TRANSPORT OF GLOBIN BY THE RENAL GLOMERULUS*

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(Received for publication, February 4, 1964)

Two generally different concepts of glomerular fluid production have been offered by various investigators. The idea of ultrafiltration based on hydrostatic pressure is exemplified by the work of Pappenheimer (1) who presents a model of passive filtration through capillary walls which are visualized as consisting of relatively rigid three-dimensional pores to which Poiseuille's law of bulk flow may be applied. A somewhat different model is exemplified by the study of Chinard, Vosburgh, and Enns (2) whose experimental results support a concept of diffusion as the primary mode of glomerular fluid production, and who state that an attempt to explain their results by the filtration hypothesis is a thermodynamic impossibility.

The term "filter" has usually been applied to whatever barrier exists between the contents of the glomerular capillaries and Bowman's space. Farquhar (3) and Farquhar, Wissig, and Palade (4) observed that the basement membrane "... is probably not a simple sieve but presumably is a gel-like structure with two fine fibrillar components embedded in an amorphous matrix." This description is in general agreement with our present findings with the additional notation that the fibrillar components are probably not permanent structures but are only the fixed representatives of a transient interaction between protein molecules making up the gel structure. Rhodin (5) postulates that the diaphragms sealing the fenestrations in endothelial cells act as a filter which can regulate the size of molecules passing through. This would account for the ultrafiltration effect described by Pappenheimer (1) with the limitation of particle size being provided by the structural "pores" of the membrane making up the diaphragm. As Rhodin (5) points out, this would take much of the burden of particle size determination from the "... structurally and functionally ill-defined basement membrane of the capillary."

The deficiency of all the mathematical and physiological models is that they

* This work was supported by an award from the United States Public Health Service, National Institutes of Health AM 01393.

must necessarily oversimplify the structural concept of the capillary wall because the experimental techniques do not permit measurement of rates of movement within the individual component parts of the wall. Although diffusion is a reasonable mechanism for passage of material through the basement membrane, the transport of substances through endothelial cells is likely to be influenced by additional factors. The movement of relatively small molecules in the glomerulus has been studied by electron microscopy with ferritin as a model protein (4) and thorotrast as a small colloidal particle (6). In the present study we have used large protein particles in the form of aggregated globin, and have noted that it, in some ways, is handled differently than the smaller molecules.

Materials and Methods

Female Sprague-Dawley rats weighing 110 to 160 gm were given intravenous injections of 0.75 gm of human globin (see appendix for preparation method) per kg of body weight. These rats were pretreated by various combinations of diet and water restriction or water loading, because they were used in experiments designed to elucidate the mechanism of acute renal failure. Other rats, as control animals, were injected without pretreatment. There was no difference between the experimental and control animals in the appearance of the various components of the glomerulus during globin transport; therefore, the descriptions presented here will be of the control animals.

The animals were anesthetized with ether and the kidneys were perfused *via* the renal artery with a fixative made as follows: (percentages are weight/volume) 2 per cent osmium tetroxide, 2 parts; 7.3 per cent polyvinylpyrrolidone, 1 part; 4 per cent sodium nitrite, 1 part. To this mixture was added just sufficient phenol red indicator to give a color (yellow) and the solution was titrated with 0.01 N sodium hydroxide to a brick red color (pH 7.3). The pH remained constant for several days at room temperature. If the fixative turned yellow during fixation it was an indication of excess acid and fresh fixative was added. In that way the characteristic changes based on acid fixation were eliminated. Examination of thick sections by light microscopy showed that approximately half of the capillaries were perfused by this method and that they were located at random. Thus the fixative had access to all of the cells of the kidney with much greater predictability than is provided by dripping of fixative on the surface, and changes within tubules produced by handling the kidney were avoided (7). The times of taking the kidneys after globin were: 1, 5, 10, and 30 minutes, 1, 2, 3, and 6 hours. Also, two animals were injected with globin which had been labeled with tritium[1] and these were sacrificed at 1 and 2 hours. After perfusion the kidneys were removed, sliced into small segments, and placed in an excess of fixative for an additional 30 minutes. The tissue was then washed in isotonic saline, dehydrated in graded ethanol solutions, and embedded in epoxy resin. Sections were cut on an LKB ultrotome, stained with lead (8), and photographed with an RCA EMU 3F or 3G electron microscope.

For light microscopy, kidneys were perfused with buffered neutral isotonic formol, and embedded in paraffin. These sections were cut at 5 microns. Proteins were stained by the bromphenol blue method. Autoradiographs were made by dipping in Ilford nuclear research emulsion L-4 diluted in the proportion of 10 gm emulsion to 20 ml distilled water (9). Exposure was optimum at 8 days with this procedure.

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[1] The preparation of globin-H^3 was carried out by the New England Nuclear Corporation, Boston. Crude globin was exposed to 3 c of tritium for 3 days at 0°C (Wilzbach procedure). The labile tritium was removed during the subsequent purification steps performed in our laboratories.

Electron microscope autoradiographs were made by applying a gelled film of Ilford L-4 emulsion directly from a loop to sections mounted on grids according to the method of Caro and van Tubergen (9). Maximum activity was reached at about 6 weeks.

OBSERVATIONS

When the purified globin used in these experiments is diluted in either distilled water or defibrinated rat plasma and warmed to 37°C, it forms a colloidal suspension which gives the solution a cloudy appearance and then progressively precipitates out of solution (also see Appendix). A similar aggregation seems to occur *in vivo* (Figs. 1 and 7). Light microscopy of sections stained by bromphenol blue demonstrates protein droplets in various positions in the walls of glomerular capillaries and in the stalk region (Fig. 1) and autoradiography at the light microscope level following tritiated globin injection demonstrates activity in the same distribution as the protein droplets (Fig. 2). Small spherical bodies of similar or smaller size, and of similar distribution are seen by electron microscopy and electron microscope autoradiography (Figs. 4, 8 to 11).

The electron density of globin aggregates after osmium tetroxide fixation is of the same order as that of erythrocytes, but there are some distinctive differences. Generally the aggregates have an irregular outline and may appear less homogeneous than erythrocytes (Figs. 3, 5, 7, and 8). The globin aggregates are not found to reach the size of erythrocytes although a section through a process of an irregularly shaped erythrocyte may provide a profile no larger than a globin aggregate. In this latter instance the criteria mentioned above are usually enough to distinguish between the two. There are thus three different ways of confirming the identification of globin *in situ*: (*a*) aggregates of a distinctive electron microscopic appearance are present after globin injection and are not present in control animals; (*b*) droplets which are stainable with protein stain are present in the injected animals but not in controls and they occupy the same position as the aggregates seen in electron micrographs; (*c*) when tritium-labeled globin is injected the resulting characteristic aggregates are seen to give positive autoradiographs in the light and electron microscopes.

The aggregated globin (hereafter called simply globin) is taken up by glomerular endothelial cells and is seen within the cells in small packets, each bounded by a membrane (Figs. 7 to 11). Dissolved globin is not identifiable within the glomerular structures so that observations on its passage must be confined to the visible aggregates. However, it may be assumed that globin also passes through the glomerulus in solution because it is to be seen in a dispersed form in the various portions of the tubule (10). Fig. 10 illustrates a second mechanism for transport through the endothelial cell. In this instance, the globin is seen within the interconnecting vesicles of irregular size and shape which seem to form channels at least part way through the cell. That these are not surface views of either fenestrae or pinocytotic vesicles can be ascertained from the following considerations: (*a*) fenestrae are regular in distribution and do not intercommunicate whereas the vesicles illustrated are irregular and inter-

connect; (*b*) surface views of pinocytotic vesicles and fenestrae must demonstrate a tangential cut of the cell membrane at some point because of the curvature of the cell (see Fig. 22 for example) whereas the cell membrane on both sides of the vesicles in question are sharp and thus must be cut transversely. The fact that the globin does not conform to the shape of these irregular vesicles suggests that it is there incidentally rather than as a packet being transported. A third type of transport is seen in the attenuated part of the endothelial cell and may be thought of as a modified phagocytosis in which the cytoplasm has begun to engulf a particle as in normal phagocytosis but because the particle diameter is so much greater than the thickness of the cytoplasm, the particle is extruded on the distal side before the lumen side has been able to reseal itself (Figs. 10 and 14). A fourth type of transport consisting of movement through fenestrae is sometimes seen, and it may involve either large or small particles. Fig. 14 illustrates a particle which separates the endothelium from the basement membrane and thus must have been engulfed by modified phagocytosis, this may be contrasted with Fig. 19 which illustrates a particle indenting the endothelium in the region of a fenestration. Forcing a particle through a fenestration would result in disruption of the diaphragm but would leave the endothelium still in contact with the basement membrane. Smaller particles are also sometimes seen in the region of fenestrations (Figs. 19 to 22). In a few places very small particles are seen in the basement membrane near an intact diaphragm (Figs. 20 and 21).

Whenever present in the endothelial cell, the globin droplet is not appreciably deformed from a sphere. At the distal border of the cell it is discharged directly into the basement membrane (Figs. 13 and 14) and still remains spherical, but is now without an enveloping membrane. As the globin traverses the basement membrane, it is either spherical or slightly flattened in a plane tangential to the basement membrane itself. (Figs. 7, 8, 11 to 13). No indication of stress or deformation in the basement membrane material is ever observed.

After traversing the basement membrane, the globin either passes directly through the space between the foot processes of the epithelial cells (Figs. 7, 15 to 17, and 19), or is seen to be piled up beneath the foot processes (Figs. 11, 14 to 16). Although the globin droplets may occasionally be seen to indent the foot processes on their basal side (Figs. 14 and 15) they have never been seen to be incorporated into the epithelial cells. As the globin passes between the foot processes it is elongated in the direction of flow (Figs. 6, 15 to 17), and during this passage the foot processes are pushed farther apart than their normal spacing. The slit membrane (11) is displaced from its normal position near the basement membrane by the passage of globin (Figs. 15 to 17) and presumably is eventually disrupted. The original relationship between epithelial cells is apparently reestablished after the passage of globin between them because very few distortions are seen except at the points where globin is obviously present (Fig. 18 shows what may be a recently disrupted slit membrane). The globin

which is originally seen beneath the foot processes eventually passes along the base near the basement membrane and makes its way between the foot processes just as described above (Figs. 11 and 16). After the globin has passed between the foot processes it becomes free in Bowman's space and resumes its original spherical form (Fig. 18).

In addition to the more or less direct path through the endothelial cells, basement membrane, and between the foot processes, the globin is also seen within the sponge fibers of the stalk (12) (Figs. 3, 5, 7, 8, 11, and 12) and is found occasionally within the stalk cells (Figs. 7 and 8). By the end of 6 hours after injection very little globin remains in the stalk region, and no further evidence of it is seen in either the capillary lumen or the structures of the glomerulus.

DISCUSSION

Particles of globin pass from the glomerular capillaries into the endothelial cells. The mechanism appears to be similar to that of erythrophagocytosis reported by Essner (13) except that no obvious thickening of the plasma membrane at the point of contact with the particle is noted. A less commonly observed path through the endothelial cell is related to a system of channels of interconnecting vesicles into which the globin is taken. These channels also exist in the absence of globin and may correspond to the "juxtacollicular vesicles" of Yamada (11). Whatever the nature of these channels may be, the globin does not conform to their contours and thus appears to be present incidentally to the existence of the channels. Globin was infrequently seen in various stages of apparent transport through fenestrations. Some of the smaller particles have a loose organization which is different from the more compact appearance of larger particles. The irregular density of the small particles could possibly reflect the first stage of aggregation (Figs. 20 and 21). Since these loosely arranged aggregates are seen on the basement membrane side of intact diaphragms, it is possible that the globin has passed through the diaphragms in a dissolved form and has only begun to aggregate after passage. It is also possible that the diaphragms have suffered small ruptures which were quickly repaired.

After traversing the endothelial cell, the globin is discharged into the basement membrane by a process similar to that of secretion (14, 15) in which the membrane surrounding the particle fuses with the plasma membrane and the particle is then expelled while the original surrounding membrane remains as a continuous part of the plasma membrane. Several stages in this process are illustrated in Fig. 13. Although the characterization of purified globin is not complete, the available evidence strongly indicates that the flocculent precipitate produced *in vitro* by warming is paralleled *in vivo* after injection. Since the precipitate is not reversible we may also assume that the aggregates seen within fixed tissue represent true particles rather than a transient, local concentration difference. Such particles would maintain their integrity throughout any inter-

action with cells or basement membrane and although they might be flexible they would be unlikely to disintegrate. There is no apparent physical deformation of the basement membrane material caused by the entry or transit of particles of globin. The molecular structure most consistent with this behavior is that of a thixotrophic gel; in its undisturbed state it is relatively stable and maintains its physical contour, but it liquifies in any localized region where a critical pressure is exceeded. Gels in general are made up of long chain molecules which have various types of intermolecular bonding. The term *micell* has been applied to the regions of ordered aggregation of several long molecules in a gel, and it is known that the tendency to form micells is increased during fixation and dehydration (16). It seems most likely that fibrils seen within the basement membrane represent the fixed representatives of highly labile micells which existed in the original gel.

Other observers have presented evidence for the passage of small dimension particles (100 to 105 A) through the basement membrane without deforming it (4, 6, 17); we have now demonstrated that this is also true for particles of much larger dimension. The movement of large particles through the basement membrane with apparent ease is not surprising because leukocytes and erythrocytes are commonly known to do this, and they leave no apparent defect in the membrane. It has also been demonstrated that sparingly soluble Bence Jones protein injected intravascularly is rapidly excreted in the urine (18). The accumulation of ferritin particles on the endothelial side of the basement membrane and their subsequent slow movement through it (4) may be explained in several ways: (*a*) this could occur if the membrane were a meshwork of relatively stable fibrils of molecular dimensions such as a filter; (*b*) the interaction of a charged gel such as a chromatographic column with charged particles would produce a layering such as that observed; (*c*) uncharged particles with a low diffusion coefficient migrating through an uncharged gel would also give results identical with those observed with ferritin. The fact that no deformation is observed with the transit of either small molecules such as ferritin or larger particles such as aggregates of globin suggests that the first possibility is unlikely and that one or both of the latter two is more consistent with the facts.

That the basement membrane offers little resistance to the passage of globin is demonstrated by the fact that the globin droplets are not deformed during their passage through it. That the globin is not in rigid spheres is seen by the fact that they are flattened while traversing the space between foot processes (compare Figs. 12 and 13 with Figs. 15 to 17). Conversely the droplets are not completely fluid because the foot processes are pushed apart to some extent by their passage. It is impossible to state if the globin is passively carried along in the general direction of flow, or if it is moved between the foot processes by a sweeping or milking motion of the cell membranes covering these processes.

The mode of attachment of one foot process to another in the region of the basement membrane has been considered to be by a "desmosome like structure

(4)," or by a "filtration slit membrane (9)." Hall (19) thought that the foot processes were contiguous in the living state and did not consider the problem of attachment. The fact that globin displaces an obvious membrane (Figs. 15 to 17) when it goes through the space between foot processes supports Yamada's interpretation (9). Rhodin (5) notes that the structure of the slit membrane most closely resembles that of the capillary diaphragm, and our observations are in accord with this concept. Lateral displacement of foot processes from each other are rarely seen except during actual passage of globin, and from this we conclude that the normal relationships are rapidly reformed.

Some other materials which pass through the glomerulus are taken up by the epithelial cells, presumably by pinocytosis (4, 17, 20, 21). When globin comes in contact with the basement membrane side of foot processes, it may indent the cell membrane (Figs. 14 to 16), but the epithelial cells are not found to incorporate it.

A small quantity of globin may be taken up by the stalk cells, but the greatest bulk of that portion passing through the stalk region goes through the sponge fibers and basement membranes. Thorotrast has been reported to follow a similar course (6). The globin eventually passes out of the stalk region from both intra- and extracellular loci so that only a small amount remains at 6 hours after injection. The apparent ease of movement of globin and Thorotrast through the basement membrane and sponge fiber material adds support to the concept of gel structure outlined above.

SUMMARY

Purified human globin injected into rats forms aggregates which are identifiable by their characteristic appearance in thin sections in the electron microscope and by their positive autoradiographs when the globin is tritiated before injection. Globin is taken up by endothelial cells of glomerular capillaries and is transported across the cell within the limits of a surrounding membrane. Globin is rarely seen to pass through fenestrations. Globin is also taken into the stalk region where it is seen usually within the sponge fibers and only occasionally within stalk cells. Globin is seen in all stages of passage through the basement membranes and sponge fibers, which are not deformed by its passage. On the basis of the findings presented here and by others, it is postulated that the basement membrane and sponge fibers consist of a thixotrophic gel.

After traversing the basement membrane, the globin passes between foot processes of the epithelial cells. The slit membranes are deformed by this passage and thus appear to be distinctive structures. The globin is next found free in Bowman's space; the earliest aggregates are seen there within 1 minute after injection.

Globin taken up in the stalk region is slowly discharged and very little is found there 6 hours postinjection.

APPENDIX

Globin preparation according to A. D. Mason (personal communication) as modified from the method of Anson and Mirsky (22).

Outdated human red blood cells obtained from the University Hospital, Syracuse, blood bank were washed and hemolyzed by alternate freezing and thawing followed by extraction of the stroma with toluene. The solution was diluted to 6 to 7 gm per cent and slowly mixed with 10 volumes of acetone to which 1.15 ml of concentrated HCl per liter of acetone had been added. After standing 15 minutes, the precipitated globin-HCl was washed to remove the liberated heme and then dried in a vacuum desiccator overnight. The precipitate was next dissolved in water and the solution slowly neutralized with 0.2 N NaOH until the first permanent precipitate formed. After standing 15 minutes, further NaOH was added until maximum precipitation took place, approximately at neutrality. The insoluble, denatured globin was removed by filtration and the filtrate brought to 40 per cent saturation with $(NH_4)_2SO_4$ and again filtered to remove any remaining denatured protein. The resultant filtrate was brought to 70 per cent saturation with $(NH_4)_2SO_4$ to precipitate the native globin which was filtered and washed. This was dissolved in water, and the solution was dialyzed against distilled water for 48 hours and then against normal saline for 24 hours to prepare it for injection.

The resulting protein concentration varied from 48 to 65 mg/ml as measured by the biuret method (23); the contaminating hemoglobin never exceeded 0.6 mg/ml as measured by the method of Hunter *et al.* (24). The globin was heat-labile, so the entire procedure was carried out at 1°C. The globin solution was stored in a deep freeze and always used within 2 weeks. Just prior to injection, the globin was thawed in cold water and the syringes used for injection were chilled.

Characteristics of Globin.—Paper electrophoresis at 0–2°C of the globin solution alone or mixed with rat plasma showed a single, relatively homogeneous band near the gamma globulin fraction, which migrated slowly at pH 8.6.

If globin solution was warmed to 37°C it became opalescent within a short time and then rapidly and irreversibly formed a flocculent precipitate. Several concentrations of globin in isotonic saline at pH 6.2 from 6 to 60 mg/ml were made up in the cold and then placed in a water bath at 37°C; the elapsed time before opalescence and flocculation was determined to be 4.5 to 14 minutes for the 6 mg solution, and 1.0 to 3.0 minutes for the 60 mg solution. It is thus obvious that increase in concentration increases the speed of aggregation of globin *in vitro*.

In the dosage given to our rats, the expected plasma concentration immediately after injection was about 10 mg/ml. While holding this concentration constant, the pH was adjusted with the following results.

pH	Opalescence time	Flocculation time
	min.	*min.*
8.1	0.25	6.5
7.5	0.75	5.0
7.3	0.75	6.0
6.1	1.50	16.0

It is thus seen that at normal blood pH the globin begins to aggregate in less than 1 minute *in vitro*. In order to examine this phenomenon *in vivo*, blood samples were taken from injected rats within 5 minutes after injection and the cells immediately centrifuged down; the plasma was opalescent at that time. Blood samples taken at 30 minutes after injection showed that the plasma had been cleared of globin as evidenced by electrophoresis (no globin band present) and lack of opalescence. The observations on the behavior of purified globin when injected into rats are in keeping with the findings of Strumia and Sample (25), who found that in their procedure for modifying globin to use as a blood expander in human patients, precipitates occurred *in vivo* if the modification was not complete enough; *i.e.*, if native globin remained in the injection solution.

BIBLIOGRAPHY

1. Pappenheimer, J. R., Passage of molecules through capillary walls, *Physiol. Rev.*, 1953, **33,** 387.
2. Chinard, F. P., Vosburgh, G. J., and Enns, T., Transcapillary exchange of water and of other substances in certain organs of the dog, *Am. J. Physiol.*, 1955, **183,** 221.
3. Farquhar, M. G., An electron microscope study of glomerular permeability, *Anat. Rec.*, 1960, **136,** 191.
4. Farquhar, M. G., Wissig, S. L., and Palade, G. E., Glomerular permeability. I. Ferritin transfer across the normal glomerular capillary wall, *J. Exp. Med.*, 1961, **113,** 47.
5. Rhodin, J. A. G., The diaphragm of capillary endothelial fenestrations, *J. Ultrastruct. Research*, 1962, **6,** 171.
6. Latta, H., and Manusbach, A. B., Relations of the centrolobular region of the glomerulus to the juxtaglomerular apparatus, *J. Ultrastruct. Research*, 1962, **6,** 562.
7. Manusbach, A. B., Madden, S. C., and Latta, H., Variations in fine structure of renal tubular epithelium under different conditions of fixation, *J. Ultrastruct. Research*, 1962, **6,** 511.
8. Dalton, A. J., and Zeigle, R. F., A simplified method of staining thin sections of biological material with lead hydroxide for electron microscopy, *J. Biophysic. and Biochem. Cytol.*, 1960, **7,** 409.
9. Caro, L. G., and van Tubergen, R. P., High-resolution autoradiography. I. Methods, *J. Cell Biol.*, 1962, **15,** 173.
10. Menefee, M. G., Mueller, C. B., Miller, T. B., Myers, J. K., and Bell, A. L., Experimental studies in acute renal failure. II. Fine structure changes in tubules associated with renal failure induced by globin, *J. Exp. Med.*, 1964, **120,** 1139.
11. Yamada, E., The fine structure of the renal glomerulus of the mouse, *J. Biophysic. and Biochem. Cytol.*, 1955, **1,** 551.
12. Jones, D. B., Mueller, C. B., and Menefee, M. G., The cellular and extracellular morphology of the glomerular stalk, *Am. J. Path.*, 1962, **41,** 373.
13. Essner, E., An electron microscopic study of erythrophagocytosis, *J. Biophysic. and Biochem. Cytol.*, 1960, **7,** 329.

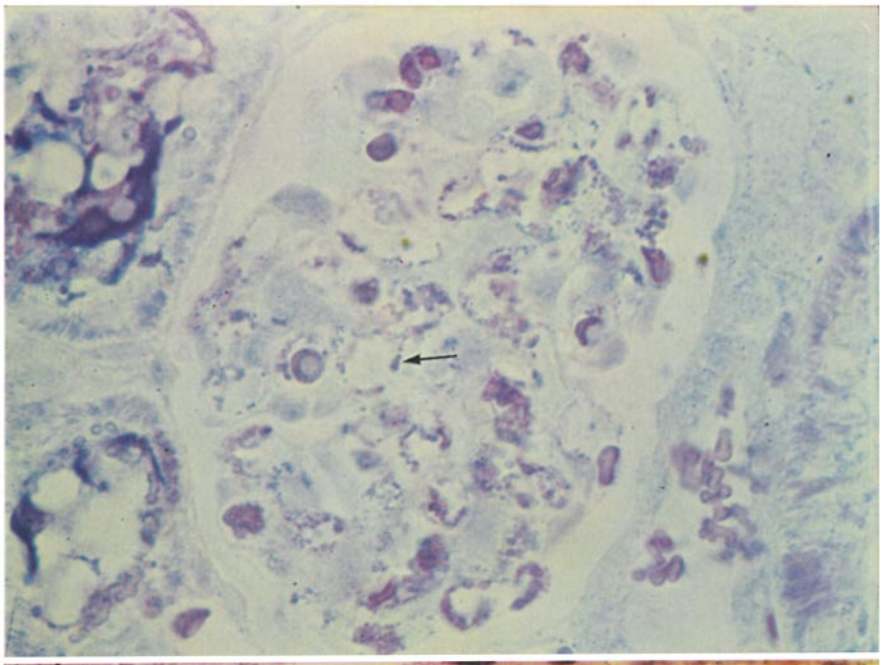
14. Palade, G. E., Functional changes in the structure of cell components, *in* Subcellular Particles, (T. Hayashi, ed.), N. Y., The Ronald Press, 1959, 64.
15. Parks, H. F., Morphological study of the extrusion of secretory materials by the parotid glands of the mouse and rat, *J. Ultrastruct. Research*, 1962, **6**, 449.
16. Frey-Wyssling, A., Submicroscopic Morphology of Protoplasm and Its Derivatives, New York, Elsevier Publishing Co., Inc., 1948.
17. James, J. A., and Ashworth, C. T., Some features of glomerular filtration and permeability revealed by electron microscopy after intraperitoneal injection of dextran into rats, *Am. J. Path.*, 1961, **38**, 515.
18. Meyer, F., and Putnam, F., The fate of injected Bence-Jones protein, *J. Exp. Med.*, 1963, **117**, 573.
19. Hall, V., The protoplasmic basis of glomerular ultrafiltration, *Am. Heart J.*, 1957, **54**, 1.
20. Farquhar, M. G., and Palade, G. E., Segregation of ferritin in glomerular protein absorption droplets, *J. Biophysic. and Biochem. Cytol.*, 1960, **7**, 297.
21. Reger, J. F., Hutt, M. P., and Neustein, H. B., The fine structure of human hemoglobinuric kidney cells with particular reference to hyalin droplets and iron micelle localization, *J. Ultrastruct. Research*, 1961, **5**, 28.
22. Anson, M., and Mirsky, A., Protein coagulation and its reversal, *J. Gen. Physiol.*, 1930, **13**, 477.
23. Weischselbaum, T. E., An accurate and rapid method for determination of proteins in small amounts of blood serum and plasma, *Am. J. Clin. Path.*, 1950, **10**, 46.
24. Hunter, F. T., Grove-Rasmussen, M., and Soutter, L., A spectrophotometric method for quantitating hemoglobin in plasma or serum, *Am. J. Clin. Path.*, 1960, **20**, 429.
25. Strumia, M. M., and Sample, A. B., Modified globin. I. Method for preparation from human erythrocytes, *J. Lab. and Clin. Med.*, 1951, **37**, 959.

EXPLANATION OF PLATES

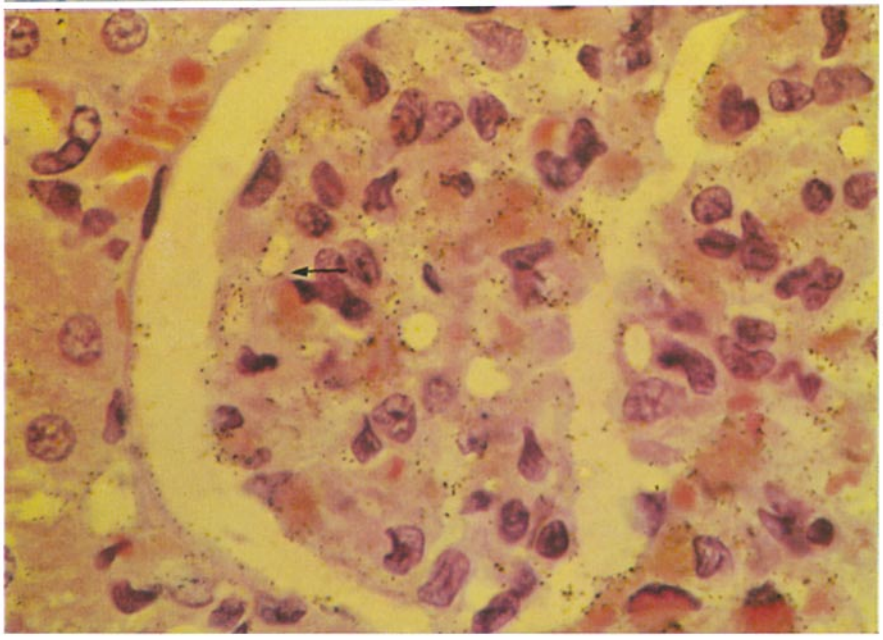
PLATE 108

FIG. 1. Kidney section taken 2 hours after injection of globin, stained with bromphenol blue and without counterstain. Droplets of protein are seen to be lined up around the periphery of many capillaries (one is at the arrow). Red blood cells as well as irregular aggregates of protein are seen in the lumens of several other capillaries. Oil immersion. × 1000.

FIG. 2. Kidney section taken 2 hours after injection of tritium-labeled globin. Exposed to photographic emulsion for 1 week and stained with hematoxylin and chromotrope. The radioactivity is seen to be concentrated in the same regions as the protein illustrated in Fig. 1. Arrow indicates a typical capillary with exposed grains overlying the periphery. Oil immersion. × 1000.



1



2

(Menefee *et al.*: Transport of globin)

PLATE 109

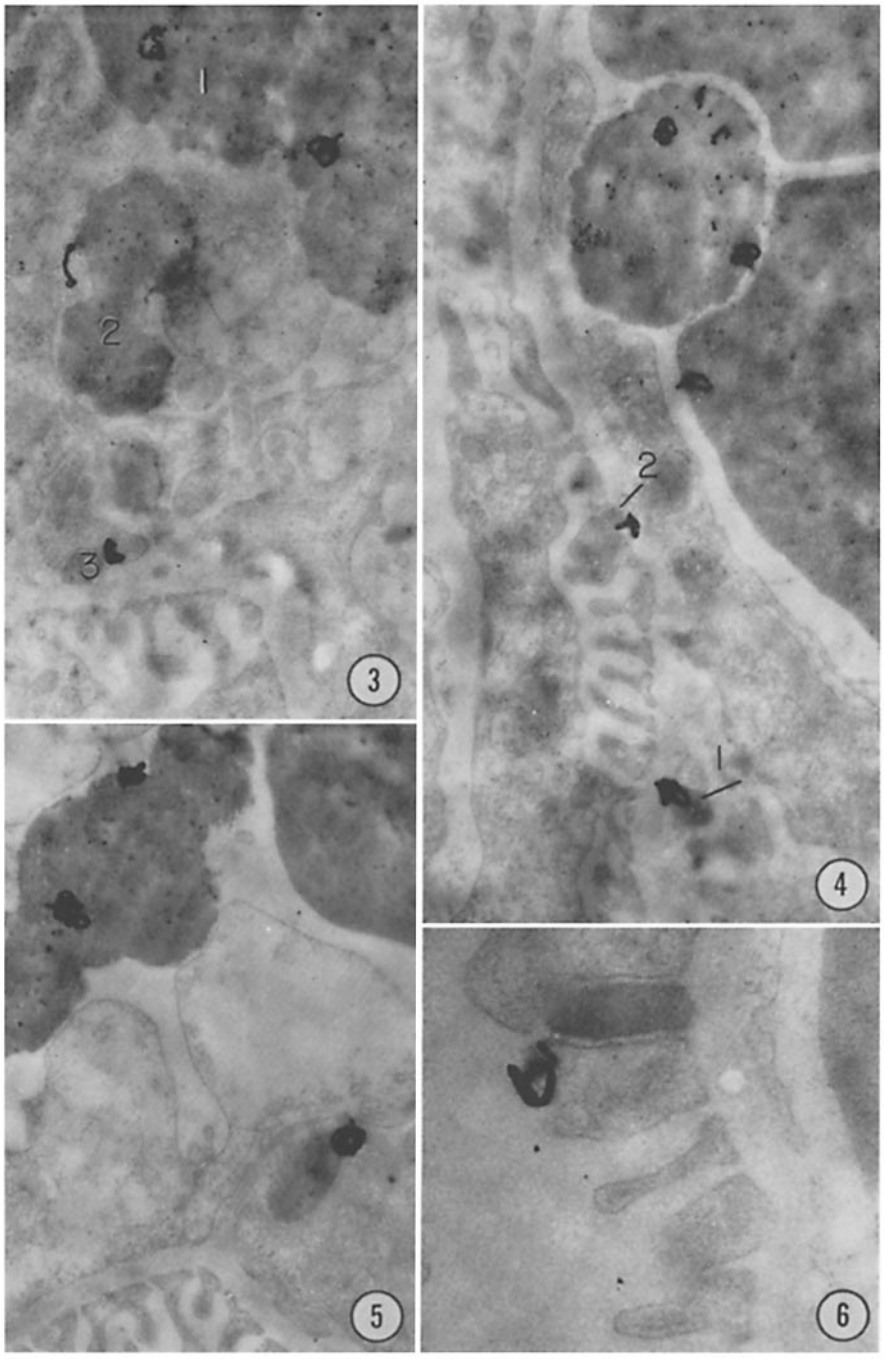
FIGS. 3 to 6. Glomeruli 1 hour after injection of tritiated globin. Electron microscope autoradiographs.

FIG. 3. Typical globin density, *1*, with overlying grains in the capillary lumen; globin in the stalk region, *2*; and globin partially within the basement membrane, *3*. × 17,000.

FIG. 4. Globin within capillary lumen at upper right. Arrow *1* at globin in stalk region; arrow *2* indicates globin between foot processes of an epithelial cell. × 14,000.

FIG. 5. Globin in capillary lumen at upper left, and in stalk region at lower right. × 23,000.

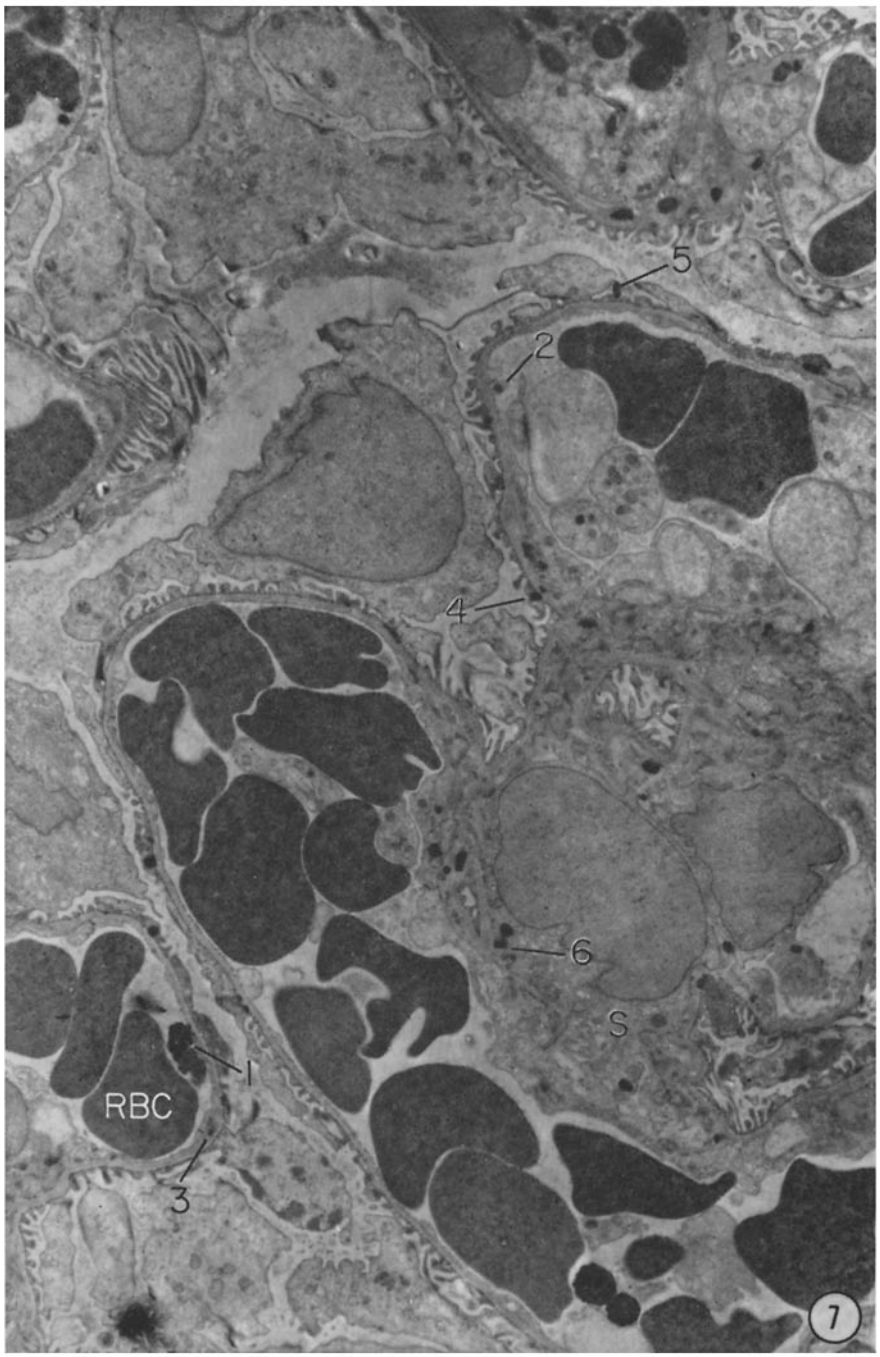
FIG. 6. Globin between two foot processes. × 43,000.



(Menefee *et al.*: Transport of globin)

PLATE 110

FIG. 7. Low power electron micrograph illustrating, at the numbered arrosw, several stages in transport of globin from the glomerular capillary lumen to Bowman's space. The appearance of globin within the capillary lumen is seen at *1* and may be compared to the appearance of red blood cells (*RBC*). At *2* globin is seen within an endothelial cell. Globin within the basement membrane is at *3*. *4* shows globin between epithelial cell foot processes, and *5* indicates globin free within Bowman's space. *6* is globin within a sponge fiber adjacent to a stalk cell (*S*). × 4000.

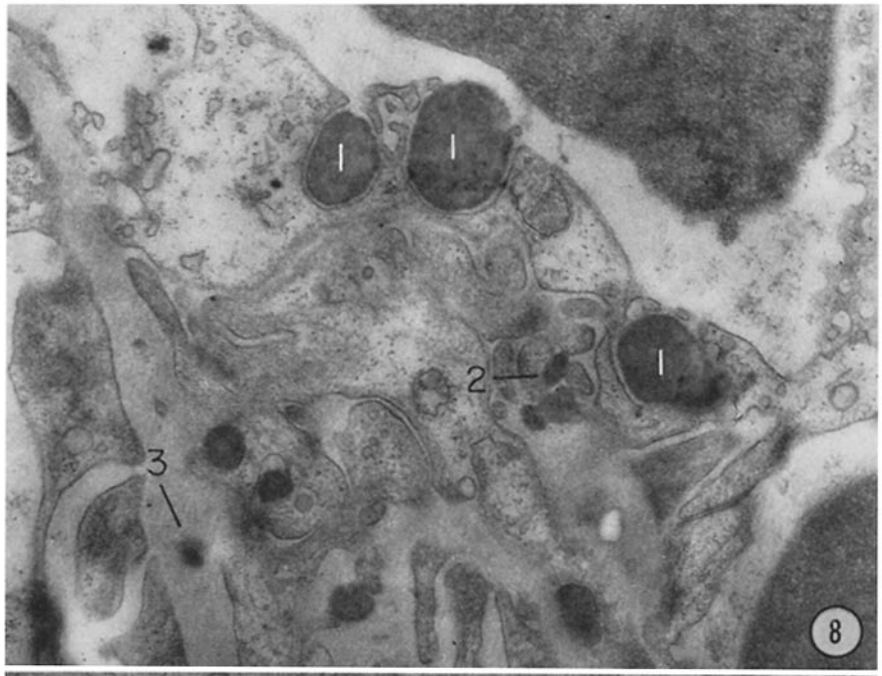


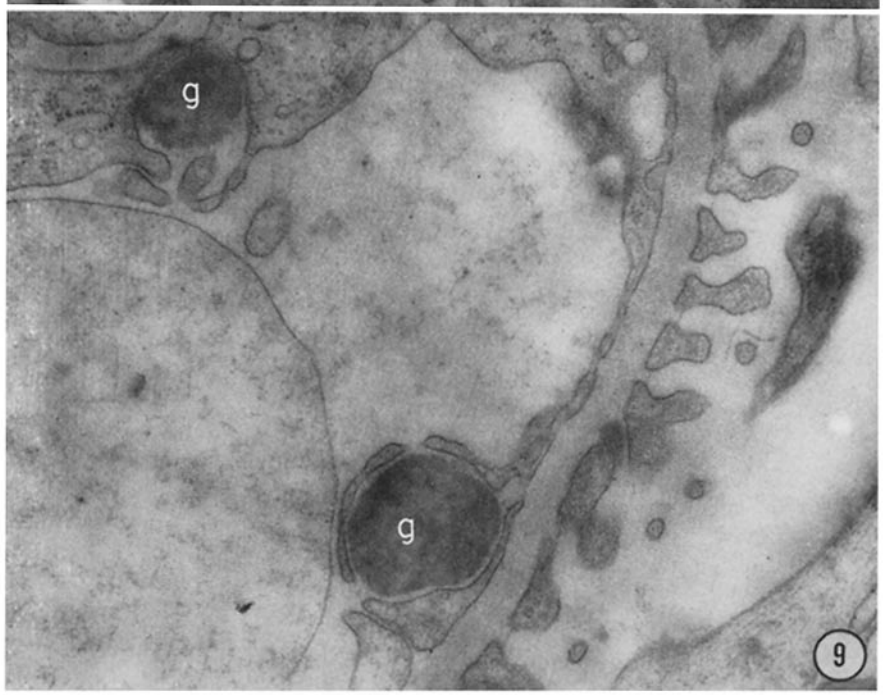
(Menefee *et al.*: Transport of globin)

PLATE 111

FIG. 8. Globin in various stages of uptake by endothelial cells is indicated at *1*. Several globin droplets are in the sponge fibers as seen at *2*. *3* indicates globin within the basement membrane. × 23,000.

FIG. 9. Two globin droplets (*g*) partially phagocyted by endothelial cells. × 34,000.



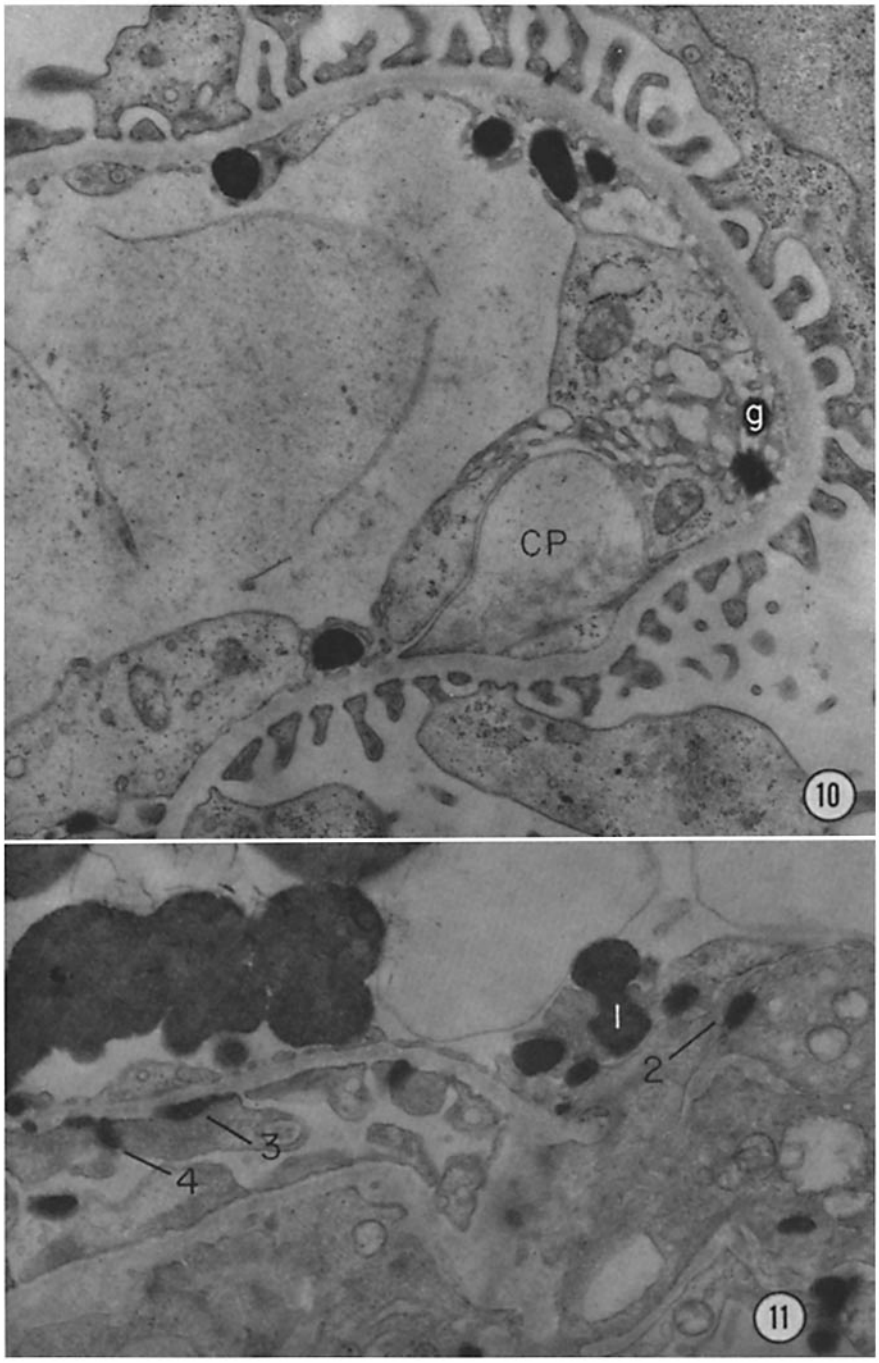


(Menefee *et al.*: Transport of globin)

PLATE 112

FIG. 10. Globin is seen in several positions within endothelial cells. A cytoplasmic probe (*CP*) is shown in relation to the juxtacollicular channels, within which globin (*g*) is seen. × 14,000.

FIG. 11. Various stages of globin transport in the glomerulus. A droplet is apparently being squeezed in the process of uptake by an endothelial cell, *1*. Globin is also seen within sponge fibers, *2*, and still other aggregates of globin are on the epithelial side of the basement membrane indenting the foot process, *3*, and between the foot processes, *4*. × 16,000.

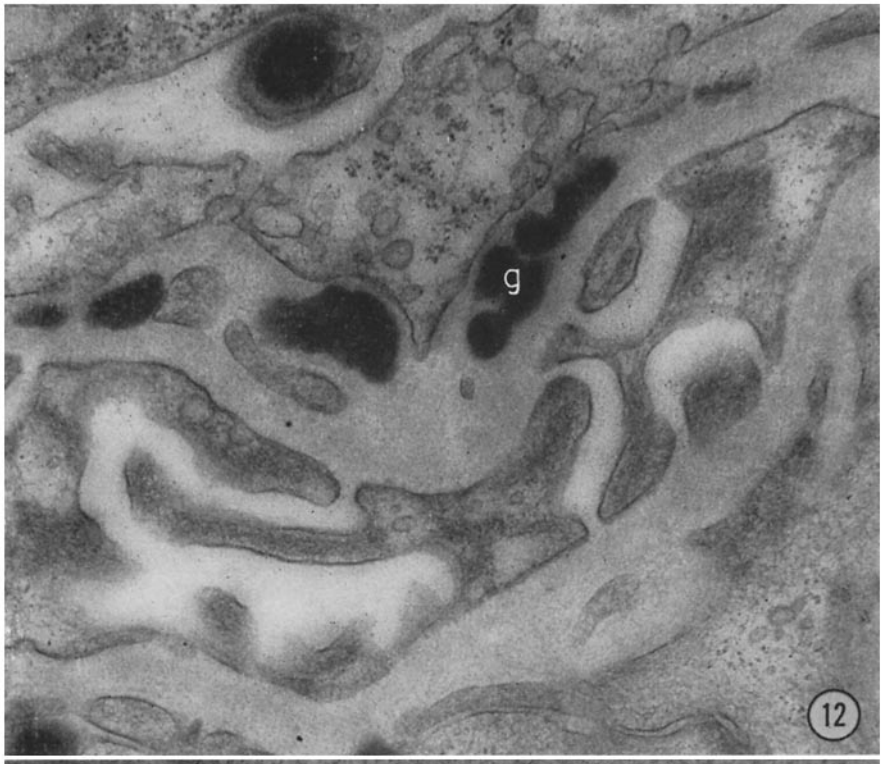


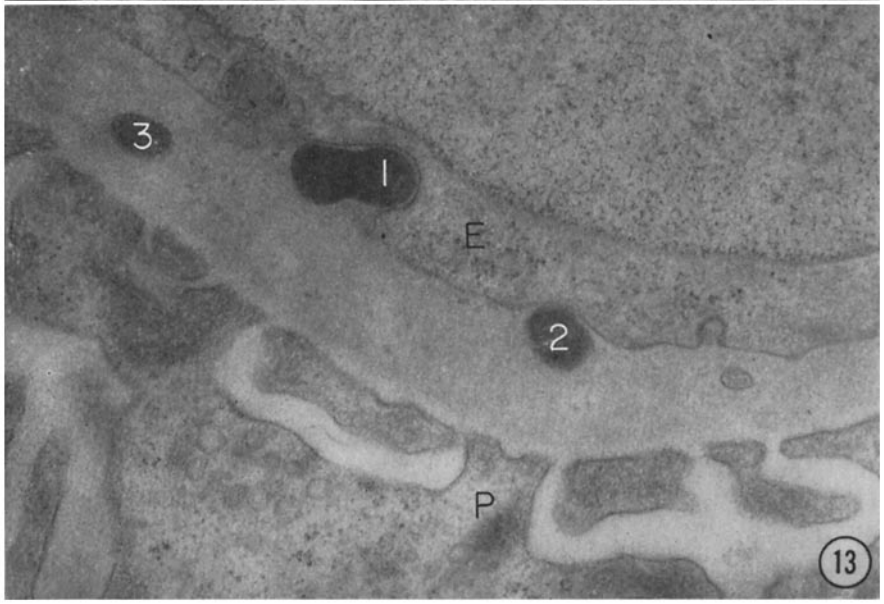
(Menefee *et al.*: Transport of globin)

PLATE 113

FIG. 12. Globin (*g*) within the basement membrane and connecting sponge fibers, neither of the latter show deformation. × 31,000.

FIG. 13. A logical sequence of globin transport from endothelial cell (*E*) into basement membrane is seen with *1* indicating a partially extruded particle, *2*, an almost completely extruded particle, and *3*, a particle part way through the basement membrane. A podocyte foot process is at *P*. × 40,000.



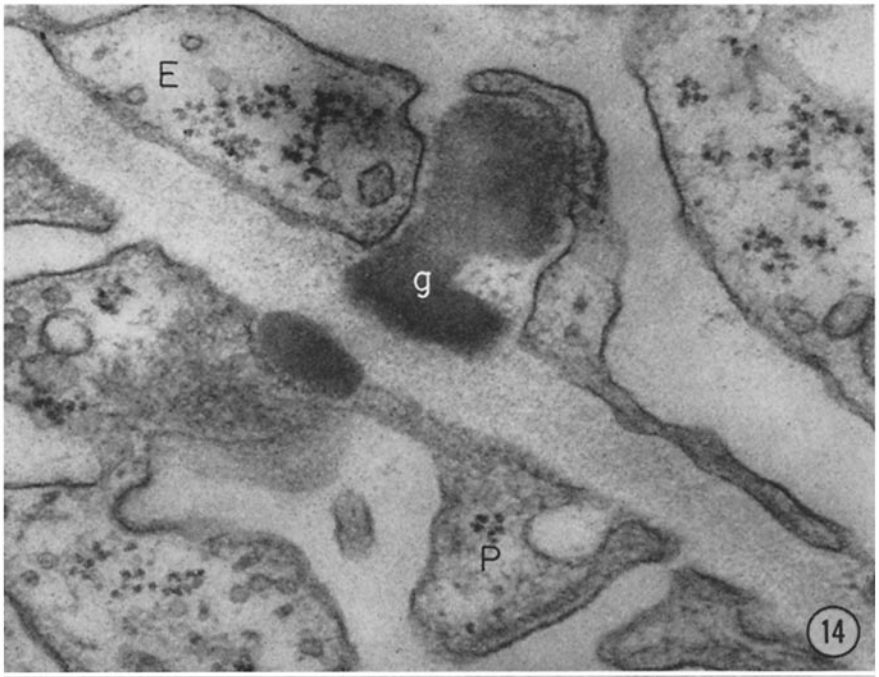


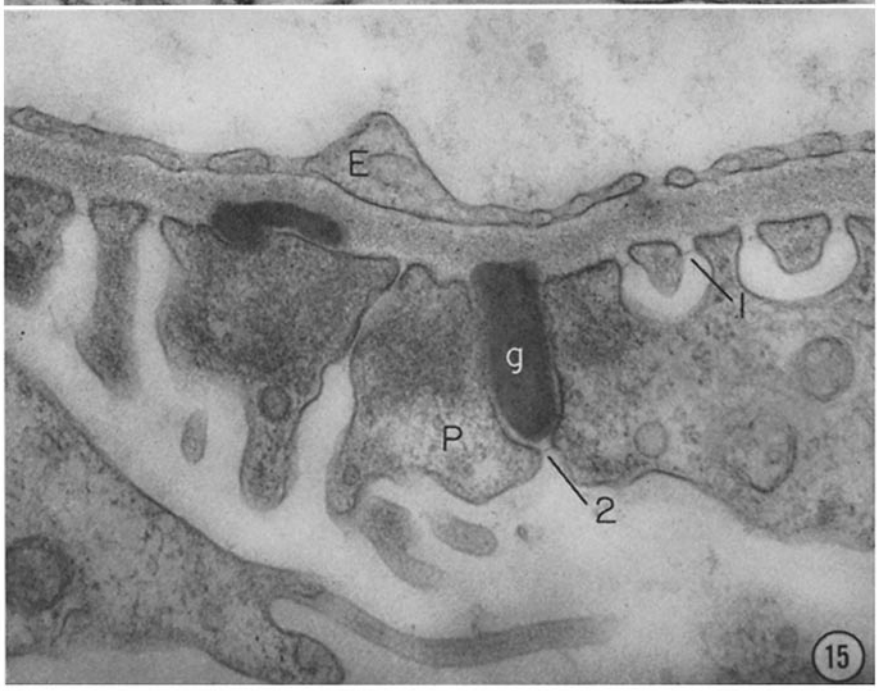
(Menefee *et al.*: Transport of globin)

PLATE 114

FIG. 14. An aggregate of globin (*g*) is seen partly surrounded by an endothelial cell (*E*) and partly within the basement membrane, while a smaller aggregate is indenting a foot process on the opposite side of the basement membrane. × 86,000.

FIG. 15. Globin (*g*) between two foot processes (*P*) displaces them laterally and displaces the related slit membrane, *2*, from its normal position, *1*, near the basement membrane. A second globin droplet is seen indenting a foot process on the side of the basement membrane away from the endothelium (*E*). × 43,000.





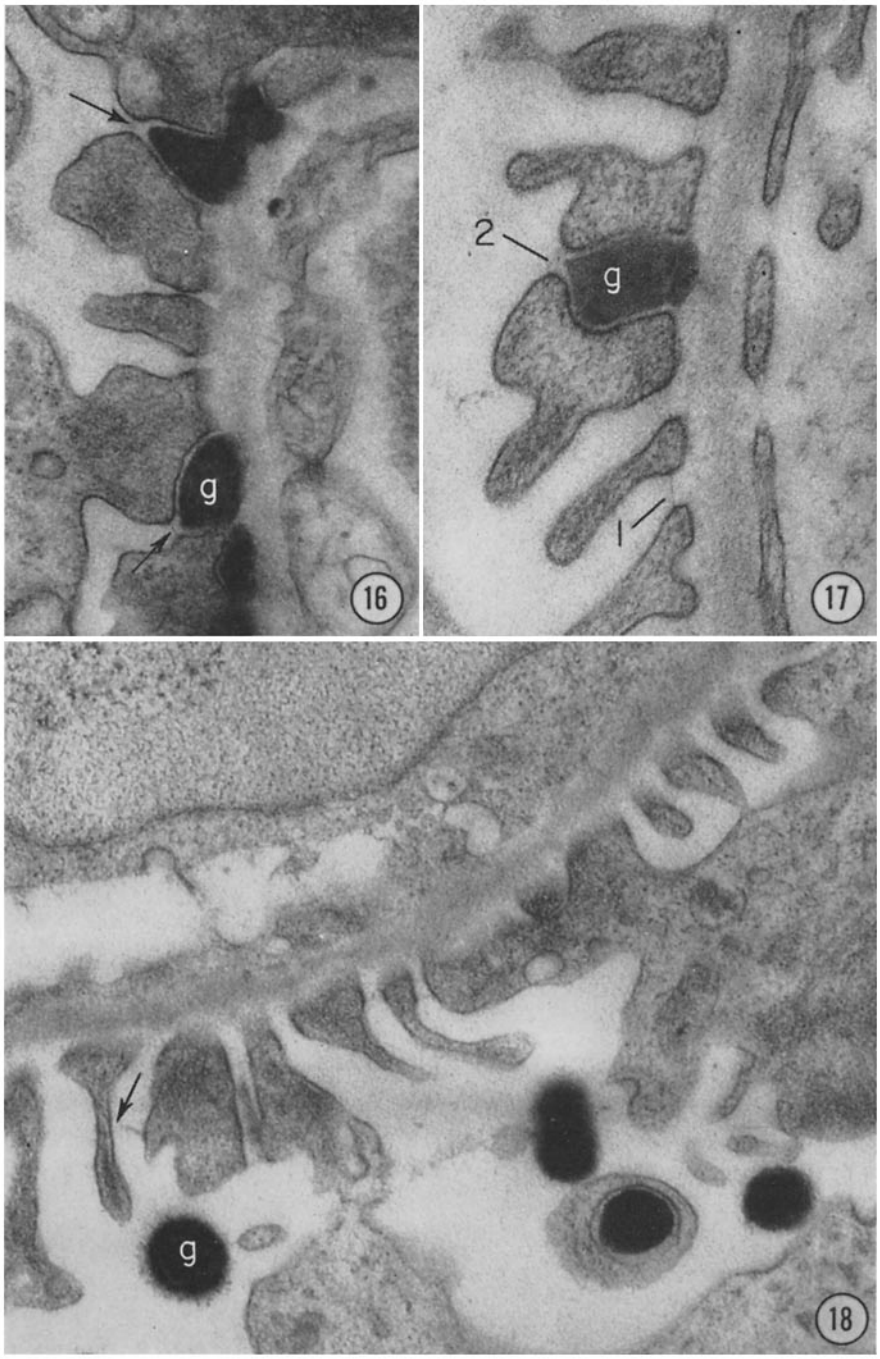
(Menefee *et al.*: Transport of globin)

PLATE 115

FIG. 16. Globin (*g*) between foot processes displacing the slit membranes (arrows). × 55,000.

FIG. 17. A comparison between the normal position of a slit membrane, *1*, and a slit membrane displaced by globin, *2*. × 63,000.

FIG. 18. Globin (*g*) is seen to be in the form of free droplets in Bowman's space. It is noted that they have resumed a rounded form in contrast to the somewhat flattened form seen in their passage between the foot processes. The arrow points to a possible rupture in a displaced slit membrane which could result from the passage of a globin aggregate. × 32,000.



(Menefee *et al.*: Transport of globin)

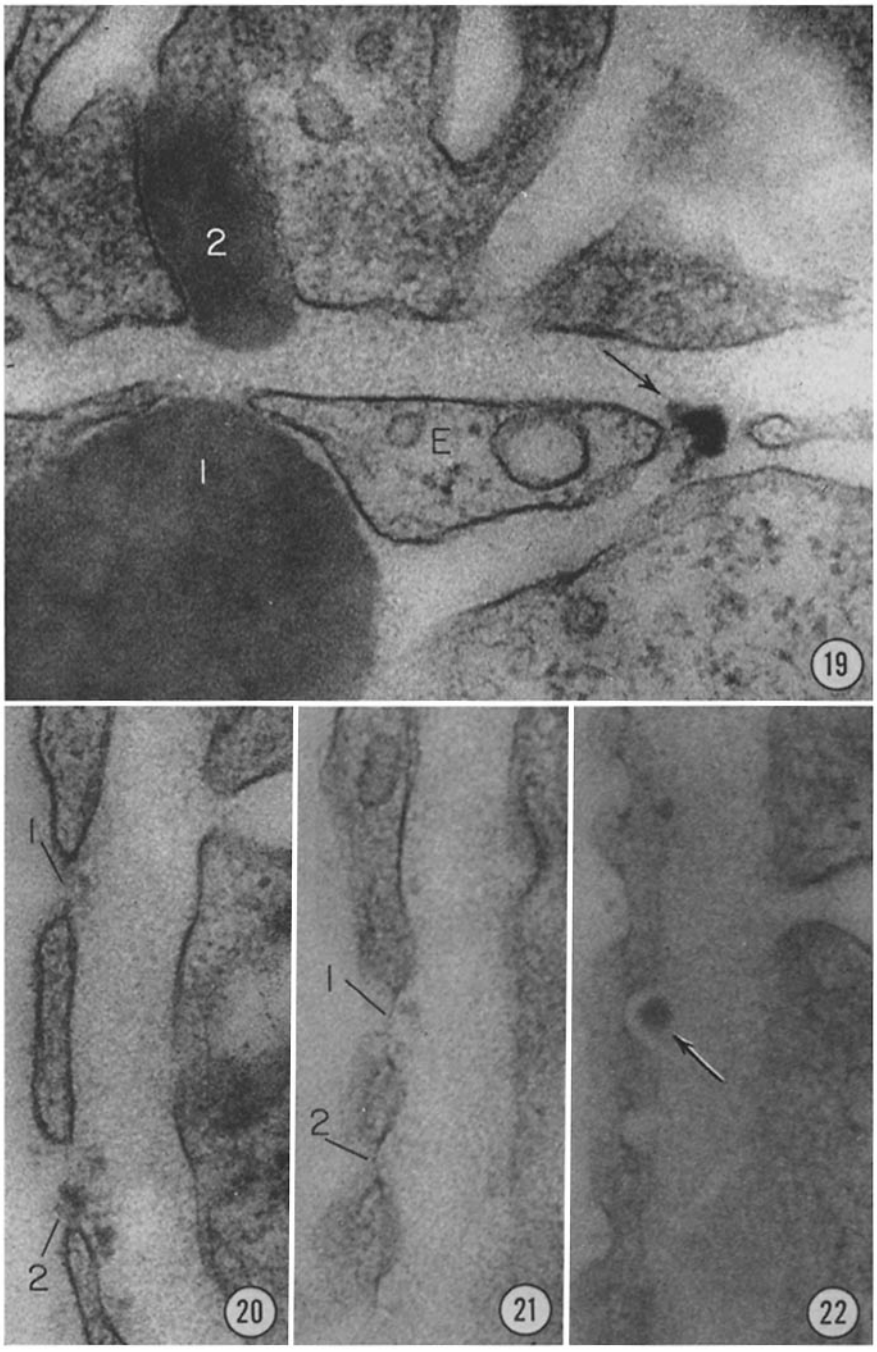
PLATE 116

FIG. 19. A globin aggregate, *1*, is in close contact with a fenestration in an endothelial cell (*E*) and another aggregate, *2*, is between foot processes on the opposite side of the basement membrane. The arrow points to a small particle of globin part way through a fenestration. × 104,000.

FIG. 20. Globin within the basement membrane near an intact diaphragm, *1*. A fenestration with no apparent diaphragm, *2*, has globin within it which also extends into the substance of the basement membrane. × 104,000.

FIG. 21. A fenestration with an intact diaphragm, *1*, has globin nearby within the basement membrane, while an adjacent fenestration with a similar intact diaphragm, *2*, has no globin. × 104,000.

FIG. 22. Tangential section of several fenestrations with globin (arrow) fitting the confines of one of them. The cell membranes of both the foot processes and the endothelial cell demonstrate the indistinct appearance characteristic of tangential sections. × 104,000.



(Menefee *et al.*: Transport of globin)