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ULTRASTRUCTURAL STUDIES OF X-RAY INDUCED GLOMERULOSCLEROSIS IN RATS SUBJECTED TO UNINEPHRECTOMY AND FOOD RESTRICTION

by

V. J. Rosen*
L. J. Cole
L. W. Wachtel**
R. S. Doggett*

U.S. NAVAL RADIOLOGICAL DEFENSE LABORATORY

SAN FRANCISCO · CALIFORNIA · 94135

EXPERIMENTAL PATHOLOGY BRANCH
L. J. Cole, Head

BIOLOGICAL AND MEDICAL SCIENCES DIVISION
E. L. Alpen, Head

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*Present address: U. S. Veterans Hospital, Los Angeles, California.

**Present address: U. S. Naval Dental School, Washington, D. C.

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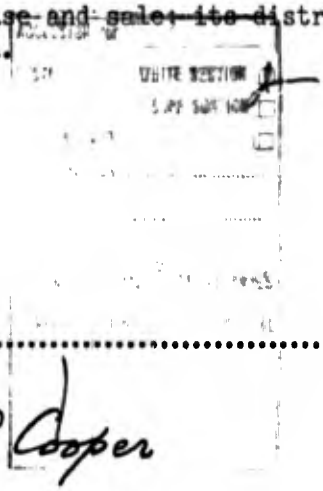

Eugene P. Cooper
Technical Director

D. C. Campbell, CAPT USN
Commanding Officer and Director

ABSTRACT

Weanling female Sprague-Dawley rats were utilized to study the effect of undernutrition on the ultrastructure of accelerated irradiation induced glomerulosclerosis. Following uninephrectomy and 2000 rad X-rays to the remaining kidney, a severe, destructive glomerulosclerosis appeared within 7-8 weeks. Food restriction to the point of significant growth retardation resulted in a marked inhibition of the genesis of the glomerular lesion. Ultrastructurally there was marked reduction in all cytoplasm organelles and a striking reduction in mesangial matrix deposition. These observations show that food restriction retards the development or manifestation of what is considered to be a direct radiation effect on the kidney; and add further confirmation to the thesis that a stimulus for growth, combined with the direct effects of radiation accelerates the development of glomerulosclerosis, while growth retardation has a profound inhibiting effect on the evolution of the lesion.

SUMMARY

The Problem:

Although the kidney is not considered to be a radiosensitive tissue (such as for example the bone marrow or lymphoid tissues) in the sense that acute cellular destruction is observed after radiation exposure, it does in fact exhibit delayed and late radiation effects which are of utmost significance for the longevity of irradiated animals. The severity of this delayed lesion, glomerulosclerosis, depends in part upon the radiation dose, and its development can be accelerated, on the one hand, by unilateral nephrectomy, and retarded, on the other, by restricted food intake. The ultimate cellular basis and pathogenesis of this radiation-induced kidney lesion has not been fully established. Such information is necessary for the eventual prevention and/or specific therapy of this important late radiation lesion—an objective which is part of the ongoing biomedical research program at NRDL. The present work represents a definitive study of the electron microscopic features of radiation-induced glomerulosclerosis, both after unilateral nephrectomy and after food restriction.

The Findings:

Groups of weanling female rats were treated as follows: 1) left unilateral nephrectomy, 2000 rad of X-rays to the right kidney, and normal diet; 2) left unilateral nephrectomy, 2000 rad to the right kidney, and restricted diet (8 g/day); 3) left unilateral nephrectomy and normal diet, nonirradiated; 4) left unilateral nephrectomy and restricted diet (8 g/day), nonirradiated; 5) normal controls. The rats were thus maintained for 8 weeks and sacrificed, and the kidneys were prepared for electron microscopy. The combination of a stimulus for renal growth (unilateral nephrectomy) and increased renal metabolic activity, combined with direct radiation to the kidney produces an accelerated glomerulosclerosis, which exhibits evidence of cell proliferation, increased mesangial matrix formation, collagen elaboration, and cell damage. In addition, some apparent direct basement membrane injury is present. When food restriction to the point of substantial whole body and parenchymal organ growth regardation is introduced, the full extent of the radiation injury is not capable of expression in spite of a stimulus for growth; i.e., uninephrectomy. There is evidence that the deposits in the mesangium are produced by mesangial and endothelial cells. The associated increased ribosomal activity in the normally fed animals

suggests active protein synthesis in the mesangial and endothelial cells. The underfed animals demonstrated a marked decrease in ribosomal activity and diminished matrix formation. These observations show that food restriction retards the development or manifestation of what is considered to be a direct radiation effect on the kidney; and add further confirmation to the thesis that a stimulus for growth, combined with the direct effects of radiation accelerates the development of glomerulosclerosis, while growth retardation has a profound inhibiting effect on the evolution of the lesion.

INTRODUCTION

A progressive intercapillary glomerulosclerosis is seen with increasing frequency in ageing rats and mice. The lesion can be induced by treating young animals by various means, including irradiation (1, 4,7) carbon tetracholoride and ethionine poisoning (9) cholesterol feeding, (2) cortisone treatment, (11) and alloxan-induced diabetes (3). A particularly severe lesion appears following either whole body or direct X-irradiation or neutron irradiation, and the lesion is further accelerated by uninephrectomy (8,10). In a previous communication (10), it was shown that advanced glomerulosclerotic lesions appear within two months following direct x-irradiation(1000 and 2000 rad) of the exteriorized kidney of weanling rats. The kidney lesions were found to be more severe if uninephrectomy, a stimulus to increased renal growth, was performed at the time of irradiation. In contrast, the lesions were significantly less severe after restricted food intake, resulting in whole body growth retardation. In the present study the ultrastructural features of an accelerated glomerulosclerosis are presented and are compared to those in animals maintained on restricted food intake.

MATERIALS AND METHODS

Animals: Female Sprague-Dawley rats, 28 days of age, were used. Animals were maintained on Purina lab chow and tap water ad libitum. Rats were divided into the following groups of four animals each: (I) left uninephrectomy, 2000 rad X-ray to the right kidney and normal diet; (II) left uninephrectomy, 2000 rad X-ray to the right kidney, and restricted diet (8 g/day); (III) left uninephrectomy and normal diet; (IV) left uninephrectomy and restricted diet (8 g/day); (V) normal controls. Animals were maintained for eight weeks and sacrificed with ether anesthesia. Animals on restricted diet were maintained in separate cages to avoid cannibalism.

Radiation: The radiation source was provided by a 250 kvp X-ray unit, 25 ma, HVL 1.28 mmCu with 0.5 mmAl and 1.0 mmCu filters, and was delivered at the rate of 200 rad/minute, at the TSD (target-to-skin distance) of 35 cm. Rats were anesthetized with ether and Nembutal and the right kidney was exteriorized through a dorsolateral incision and irradiated through a lead shield, utilizing two 1.5 mm lead sheets, absorbing 97% of the radiation dose.

Electron Microscopy: Kidneys were fixed in situ with cold (0-4 C) S-Collidine buffered paraformaldehyde (pH 7.4), quickly excised and split. Half of each kidney was placed in formalin-acetic acid-alcohol

for light microscopy, and the other half was minced into 0.5 - 1.0 mm cubes and fixed in the paraformaldehyde for 2 - 4 days. The tissue was post-fixed in 2% S-Collidine buffered osmium tetroxide, dehydrated in a series of graded alcohol and propylene oxide, and then embedded in Epon 812. All sections were cut with glass knives on a Porter-Blum MT-2 ultramicrotome and mounted on formvar coated 200 mesh copper grids. Sections were double stained with uranyl acetate and lead citrate and examined with an RCA EMU-3H electron microscope. In addition, 0.5 μ sections were stained with paragon 1301 frozen section stain for orientation purposes. Material was also embedded in paraffin and sections were stained with hematoxylin and eosin, Periodic acid-Schiff reaction, and colloidal iron.

RESULTS

The body weight and renal weights of the rats at the time of sacrifice are shown in Table I.

The body weight and renal weight retardation is apparent in both groups of animals subjected to food restriction.

Light Microscopy: The light microscopic features of radiation induced glomerulosclerosis have been described in detail. The lesions produced by direct radiation and uninephrectomy and normal diet (gp I) were advanced at 7-8 weeks postradiation and showed irregularity of basement membranes, increased mesangial deposits, proliferation of mesangial and endothelial cells, and obliteration of many capillaries (Fig. 1). The glomeruli were large and tubules were dilated. Kidneys from the animals maintained on a restricted diet (gp II) showed only mild changes (Fig. 2). Some apparent basement membrane thickening was present, and centrolobular widening was noted, but the differences between these groups were readily apparent. Other than enlargement of nephrons, animals in group III had normal glomeruli; those in animals in group IV were unremarkable.

Electron Microscopy: Glomeruli from rats in group I (Uninephrectomy plus 2000 rad to remaining kidney and normal diet) showed striking alterations in the centrilobular regions, and less prominent basement membrane changes. Some areas showed splitting of basement membrane structure, with cell fragments apparently interposed between the basement membrane material (Fig. 3). In other regions, irregularity and variability in the density of basement membrane was apparent. Capillary lumina were reduced in caliber by proliferated endothelial cells as well as subjacent mesangial cells (Fig. 4,5). Endothelial cells contained large numbers of free RNP granules and showed a hypertrophy of the short segmented rough endoplasmic reticulum as well (Fig. 6).

TABLE I

AVERAGE BODY WEIGHTS AND RENAL WEIGHTS IN THE GROUPS OF RATS STUDIED

GROUP NO.	TREATMENT	AVERAGE BODY WEIGHT	AVERAGE RIGHT KIDNEY WT.
I	2000 rad to rt kidney and left uninephrectomy Normal diet	230 g	185 mg
II	2000 rad to rt kidney and left uninephrectomy Restricted diet	140 g	120 mg
III	Left uninephrectomy and normal diet	240 g	230 mg
IV	Left uninephrectomy and restricted diet	135 g	122 mg
V	Normal control	230 g	200 mg

The body weight and renal weight retardation is apparent in both groups of animals subjected to food restriction.

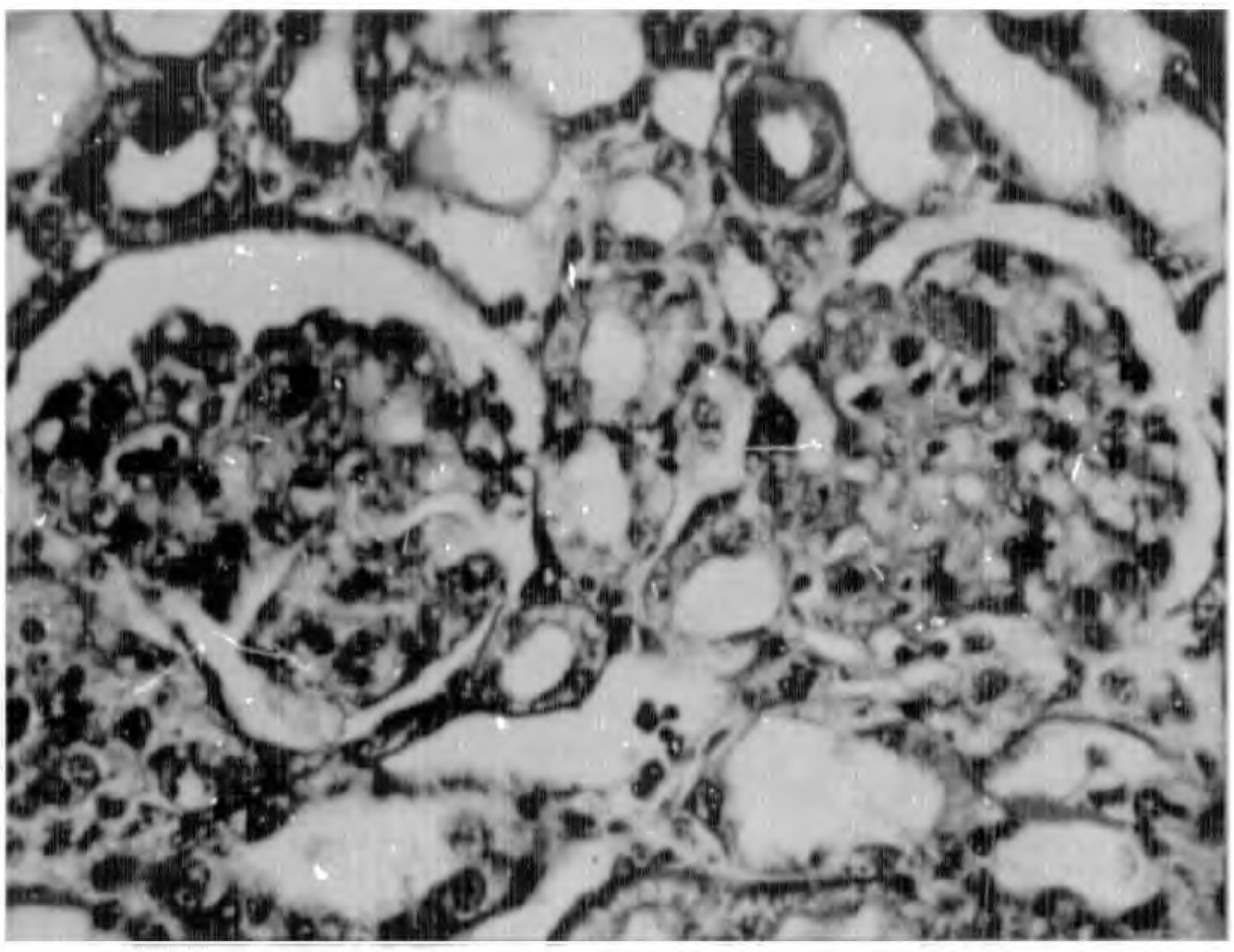

Figure 1. Photomicrograph of enlarged glomeruli from a rat in group I. Note the large mesangial deposits, focal basement membrane thickening, obliteration of capillary loops, and pleomorphism of cell nuclei. Colloidal iron X 420.

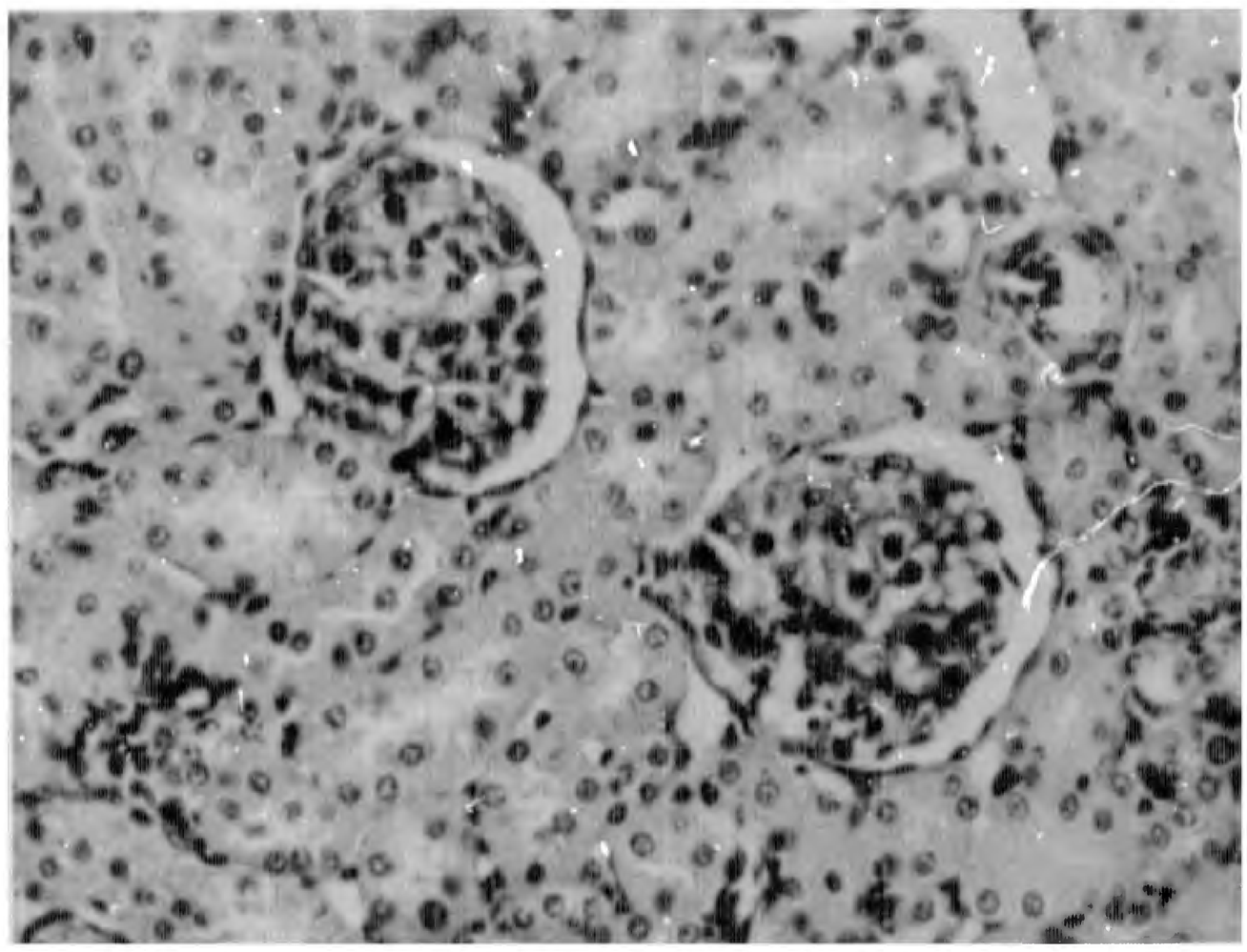

Figure 2. Glomeruli from rat in group II. Note some mild mesangial thickening and cell proliferation. Glomeruli are significantly smaller than those in group I. Colloidal iron X 420.

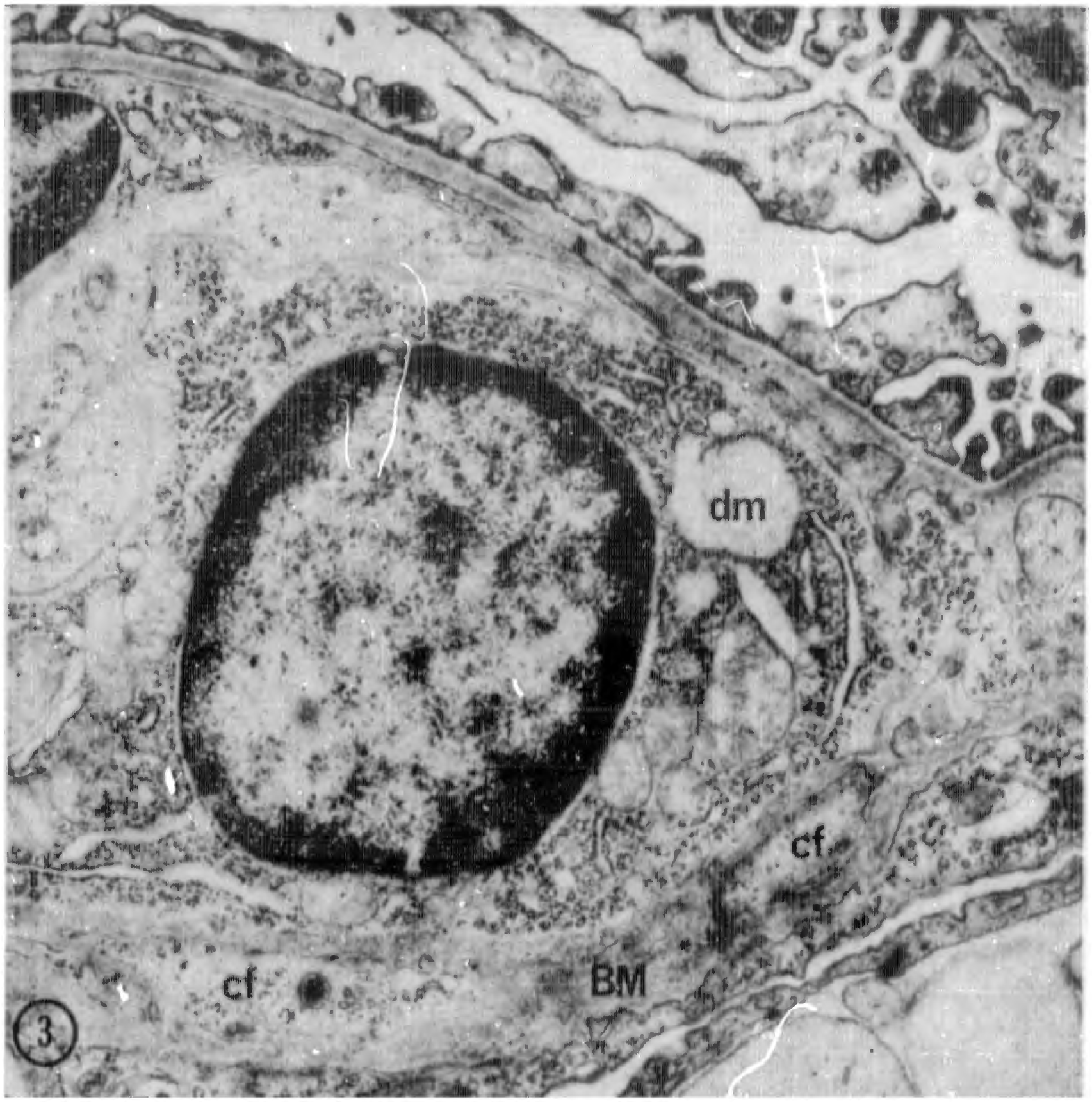

Figure 3. Portion of a glomerulus from irradiated, uninephrectomized, normally fed rat (gp I). The basement membrane (BM) is split and cell fragments (cf) are present. A degenerating, swollen mitochondrion (dm) is seen in a zone of prominent cytoplasmic free ribosomes. Many large mitochondria are present. Epithelial foot process fusion is observable over the damaged basement membrane. X 16,800.

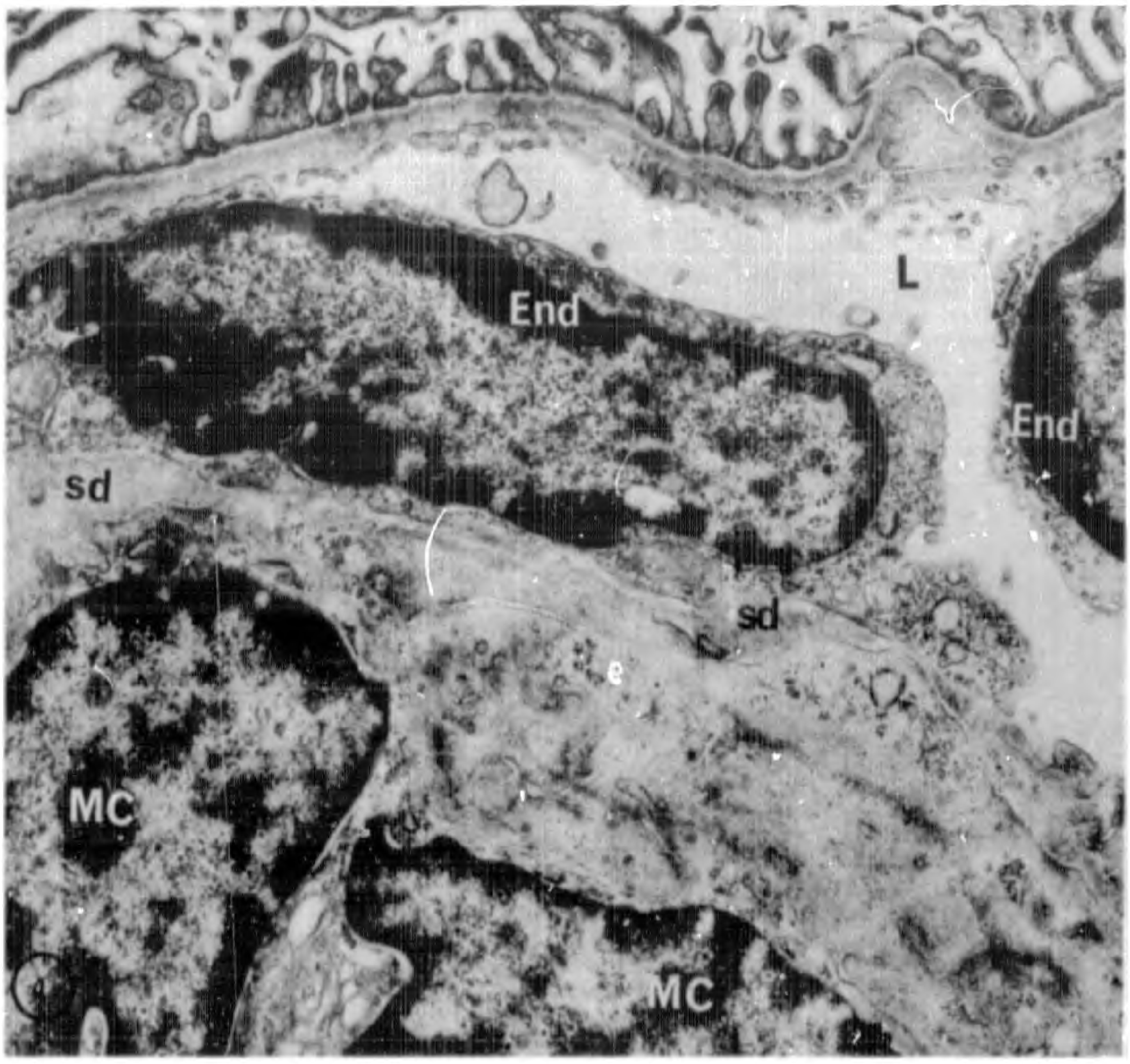

Figure 4. Capillary loop from glomerulus in Fig. 3, showing an endothelial cell (End) elevated above two mesangial cells (MC). Subendothelial deposits of mesangial matrix material (sd) are noted. X 16,800.

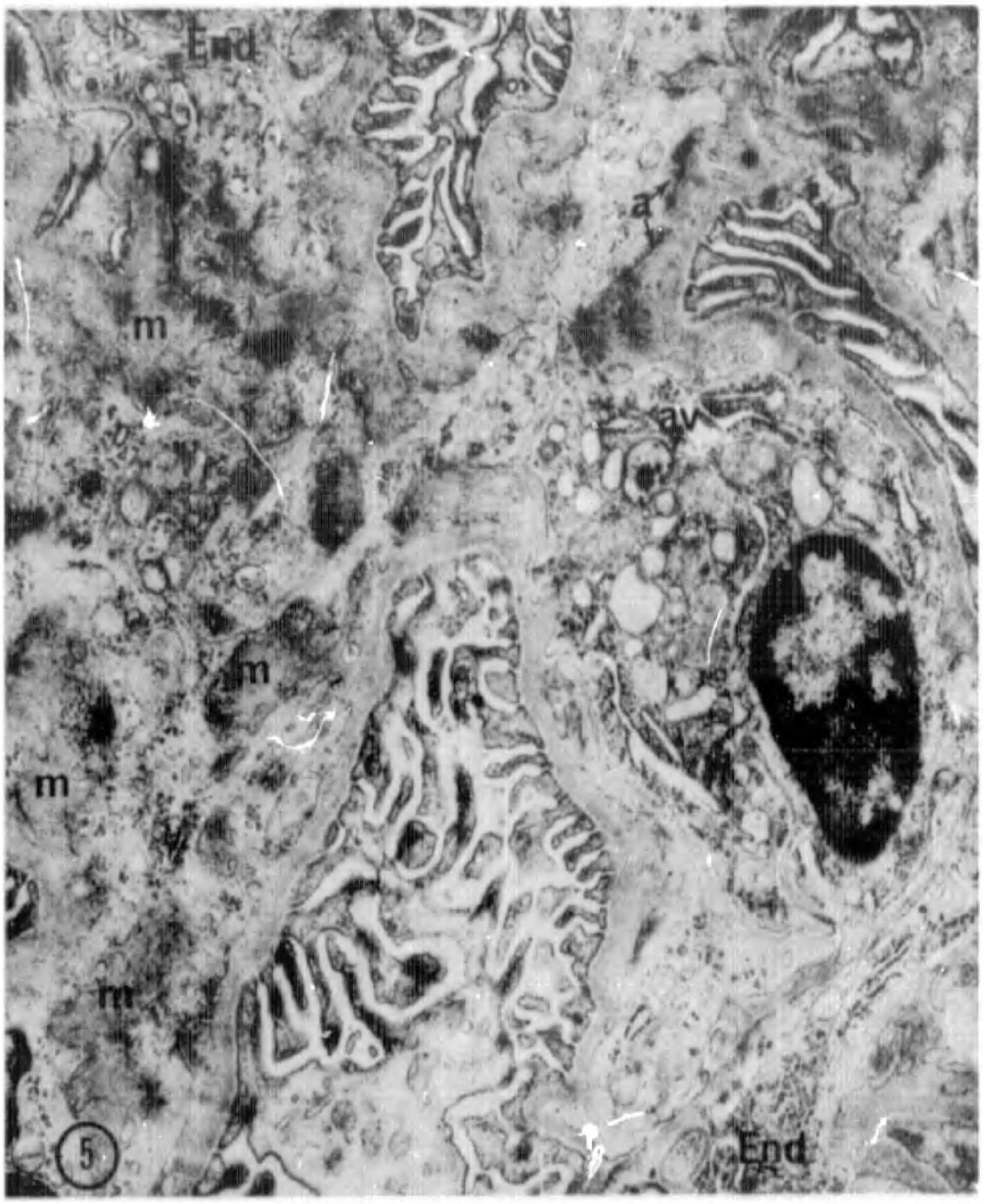

Figure 5. Centrilobular region of a glomerulus of group I, showing multiple large deposits of mesangial matrix (m). An autophagic vacuole (av) is seen. Anchor structures (a) in mesangial cells are noted. End, endothelial cell. X 12,000.

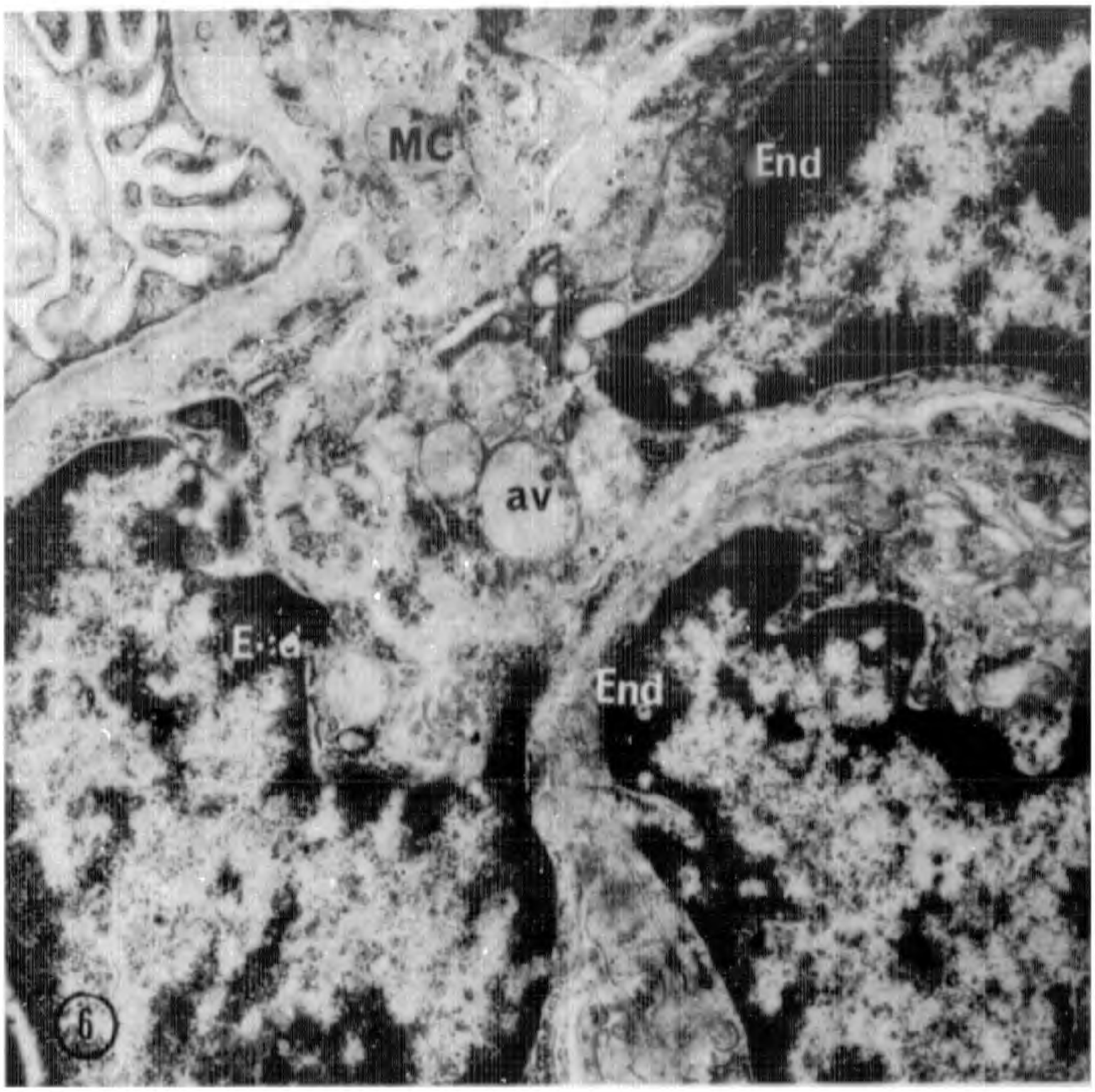

Figure 6. Some glomerulus as in Fig. 5, showing proliferated endothelial cells (End). An autophagic vacuole (av) is seen adjacent to several large mitochondria. Nuclei shows marked coarsening of chromatin. MC, mesangial cell. X 16,800.

Mitochondria appeared large, and abnormal forms were seen in both endothelial and mesangial cells. Various autophagic vacuoles were present. Mesangial cells could be identified in many instances by the presence of characteristic "anchor" sites on the inner surfaces of the plasma membranes, apparently attaching to basement membranes and mesangial matrix. Fine cytoplasmic fibrils resembling myofibrils tailed off from these anchor sites. Mesangial cells and endothelium had a complex interdigitation of cell processes and between these cells were irregular deposits of mesangial matrix, markedly increased in amount. The matrix deposits were closely related to basement membrane material, especially to the subendothelial space region. The borders of mesangial cells often blended with the mesangial matrix. In the mesangial matrix, and related to basement membrane were occasional clusters of fibrils having characteristics of collagen (Fig. 7,8). Subendothelial dense deposits, occasionally more dense than the usual mesangial matrix, were present. Epithelial cells showed occasional, but highly variable foot process fusion, and the cells contained numerous large cytosomes bounded by a rim of ribosomes(Fig. 9). The rough endoplasmic reticulum was hypertrophied, and the Golgi complexes were prominent. Nuclei of endothelial and mesangial cells showed a coarsening of the chromatin material, correlating with the "atypia" seen by light microscopy in these kidneys. Endothelial and mesangial cells also contained a prominent Golgi apparatus.

Glomeruli from rats in group II (uninephrectomy plus 2000 rad to remaining kidney and a restricted diet) showed minimal changes. Basement membranes were occasionally thickened and blurred (Fig. 10). Mesangial deposits were small and only rare subendothelial deposits were noted (Fig. 11, 12). All cells showed reduced rough endoplasmic reticulum and the mitochondria were small and extremely sparse. Again, mesangial cells could be identified by their anchor structures (Fig. 13). Epithelial cells had sparse organelles, and no cytosomes were observed. Foot processes were occasionally fused. No collagen fibers could be identified. Nuclei showed less clumping of the chromatin material.

Micrographs of the animals in groups III and IV showed no significant alterations from normal as far as mesangial matrix was concerned, although the underfed (group IV) rats showed a striking reduction in all cytoplasmic organelles in the glomerular cells.

DISCUSSION

The combination of a stimulus for renal growth and increased renal metabolic activity, combined with direct radiation to the kidney produces an accelerated glomerulosclerosis, which exhibits evidence of

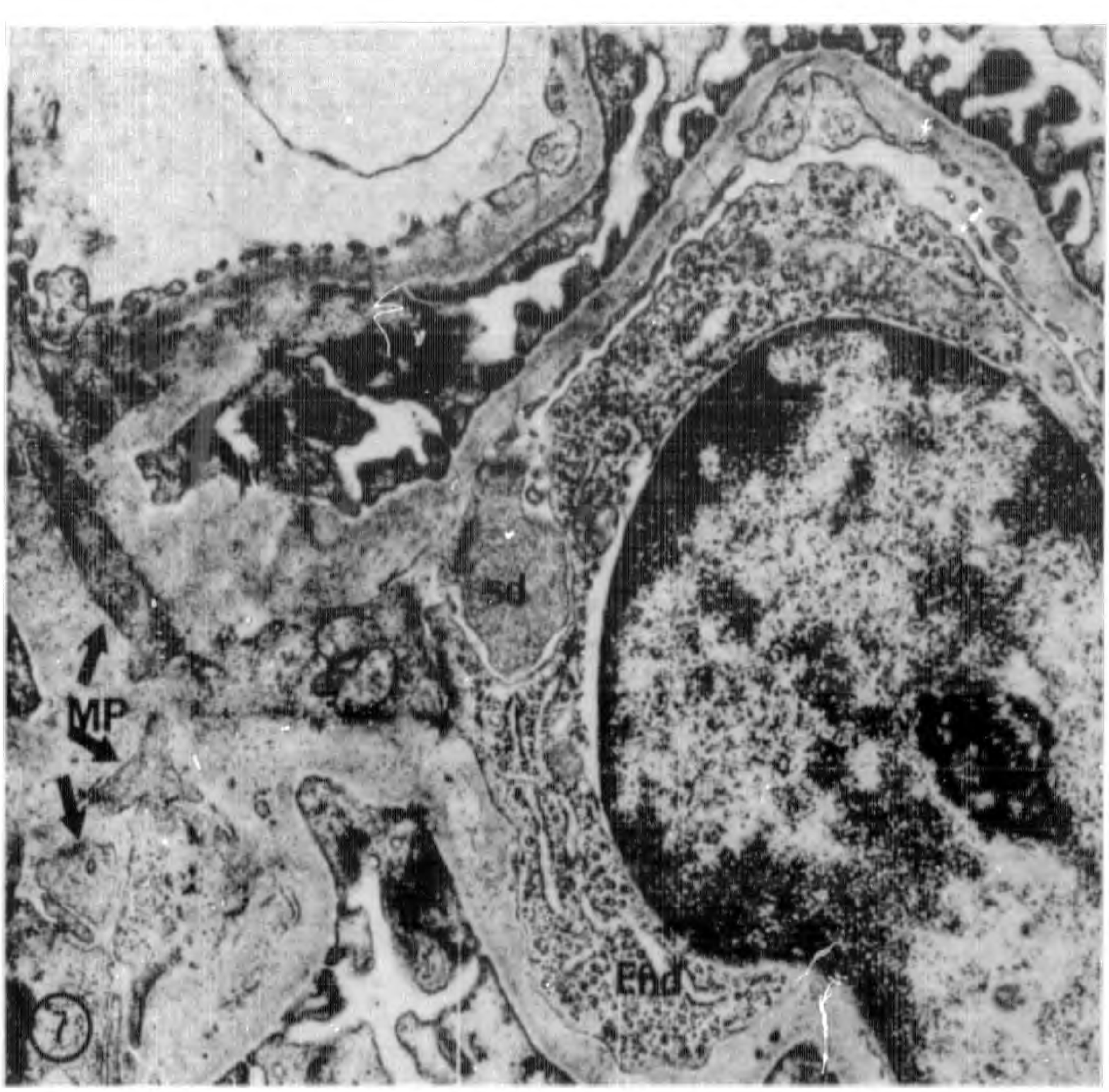

Figure 7. This glomerulus from a rat in group I shows a prominent sub-endothelial deposit (sd). Mesangial pseudopods (MP) are noted coarsing through the mottled matrix. . The endothelial cell (End) has a prominent rough endoplasmic reticulum and numerous polyribosomes. A few collagen fibers are seen in the mesangial region below the basement membrane (small arrow. X 16,800.

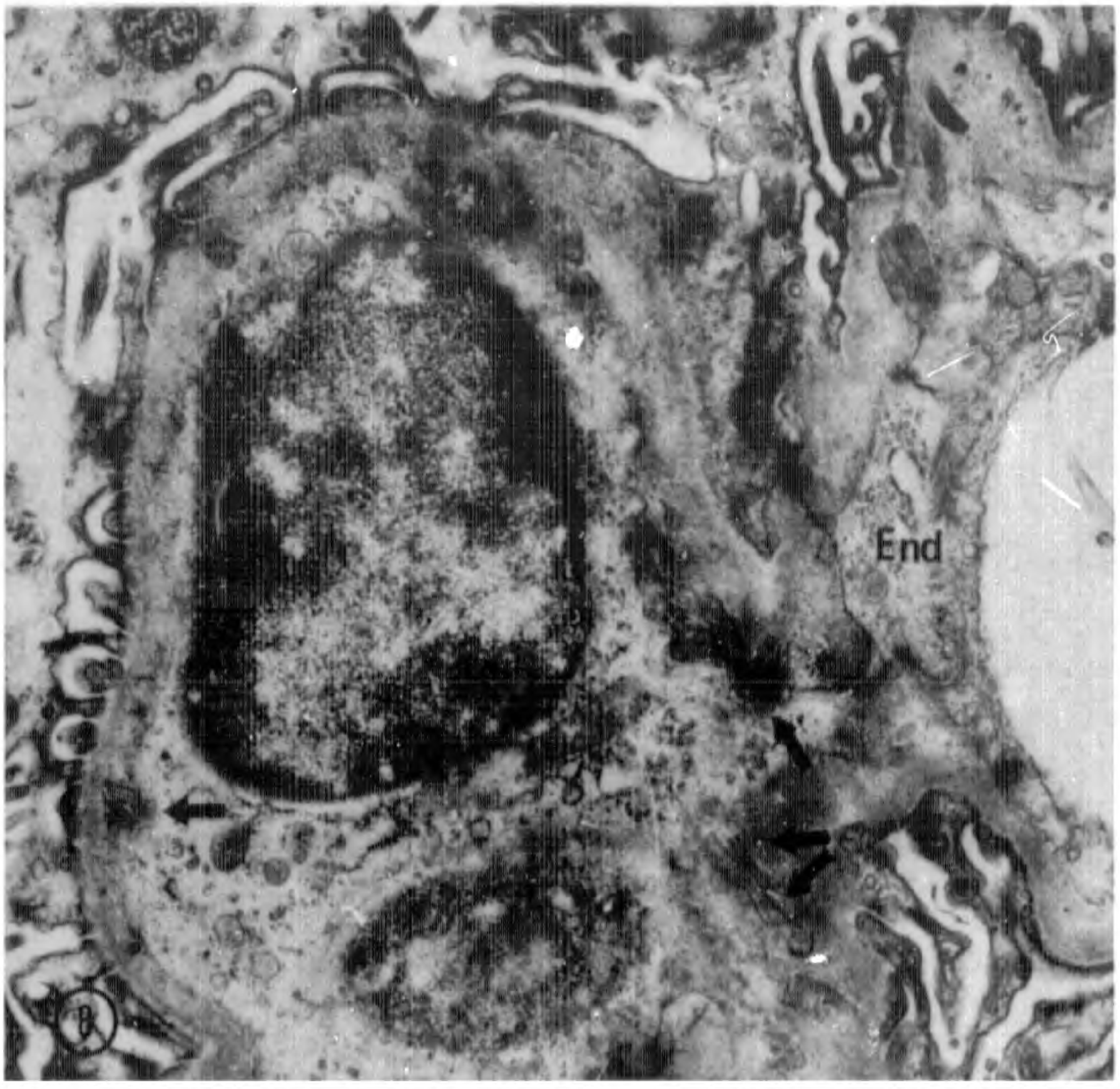

Figure 8. Mesangial region from group I rat, showing bundles of collagen fibrils. X 16,800.

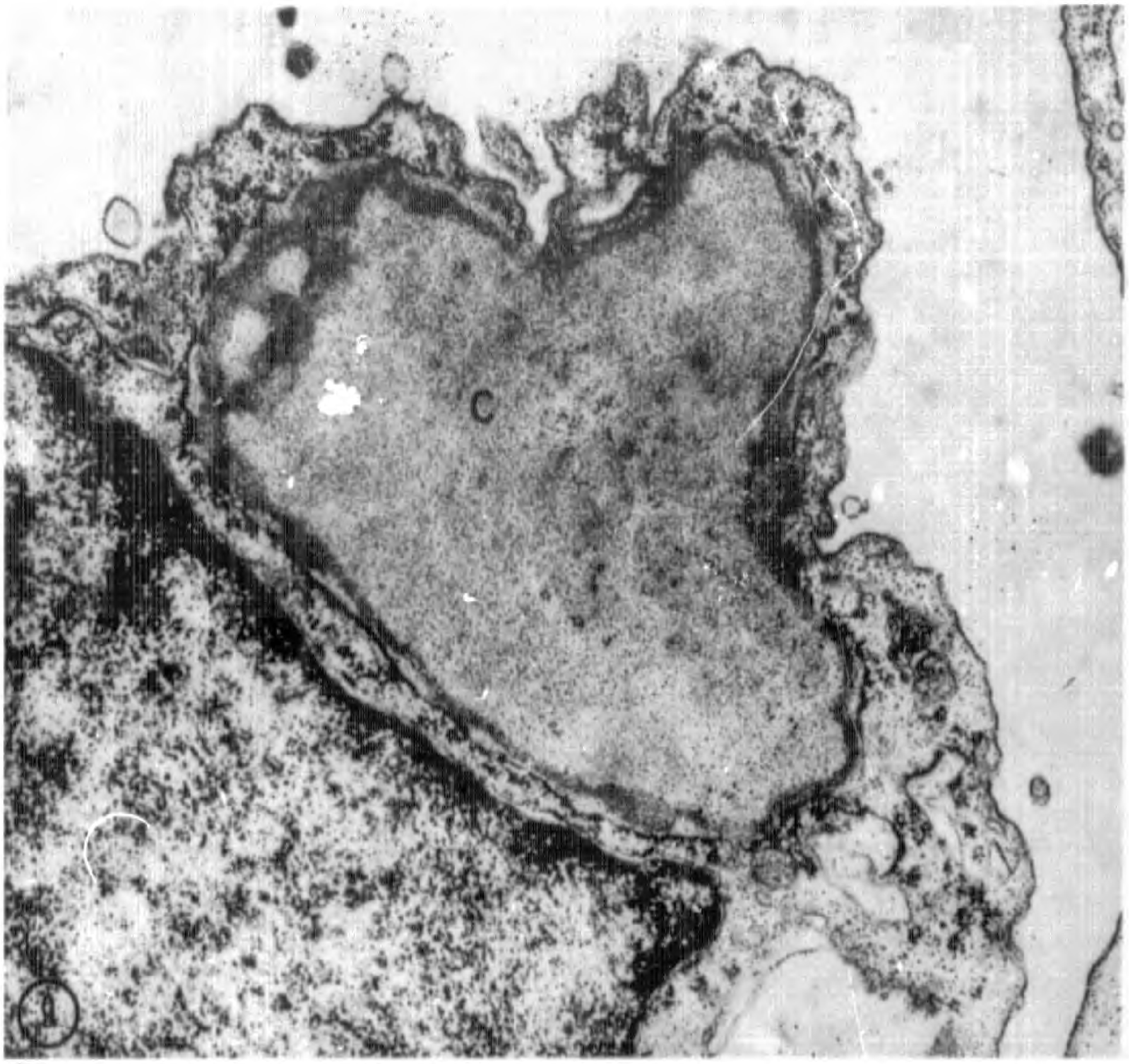

Figure 9. A large cytosome is present in a podocyte from group I. Note the prominent ribosomal ring around the structure. X 24,000.

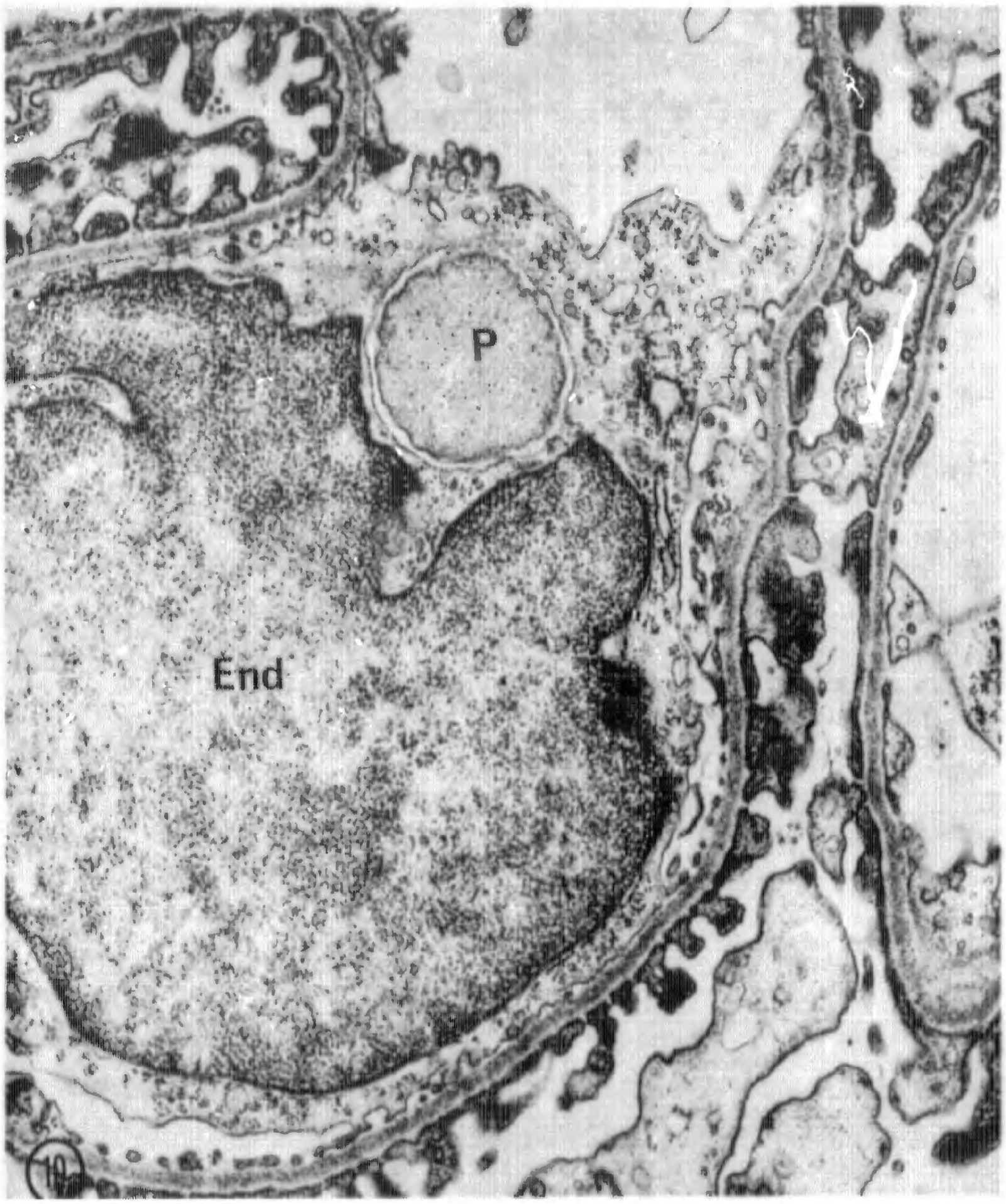

Figure 10. An endothelial cell from a glomerulus of a group II rat. Note the sparse organelles. A pseudopod from a subjacent mesangial cell (P) is present. The basement membrane is intact except in the loop at the lower right where it appears reduplicated. X 16,800.

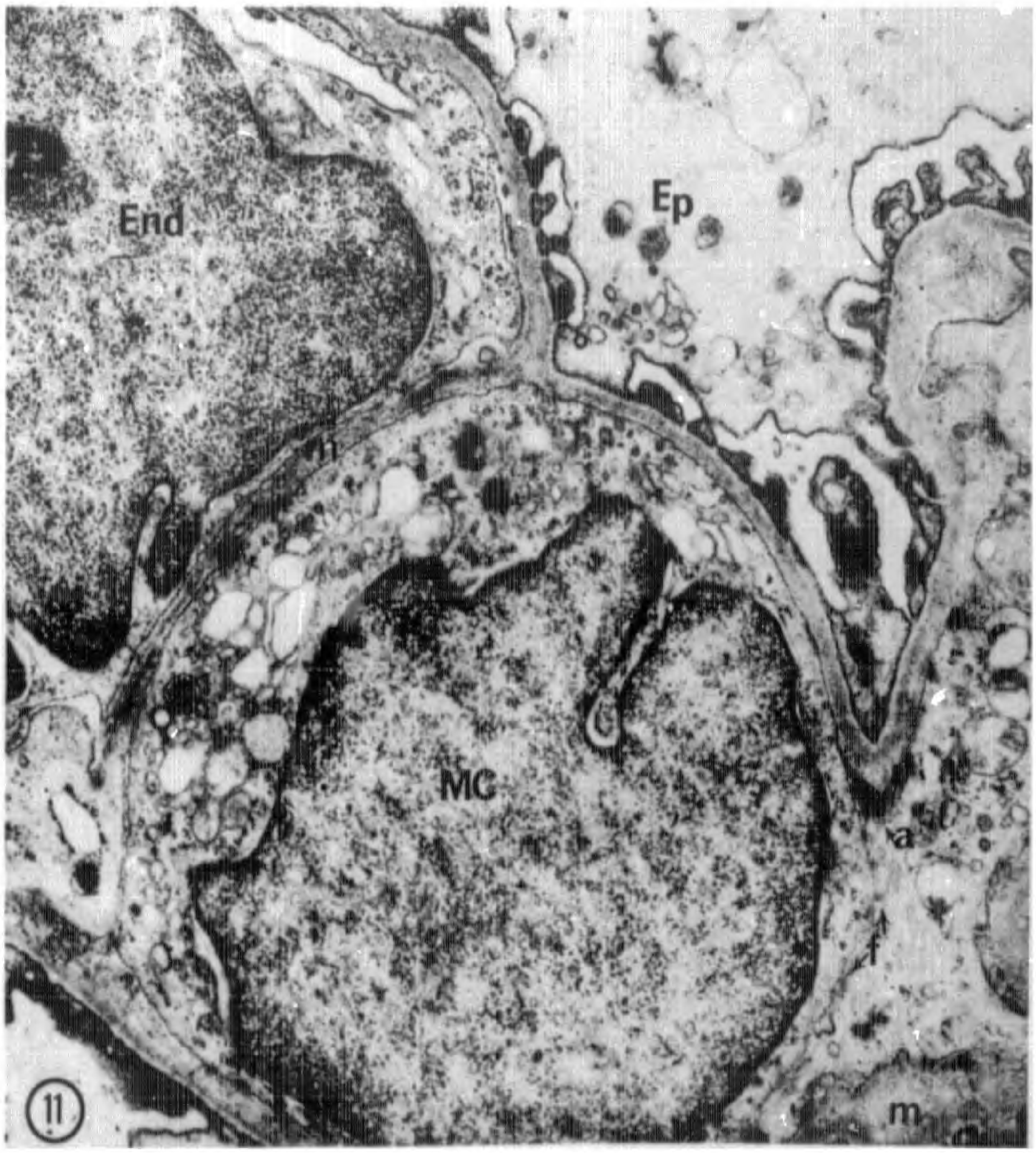

Figure 11. Mesangial region from a group II glomerulus. Note the sparse matrix (m). An anchor site (a) is prominent and attached fibrils (f) are seen. Organelles are sparse. Ep, epithelial cell. X 16,800.

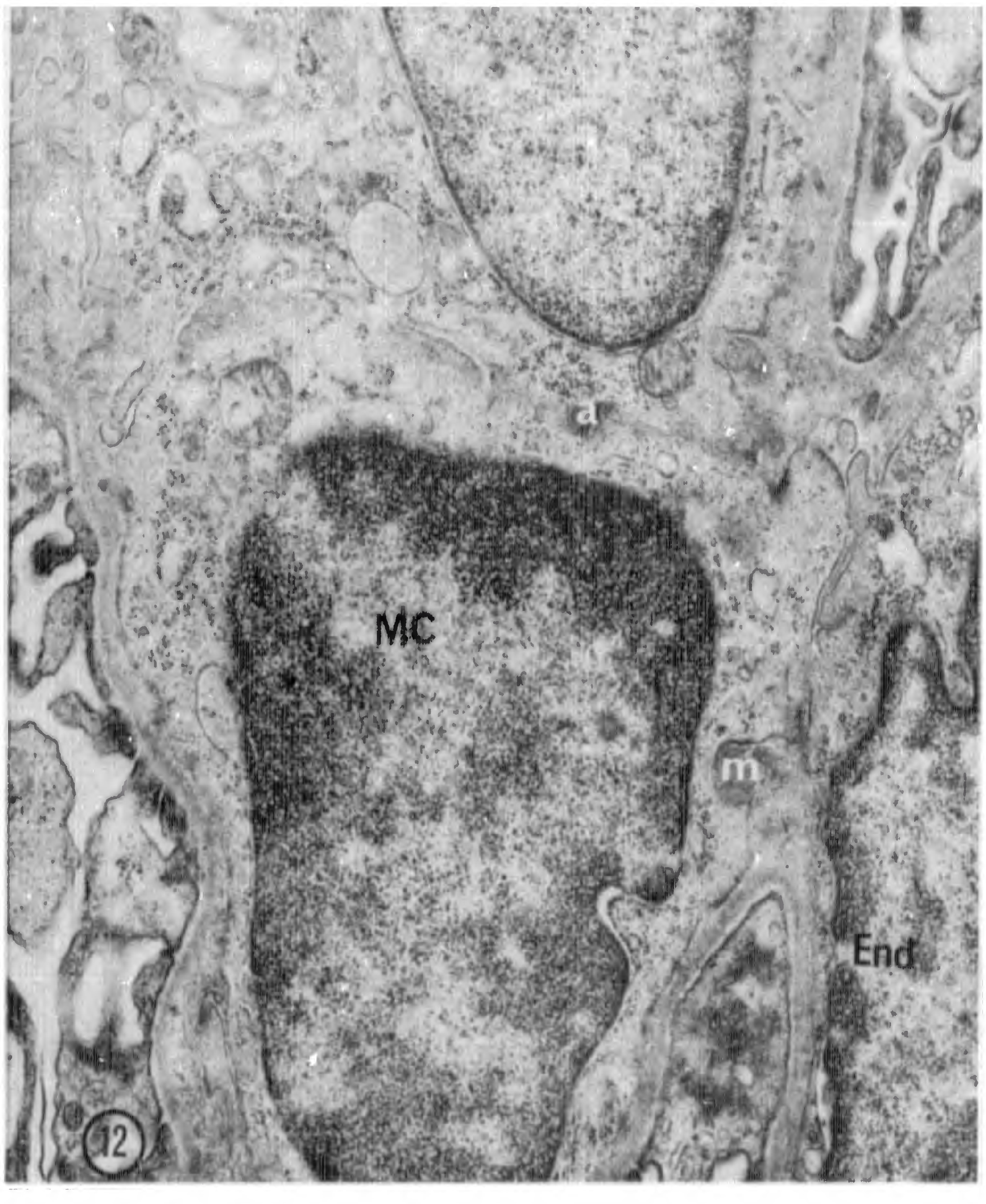

Figure 12. Mesangial cell (MC) of a group II rat, showing few organelles. An anchor (a) is present. The matrix (m) is sparse. X 16,800.

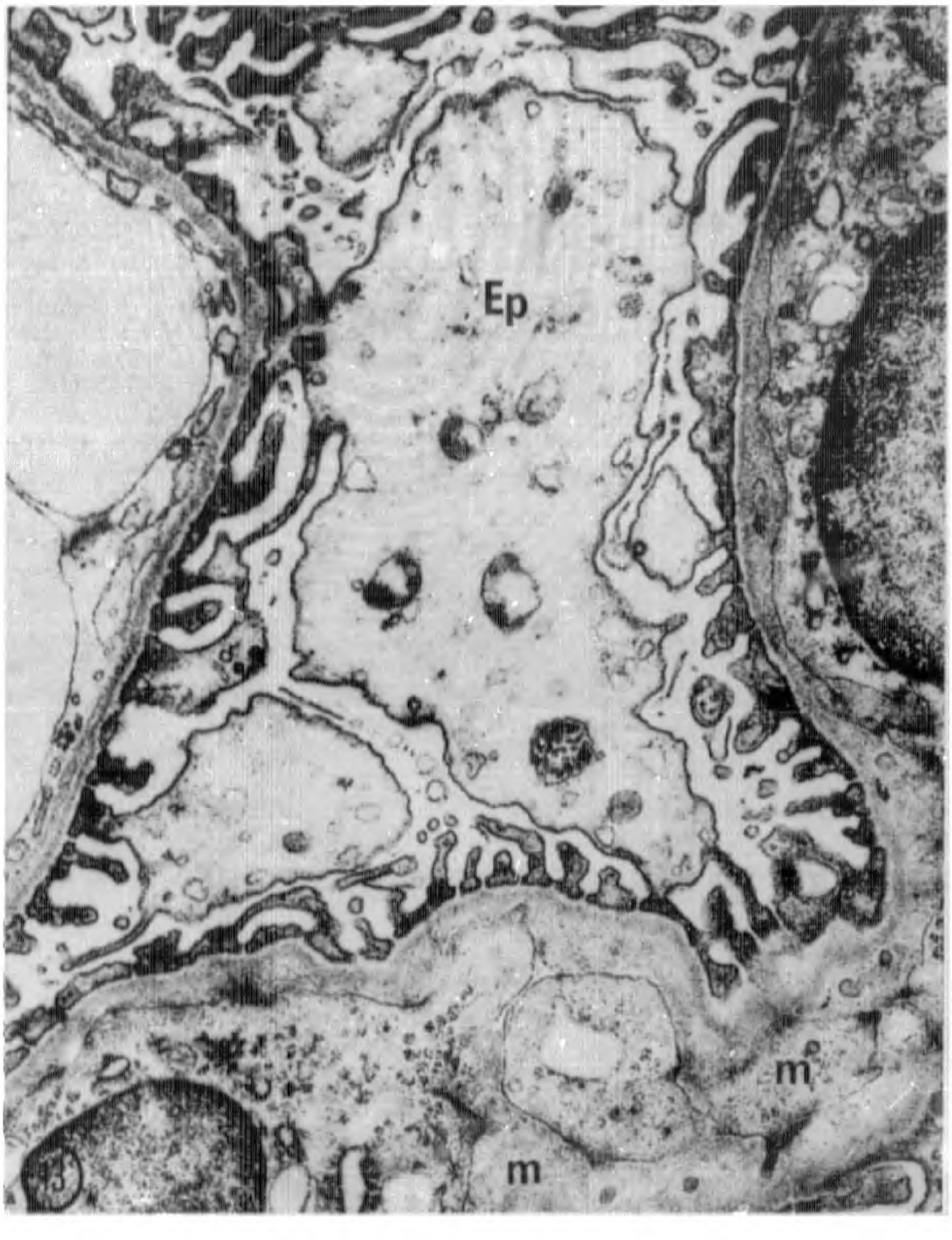

Figure 13. Glomerulus from a group II rat. The mesangial cytoplasm (M) has several anchors. The matrix (m) is irregular and of variable density. The epithelial cell (Ep) has sparse organelles. X 16,800.

cell proliferation, increased mesangial matrix formation, collagen elaboration, and cell damage. In addition, some apparent direct basement membrane injury is present. When food restriction to the point of substantial whole body and parenchymal organ growth regardation is introduced, the full extent of the radiation injury is not capable of expression in spite of a stimulus for growth; i.e., uninephrectomy.

There is evidence that the deposits in the mesangium are produced by mesangial and endothelial cells. The associated increased ribosomal activity in the normally fed animals suggests active protein synthesis in the mesangial and endothelial cells. The underfed animals demonstrated a marked decrease in ribosomal activity and diminished matrix formation.

The observation that underfeeding was also associated with diminished numbers and size of mitochondria implies that the oxidative metabolism of the glomerular cell population was operating at substantially reduced levels. This suggests that the increased mesangial deposits, a major manifestation of the lesion, are in part quantitatively related to the metabolic activities of the cells.

There is evidence that immunoglobulins may be present in the mesangial matrix material following radiation (6). Underfeeding may also decrease the amount of protein material available for synthesis of immunoglobulins and other substances found in the matrix.

It may be concluded from our studies on radiation-induced glomerulosclerosis that, at least in weanling rats, the progression of these lesions are modifiable by factors or conditions which alter kidney growth. This conclusion is directly substantiated by the present electron microscopic observations. It will be our aim, next, to ascertain the role of immunological factors in the development of this delayed radiation-induced kidney pathology.

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Victor J. Rosen Louis W. Wachtel
Leonard J. Cole Reuben S. Doggett

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13. ABSTRACT

Weanling female Sprague-Dawley rats were utilized to study the effect of undernutrition on the ultrastructure of accelerated irradiation induced glomerulosclerosis. Following uninephrectomy and 2000 rad X-rays to the remaining kidney, a severe, destructive glomerulosclerosis appeared within 7-8 weeks. Food restriction to the point of significant growth retardation resulted in a marked inhibition of the genesis of the glomerular lesion. Ultrastructurally there was marked reduction in all cytoplasm organelles and a striking reduction in mesangial matrix deposition. These observations show that food restriction retards the development or manifestation of what is considered to be a direct radiation effect on the kidney; and add further confirmation to the thesis that a stimulus for growth, combined with the direct effects of radiation accelerates the development of glomerulosclerosis, while growth retardation has a profound inhibiting effect on the evolution of the lesion.

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