GLOMERULAR PERMEABILITY

ULTRASTRUCTURAL CYTOCHEMICAL STUDIES USING PEROXIDASES AS PROTEIN TRACERS*

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The morphologic study of glomerular protein transport is limited by the fact that most proteins are not sufficiently electron opaque to be identified in electron micrographs. Farquhar, Wissig, and Palade (2) have approached this problem by the use of ferritin, a protein containing a core of tightly bound micelles of ferric hydroxide which serves as an electron-opaque marker of characteristic shape. The ferritin molecule, the protein part of which has a molecular weight of about 462,000 (3), is too large to be excreted in appreciable amounts by the normal glomerulus (4–6). Thus, it is difficult to relate results obtained with ferritin to the glomerular transport of the much smaller protein molecules which appear in the glomerular filtrate in significant quantities. Menefee and his coworkers (7) have studied the glomerular transport of globin. Unfortunately, soluble globin cannot be identified in electron micrographs; the protein only is visible when in the form of large aggregates.

Recently, we have reported an ultrastructural cytochemical method which permits the use of peroxidases as tracers in the study of the morphologic aspects of protein transport (8–10). In this technique, peroxidases catalyze the oxidation of 3,3′-diaminobenzidine by H_2O_2, yielding an insoluble, brown, electron-opaque reaction product at the sites of enzymatic activity (i.e., the location of the peroxidase molecules). This report deals with the transport of two intravenously injected peroxidases in the mouse renal glomerulus. One, horseradish peroxidase (HRPO), has a molecular weight of about 40,000 (11) and is excreted rapidly by the glomerulus (9, 12). The other, human myeloperoxidase (MPO), has a molecular weight of 160,000 to 180,000 (14) and is apparently largely retained by the glomerular filter, as would be expected from its relatively large

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molecular size. Results with the two proteins will be compared and discussed in terms of the possible functions of the different components of the glomerular wall.

Materials and Methods

Twenty-three white mice weighing about 25 g each were injected intravenously with 4 to 5 mg horseradish peroxidase (type II, Sigma Chemical Co., St. Louis) dissolved in 0.5 ml 0.15 M NaCl solution. The mice were killed by stunning and neck dislocation at various intervals of time, ranging from 1½ to 60 min after injection. Five mice were injected intravenously with 2 to 4 mg human myeloperoxidase (13) (kindly supplied by Dr. Julius Schultz, Department of Biological Chemistry, Hahnemann Medical College, Philadelphia) dissolved in 0.5 ml 0.15 M NaCl solution and killed at intervals of 5, 12, 20, 30, and 45 min after injection.

The kidneys were removed, cut into strips (limited, when possible, to cortex) 2 to 3 mm thick, and fixed for 3 to 3½ hr at room temperature in a formaldehyde-glutaraldehyde fixative (15). To prepare this fixative, 2 g paraformaldehyde was added to 25 ml water in a flask, and the mixture was heated to 60° to 70°C with constant stirring. Two or three drops of sodium hydroxide, 1 N, were added slowly until the solution became clear (16). The solution was then cooled by holding the flask under running tap water. 5 ml 50% glutaraldehyde (biological grade, Fisher Scientific Co., Pittsburgh), 20 ml cacodylate buffer, pH 7.2, 0.2 M, and 25 mg calcium chloride were then added and thoroughly mixed. This fixative was used full strength in some experiments. In others, it was diluted 1:1 with cacodylate buffer, 0.1 M, pH 7.2. After fixation, the tissues were washed overnight in 0.1 M cacodylate buffer, pH 7.2.

Frozen sections 40 μ thick were cut on a Leitz freezing microtome equipped with a Pel-Cool thermoelectric freezing stage. Other sections about 40 μ thick were cut without freezing on a Smith and Farquhar tissue sectioner. Localization of activity was the same in tissue sectioned by either method, but structural preservation was better in unfrozen tissue cut on the tissue sectioner.

The sections were incubated at room temperature for 10 to 30 min in a medium consisting of 5 mg 3,3′-diaminobenzidine tetrahydrochloride (Sigma Chemical Co.), 10 ml tris-HCl buffer pH 7.6, and 0.1 ml 1% H_2O_2.

After incubation, the sections were washed in 3 changes of distilled water and postfixed for 90 min in 1.3% osmium tetroxide in s-collidine buffer, pH 7.2 (17). They were then dehydrated in ethanol and embedded in Epon 812 (18) or Araldite (18). Sections 0.5 to 1 μ thick were cut and examined without counterstaining by light microscopy. The reaction product in such "thick" sections was seen as dark brown deposits. Thin sections were cut on a Porter-Blum MT-1 ultramicrotome or an LKB ultrotome, stained with lead (19), and examined in an RCA EMU-3F electron microscope. Although the reaction product was easily seen in sections not stained with lead, sections were usually counterstained so that tissue structure also could be clearly visualized. Alkaline lead solutions did not disturb the reaction product.

The rationale of the cytochemical method, and the control experiments done to test its validity, have been described in detail elsewhere (9).

In the doses used in these experiments, neither HRPO nor MPO caused evident toxicity in the mouse during the period of observation. The mice did not appear ill, and the fine structure of glomeruli from injected mice was not detectably different from that of normal uninjected controls. Moreover, it has been shown that HRPO does not increase vascular permeability when injected into the mouse, whereas in the rat and guinea pig, in high dosage, it acts as a histamine liberator (20).

RESULTS

Controls.—Control experiments in this investigation were of two types. In the first, kidneys from normal uninjected mice were incubated in the full cytochemical medium. When observed by light and electron microscopy (Figs. 3 and 4), reaction product was seen only in erythrocytes. In the second type, kidneys from mice injected with horseradish peroxidase and killed at various intervals were incubated in incomplete media, lacking either 3,3′-diaminobenzidine or H_2O_2. No reaction product was seen.

Our observations on glomerular fine structure will not be described here, since they agree with those of Yamada (21) in the mouse and Farquhar and her associates (2, 22–24) in the rat.

Horseradish Peroxidase

Light Microscopy.—The brown reaction product is clearly visible by light microscopy (Fig. 1), and this technique was useful in studying the temporal aspects of peroxidase filtration, as well as in selecting tissue for electron microscopy. In kidneys of mice killed as early as 90 sec after HRPO injection, glomeruli were strongly stained and reaction product was present in the tubular lumens and in early phagosomes at the base of the brush borders, indicating that the glomerular filtrate already contained significant amounts of HRPO. At various intervals up to 15 min after injection (Fig. 1), the appearance of the glomeruli and tubules was similar, except that the size and number of the phagosomes in the proximal tubules had increased progressively. At intervals after 15 min, there was a progressive decrease in the amount of reaction product in the glomeruli and in the tubular lumens. At 30 min after injection, faint staining of glomerular capillary lumens and basement membranes and of proximal tubular brush borders still was visible, but by 45 min after injection usually none was detectable in these sites. Activity persisted in many phagosomes in the proximal tubules. The electron microscopic appearances of tubular uptake of HRPO have been described previously (9).

Electron Microscopy.—Glomeruli from mice killed 90 sec to 15 min after HRPO injection were of similar appearance and will be described together (Figs. 5 to 7). Reaction product was consistently seen in the basement membranes and often was noted in the lumen itself. In many areas, it also could be seen within the endothelial fenestrae, apparently continuous with that in the lumen and with that in the basement membrane (Figs. 5 and 6). Occasional endothelial vesicles also contained reaction product (Figs. 5 and 6). Erythrocytes in capillary lumens usually contained reaction product because of the peroxidatic activity of their hemoglobin.

The basement membrane contained large amounts of reaction product throughout its entire thickness. In lightly reacted areas, however, there some-

times appeared to be a greater concentration of reaction product in the portion of basement membrane adjacent to the epithelial foot processes. Much reaction product was seen on the epithelial cell membranes and free between the foot processes and in other parts of the urinary space (Figs. 5 to 7). A few epithelial vesicles contained reaction product, but these were distinctly uncommon. In the mesangial areas, the spongy areas of basement membrane–like material contained reaction product and appeared to be of similar opacity to the capillary basement membrane itself (Fig. 7). Much reaction product was present on the epithelial membranes and between the foot processes abutting on mesangial areas. Reaction product was present in occasional vesicles and vacuoles in mesangial cells.

At intervals longer than 15 min after injection, deposition of reaction product in capillary lumens, basement membranes, and on epithelial membranes and in the urinary space became progressively less. There was no detectable increase in the number of epithelial vesicles containing reaction product. Peroxidase uptake by mesangial cells was increased at intervals greater than 15 min after injection. By 45 min after injection, reaction product usually was observed only in erythrocytes, in mesangial cells and in rare epithelial vesicles (Fig. 8).

Electron microscopic evidence of proximal tubular HRPO reabsorption, reported previously (9), appeared to be maximal at the same intervals at which large amounts of HRPO appeared to be passing into the glomerular filtrate. At intervals from 1½ to 20 min after injection there was evidence of much HRPO reabsorption, with staining of the brush border membranes, filling of the system of apical tubular invaginations, and progressive concentration of reaction product in the apical vacuoles. At longer intervals, active HRPO reabsorption, as manifested by the presence of reaction product on brush border membranes and in apical tubular invaginations, appeared to decrease. At intervals greater than 30 min after injection, reaction product usually was found only in dense "absorption droplets," which presumably formed from apical vacuoles by progressive concentration of their contents. As mentioned previously, at intervals longer than 30 min after injection, deposition of reaction product in glomerular basement membranes and on epithelial membranes was seldom observed.

Human Myeloperoxidase

Light Microscopy.—Reaction product was visible as a fine line along the course of the glomerular basement membrane 5 min after MPO injection. Apart from the usual deposition on erythrocytes, reaction product was not apparent in any other site; none was seen in the tubules. After 12 and 20 min (Fig. 2), the appearance of the glomerular basement membranes was similar, except that somewhat larger amounts of reaction product were present. In addition, reaction product, some of which appeared to be segregated in small

phagosomes, was present in mesangial areas (Fig. 2). A few small phagosomes were seen beneath the brush borders of some of the proximal tubules. After 30 min, staining of the glomerular basement membranes was less intense, deposition of reaction product in mesangial areas persisted, and mesangial phagosomes appeared larger and more numerous. After 45 min, glomerular reaction product was visible only in mesangial phagsomes and in erythrocytes. A few small phagosomes were present in some of the proximal tubules.

Electron Microscopy.—In the mouse glomerulus 5 min after MPO injection small amounts of reaction product were present throughout the basement membrane. However, the concentration of reaction product was much greater in that part of the basement membrane immediately adjacent to the epithelial foot processes. There also was reaction product in the epithelial slits.[1] When examined at low magnifications, there thus appeared to be a dark line of reaction product outlining the junction between the basement membrane and the epithelium (Fig. 9). In the mesangial regions, the spongy areas often contained moderate amounts of reaction product (Fig. 10) and occasional evidence of MPO uptake in mesangial vesicles and vacuoles was observed. There was no evidence of MPO uptake by epithelial cells at this stage. 12 and 20 min after MPO injection (Figs. 11 to 16) there were increased amounts of reaction product throughout the basement membrane. As seen after 5 min, the accumulation of reaction product was much greater in that portion of the basement membrane adjacent to the epithelium. Very dense deposits of reaction product were present in the epithelial slits, and in favorable sections the accumulation sometimes appeared to end at the level of the slit membranes[2] between the foot processes (Fig. 16). Only very occasional evidence of vesicular uptake of MPO by the epithelium was observed. In the mesangial regions, the spongy areas contained dense accumulations of reaction product (Figs. 11, 15, and 16) and there was increasing evidence of uptake of MPO in vesicles and vacuoles by mesangial cells (Fig. 11). Rare epithelial vesicles contained reaction product. By 30 min after MPO injection deposition of reaction product throughout the basement membrane was considerably decreased, and the evidence of accumulation against the epithelium, although still present, was much less striking. The mesangial spongy areas still contained large amounts of reaction product (much more than was present in the basement membrane of the glomerular wall at this stage) and many mesangial cell vesicles and vacuoles contained reaction

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[1] In agreement with Farquhar, Wissig, and Palade (2), we consider the epithelial slit to be that portion of the space between the foot processes extending from the level of the basement membrane to the level of the slit membrane.

[2] The nature of the slit membrane is not known. Although in suitably oriented sections the MPO reaction product appears to stop at the level of the slit membrane, we do not mean to imply that the slit membrane is necessarily the structural basis for restriction of passage through the epithelial slits.

product. After 45 min reaction product was observed principally in phagosomes in mesangial cells. Small amounts of reaction product were present in occasional spongy areas. No reaction product was observed in the basement membrane of the glomerular wall, or in the epithelial slits. Evidence of epithelial uptake of MPO was rare, and did not appear increased over that seen after 20 min.

DISCUSSION

It is clear that morphologic observations on glomerular protein transport vary considerably, depending on which tracer is used. The differences in the results obtained with HRPO, MPO, and ferritin are probably due, in large part,

TABLE I

Molecular Weight and Glomerular Clearance Data for Some Representative Substances

Substance (reference)	Molecular weight	Glomerular clearance (creatinine = 1)
Ferritin (3)	462,000*	‡
Myeloperoxidase (14)	160,000–180,000	‡
7S gamma globulin (26)	160,000	‡
Plasma albumin (25)	69,000	<0.01
Hemoglobin (25)	68,000	0.03–0.1
Egg albumin (25)	43,500	0.22
Horseradish peroxidase (11)	40,000	‡
Bence Jones protein (27, 28)	22,000–44,000	‡
Myoglobin (25)	17,000	0.75
Inulin (25)	5,500	0.97+

* This value represents the molecular weight of the protein portion of the molecule.
‡ Glomerular clearance data unavailable for these substances.

to the great differences in size of their molecules (see Table I). Physiological studies (4–6, 25) have shown that the permeability of the glomerulus to a protein is related to the dimensions of its molecules (and thus, roughly, to its molecular weight). In general, although there is some species variation, proteins with molecular weights greater than about 70,000 do not appear in the glomerular filtrate in appreciable amounts. The permeability of the glomerulus to smaller protein molecules increases with decreasing molecular weight (see Table I). Similar findings have been obtained with dextrans of various sizes (29).

The present observations indicate that HRPO passes rapidly from the capillary lumen through the endothelial fenestrae, across the basement membrane, and through the epithelial slits into the urinary space. The site of the filtration barrier could not be demonstrated conclusively with this small tracer. However, the fact that an apparent concentration of reaction product along the epithelial

membranes and in the slits sometimes was observed in lightly reacted areas suggests that some restriction to passage may exist in the epithelial slits.

MPO also apparently passes rapidly through the endothelial fenestrae and across the basement membrane. However, it then appears to encounter a barrier at the level of the epithelial slits. The barrier is not complete, and some peroxidase activity eventually appears in the glomerular filtrate. This probably represents, at least in part, the slow diffusion of some intact MPO molecules through the slits. It is possible that the MPO preparations used contained smaller fragments having peroxidase activity. However, this seems unlikely since MPO prepared by the method of Schultz and Shmuckler appears homogeneous in the ultracentrifuge (13). The eventual fate of the MPO blocked by the epithelial filtration barrier is uncertain. An unknown, but apparently large, proportion is taken up by the mesangial cells. Some of the rest may diffuse back through the basement membrane and reenter the blood, subsequently to be removed by the reticuloendothelial system. It seems unlikely that much of the MPO reaches the glomerular filtrate, since the cytochemical evidence of tubular peroxidase reabsorption is so slight. However, we cannot sustain this statement completely because urinary peroxidase activity was not measured, and it is conceivable that for some reason MPO is reabsorbed less readily by the renal tubules than is HRPO. This seems unlikely, however, since MPO certainly is pinocytosed readily by other cells, such as mesangial cells.

Uptake of both HRPO and MPO is prominent in mesangial cells, a finding which agrees with the observations of Farquhar and Palade (24) that mesangial cells are active in the removal of ferritin from the basement membrane, and supports their hypothesis that an important function of these cells is the incorporation and disposal of filtration residues. Neither endothelial nor epithelial pinocytosis appears to play a major role in the passage of HRPO or MPO across the glomerular wall.

In their studies of glomerular protein transfer using ferritin as a tracer, Farquhar and her associates (2, 22–24) made observations which differed from those obtained with the peroxidases in two main respects. First, they found that the glomerular basement membrane was a significant barrier to the ferritin molecules. Second, they found that most of those ferritin molecules which did pass through the basement membrane were taken up in vesicles and vacuoles by the epithelial cells.

In view of the dissimilar results obtained with the various protein tracers, it seems worthwhile to reexamine the glomerular filtration hypothesis proposed by Farquhar, Wissig, and Palade (2) on the basis of their observations with ferritin. The first main point of their hypothesis was that the basement membrane is the principal glomerular filtration barrier, responsible for the differential permeability of the glomerulus to proteins of varying molecular size. Their studies demonstrate clearly that the glomerular basement membrane is not freely permeable to ferritin. Ferritin, however, is an extremely large molecule, and its impedance by the basement membrane should not be the basis for generalization to the site of the filtration barrier for smaller pro-

teins. Their data do not exclude the possibility that a further barrier exists distal to the basement membrane (e.g., in the epithelial slits). In fact, the extensive epithelial uptake of ferritin which they observed suggests that a proportion of the ferritin which crosses the basement membrane is further impeded distally, with the result that the ferritin molecules remain in contact with the epithelial membranes for an appreciable time. The observations with MPO indicate that the main filtration barrier to this protein is distal to the basement membrane, located in the epithelial slits. In view of the above, it seems likely that the glomerular basement membrane acts as a "coarse" filter to exclude very large molecules (and, probably, to retard somewhat the passage of smaller molecules). The epithelial slits appear to act as a "fine" filter (i.e., the epithelial slits are the principal filtration barrier) and thus actually determine the nature of the protein content of the glomerular filtrate. The present findings thus lend support to the suggestion originally made by Hall (30) and later by Landis and Pappenheimer (31) that the epithelial "slit pores" are the principal site of glomerular protein filtration.

The second main point in the hypothesis of Farquhar et al. (2) was that the glomerular epithelium acts as a monitor to recover, at least in part, the protein which manages to leak through the basement membrane. However, in contrast to the striking epithelial uptake of ferritin, evidence of HRPO or MPO within epithelial cells was minimal. The reasons for the lack of peroxidase uptake by the epithelium are not entirely clear. On the basis of our earlier studies with HRPO, we considered the lack of uptake of this protein probably to be caused by the rapid removal of the HRPO through the slits into the urinary space, thus allowing only brief contact between the HRPO and the epithelial membranes. However our subsequent demonstration that epithelial uptake of MPO also is minimal raises some doubts about this explanation, since the larger MPO molecules might possibly remain against the epithelium for an appreciable time. One possible explanation, mentioned previously, is that the MPO molecules, upon being impeded at the epithelial slits might rapidly diffuse back across the basement membrane into the blood. The ferritin molecules, presumably, would follow this path less readily since the basement membrane is relatively impermeable to them. It is also conceivable that the peroxidase molecules for some reason have a reduced susceptibility to epithelial uptake. However, this seems unlikely since they are taken up readily by pinocytosis in other cells (e.g., mesangial cells). A further factor possibly complicating the interpretation of the lack of epithelial uptake of MPO is the fact that the doses of HRPO used were considerably greater, when calculated on the basis of molarity, than were the doses of MPO. This discrepancy was unavoidable. The HRPO experiments were done first, with a dose of enzyme considered optimal for the cytochemical procedure; when the MPO experiments were done subsequently, the limited amounts of MPO available precluded the use of an equivalently large dose. It is possible that with a larger dose of MPO, there would have been evidence of increased epithelial uptake relative to that seen with HRPO. In fact, however, the doses of MPO used were sufficiently high to give strong activities in basement membranes and mesangial cells. In any event, the present results cast doubt on the significance of the glomerular epithelium as a "monitor" for smaller protein molecules.

It is of interest that the molecular weight of the dimer form of Bence Jones proteins is similar to that of HRPO. The Bence Jones proteins, which exist both as monomers and dimers of molecular weights 22,000 and 44,000 respectively (27, 28), are rapidly excreted by the glomerulus (32). Thus, these studies using HRPO as a tracer are relevant to the mechanism of excretion of these small proteins. Many of the plasma globulins are similar in molecular weight to MPO, and these studies using this tracer should contribute to an understanding of the structural basis by which these proteins are retained by the normal glomerulus.

SUMMARY

1. Glomerular permeability was studied by ultrastructural cytochemistry, using as protein tracers two intravenously injected peroxidases of differing molecular weight.

2. Horseradish peroxidase (molecular weight 40,000) passed rapidly through the endothelial fenestrae, across the basement membrane, and through the epithelial slits into the urinary space. Human myeloperoxidase (molecular weight 160,000 to 180,000) also passed rapidly through the endothelial fenestrae and across the basement membrane, but was impeded at the level of the epithelial slits. Both proteins were taken up in large amounts by the mesangial cells.

3. The present findings indicate that the epithelial slits are the primary filtration barrier responsible for the differential permeability to proteins of varying molecular size.

4. The observations also support the concept that an important function of the mesangial cells is the incorporation and disposal of glomerular filtration residues.

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EXPLANATION OF PLATES

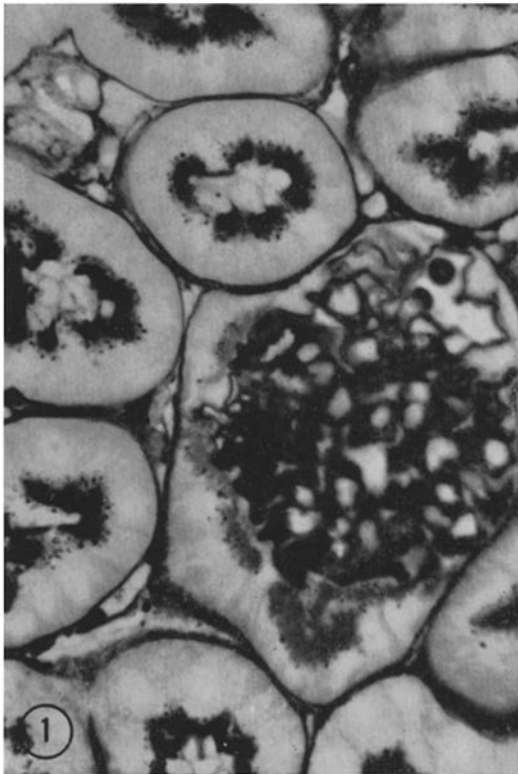
PLATE 113

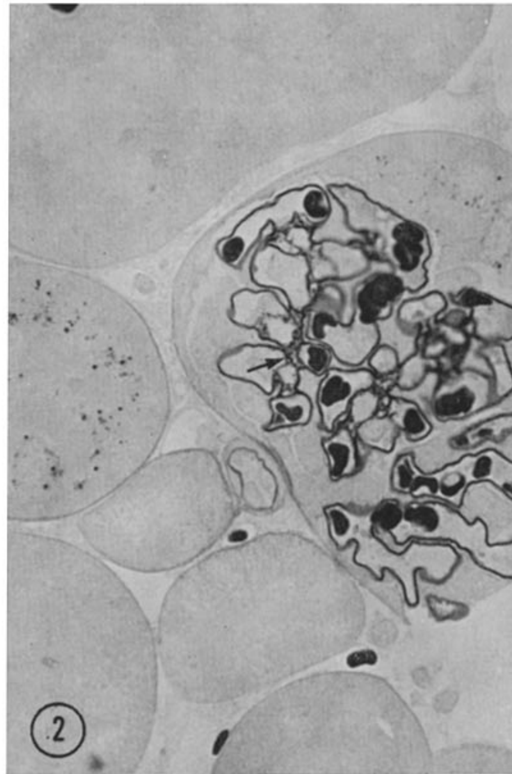
FIG. 1. Light micrograph of kidney from mouse killed 3 min after injection of HRPO, treated as described in text. In the glomerulus, reaction product is present in capillary lumens, in basement membranes, and in erythrocytes. In the adjacent proximal tubules, much reaction product is present on the brush borders and in early phagosomes at the apices of the tubular cells. In the mouse, a portion of the proximal tubule lies within the glomerular capsule and similar sites of tubular staining are seen at the lower left pole of the glomerulus. × 900.

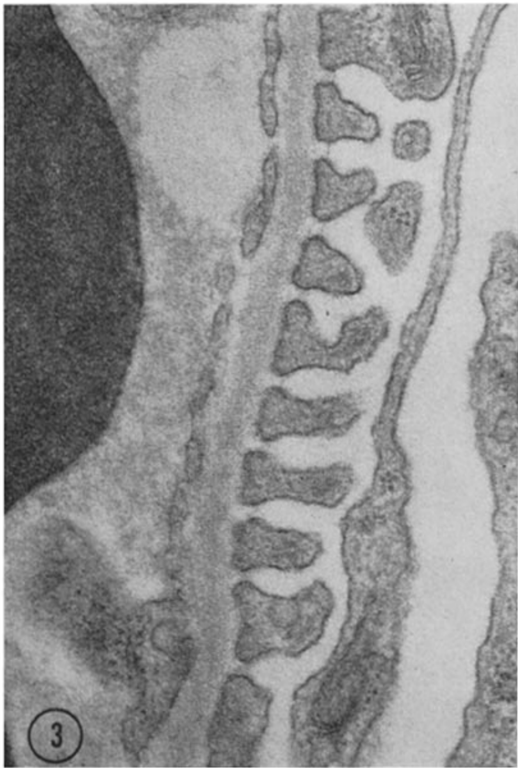
FIG. 2. Light micrograph of kidney from mouse killed 20 min after MPO injection. In the glomerulus, reaction product is visible in the basement membranes, in mesangial regions (arrow) and in erythrocytes. Phagosomes are present in two of the proximal tubules; in several others, none are visible. Compare the small amount of tubular activity in this section with the large amounts present in the kidney of the mouse injected with HRPO and killed much earlier, shown in Fig. 1. × 900.

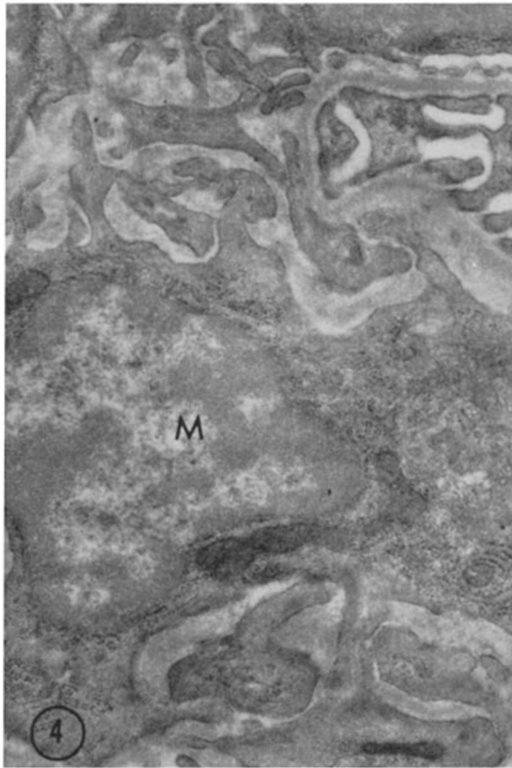
FIG. 3. Electron micrograph of glomerular wall from uninjected control mouse, incubated for peroxidase activity as described in text. Reaction product is present only on the erythrocyte in the lumen of the capillary at left. Note the lack of electron-opaque material in the basement membrane and in the epithelial slits. × 42,000.

FIG. 4. Electron micrograph of glomerular mesangial region from the same control mouse as Fig. 3. No reaction product is present in the spongy areas or in any of the ramifications of the mesangial cells (*M*). × 21,000.





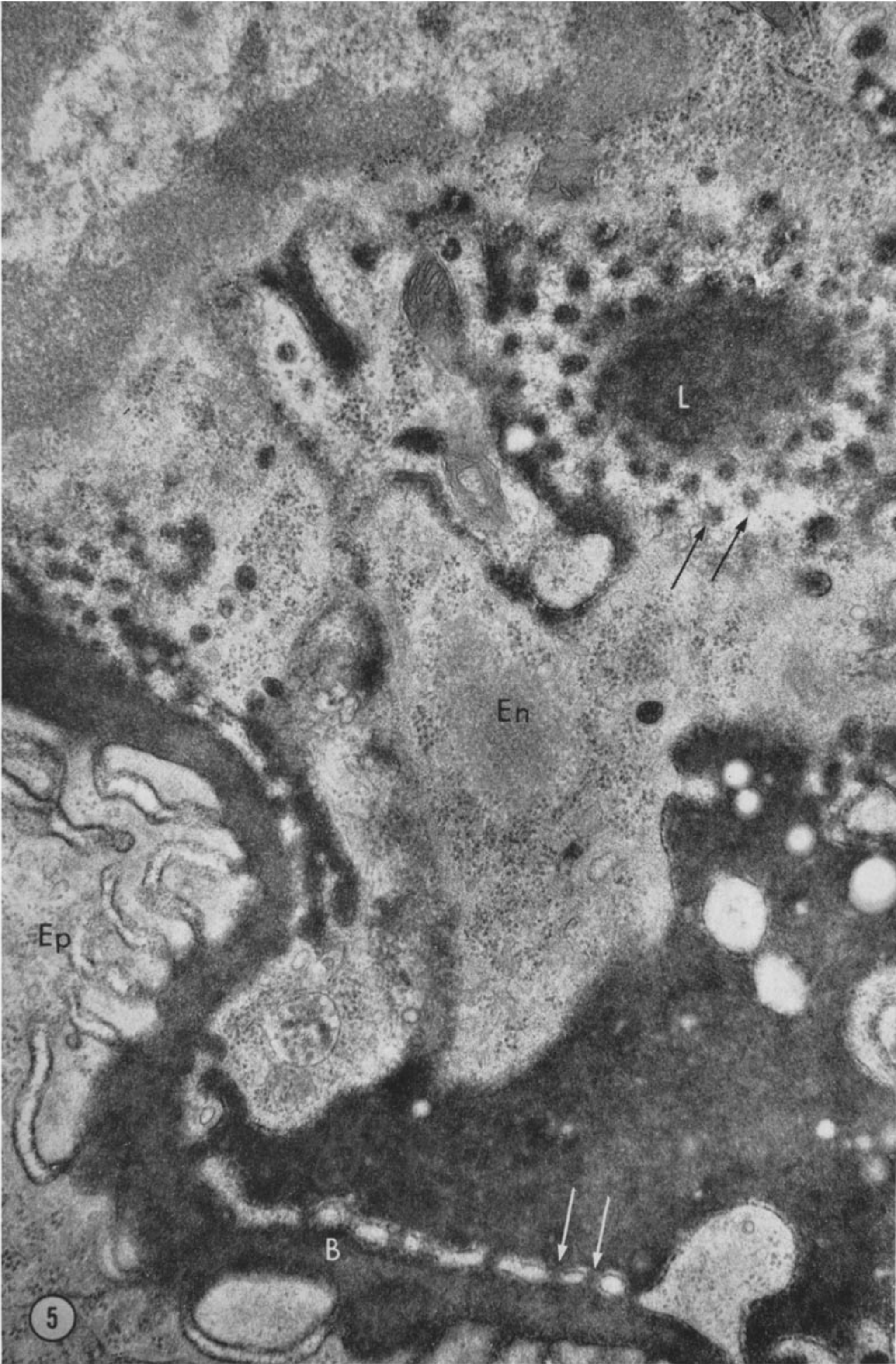




(Graham and Karnovsky: Glomerular permeability)

PLATE 114

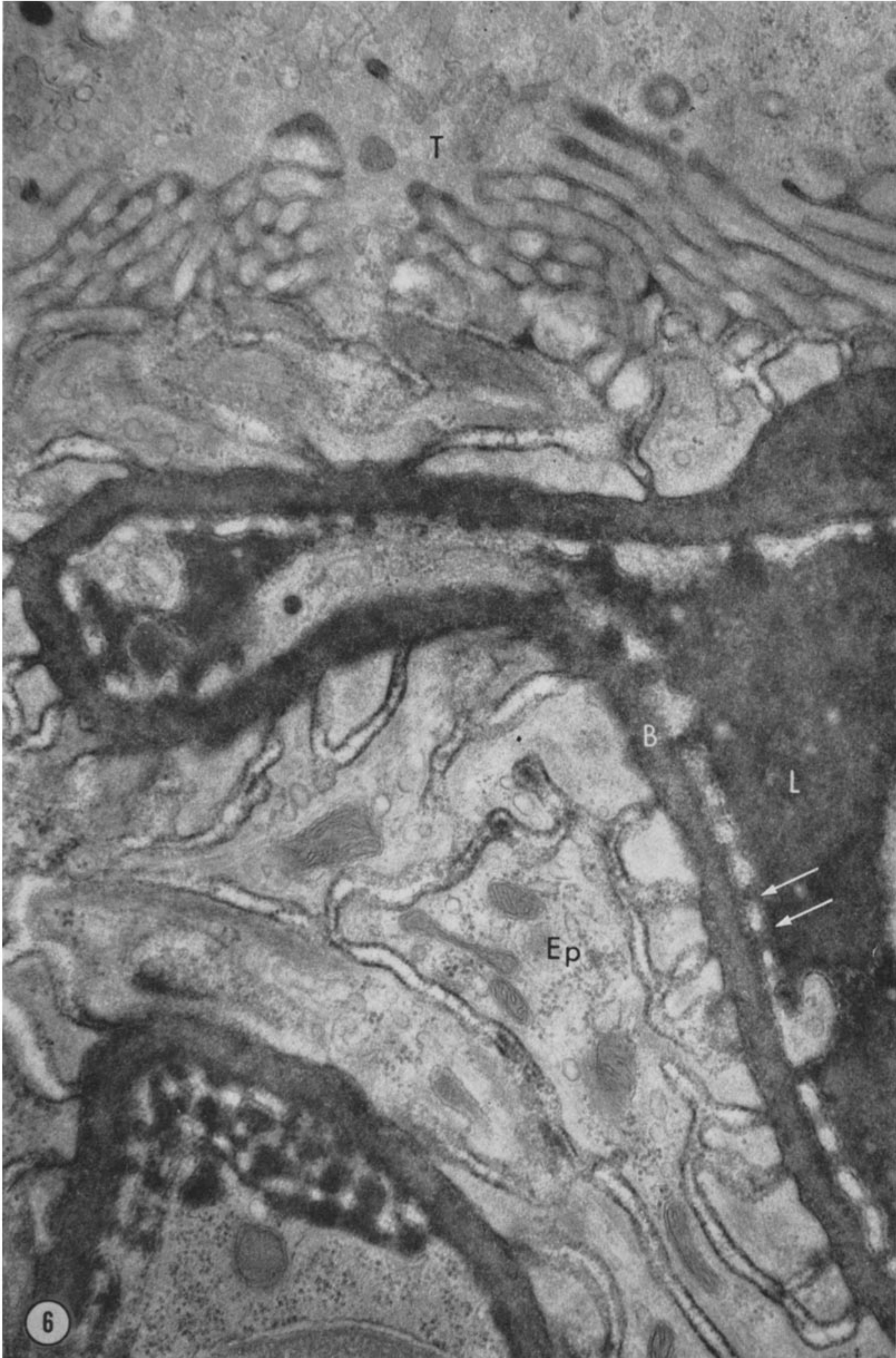
FIG. 5. Electron micrograph of glomerulus from mouse killed 5 min after injection of HRPO. Opaque reaction product is present in the capillary lumen (*L*). The endothelial cell (*En*) contains several vesicles filled with reaction product. The endothelial fenestrae are filled with reaction product, and can be seen both in normal (white arrows) and tangential (black arrows) planes of section. The basement membrane (*B*) contains reaction product throughout, and reaction product is present on the membranes of the foot processes of the epithelial cell (*Ep*), and in the urinary spaces. × 25,000.



(Graham and Karnovsky: Glomerular permeability)

PLATE 115

FIG. 6. Electron micrograph of glomerulus from mouse killed 3 min after HRPO injection. Reaction product is present in the capillary lumen (*L*), in the endothelial fenestrae (arrows), and in the basement membrane (*B*). Reaction product outlines the membranes of the epithelial cell (*Ep*), and some reaction product can be seen free within the urinary space. No reaction product is apparent in epithelial vesicles. A portion of the proximal tubule (*T*) lies within the glomerular capsule in the mouse, and reaction product can be seen on the brush border membranes, and in vesicles and apical tubular invaginations. $\times$ 25,000.

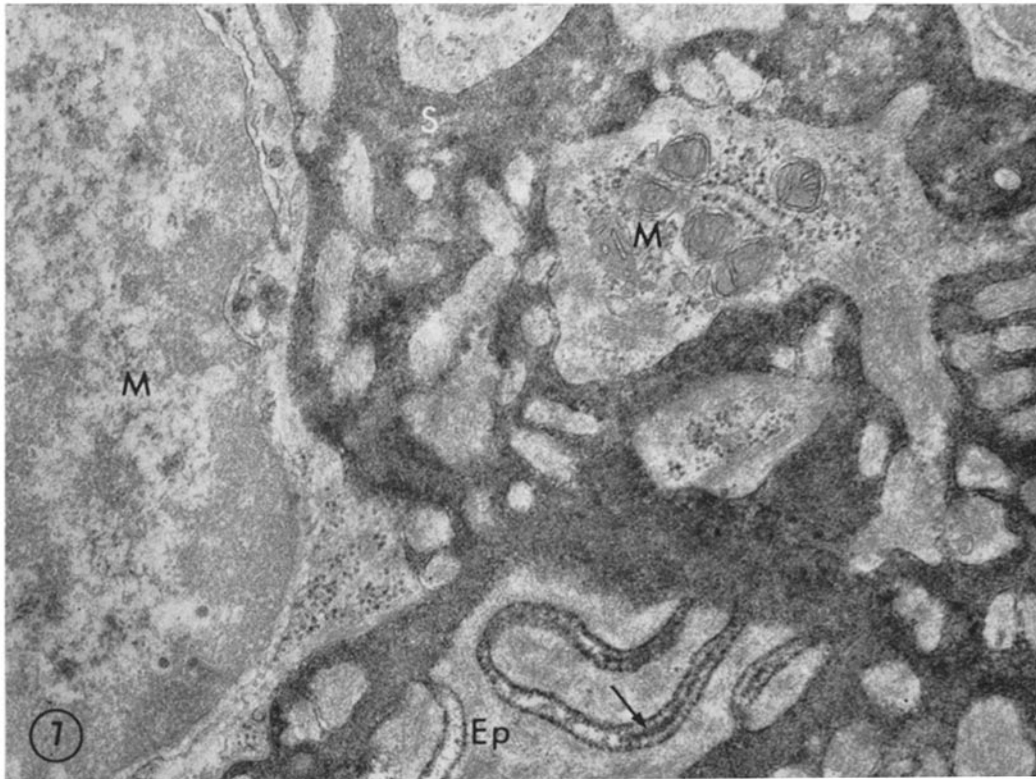


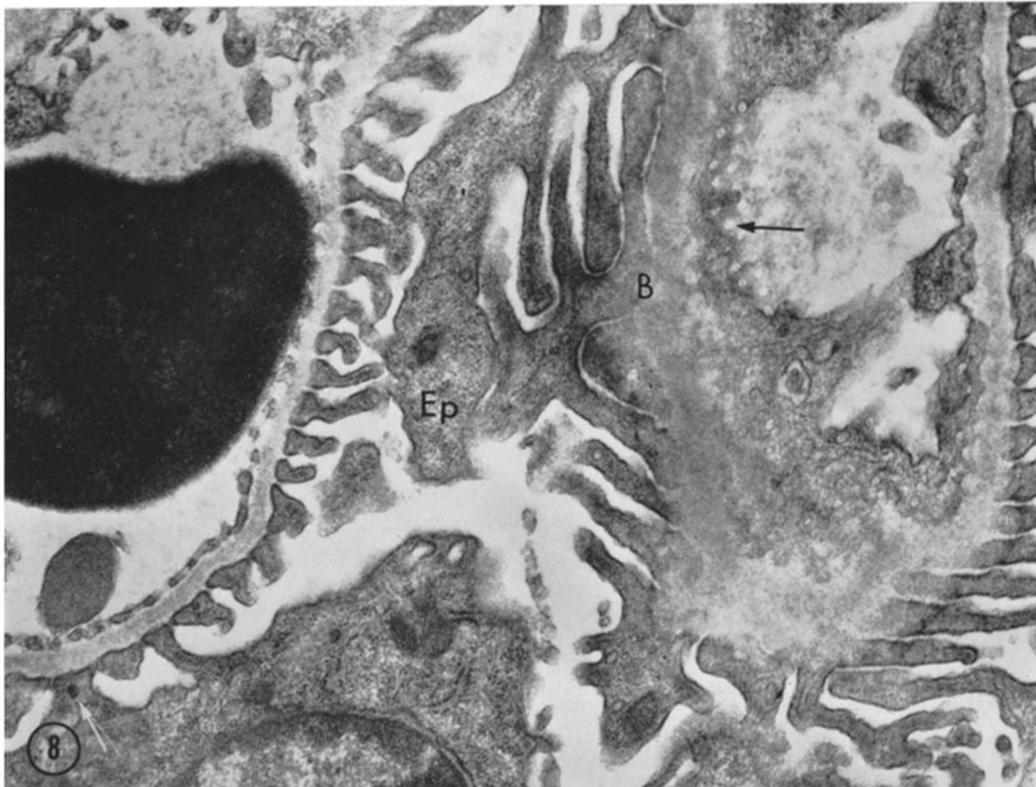
(Graham and Karnovsky: Glomerular permeability)

PLATE 116

FIG. 7. Electron micrograph of mesangial area of mouse killed 5 min after injection of HRPO. The spongy areas (*S*) contain large amounts of reaction product. No evidence of uptake by the mesangial cells (*M*) is apparent in this field. The membranes of the epithelial cell (*Ep*) are outlined with reaction product, and reaction product is present in the urinary space between the foot processes (arrow). No reaction product is visible within the epithelial cell. × 25,000.

FIG. 8. Electron micrograph of glomerulus from mouse killed 45 min after injection of HRPO. No reaction product is present in the endothelial fenestrae (black arrow), in the basement membrane (*B*), or on the membranes of the epithelial cells (*Ep*). One epithelial vesicle may contain reaction product (white arrow). Much reaction product is present in the erythrocyte in the capillary lumen. × 22,000.





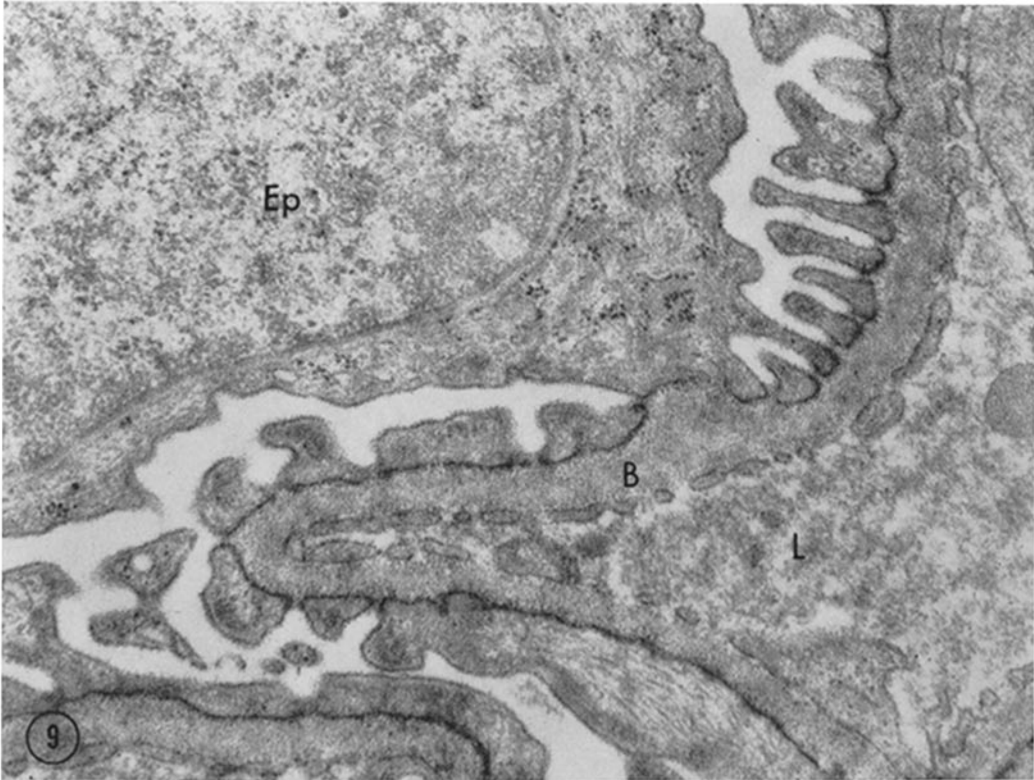
(Graham and Karnovsky: Glomerular permeability)

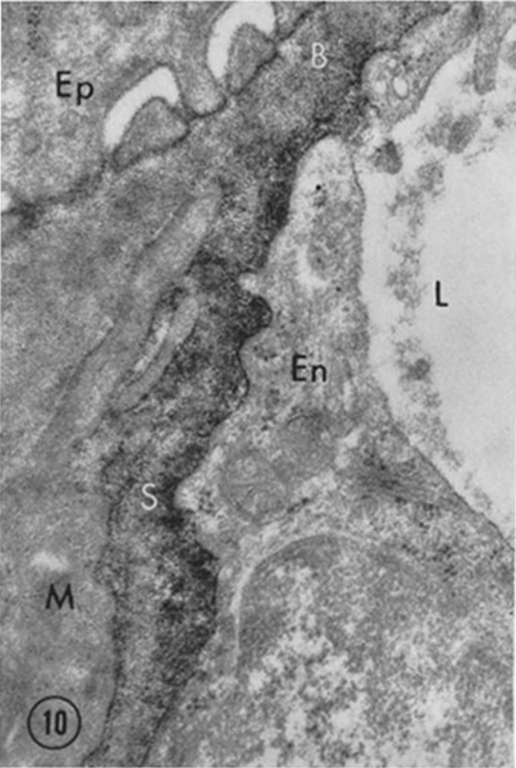
PLATE 117

FIG. 9. Electron micrograph of glomerulus from mouse killed 5 min after injection of MPO. Some granular reaction product is present in the capillary lumen (*L*). Some reaction product is present throughout the basement membrane (*B*), but it is chiefly concentrated along the junction between the basement membrane and the foot processes of the epithelial cell (*Ep*). × 28,000.

FIG. 10. Electron micrograph of glomerulus from mouse killed 5 min after MPO injection. The basement membrane (*B*) contains reaction product, and the greater concentration at the junction between the basement membrane and the foot processes of the epithelial cell (*Ep*) is again observed. A mesangial cell (*M*) is at the left. The spongy area (*S*) contains considerably more reaction product than does the adjacent basement membrane. An endothelial cell (*En*) and a capillary lumen (*L*) are seen to the right. × 28,000.

FIG. 11. Electron micrograph of a glomerular mesangial region from a mouse killed 20 min after injection of MPO. The spongy areas (*S*) contain dense reaction product. Reaction product is present in several vesicles and vacuoles in the mesangial cells (*M*). × 27,000.


Ep
B
L
9


Ep
B
L
En
S
M
10

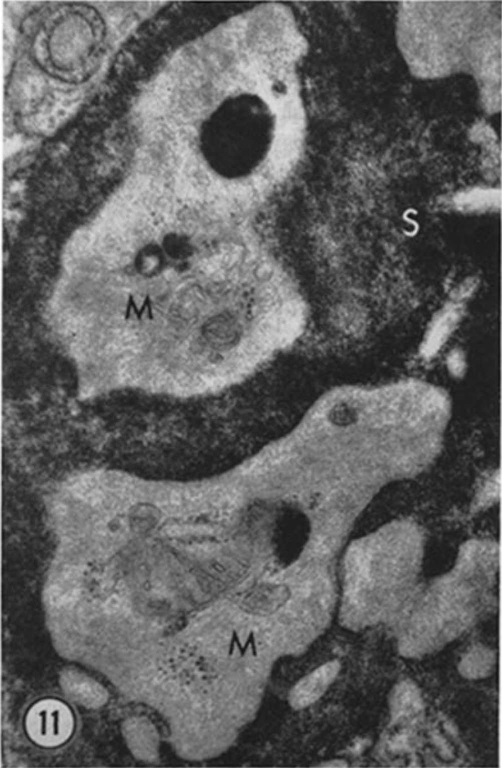
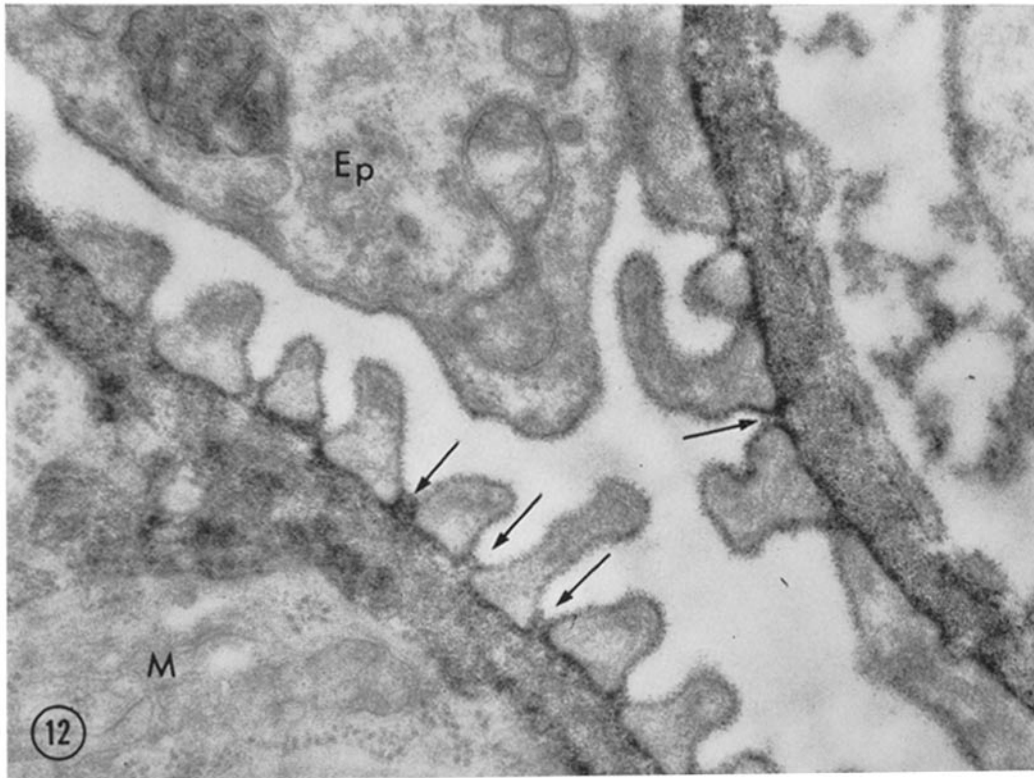
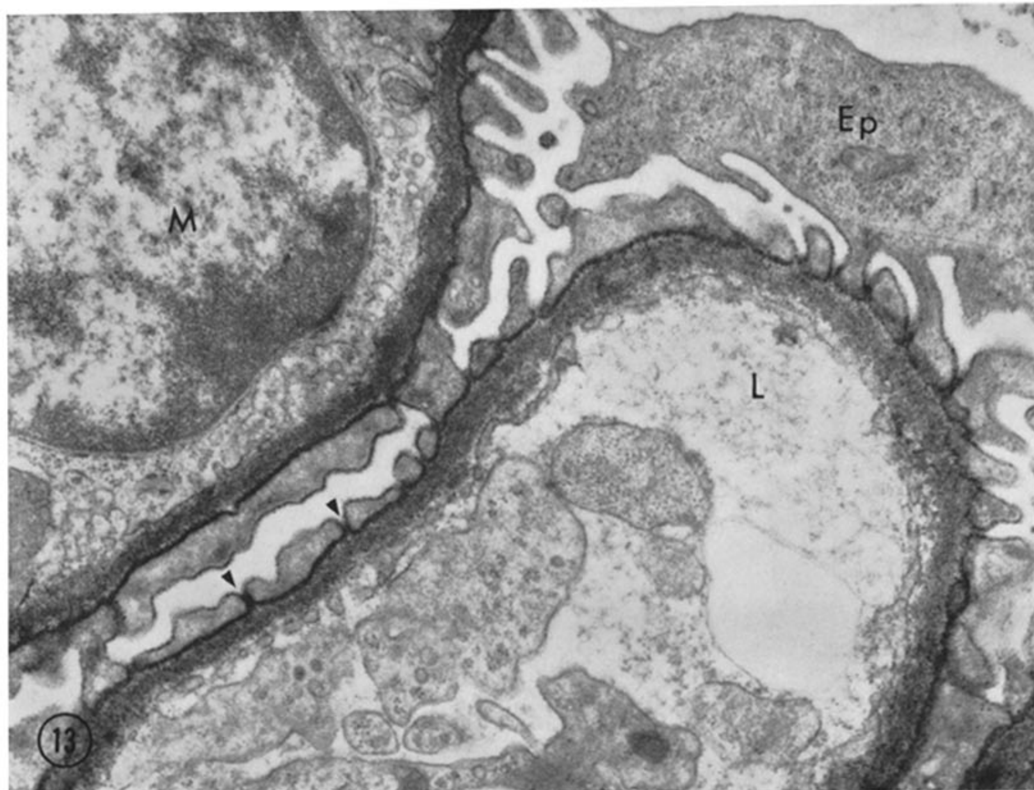

S
M
M
11

PLATE 118

FIG. 12. Electron micrograph of glomerulus from mouse killed 12 min after MPO injection. Reaction product in the basement membrane is concentrated in the area adjacent to the foot processes of the epithelial cell (*Ep*) and in the epithelial slits (arrows). A mesangial cell (*M*) is at the lower left. × 41,000.

FIG. 13. Electron micrograph of glomerulus from mouse killed 20 min after injection of MPO. Reaction product is concentrated at the junction between the basement membrane (*B*) and the epithelial foot processes, and in the epithelial slits (arrows). No reaction product is present in vesicles or vacuoles in the epithelial cells (*Ep*). A capillary lumen (*L*) is at the lower right. × 22,000.



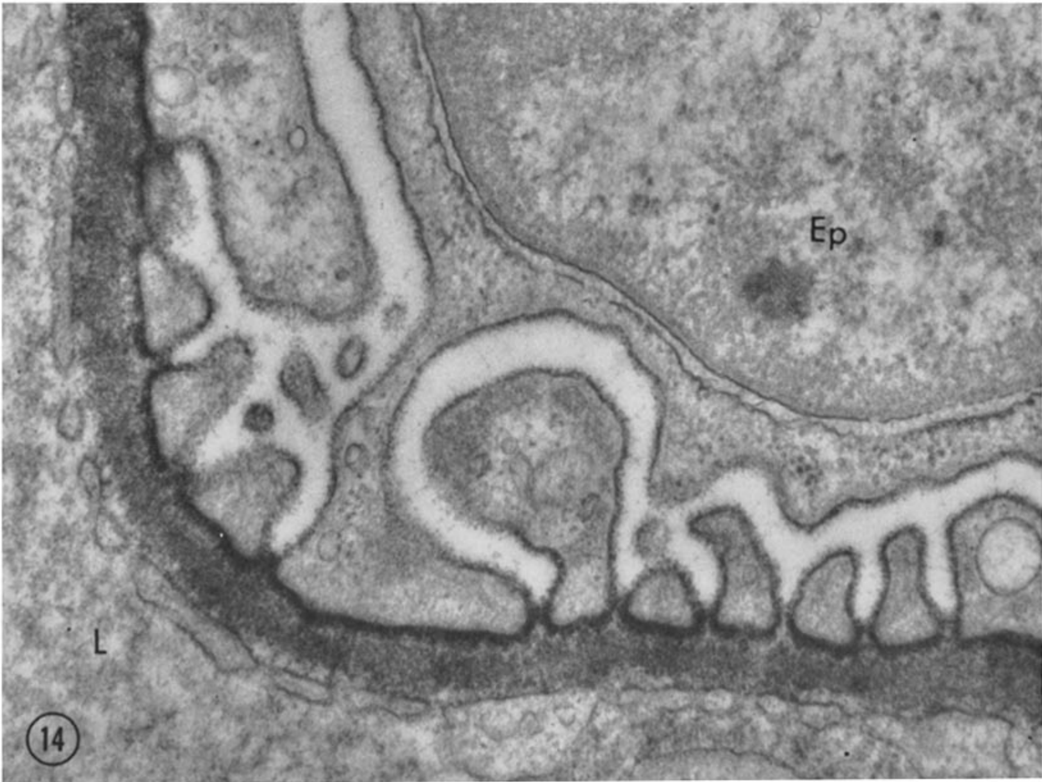


(Graham and Karnovsky: Glomerular permeability)

Plate 119

Fig. 14. Electron micrograph of kidney from mouse killed 20 min after MPO injection. Some granular reaction product is present in the lumen (*L*). Reaction product, present throughout the basement membrane, is concentrated adjacent to the foot processes, and in the epithelial slits. An epithelial cell body (*Ep*) is in the upper right. × 41,000.

Fig. 15. Electron micrograph of glomerulus from mouse killed 20 min after injection of MPO. The basement membrane (*B*) contains much reaction product throughout its thickness, but concentration at the junction between the basement membrane and the epithelial foot processes, and in the epithelial slits (arrows) is clearly seen. A spongy area (*S*) contains reaction product. No reaction product is apparent within the epithelial cells (*Ep*) or the mesangial cell (*M*). × 41,000.


Ep
L
14

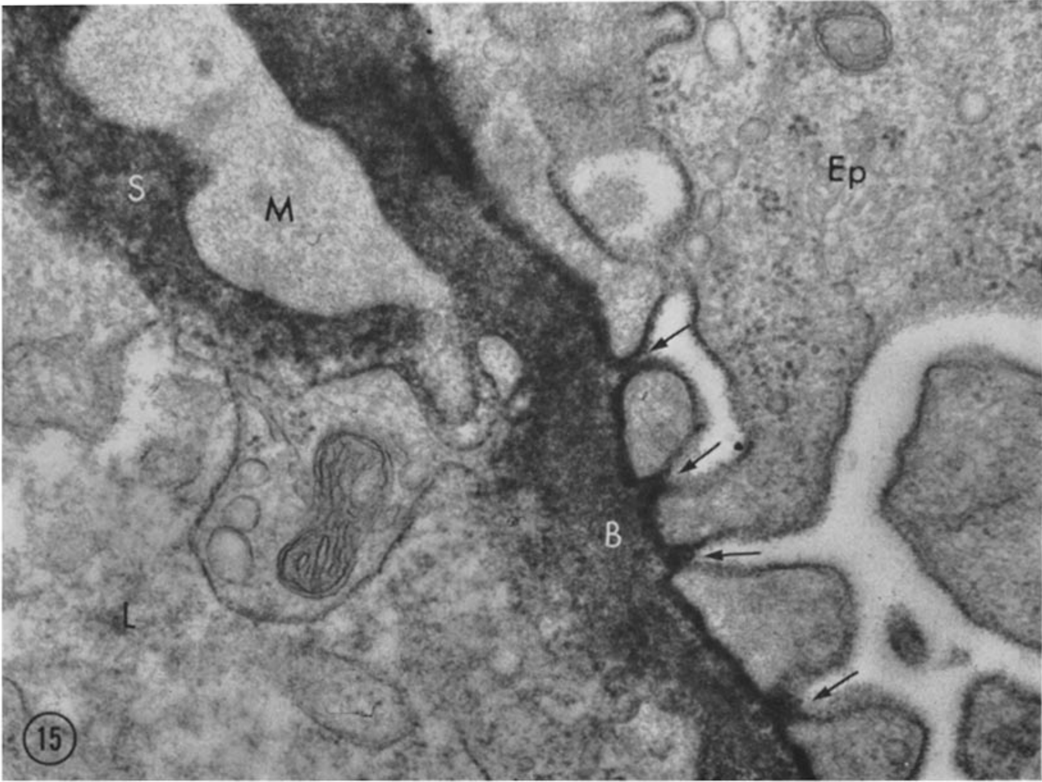
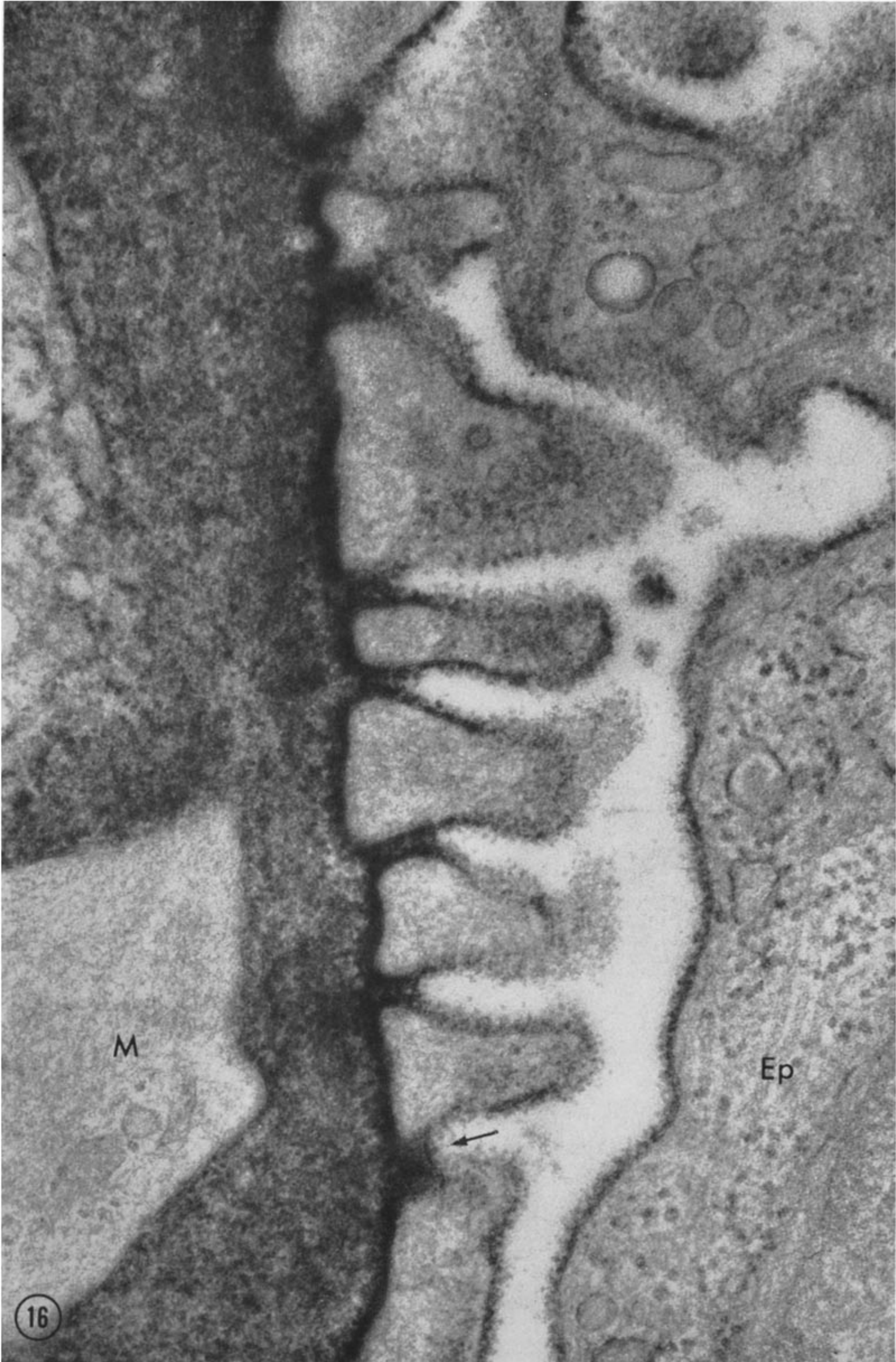

S
M
Ep
B
L
15

PLATE 120

FIG. 16. Electron micrograph of glomerulus from mouse killed 20 min after MPO injection. The concentration of reaction product adjacent to the epithelium, and in the epithelial slits is seen here at higher magnification. The accumulation of reaction product in one of the slits (arrow) appears to stop at the level of the slit membrane. Some reaction product is present on the membranes of the epithelial cell (*Ep*) A portion of a mesangial cell (*M*) is at the lower left. × 88,000.


M
Ep
16