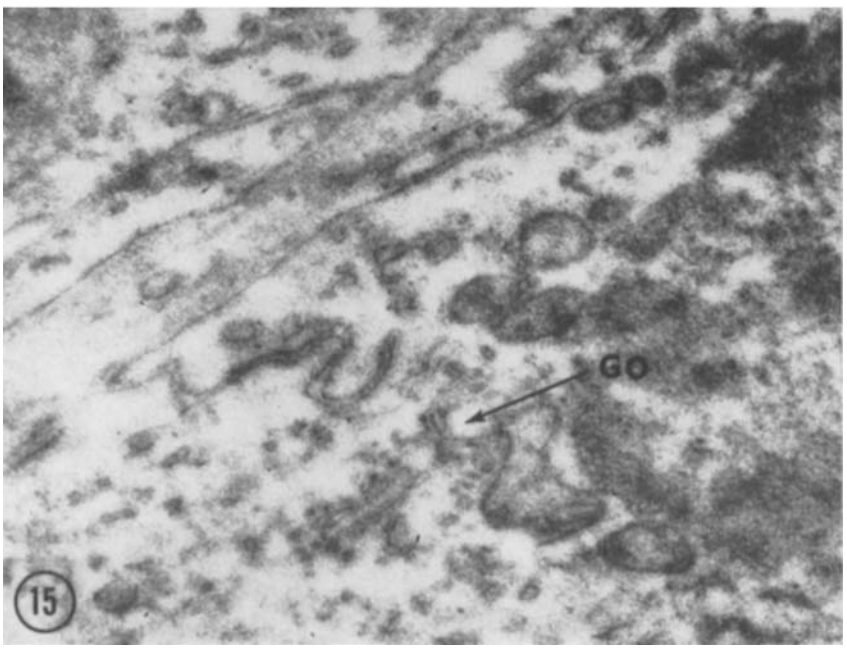
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