A STEREOREOLOGICAL STUDY OF THE
GLOMERULAR FILTER IN THE RAT

Morphometry of the Slit Diaphragm and Basement Membrane

STEPHEN M. SHEA and ASHTON B. MORRISON

From the Department of Pathology, College of Medicine and Dentistry of New Jersey, Rutgers Medical School, Piscataway, New Jersey 08854

ABSTRACT

Kidney from normal male albino rats, of body weight 170-200 g, was fixed by arterial perfusion with buffered tannic acid-glutaraldehyde, and postfixed with osmium tetroxide. Random and isotropic ultrathin sections from 23 different glomeruli from five rats were mounted on slot grids for staining and electron microscopy. Prints of whole glomeruli at a magnification of 3,909 were analyzed by stereological methods. The mean glomerular volume was \( (8.048 \pm 0.474) \times 10^4 \) \( \mu \text{m}^3 \) if the glomeruli are treated as spheres. The area of the basement membrane was \( 0.281 \pm 0.017 \text{ mm}^2 \) per glomerulus, of which \( 0.184 \pm 0.011 \text{ mm}^2 \) represents peripheral basement membrane. The aggregate epithelial slit length per glomerulus was \( 65.19 \pm 3.84 \text{ cm} \), of which \( 48.69 \pm 2.87 \text{ cm} \) represents epithelial slits abutting on the peripheral basement membrane. Assuming that a slit diaphragm is 390 Å wide, and that the pores of the slit diaphragm represent 26% of its area, the mean pore area is \( 3.96 \text{ cm}^2 \), of which \( 2.96 \text{ cm}^2 \) represents the area of peripheral pores. These findings are discussed in the context of the hydrodynamic theory of glomerular ultrafiltration. We conclude that the porous substructure of the glomerular slit diaphragm is significant in determining the hydraulic conductivity of the glomerulus and hence also solute flux during ultrafiltration.
regions, or reflected over the mesangium (Fig. 1). The
the epithelial cytoplasm, or to nonattenuated or nuclear
of the glomerulus. Slits were classified as peripheral or
within the convex outline circumscribed to the image of
stretched by exposure to chloroform vapor, and stained sequentially with aqueous
embedding mixture no. 1). Large, thin blocks of renal cortex
were obtained by cutting randomly oriented tissue slices
2-4-mm wide and 0.5-mm thick with a razor blade after the
initial perfusion fixation. After postfixation these
blocks were embedded in inverted capsules so as to offer
an extensive block face. Random and isotropic ultrathin
sections were obtained by mounting large sections from
such blocks, each showing several glomeruli, on Form-
var-coated slot grids (36). Sections were cut with dia-
mond knives on a Dupont-Sorvall MT-2 or AO-Reichert
OMU-3 ultramicrotome, stretched by exposure to chlo-
roform vapor, and stained sequentially with aqueous
uranyl acetate and lead citrate. Electron micrographs of
whole glomeruli were made in a Philips 300 electron
microscope after preliminary clearing and stabilization
of the image by deliberate exposure of the area of interest
to the electron beam near cross-over (35). Micrographs
were calibrated by use of a diffraction grating replica
having 28,800 lines to the inch (Ladd Research Indus-
tries, Inc., Burlington, Vt.). No allowance was made for
possible section compression. Electron magnifications
were found to be reproducible within ± 1.3%. Micro-
graphs were made at low magnification (× 420) of all
glomeruli showing in a single section of each block, and
printed at a final magnification of 3,909.

Stereological Methods
The methods of stereological analysis are described
and discussed in detail in the Appendix. For reasons
which will be discussed there, direct integration of areas
and of contour lengths was employed for estimates of
length and surface density of the glomerular slit and
basement membrane, respectively, and of mean glomeru-
lar volume. The equipment used was essentially an
electronic planimeter and map measure, consisting of a
digitizer with platen and cursor (HP-9864) used as
peripheral equipment with a programmable digital calcu-
lator (HP-9810A) (Hewlett-Packard Co., Palo Alto,
Calif.). Convex contours were circumscribed to cali-
bored micrographs of the glomeruli by use of a rule and
of a fiber-tipped pen with a water-proof ink (Sanford Ink
Co., New York). Quantitative stereological data were
obtained as follows.
(a) The length density of the glomerular slit was
obtained from individual micrographs, each of an entire
section of a distinct glomerulus, by direct enumeration of
slits and measurement of the area, on the same print,
within the convex outline circumscribed to the image of
the glomerulus. Slits were classified as peripheral or
nonperipheral, according to whether they were related to
basement membrane contiguous to attenuated endo-
thalial cytoplasm, or to nonattenuated or nuclear
regions, or reflected over the mesangium (Fig. 1). The
length densities for the glomerular slits were obtained
from the relation (34)

\[ L_v = 2Q_a, \]

where \( Q_a \) represents the number of slits per unit area
within the convex outline circumscribed to the glomeru-
lar profile. The mean densities were then determined
from estimates made on individual glomerular sections.
(b) The surface density, peripheral or nonperipheral,
of the basement membrane was estimated from the
relation (34)

\[ S_v = 4\pi \cdot L_a, \]

where \( L_a \) is the relevant contour length per unit area
determined by measuring directly the aggregate length of
peripheral or nonperipheral basement membrane in a
calibrated print of an entire glomerular section, as well as
the area within the convex outline circumscribed to the
image of the glomerulus. The mean densities were then
determined from estimates made on individual glomeru-
lar sections.
The mean glomerular volume was estimated from
direct measurements of the perimeter and area of
calibrated prints of all glomeruli studied, as described
and discussed in the Appendix. Similarly, aggregate
values for glomerular slit length and basement mem-
brane surface area were obtained as described in that
Appendix.

RESULTS
The mean glomerular diameter, estimated in ac-
cordance with Eq. A3, was 135 \( \mu \)m. The corre-
sponding estimate of mean glomerular volume
(from Eq. A4) was found to be \((8.048 ± 0.474) \times
density of the glomerular slit and the glomerular basement
membrane are tabulated in Table 1, and in conjunction
with the estimate of mean glomerular volume give
the aggregate lengths and areas presented in the
same table. If we assume that the glomerular slit
diaphragm is 390 \( \AA \) wide (28) and that it is
occupied by pores to the extent of 26% of its area
(28), and if we assume in addition that the number
of glomeruli per rat is 60,000 (20, 26), we obtain an
aggregate slit diaphragm pore area of 2.96 \( \text{cm}^2 \)
in relation to the peripheral basement membrane,
and a total aggregate pore area of 3.96 \( \text{cm}^2 \). Thus,
the peripheral pores represent 2.68% of the surface
area of the peripheral glomerular basement mem-
brane, while the total pore area represents 3.34% of
the whole surface area of the basement mem-
brane.

DISCUSSION
The few published stereological electron micro-
graph studies of the glomerulus include a study by
FIGURE 1 Portion of a print of a whole glomerular section at the magnification used for stereological analysis. Capillary lumen (Cap), urinary space (US). Transition between peripheral and nonperipheral regions of basement membrane (paired long arrows); note densely staining lamina densa. Foot processes (short arrows) and slits. × 3,900.

Österby of normal human glomerular basement membrane thickness (21), her planimetric studies of the mesangial fractional volume (22), and a recent study by intercept counting of the surface density of the normal peripheral glomerular basement membrane in the rat by Pinto and Brewer

| Table 1 |
|-----------------|----------------|----------------|----------------|----------------|
| Stereological Parameters of the Glomerular Basement Membrane and Epithelial Slits |
|                | Aggregate Aggregate Aggregate Aggregate Aggregate Aggregate |
|                | surface area of basement membrane | length of glomerular slit per glomerulus | length of glomerular slit per 60,000 glomeruli | aggregate length of glomerular slit per 60,000 glomeruli | aggregate length of glomerular slit per diaphragm |
| Peripheral surface | µm⁻¹ | mm² | cm² | µm⁻¹ | cm | km | cm² |
| Surface density of basement membrane | 0.229 | 0.184 | 110.6 | 0.605 | 48.69 | 29.21 | 11.39 |
| (±0.005) | (±0.011) | (±6.5) | (±0.036) | (±2.87) | (±1.72) |
| Nonperipheral surface | 0.121 | 0.097 | 58.4 | 0.205 | 16.50 | 9.90 | 3.86 |
| Surface density of basement membrane | 0.121 | 0.097 | 58.4 | 0.205 | 16.50 | 9.90 | 3.86 |
| (±0.006) | (±0.006) | (±3.4) | (±0.012) | (±0.97) | (±0.58) | (±0.23) |
| Total surface | 0.350 | 0.281 | 169.0 | 0.810 | 65.19 | 39.11 | 15.25 |
| Surface density of basement membrane | 0.350 | 0.281 | 169.0 | 0.810 | 65.19 | 39.11 | 15.25 |
| (±0.008) | (±0.017) | (±9.9) | (±0.048) | (±3.84) | (±2.35) | (±0.90) |
endothelium, for the rat, cited by Renkin and this paper (see Appendix), and upon which the per diameter and hence also the glomerular volume estimates which we obtain by the methods of Table I.

Our estimate of the aggregate surface area of the basement membrane per glomerulus (0.184 ± 0.011 mm² for the peripheral basement membrane and 0.281 ± 0.017 mm² for the total basement membrane surface) is similar to the value of 0.19 mm² for the area of the glomerular capillary endothelium, for the rat, cited by Renkin and Gilmore (26) from Kirkman and Stowell (13); the latter estimate was based on serial sectioning of several glomeruli combined with light microscopy. Again, our estimate of the glomerular mean caliper diameter of 135 μm is in agreement with our value of 0.229 ± 0.005 μm⁻¹ (Table I).

The significance of the measurement of the aggregate pore area of the slit diaphragm in relation to the identification of the anatomical site in the glomerular epithelium for the passage of water and solutes, and in the assessment of the contribution of these pores to resistance to hydraulic flow. While it has been suggested that, for myocardial (37) and muscle (23) capillaries, the aggregate filtration coefficient for endothelial cells for water may be comparable with or even greater than that for the “small pores” (32), the contribution of the permeability to water of the podocyte plasmalemma hardly seems likely to be significant for glomerular filtration, since the glomerulus has a hydraulic conductivity 40–500 times greater (11, 14) than that of muscle capillaries. Accordingly, it seems reasonable to consider only the pores in the glomerular slit diaphragm as representing the path for water and solute across the glomerular epithelium, and thus to regard the glomerular filter as a multilayered structure, in which, in the peripheral part of the membrane, water and solute traverse sequentially the endothelial fenestrations, then a porous barrier in the basement membrane, and finally the pores of the glomerular slit diaphragm.

Perl (24) has considered theoretically a model of the skeletal muscle capillary according to which it is assumed, on the basis of the ultrastructure of the interendothelial cell junctions (9, 10), that the “small” pores are represented by two slits of different width, arranged in series. In such circumstances, as this author points out (24), a “pore area per unit path length” must be defined in relation to an “effective” path length for the whole membrane, and the effective path lengths, Δx, for diffusion and for filtration will in general differ. It can be shown that Eq. 41 of Perl (24) can be applied in an alternative form appropriate to the computation of an effective glomerular path length for filtration, if we write for the latter

$$\Delta x = l_i + \frac{w^2_{11}}{w_{hi}} A_{11} l_{11},$$

where the symbols are as defined in Table II. As noted in that table, we have assumed that the pores in the region of the basement membrane are somewhat more restrictive than those of the slit diaphragm, in accordance with the findings of Caulfield and Farehaut (1) with graded dextrans, and of Ryan and Karnovsky (29, 30) with endogenous albumin. Specifically, it is assumed that the pores of the basement membrane are hydraulically equivalent to slits of width 30 Å, or to cylindrical pores of radius 24.5 Å.

The substitution of the values of Table II in Eq. 4 gives an effective filtration path length of Δxₜ = 70 Å + 83 Å = 153 Å, in relation to the aggregate area of the pores of the peripheral slit diaphragm, where lᵢ = 70 Å represents the length of the slit.
diaphragm pores, i.e., the thickness of the slit diaphragm in the region of its cross bridges (28). As Perl points out (24), the contribution of the terms in an equation such as Eq. 4 to an aggregate effective path length may be regarded as the sum of resistances in series. Thus, the values assumed in Table II suggest that the slit diaphragm pores may contribute approximately half of the resistance to hydraulic flow for the glomerular membrane. The contribution of the endothelial fenestrae could be represented by a third term analogous to the second on the right-hand side of Eq. 4, but in view of their relatively extensive area (about 20% of the fenestrated endothelium [4]), their wide radius (400 Å [11, 27]), and shallow depth (400 Å [27]) and of the fact that glomerular endothelial fenestrae usually lack a diaphragm (11), the contribution of the fenestrae to Δx would be trivial, and indeed Renkin and Gilmore (26) conclude that the fenestrated endothelium contributes a negligible part of the total hydraulic resistance of the glomerular membrane.

The above estimate of an effective filtration path length of Δx = 153 Å is not commensurable with the path length implied in Renkin and Gilmore’s (26) estimate of pore area per unit path length, of A/Δx = 2.5 × 10^5 cm/g kidney, or 3.25 × 10^6 cm/ rat, where Δx was defined in Eq. 16 of that article as a diffusion path length. However, the slit diaphragm pore area and its associated effective filtration path length can be used in conjunction with Eq. 25 of Renkin and Gilmore (26) to compute directly an ultrafiltration coefficient, Kf. This equation can be written in our notation as

\[ K_f = \frac{A_1}{\Delta x} \cdot \frac{w_1^2}{12 \eta} \]  

where w1 represents the slit width (40 Å) of the slit diaphragm pore (28), A1 is as defined in Table II, Δx = 153 Å, and η represents the viscosity of water at 37°C (0.007 P). These values give an ultrafiltration coefficient of Kf = 3.685 × 10^{-8} cm³/dyne·s, or Kf = 3.33 × 10^{-8} cm³/dyne·s·cm² expressed per unit area of peripheral basement membrane (Table I); this can also be expressed as Kf = 44.4 nl/s·mm Hg·cm². The latter value is not inconsistent with a recent estimate of Kf = 41 nl/s·mm Hg·cm² obtained by Deen et al. (2) by micropuncture studies in superficial glomeruli of Munich-Wistar rats.

Clearly, the basement membrane is the site of significant restriction of hydraulic flow in steady-state filtration. It appears to be the interpretation of Caulfield and Farquhar (1) that solute restriction is a property of the structural protein of the basement membrane alone. However, Renkin and Gilmore (26) calculate that a filter layer consisting of a fiber meshwork that was sufficiently restrictive of protein solute would be excessively restrictive of hydraulic flow, and accordingly rule out the basement membrane as a filter, either alone or in series with another layer, and ascribe restriction of both solute and hydraulic flow to a layer of cylindrical pores assumed to exist at the level of the epithelial slits. This argument creates some difficulty for a purely structural model of solute restriction by the basement membrane; possibly the basement membrane should be regarded as a cross-linked gel (cf. Laurent [16]). Alternatively, the paradox could be resolved in terms of the “concentration polarization” hypothesis of Ryan and Karnovsky (29, 30), according to which the structural pores of the basement membrane are rather large, but during ultrafiltration restrict very large plasma protein molecules to form an additional and more restrictive barrier between the glomerular basement membrane and the endothe-lium. Whatever the direct contribution of the porous substructure of the glomerular slit diaphragm to solute restriction (28–30), its aggregate area is consistent with a major function in determining the hydraulic conductivity of the glomerulus and hence solute flux during ultrafiltration.

APPENDIX

Stereological Techniques and Discussion

The aggregate length of the glomerular slit and the aggregate area of the glomerular basement membrane are estimated in two stages. First, the length density of the glomerular slit and the surface density of the glomerular basement membrane are determined with considerable precision from individual micrographs by the standard stereological relations given above in Eq. 1 and Eq. 2 (34), and expressed in terms of mean values with their standard errors (Table I). Secondly, a less precise estimate of the mean glomerular volume is obtained from observations on all the micrographs, together with an approximate estimate of its standard error. Finally, the aggregate values are obtained as the product of these estimates of density and volume, and tabulated with error estimates based on that of glomerular volume (Table I).

To describe the procedure used for estimating
TABLE II
Effective Filtration Path Length, $\Delta x$: Definition of Parameters

| Symbol | Definition | Value assumed | Reference |
|--------|------------|---------------|-----------|
| $l_1$  | Length of path through slit diaphragm pores | 70 Å | (28) |
| $l_{11}$ | Length of path through basement membrane pores | 1,400 Å | (1, 11) |
| $w/w_1$ | Ratio of effective widths of slit diaphragm to basement membrane pores | 1.33* | See text |
| $A_1$  | Aggregate area of peripheral slit diaphragm pores | 2.96 cm$^2$ | Table I |
| $A_{11}$ | Aggregate area of peripheral basement membrane pores (liquid fraction $\times$ basement membrane area) | Liquid fraction; 0.8; basement membrane area, 110.6 cm$^2$; product, 88.48 cm$^2$ | (26) |

* This assumes that the slit diaphragm pores (28) are elongated slits of width $w_1 = 40$ Å.

mean glomerular volume, we use a notation modified from Miles (18). First, there is a general relation, valid for convex bodies of all distributions of shape and size

$$E_s(V) = E_s(A) \times E_s(M_1), \tag{A1}$$

(Miles, Eq. 6.7 and 8.8 [18]), relating the mean volume to the product of the mean area of isotropic random sections and the mean projection on to an isotropically oriented line or "mean caliper diameter" (8, 18); the subscripts refer to numbers of dimensions. This relation was used in effect by Fullman (5) to obtain an expression for the mean volume of spheres from a population of spheres of varying radii. If $D$ represents the diameters of the circles representing random sections of such spheres, then Fullman (5) found the mean caliper diameter to be given by

$$E(M) = \frac{\pi}{2} \cdot \frac{1}{E(D^{-1})}, \tag{A2}$$

if we use the above notation, dropping subscripts.

An equivalent expression to Eq. A2 is

$$E(M) = \frac{1}{2E(B^{-1})}, \tag{A3}$$

where $B$ represents the perimeters of the circular section profiles. The expression for volume equivalent to Eq. A3 is

$$E(V) = \frac{E(A)}{2E(B^{-1})}, \tag{A4}$$

which is the expression we use to compute the mean glomerular volume.

An estimate of random (but not of systematic) error in this estimate of mean glomerular volume is obtained from the approximation

$$\text{SE } E(V) \approx \frac{1}{2E(B^{-1})} \sqrt{\frac{\sum_{i=1}^{n} (A_i - \bar{A})^2}{n(n-1)}}. \tag{A5}$$

Significant systematic errors may be involved in treating nonspherical particles as spheres in this way to estimate the mean caliper diameter. De Hoff and Rhines (3) have computed the errors for volume estimates of prolate and oblate spheroids, with generating ellipses of various axial ratios, consequent on applying Fullman's (5) expression for the mean caliper diameter (Eq. A2 above), and of substituting the major or minor diameters of elliptical cuts for $D$ in that equation. In either case, there was appreciable underestimation of the mean volume of prolate spheroids and there was overestimation of the mean volume of oblate spheroids if the ratio of the minor to the major axis of the generating ellipse was <0.8. The spherical approximation would be less susceptible to error if it could be assumed that one is dealing with a mixture of particles of varying shape, some nearly spherical, some flattened and some elongated, as is apparently the case with glomeruli (13).

The choice of the stereological methods in this study was in large part imposed by the material. The estimation of mean glomerular volume from numerical density and volume fraction by point
counting would be difficult in a tissue as nonuniform as kidney. The solution adopted involved the use of semiautomatic methods of planimetry and contour measurement, which were then used to estimate surface densities as well. In the case of the glomerular slit, it proved impracticable to measure the area of slit abutting upon the basement membrane by intercept counting, as can be understood when one considers the relation of slit width to section thickness, in the case of obliquely oriented slits. Instead, the length density of the glomerular slit was readily determined by direct enumeration and the caliper diameter of a body for use in the analysis of the number of particles per unit volume. Proceedings of the Second International Congress for Stereology, Chicago, 1971. Hans Elias, editor. Springer-Verlag New York Inc., New York. 211–215.

9. Karnovsky, M. J. 1967. The ultrastructural basis of capillary permeability studied with peroxidase as a tracer. J. Cell Biol. 35:213–236.

10. Karnovsky, M. J. 1970. Morphology of capillaries with special reference to muscle capillaries. In: Capillary Permeability. The Transfer of Molecules and Ions Between Capillary Blood and Tissue. Proceedings of the Alfred Benzon Symposium II, Copenhagen, 1969. C. Crone and N. A. Lassen, editors. Academic Press, Inc., New York. 341–350.

11. Karnovsky, M. J., and S. K. Ainsworth. 1972. The structural basis of glomerular filtration. In: Advances in Nephrology. J. Hamburger, J. Crosnier, and M. H. Maxwell, editors. Year Book Medical Publishers, Inc., Chicago. 35–60.

12. Karnovsky, M. J., and G. B. Ryan. 1975. Substructure of the glomerular slit diaphragm in freeze-fractured normal rat kidney. J. Cell Biol. 65:233–236.

We thank Miss Synthia Sun for excellent technical assistance.

This investigation was supported by research grant AM-13495 from the National Institutes of Health.

Received for publication 30 April 1975, and in revised form 17 July 1975.

REFERENCES

1. Caulfield, J. P., and M. G. Farquhar. 1974. The permeability of glomerular capillaries to graded dextrans. Identification of the basement membrane as the primary filtration barrier. J. Cell Biol. 63:883–903.

2. Deen, W. M., J. L. Troy, C. R. Robertson, and B. M. Brenner. 1973. Dynamics of glomerular ultrafiltration in the rat. IV. Determination of the ultrafiltration coefficient. J. Clin. Invest. 52:1500–1508.

3. de Hoff, R. T., and F. N. Rhines. 1961. Determination of number of particles per unit volume from measurements made on random plane sections: the general cylinder and ellipsoid. Trans. Am. Inst. Min. Met. Eng. 221:975–982.

4. Friederici, H. H. R. 1968. The tridimensional ultrastructure of fenestrated capillaries. J. Ultrastruct. Res. 23:444–456.

5. Fullman, R. L. 1953. Measurement of particle sizes in opaque bodies. Trans. AIME. 197:447–452.

6. Futaseku, Y., V Mizuhira, and H. Nakamura. 1972. A new fixation method using tannic acid for electron microscopy and some observations of biological specimens. In: Histochemistry and Cytochemistry. Proceedings of the Fourth International Congress of Histochemistry and Cytochemistry, Kyoto, Japan, 1972. T. Takeuchi, K. Ogawa, and S. Fujita, editors. 155–156.

7. Graham, R. C., and M. J. Karnovsky. 1966. Glomerular permeability. Ultrastructural cytochemical studies using peroxidases as protein tracers. J. Exp. Med. 124:1123–1134.

8. Hilliard, J. E. 1971. The calculation of the mean caliper diameter of a body for use in the analysis of the number of particles per unit volume. In: Stereology. Proceedings of the Second International Congress for Stereology, Chicago, 1971. Hans Elias, editor. Springer-Verlag New York Inc., New York. 211–215.

9. Karnovsky, M. J. 1967. The ultrastructural basis of capillary permeability studied with peroxidase as a tracer. J. Cell Biol. 35:213–236.

10. Karnovsky, M. J. 1970. Morphology of capillaries with special reference to muscle capillaries. In: Capillary Permeability. The Transfer of Molecules and Ions Between Capillary Blood and Tissue. Proceedings of the Alfred Benzon Symposium II, Copenhagen, 1969. C. Crone and N. A. Lassen, editors. Academic Press, Inc., New York. 341–350.

11. Karnovsky, M. J., and S. K. Ainsworth. 1972. The structural basis of glomerular filtration. In: Advances in Nephrology. J. Hamburger, J. Crosnier, and M. H. Maxwell, editors. Year Book Medical Publishers, Inc., Chicago. 35–60.

12. Karnovsky, M. J., and G. B. Ryan. 1975. Substructure of the glomerular slit diaphragm in freeze-fractured normal rat kidney. J. Cell Biol. 65:233–236.

13. Kirkman, A., and R. E. Stowell. 1942. Renal filtration surface in the albino rat. Anat. Rec. 82:373–389.

14. Landis, E. M., and J. R. Pappenheimer. 1963. Exchanges of substances through the capillary walls. Hand. Physiol. Sec. 2:961–1034.

15. Latta, H. 1973. Ultrastructure of the glomerulus and juxtaglomerular apparatus. Hand. Physiol. Sec. 8:1–29.

16. Laurent, T. C. 1970. The structure and function of the intercellular polysaccharides in connective tissues. In: Capillary Permeability. The Transfer of Molecules and Ions Between Capillary Blood and Tissue. Proceedings of the Alfred Benzon Symposium II, Copenhagen, 1969. C. Crone and N. A. Lassen, editors. Academic Press, Inc., New York. 261–277.

17. Maunsbach, A. B. 1966. Perfusion fixation of the kidney. J. Ultrastruct. Res. 15:242–282.

18. Miles, R. E. 1972. Multi-dimensional perspectives on stereology. J. Microsc. (Oxf.). 95:181–195.

19. Mollenhauer, H. H. 1964. Plastic embedding mixture for use in electron microscopy. Stain Technol. 39:111–114.

20. Morrison, A. B., and R. M. Howard. 1966. The functional capacity of hypertrophied nephrons. Effect of partial nephrectomy on the clearance of inulin and PAH in the rat. J. Exp. Med. 123:829–844.

21. Östberg, R. 1971. Quantitative electron microscopy of the basement membrane. Lab. Invest. 25:15–24.

22. Östberg, R. 1973. A quantitative electron microscopic study of mesangial regions in glomeruli from patients with short term juvenile diabetes mellitus.
23. PAPPENHEIMER, J. R. 1970. Osmotic reflection coefficients in capillary membranes. In Capillary Permeability. The Transfer of Molecules and Ions Between Capillary Blood and Tissue. Proceedings of the Alfred Benzon Symposium II, Copenhagen, 1969. C. Crone and N. A. Lassen, editors. Academic Press, Inc., New York. 278–286.

24. PEAL, W. 1971. Modified filtration-permeability model of transcapillary transport—a solution of the Pappenheimer pore puzzle? Microvasc. Res. 3:233–251.

25. PINTO, J. A., and D. B. BREWER. 1974. Glomerular morphometry. I. Combined light and electron microscopic studies in normal rats. Lab. Invest. 30:657–663.

26. RENKIN, E. M., and J. P. GILMORE. 1973. Glomerular filtration. Hand. Physiol. Sec. 8:185–248.

27. RHODIN, J. A. G. 1962. The diaphragm of capillary endothelial fenestrations. J. Ultrastruct. Res. 6:171–185.

28. RODEWALD, R., and M. J. KARNOVSKY. 1974. Porous substructure of the glomerular slit diaphragm in the rat and mouse. J. Cell Biol. 60:423–433.

29. RYAN, G. B., and M. J. KARNOVSKY. 1975. The distribution of endogenous albumin in the rat glomerulus. Fed. Proc. 34:877 a. (Abstr.)

30. RYAN, G. B., and M. J. KARNOVSKY. 1975. Morphological aspects of glomerular permeability to proteins. VIth International Congress of Nephrology, Florence, Italy, 1975. In press. (Abstr.)

31. SHEA, S. M., and A. B. MORRISON. 1975. The site of the glomerular filter. A stereological study. VIth International Congress of Nephrology, Florence, Italy, 1975. In press. (Abstr.)

32. TOSTERSON, D. C. 1970. Closing discussion. In Capillary Permeability. The Transfer of Molecules and Ions Between Capillary Blood and Tissue. Proceedings of the Alfred Benzon Symposium II, Copenhagen, 1969. C. Crone and N. A. Lassen, editors. Academic Press, Inc., New York. 658–664.

33. VENKATACHALAM, M. A., M. J. KARNOVSKY, H. D. FAIMI, and R. S. COTRAN. 1970. An ultrastructural study of glomerular permeability using catalase and peroxidase as tracer proteins. J. Exp. Med. 132:1153–1167.

34. WEIBEL, E. R., and R. P. BOLENDER. 1973. Stereological techniques in electron microscopic morphometry. In Principles and Techniques of Electron Microscopy M. A. Hayat, editor. Van Nostrand Reinhold Company, New York. 3:239–291.

35. YANG, G. C. H., and A. B. MORRISON. 1975. Stabilization and clarification of sections mounted on Formvar-coated slot grids by deliberate irradiation in the electron beam. J. Microsc. (Oxf.). 103:187–194.

36. YANG, G. C. H., and S. M. SHEA. 1975. The precise measurement of the thickness of ultrathin sections by a “re-sectioned section” technique. J. Microsc. (Oxf.). In press.

37. YUDILEVICH, D. L., and O. A. ALVAREZ. 1967. Water, sodium and thiourea transcapillary diffusion in the dog heart. Am. J. Physiol. 213:308–314.