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Electron Microscopy Studies of Papillary Interstitial Granules in Normal Human Kidneys

ANIL K. MANDAL, M.D., JOHN A. NORDQUIST, M.S., MATTHEW W. THIGPEN, P.A., and THOMAS M. JAMES, M.D.

Research and Medical Services, Veterans Administration Hospital, Department of Medicine, University of Oklahoma College of Medicine, Oklahoma City, OK 73104
and
Department of Pathology, Washington University School of Medicine St. Louis, MO 63110

ABSTRACT

The papillae of five kidneys from four heart-beating cadaver donors (one child and three young adults) were studied using electron microscopy (EM) technique. The kidneys were made available for EM study after they were found unsuitable for transplantation owing to low antigen match. All papillae had interstitial cells (IC), stellate processes, and intracellular and free granules. The three types of granules observed were (1) homogeneously dark, (2) gray and (3) layered; the free (outside the IC) granules were identical to the granules within the IC. The child kidney had more IC but fewer granules than the adult kidneys. The granules are ultrastructurally identical to those found in the rat renal papilla. Since rat renal papillary granules have been implicated as the source of renal vasodepressor substance(s), this ultrastructural resemblance suggests that human papillary interstitial granules likewise may be the source of renal vasodepressor substance.

Introduction

The medullary and papillary interstitial cells in kidneys from normotensive and hypertensive rats have been studied by numerous individuals.[1,4,8,9,11,12,16,17,21] These interstitial cells (IC) have been implicated as the source of the renal vasodepressor substance(s) because medullary transplants reduced blood pressure[13,14] and IC granularity was found to be reduced in experimentally induced hypertensive rats[4,11,12,21] and in spontaneously hypertensive rats.[8,9] High concentrations of prostaglandins (PGA and PGE) have been found in the human renal papilla.[23] Interstitial cells in the human medulla have been described by other investigators.[3] The purpose of this report is two-fold: (1) to document papillary interstitial granules in the normal human kidney, and (2) to delineate the similarities between these granules and rat papillary granules.

Materials and Methods

Five kidneys from four heart-beating cadaver donors were made available for

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TABLE I

Clinical and Laboratory Information (24 Hours Prior to Death)

No	Name	Age, Sex & Race	Average Blood Pressure (mm Hg)	Urine Output ml/hour	Urinalysis	Serum Urea Nitrogen	Serum Creatinine mg/dl	Serum Na+/K+ mEq/L
1	D.F.	20 WM	110/80	100	Protein-negative Hyaline casts (15-20/HPF)	22	-	137/3.4
2	H.L.	30 WM	100/70	100	Protein-trace	12	1.6	122/3.3
3	B.C.	21 WM	100/60	75	Protein-negative RBC (8-12/HPF)	16	1.7	144/4.4
4	K.E.	6 WF	100/78	40	Protein 1+	19	0.4	143/3.8

electron microscopy (EM) studies after they were found unsuitable for transplantation. These kidneys were obtained from one child and three adults admitted to the University of Oklahoma Hospital. The causes for hospital admission included aspiration in the child and trauma in the adults. None had history of kidney disease or hypertension. Clinical and laboratory information 24 hours prior to death (cerebral death) is listed in table I. Nephrectomy was performed by en bloc technique. The adult kidneys were perfused with Collins Solution (artificial plasma) in a pulsatile perfusion apparatus (Waters X-100, console module) for a period of one to 12 hours. The child kidney was placed in "slush" for 19 hours. Cold non-perfusion time did not exceed 90 minutes for any of the adult kidneys. After the kidneys were deemed unsuitable for transplantation, they were taken off the perfusion apparatus or out of "slush" and brought immediately to the EM Laboratory. Each kidney was cut transversely and the papillae were dissected out. One half to one mm pieces of papillae and of cortex were fixed in four percent glutaraldehyde with phosphate (PO_4) buffer (pH 7.2) for EM study. A wedge extending from the cortex to the papilla was fixed in 10 percent formalin for light microscopy (LM) study.

LM Study

Three to four micron sections were stained with hematoxylin and eosin (H & E).

EM Study

The tissues were post-fixed in one percent osmic acid with PO_4 buffer (pH 7.2), dehydrated in alcohol solutions and embedded in Spurr low viscosity media.[18] Thick sections were cut from the blocks, stained with methylene blue and azure II and examined with light microscope to select optimal areas for EM. When IC were recognized, thin (300°A) sections were cut and collected on copper and gold grids. Also, thin sections from glomeruli were collected on copper grids. The sections on the copper grids were stained with uranyl acetate and lead citrate (UA + LC). The sections on the gold grids were stained with one percent periodic acid and methenamine silver (PAMS).[9] Interstitial cells were photographed on 2 × 2 inch film; 8 × 10 inch prints were made for analysis of the findings.

Results

LM Study

All kidneys were morphologically normal. The interstitial space was not discern-

ible in the H&E stained sections of the papillae. However, methylene blue-azure II stained thick sections displayed cells within the interstitial space, some of which contained granules (figure 1).

EM Study

In unstained sections of the papilla, the interstitial cell and its granules were discernible but the granular characteristics could not be determined.

In UA + LC stained sections, two types of cells were found in the interstitium, IC and fibroblasts. The IC were characterized by long cytoplasmic processes, many rough-surfaced endoplasmic reticulum, increased number of ribosomes and one or more granules within the cell (figures 2, 3, and 4). Numerous stellate processes containing one or more granules were observed throughout the interstitium (figure 5). The child kidney contained more IC, fewer granules and fewer stellate processes than the adult kidneys.

Granular Morphology

Three types of granules were observed. They were homogeneously dark (figures 2 and 4), gray (figures 3 and 5) and layered (figures 3 and 4). The layered granule exhibits a denser central part than the peripheral part (figures 3 and 4) and an outer membrane is often discernible (figure 4). The size of the intracellular granule varied from 0.35 to 1.03 μ and their average size was 0.7 μ. All types of granules exhibited a smooth outer border. In PAMS stained sections, the granules exhibited silver deposits as dark specks (figures 6, 7 and 8). No membrane surrounding any granule was observed in the PAMS stained section. The layered granules were infrequent, especially in the adults, and the layering was subtle (figure 4). Granules were observed marginating and protruding through the cell membrane (figure 8).

In addition to being located within the interstitial cells, granules were found within the tubular epithelium (figure 9),

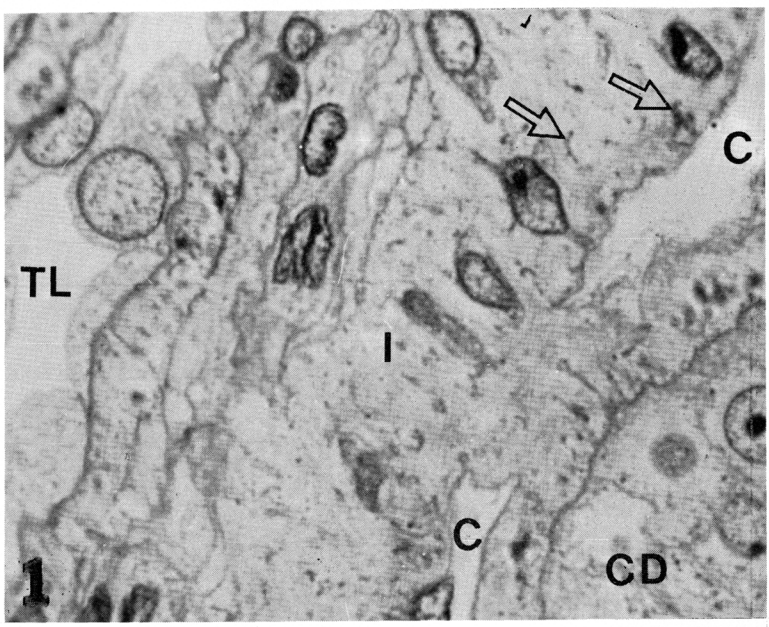


FIGURE 1. Light micrograph of the thick sections from the papilla of the child kidney. Cuboidal cells line the thin limb (TL); collecting ducts (CD) reveal columnar epithelial lining and the interstitium (I) contains several interstitial cells. The dark particles (arrows) represent granules. Two capillaries (C) are seen. (Methylene Blue-Azure II × 800)

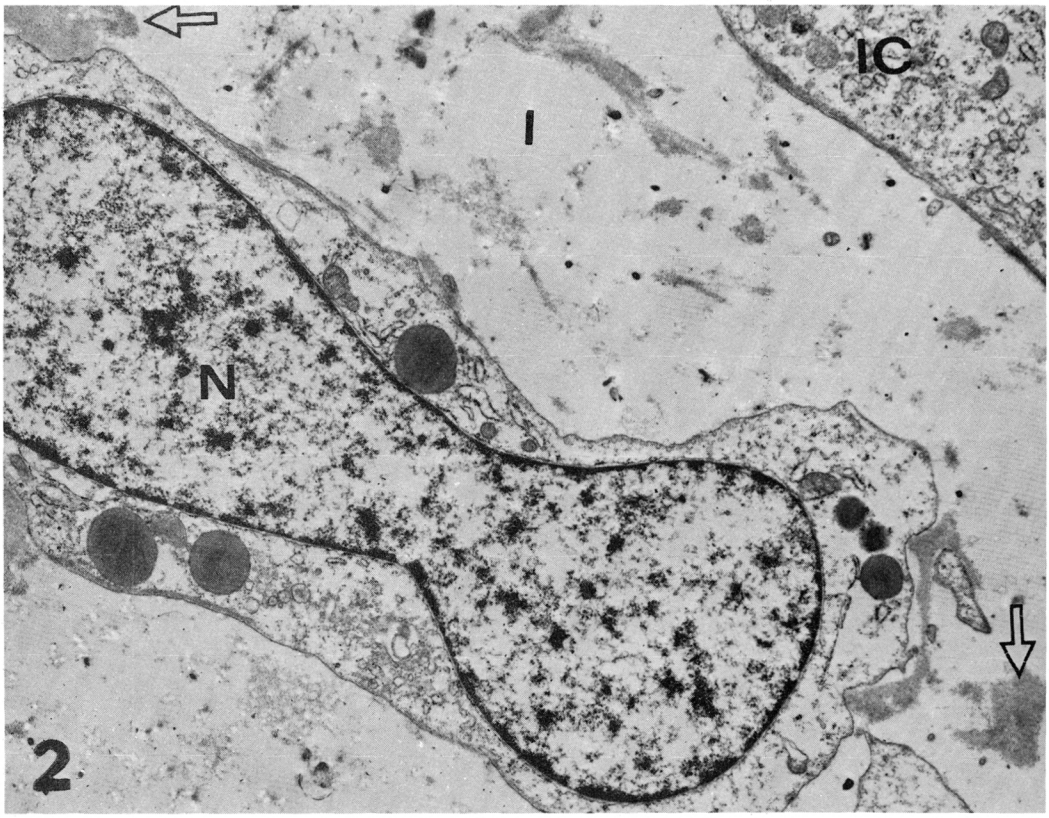


FIGURE 2. An interstitial cell from an adult kidney shows a large nucleus (N) and several homogeneously dark granules around the nucleus. A part of another interstitial cell (IC) is seen at the top (right). In the interstitium (I), collagen fibers and smooth homogeneous material (arrows) adjacent to the interstitial cell are seen. (UA + LC × 6,000)

free in the interstitium (figure 10) and within the capillary endothelial cell (figure 11). The free (outside the IC) granules were found to be located adjacent to interstitial cells or stellate processes (figure 10) and along tubular and capillary basement membranes or within the tubule and the capillary. The size of the free granule varied from 0.24 μ to 0.97 μ with an average of 0.72 μ. The ultrastructure of the free granule was identical to that of the granule within the IC. In the thin limb of the loop of Henle and distal tubule, lipid droplets resembling gray granules, cytosomes and a membrane bound lipofuscin pigment were observed. These were similar to structures described by Bulgar et al.[3] Silver staining distinguished granules from cytosomes in the tubules. Interstitial collagen fibers were found in all kidneys but more so in the adult kidneys (figures 2 and 3).

One cortical glomerulus was studied from Patients # 1 and # 3. Both appeared normal.

CLINICAL

All patients appeared healthy by physical and laboratory examination. The several hyaline casts and mild microhematuria in Patients #1 and #3, respectively, were difficult to interpret in the light of the normal renal morphology (LM and EM studies). Since neither patient's urine was examined by a member of the re-

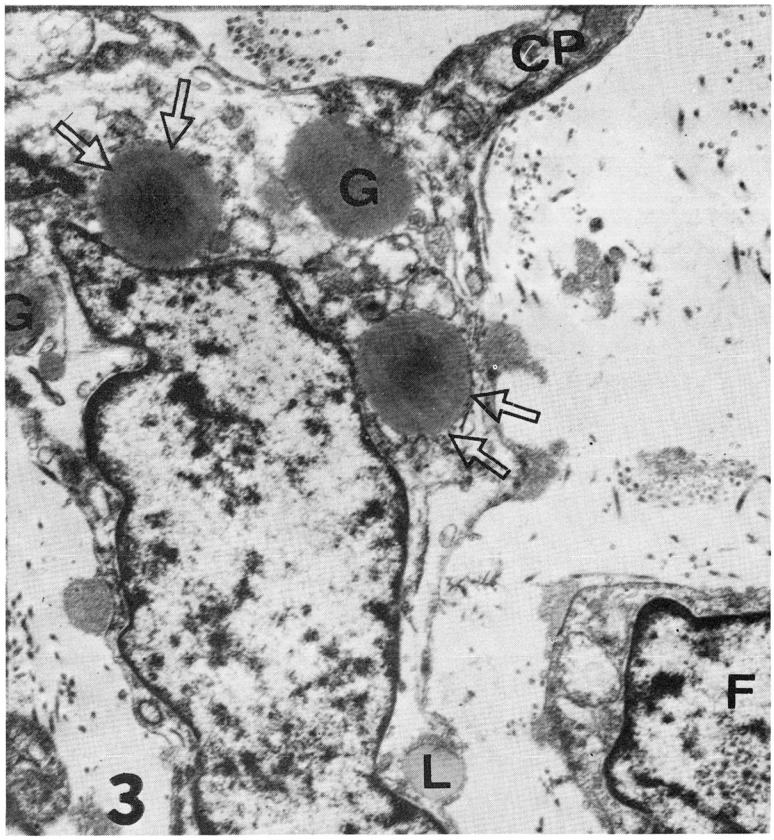


FIGURE 3. An interstitial cell from the child's kidney demonstrates long cytoplasmic process (CP), two layered granules (pointing arrows) and two gray granules (G). A lipid droplet (L) is shown. The adjacent cell appears to be a fibroblast (F). (UA + LC × 9,000)

search team, the possibility of laboratory error cannot be ruled out.

Discussion

This study describes two types of cells in the papillary interstitium of normotensive, healthy individuals. The interstitial cell (IC) contains more organelles and a variable number of granules, while the fibroblast contains fewer organelles and no granules. As in the rat, three types of granules were observed in the human renal papilla: (1) homogeneously dark, (2) gray and (3) layered. They were found within the IC, free in the interstitium, in the tubules and in the endothelial cells and lumina of capillaries. Ultrastructurally, the free granules were identical to those inside the IC.

Since free granules are ultrastructurally identical to those within the IC, it is assumed that they originate in the IC and are somehow expelled. Mechanical rupture is an unlikely mechanism as other organelles were not observed with the granules and granules were found adjacent to intact cells. They are more likely secreted through membrane fusion as demonstrated in the rat[9] and in man (figure 8). In support of this is our finding of granular margination to cell membrane with elevation of the cellular membrane (figure 8). A similar process has been described for mast cell granule secretion.[5,10]

The papillary interstitial granules described herein are strikingly similar to those in the spontaneously hypertensive rat and normotensive Wistar rat.[8,9] They also resemble the granules in the medul-

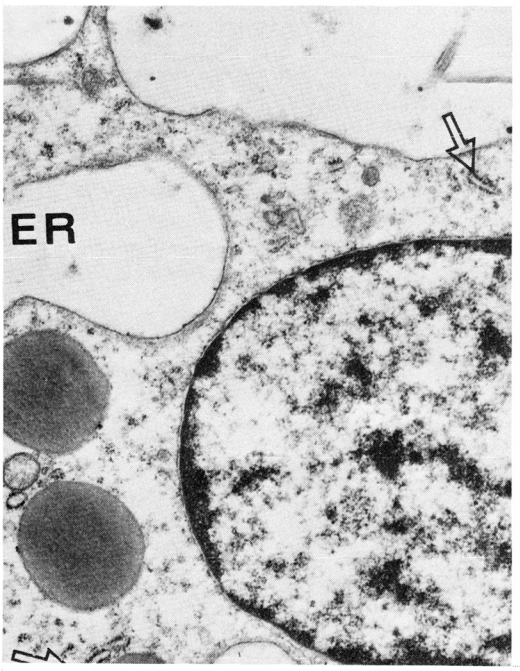


FIGURE 4. This interstitial cell from an adult kidney exhibits widely dilated endoplasmic reticulum (ER) which appears to be cistern, and three different granules; the lower two granules are layered and the upper granule is homogeneously dark. In addition, many rough-surfaced endoplasmic reticulum (arrows) also are seen. (UA + LC × 15,000)

lary interstitial cells grown in tissue culture.[14] Bulgar et al[3] found interstitial cells similar to those reported in this study which contained lipid droplets. These lipid droplets are unlike the granules described in this report. Although most evidence indicates that lipid is the major constituent of rat renal granules, some glycoproteins cannot be excluded owing to intense silver staining.[9] Intense staining of human papillary interstitial granules with silver (figures 6, 7, and 10) suggests the presence of glycoprotein

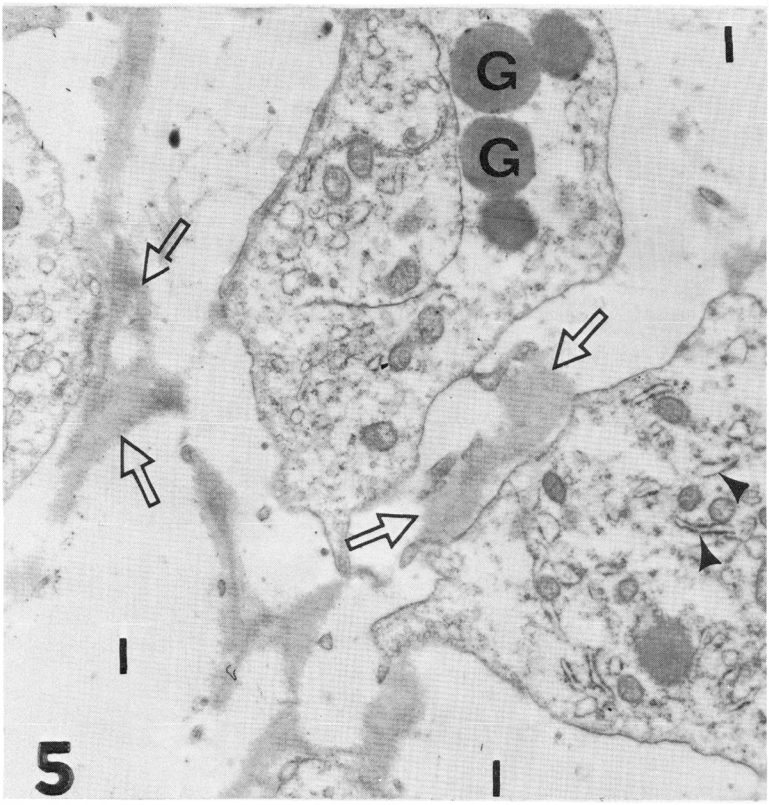


FIGURE 5. Several stellate processes reveal many rough surfaced endoplasmic reticulum (arrowheads) and gray granules (G). The smooth homogeneous material (pointing arrows) found within the interstitium (I) and adjacent to the stellate processes appears to be mucopolysaccharides. (UA + LC × 5,000).

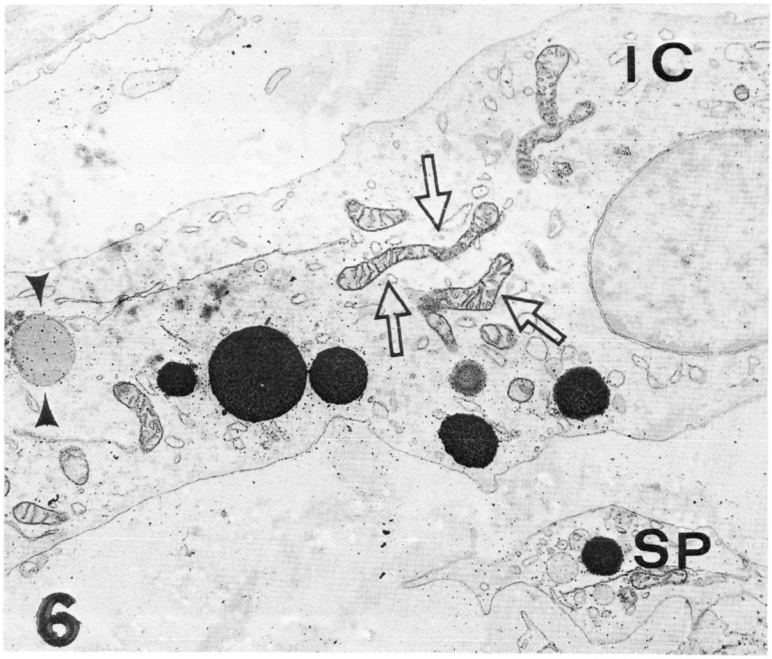


FIGURE 6. The silver positive granules located within the interstitial cell (IC) and stellate processes (SP) exhibit silver specks. The lipid droplet (arrowheads) is non-silver positive. The mitochondria (arrows) within the IC stand out. (PAMS × 5,000)

moieties in these granules. The compounds isolated from extract of the renal medulla, known as medullin,[6] and of the renomedullary interstitial cells grown in tissue culture are consistent with prostaglandins.[15] Vance et al[23] found a high concentration of prostaglandins in the human renal papilla. This study confirming the presence of granules in the human renal papilla provides further evidence that these granules may be the source of renal prostaglandins.

Although studies of transplanted renal medullae by Muirhead and colleagues[14] strongly support that the medullary interstitial cells mediate a potent antihypertensive action, others cast doubt on the intrarenal prostaglandins as the renomedullary antihypertensive factor.[20] The fall in blood pressure observed following renal transplantation may be due to a vasodepressor substance in the renal

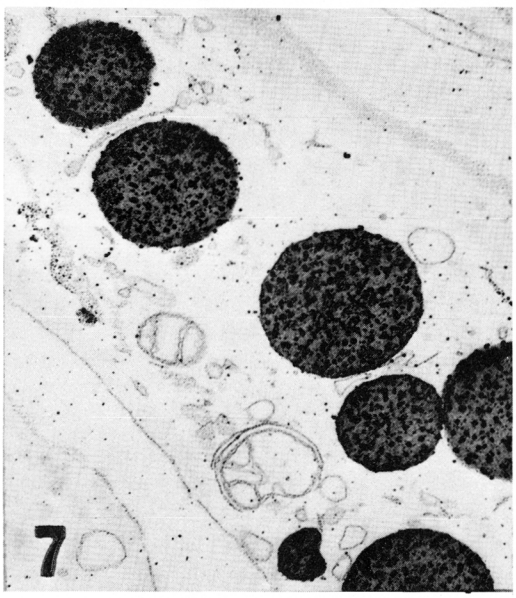


FIGURE 7. A magnified view clearly discerns the silver positive granules with abundant silver specks uniformly distributed throughout the granules. (PAMS × 22,000)

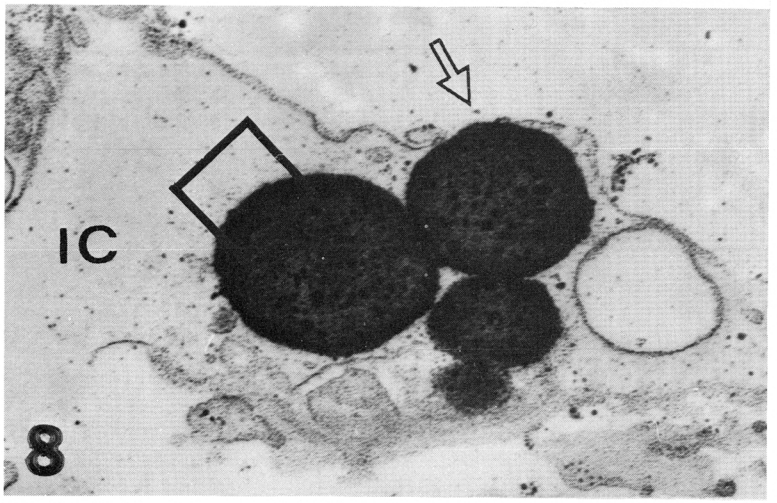


FIGURE 8. Several silver positive granules within an interstitial cell (IC) reveal layering with the central part containing silver beads and a homogeneous electron-dense thick rim at the periphery (square). Also seen is the fusion of a granule with the cellular membrane producing a protruding effect (arrow). This may be the mechanism to document the free granule. (PAMS × 47,000)

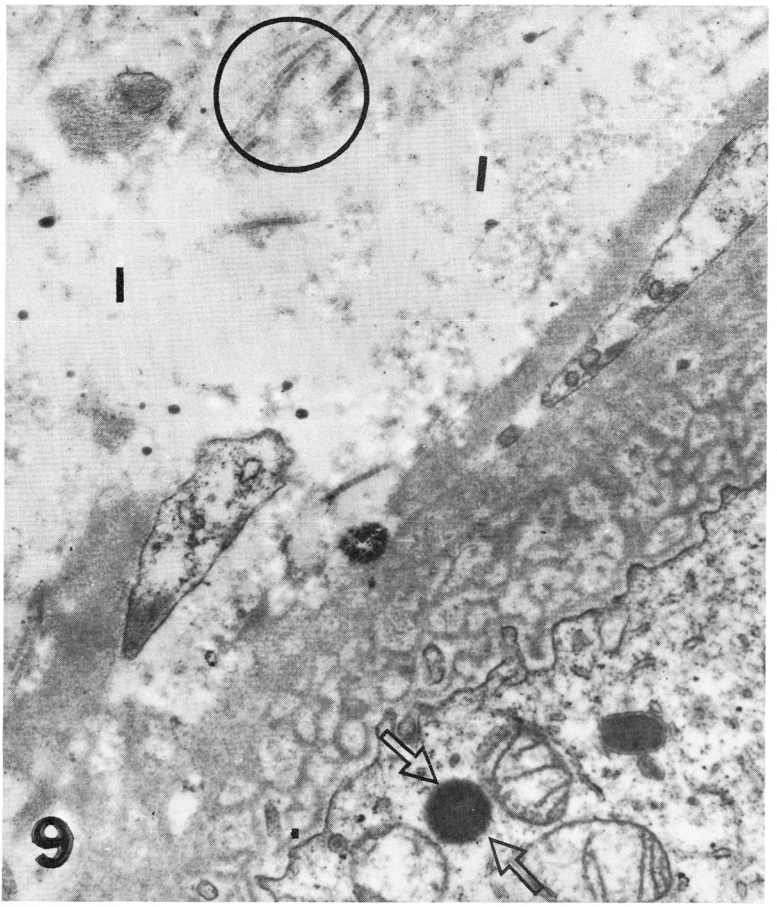
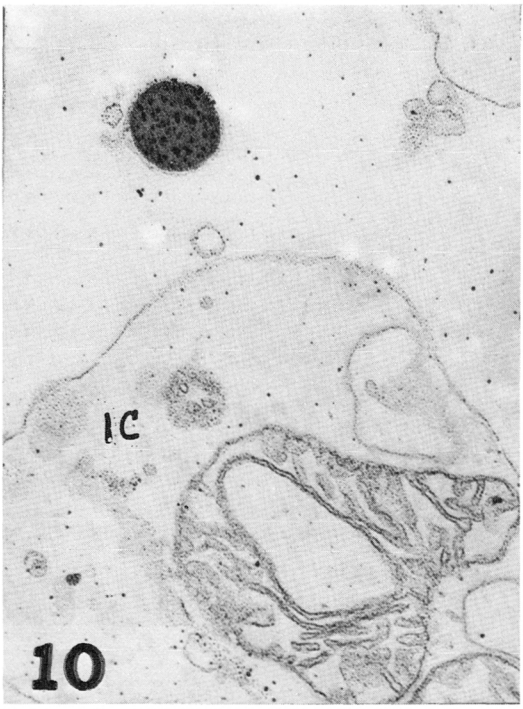


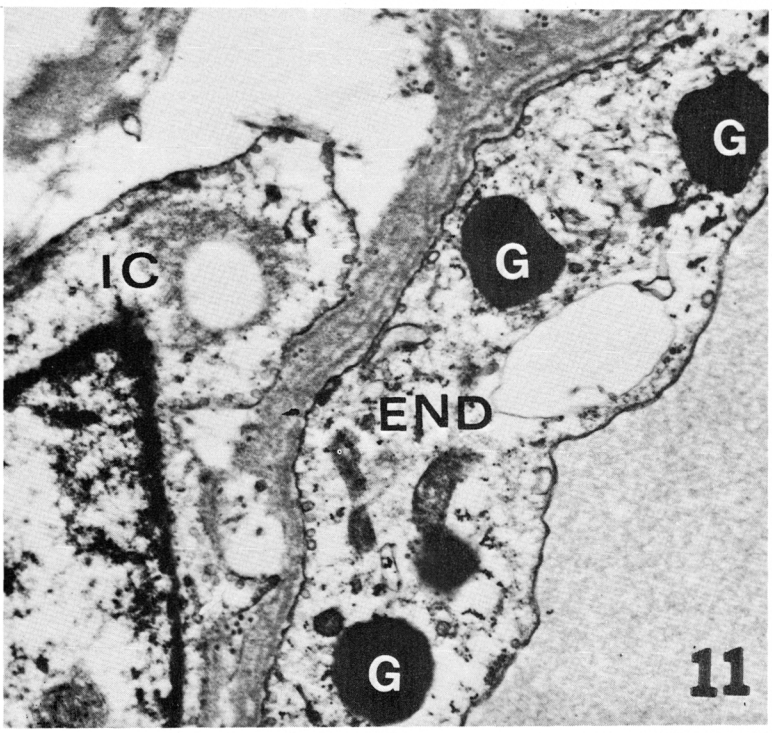
FIGURE 9. A tubular epithelial cell demonstrates the presence of a granule (pointing arrows). Within the interstitium (I) collagen fibers are shown (circle). (UA + LC × 18,000)

FIGURE 10. Shows a free granule adjacent to an interstitial cell (IC). This free granule is silver positive and exhibits silver specks within the granule. (PAMS × 30,000).



papillae.[7] Papillary interstitial cells and their granules may be the source of this substance.[22] IC granularity is decreased in the human malignant hypertensive kidney,[12] further implicating a possible role in hypertension. Lerman and colleagues[7] demonstrated that the renal medullary fibroma is formed by a tumorous transformation of renomedullary interstitial cells. By EM study, the cells and the granules in the fibroma resemble those in the rat[8,9] and in the normal human renal papillae demonstrated in this study. However, in another study, the lack of difference in the systolic blood pressure between patients with and without fibroma of the renal medulla casts some doubt upon the pro-

FIGURE 11. Located within the endothelial cell (END) of this peritubular capillary are the homogeneously dark granules (G). An interstitial cell (IC) juxtaposed to the capillary also is shown. Interstitium (I). (UA + LC × 22,000)



posed vasodepressor function of the IC.[19] Additional study is needed to elucidate the function of the renal papillary granules in hypertension.

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