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Distribution of endogenous albumin in the rat glomerulus: Role of hemodynamic factors in glomerular barrier function

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Distribution of endogenous albumin in the rat glomerulus: Role of hemodynamic factors in glomerular barrier function. Using an ultrastructural immunoperoxidase technique, the distribution of endogenous albumin in the rat glomerulus was delineated under normal and abnormal hemodynamic conditions. Superficial glomeruli in anesthetized Munich-Wistar rats were rapidly fixed *in situ* by applying glutaraldehyde to the renal surface. Fixed tissue slices were treated with anti-rat albumin Fab fragments conjugated to horseradish peroxidase (HRP), and were then subjected to the Graham-Karnovsky ultrastructural peroxidase localization procedure. During normal blood flow, dense reaction product specific for albumin was largely confined to the glomerular capillary lumen and endothelial fenestrae, with only small amounts detectable in the *lamina rara interna*, and none deeper in the basement membrane (GBM) or in the urinary space. If cortical tissue was subjected to routine immersion fixation, or if fixation was performed *in situ* after ligation of the renal artery, reaction product was detected throughout the GBM and in the urinary space. If fixation was performed *in situ* after ligation of the renal artery and vein (or artery, vein and ureter), reaction product was found in the GBM and, in very large amounts, in the urinary space. If blood flow was restored for ten minutes after five minutes of renal pedicle (artery and vein) occlusion, the distribution of albumin returned to normal. Thus, glomerular barrier function depends upon the maintenance of normal blood flow conditions.

Distribution de l'albumine endogène dans le glomérule du rat: rôle des facteurs hémodynamiques dans la fonction de barrière glomérulaire. La distribution de l'albumine endogène dans le glomérule du rat a été précisée dans des conditions hémodynamiques normales et anormales au moyen d'une technique ultrastructurale utilisant l'immunoperoxidase. Les glomérules superficiels de rats Munich-Wistar ont été rapidement fixés in situ par application de glutaraldéhyde à la surface du rein. Des tranches de tissu fixé ont été traitées par des fragments Fab anti-albumine de rat conjugués à de la peroxydase du raifort (HRP) puis soumises au procédé de localisation ultrastructurale de la peroxydase de Graham-Karnovsky. Quand le débit sanguin est normal, le produit dense de réaction spécifique de l'albumine est principalement localisé dans les lumières des capillaires glomérulaires et les fenêtres endothéliales. De faibles quantités sont détectables dans la lamina rara interna alors que plus profondément dans la membrane basale (GBM) ou dans l'espace urinaire il n'y a pas de produit de réaction. Quand le tissu cortical est soumis à la fixation par immersion, ou quand la fixation est réalisée in situ mais après ligature de l'artère rénale, le produit de réaction est trouvé dans toute la GBM et dans l'espace urinaire. Si la fixation est réalisée in situ après la ligature de l'artère et de la veine rénales (ou de l'artère, de la veine et de l'uretère), le produit de réaction est détecté dans la GBM et, en grande quantité, dans l'espace urinaire. Quand le débit sanguin est rétabli pendant dix minutes après cinq minutes d'occlusion du pédicule rénal (artère et veine), la distribution de l'albumine redevient normale. Ainsi la fonction de la barrière glomérulaire est dépendante du maintien des conditions normales de débit sanguin.

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The glomerular capillary wall consists of three structural layers. The vessel lumen is lined by a fenestrated endothelium. Outside this is the basement membrane (GBM) with an electron-dense central region (*lamina densa*) and electron-lucent zones on each side (*lamina rara interna* and *lamina rara externa*). The outermost layer is composed of interdigitating epithelial podocytes; the interpodocyte slits are traversed by a diaphragm.

There has been significant controversy over which of these layers restricts the passage of plasma proteins to the urinary space during normal ultrafiltration. The usual approach in such investigations has been to inject exogenous macromolecular tracers into the circulation and to observe their distribution in the glomerular wall. Because ferritin molecules showed little penetration beyond the *lamina rara interna*, Farquhar, Wissig and Palade [1] proposed that glomerular barrier function could be attributed solely to the GBM. More recently, Caulfield and Farquhar [2] came to the same conclusion using dextrans as tracer particles. Another view is that the epithelial layer—more specifically, the interpodocyte slit region—also plays a role in barrier function [3]. Karnovsky and

his associates [4–6] suggested that the GBM is a coarse filter for large proteins, while the interpodocyte slits contain smaller pores (probably in the diaphragm [7]) which restrict the passage of smaller proteins. This concept largely arose from studies using enzymatic tracers, such as catalase, which penetrated the GBM but did not traverse the slits [5]

In this paper, we have used an ultrastructural immunoperoxidase technique to delineate the distribution of endogenous albumin in the rat glomerulus under different hemodynamic conditions. We present evidence for a concept that stresses the role of normal blood flow in the maintenance of glomerular barrier function.

Methods

Animals. Male Munich-Wistar rats, weighing 200 to 300 g, were obtained from Dr. C. P. Lechene, Department of Physiology, Harvard Medical School. As found by Dr. Klaus Thurau of the University of Munich, Germany, rats of this strain have glomeruli at or close to the renal surface.

Preparation of anti-rat albumin Fab-HRP conjugate. The IgG fraction of rabbit anti-rat albumin serum was obtained from Cappel Laboratories, Inc., Downington, PA. When tested by immunoelectrophoresis against rat serum, this fraction gave a single line, corresponding to chromatographically purified rat albumin (Cappel Laboratories, Inc.). After purification by DEAE-cellulose chromatography (with 0.0175M sodium phosphate buffer, pH 8.08), the IgG fraction was digested with papain (Worthington Biochemical Corp., Freehold, NJ) to produce Fab fragments [8]. These Fab fragments were purified by CM-cellulose chromatography (with 0.01M acetate buffer, pH 5.5, to elute Fab I, followed by 0.035M acetate buffer, pH 5.5, to elute Fab II). Fab I fragments were concentrated to 20 mg/ml and then conjugated to HRP (horseradish peroxidase, Type VI, RZ 3.0; Sigma Chemical Co., St. Louis, MO) according to the procedure of Nakane and Kawaoi [9].

Experimental procedures. Rats were anesthetized with sodium pentobarbital i.p. (Nembutal; Abbott Laboratories, North Chicago, IL) 5 mg/100 g of body wt. The abdomen was opened, the ventral aspect of each kidney was exposed and the renal capsule was gently stripped from the surface using fine forceps under a dissecting microscope. Great care was taken to avoid interference with the vascular pedicle, or displacement of the kidney from its normal position. In three rats, fixation was performed under "good blood flow" conditions (i.e., the vascular pedicle was left undisturbed throughout the period of fixation): 2% glutaraldehyde in 0.1M phosphate buffer, pH 7.2, at room temperature, was dripped continually onto the renal surface *in situ* for one hour; the animals showed no apparent ill-effects and maintained normal respiration throughout this time, and, when the kidney was removed at the end of the fixation period, brisk bleeding occurred from the renal pedicle. The superficial rim of fixed cortical tissue, approximately 1 mm deep, was diced with razor blades into elongated pieces to facilitate orientation of the blocks and identification of the renal surface. After fixation in 2% glutaraldehyde for an additional four hours, the tissue was washed overnight at 4°C in phosphate buffer containing 0.1M sucrose.

In other animals, fixation was performed after stopping renal blood flow in various ways: (*a*) Tissue was treated simply by routine immersion fixation (three rats): the kidneys were exposed as above, again taking care not to disturb the renal pedicle, and a piece of tissue was sliced from the cortical surface and quickly cut into blocks in 2% glutaraldehyde in phosphate buffer; fixation was continued for five hours before washing in phosphate buffer containing sucrose. (*b*) Fixation was performed *in situ,* as above, commencing five minutes after ligation of the renal artery, using 4–0 surgical silk; the renal vein was left undisturbed (two rats). (*c*) Fixation was performed *in situ,* commencing five minutes after simultaneous ligation of the renal artery and vein (two rats). (*d*) Fixation was performed *in situ,* commencing five minutes after simultaneous ligation of the renal artery, vein and ureter (one rat). (*e*) Fixation was performed *in situ* under "reflow" conditions, i.e. commencing ten minutes after a five-minute period of ligation of the renal artery and vein; the ligature was removed using iris scissors (two rats).

Ultrastructural immunoperoxidase reaction. Pieces of fixed superficial cortical tissue were chopped into sections 40 μm thick, using a Smith-Farquhar TC-2 tissue sectioner (Ivan Sorvall, Inc., Norwalk, CT). The sections were incubated for two hours in the anti-rat albumin Fab-HRP conjugate, diluted 1:5 with 0.1M phosphate-buffered saline (PBS), pH 7.2, at room temperature. After washing for one hour with PBS (three washings of 20 min each), a peroxidase reaction was performed, using a modification of the Graham-Karnovsky method [10]: sections were initially incubated for 15 min at room temperature in 10 ml of 0.1M phosphate buffer, pH 7.2, containing 10 mg of 3,3′-diaminobenzidine tetrahydrochloride dihydrate (DAB) (D1240; Aldrich Chemical Co., Inc., Milwaukee, WI); this was followed by incubation for 15 min with a fresh batch of DAB to which was

added 0.2 ml of 1% hydrogen peroxide. The sections were then washed with phosphate buffer for 30 min (three washings of 10 min each), postfixed in 2% aqueous osmium tetroxide for one hour at room temperature, dehydrated in a graded series of ethanol at 4°C and embedded in epoxy resin (Epon 812). Thick sections were cut with glass knives on an LKB Ultrotome III (LKB Instruments, Inc., Bromma, Sweden) and were examined, unstained, by light microscopy. Ultrathin sections were cut, using diamond knives, from blocks selected for the presence of glomeruli close to the renal surface (i.e., in which the outer margin of the glomerulus was less than one tubule width from the surface). In each animal, 15 to 45 blocks were cut; in each animal, two to six suitable glomeruli were found. Sections were examined, without additional staining, at 60 kv in an electron microscope (Philips EM200).

Control reactions. Instead of incubation in the rabbit anti-rat albumin Fab-HRP conjugate as above, tissue sections (from each of the above groups) were incubated in the following: (*a*) nonspecific rabbit Fab-HRP conjugate, prepared exactly as above from normal rabbit serum; (*b*) albumin-absorbed rabbit anti-rat albumin Fab-HRP conjugate, prepared by exposing the conjugate to glutaraldehyde-insolubilized albumin, according to the method of Avrameas and Ternynck [11]; the final dilution of the absorbed conjugate was adjusted to give the same protein concentration as that used for the unabsorbed conjugate; (*c*) HRP alone, 1 mg/ml in PBS; (*d*) PBS alone. After the sections were washed, the Graham-Karnovsky peroxidase reaction was performed as above.

Results

In superficial glomeruli of Munich-Wistar rats, the distribution of electron-dense reaction product, specific for rat albumin, varied with the renal hemodynamic status at the time of fixation.

Good blood flow. In glomeruli fixed *in situ* during good blood flow, black reaction product was largely confined to the glomerular capillary lumen and endothelial fenestrae, with only small amounts detectable in the *lamina rara interna,* and none deeper in the GBM or in the urinary space (Fig. 1); staining was also found between cells in the mesangium (Fig. 1), and in cortical interstitial tissues. The specificity of the immunoperoxidase reaction was demonstrated in this and subsequent experiments by the lack of staining in tissue treated with nonspecific Fab-HRP (Fig. 2), with albumin-absorbed anti-albumin Fab-HRP, or with HRP alone.

Routine immersion fixation. If pieces of cortical tissue were fixed by immersion, reaction product was present in the glomerular capillary lumen, and throughout the GBM; some was also detected in the urinary space (Fig. 3). In contrast with the uniformly dense staining seen inside vessels fixed during good flow (Fig. 1), immersion-fixed tissue showed clumped reaction product (Fig. 3).

Ligated renal artery. In glomeruli fixed *in situ* after ligation of the renal artery, the distribution of albumin was indistinguishable from that seen following immersion fixation (see Fig. 3), i.e., clumped reaction product was found in the capillary lumen, in the GBM, and in the urinary space.

Ligated renal artery and vein. In glomeruli fixed *in situ* after simultaneous ligation of the renal artery and vein, clumped reaction product was found in the capillary lumen and throughout the GBM; in such circumstances, very large amounts were also present in the urinary space (Fig. 4).

Ligated renal artery, vein and ureter. In glomeruli fixed *in situ* after simultaneous ligation of the renal artery, vein and ureter, the distribution of reaction product was identical to that observed after ligation of the artery and vein, without the ureter (see Fig. 4).

Restored blood flow. If blood flow was stopped for five minutes (by ligating the renal artery and vein) and then restored (by removing the ligature) for ten minutes before commencing fixation *in situ,* the distribution of reaction product in the glomerulus was the same as that seen during good flow, i.e., uniform reaction product was found in the capillary lumen but did not penetrate significantly beyond the endothelial fenestrae (Fig. 5). Slight residual staining of epithelial cell margins was present (Fig. 5), but no free clumps of reaction product were found in the urinary space. Such "reflow" experiments resulted in the appearance of granular reaction product in vacuoles in parietal epithelial cells of Bowman's capsule, and in vacuoles in proximal convoluted tubular epithelium.

Discussion

Our findings can be summarized as follows: (*a*) During good blood flow, plasma albumin does not significantly penetrate beyond the endothelial layer of the glomerular capillary wall. (*b*) If blood flow stops, albumin penetrates the GBM and enters the urinary space. (*c*) If the renal vein is ligated at the same time as the renal artery, particularly large amounts of albumin enter the urinary space. (*d*) If blood flow is restored after a period of ligation of the renal artery and vein, the distribution of albumin in the glomer-

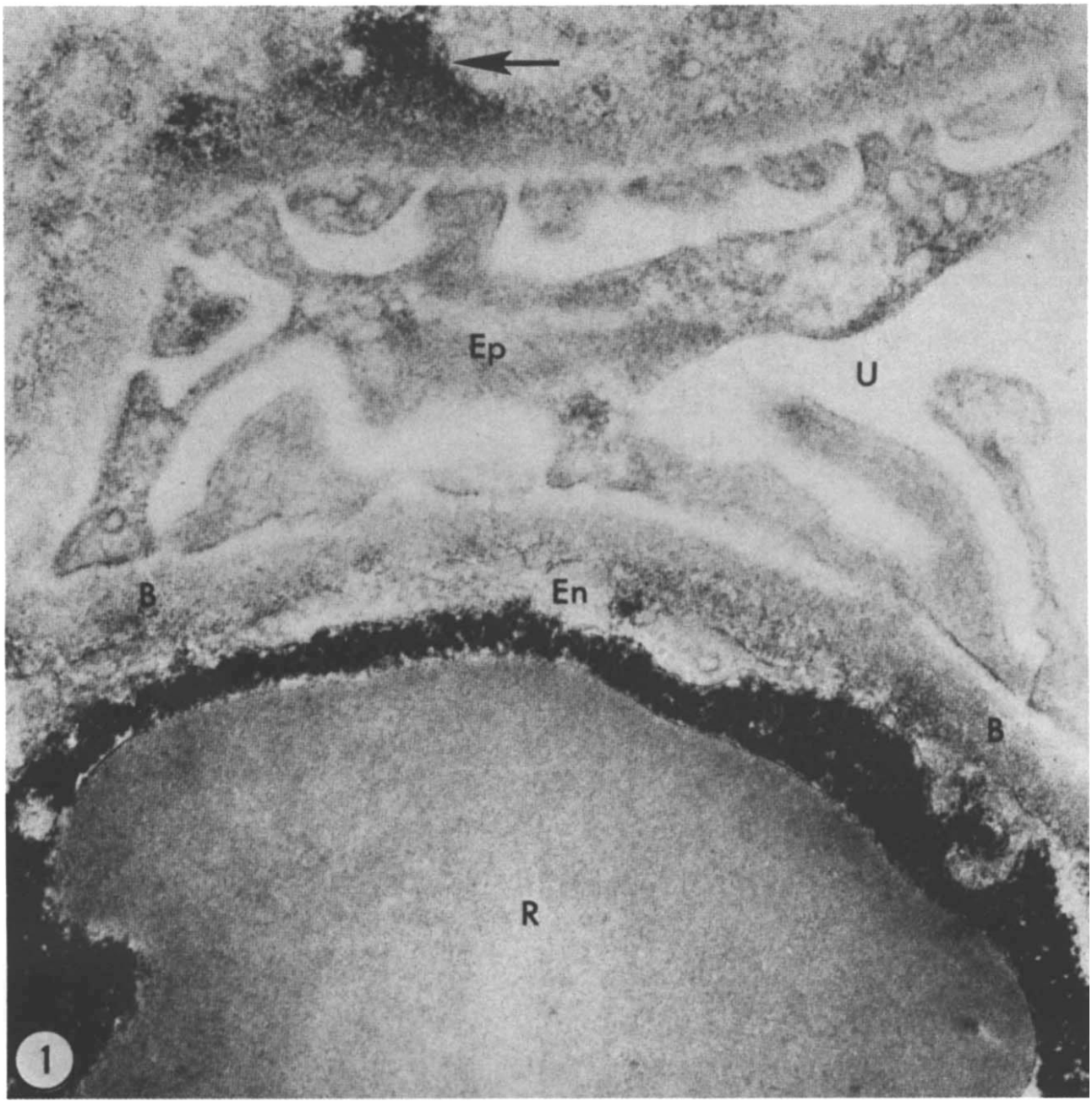

Fig. 1. *Electron micrograph of superficial glomerulus fixed* in situ *during normal blood flow, and then treated with anti-albumin Fab-HRP conjugate (×43,000).* Black reaction product specific for albumin is confined to the capillary lumen and endothelial fenestrae, with only small amounts in the *lamina rara interna,* and none detectable deeper in the basement membrance (B) or in the urinary space (U). The *arrow* indicates labelling in the mesangium. En, endothelium; Ep, epithelial cell; R, erythrocyte in capillary lumen.

ulus quickly returns to normal. These results provide ultrastructural evidence that glomerular barrier function is critically dependent upon the maintenance of normal hemodynamic conditions.

In these experiments, we took advantage of the availability of the Munich-Wistar strain of rats, in which superficially placed glomeruli are amenable to rapid fixation *in situ* under various, controlled conditions. Such glomeruli have also been used by Brenner, Troy and Daugharty [12] to directly measure glomerular capillary pressures. It could legitimately be argued that the findings in these glomeruli do not necessarily reflect the status of deeper glomeruli or glomeruli of other strains. On the other hand, superficial glomeruli are morphologically indistinguishable from other glomeruli, and, as yet, no functional dif-

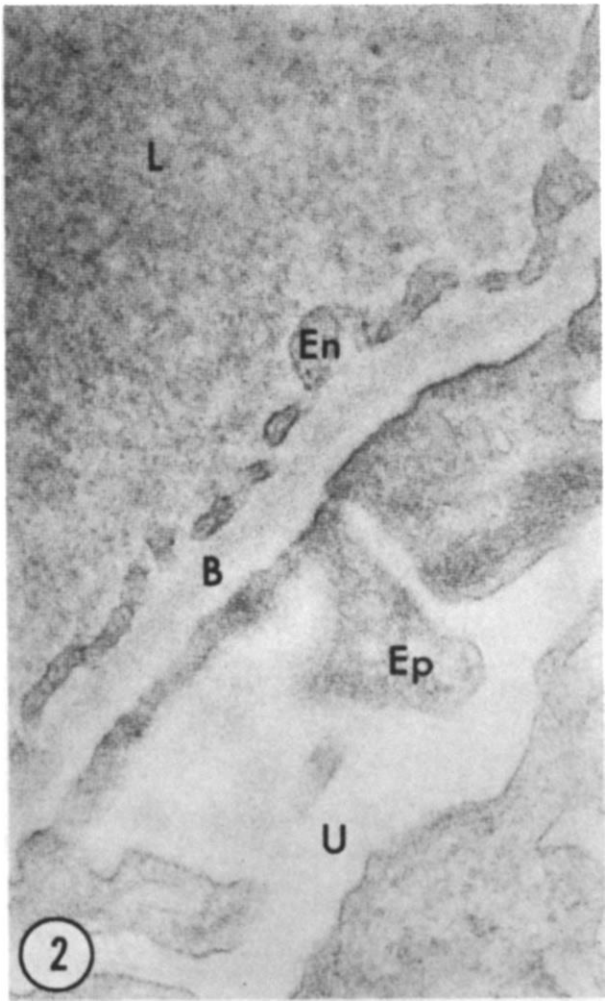

Fig. 2. *Electron micrograph of superficial glomerulus fixed* in situ *during normal blood flow, and then treated with nonspecific Fab-HRP conjugate (×36,000).* No reaction product is present. L, capillary lumen; En, endothelium; B, basement membrane; Ep, epithelium; U, urinary space.

ferences have been reported. We chose to study the distribution of endogenous albumin because it is the smallest of the major plasma proteins held up by the glomerular capillary wall [13]. This approach allowed us to examine the glomerular distribution of macromolecules under steady-state conditions, thereby obviating the problem of changes that might occur with sampling at various times after the injection of an exogenous tracer. We also avoided other objections inherent to the use of exogenous tracers, such as effects on blood volume and osmolality, and systemic histamine release (e.g., with HRP [14] or dextrans [15]).

How is albumin retained within the glomerular capillary lumen during normal ultrafiltration? Conversely, what change occurs in the glomerular capillary wall as blood flow stops, such that albumin can penetrate to the urinary space? Several hypotheses could explain these observations:

1) During normal blood flow, structural pores in the GBM may be too small to allow albumin to penetrate, but dilatation of these pores may somehow occur as blood flow slows. Such dilatation could be explained, somewhat simplistically, by postulating a loosening of a compacted feltwork of fibers in the GBM as the hydrostatic pressure falls.

2) The GBM may have structural pores that are sufficiently large to allow the passage of albumin molecules, except that, during normal blood flow and ultrafiltration (and hence normal hydrodynamic flux across the GBM), such passage may be limited by molecular sieving [16, 17] (according to which theory, the concentration of larger molecules in the filtrate is lower at higher filtration rates). As has been shown for various exogenous tracers [16, 18–20], with diminution of convective transport across the glomerular wall, albumin molecules can be expected to approach diffusion equilibrium with the ultrafiltrate.

3) The GBM may have structural pores that are sufficiently large to allow the passage of albumin, except that, during normal blood flow, an additional barrier (with smaller pores) is set up between the GBM and the endothelium. Such a functionally dependent barrier could arise if very large plasma proteins failed to penetrate coarse pores in the *lamina densa* of the GBM during normal ultrafiltration, and therefore collected in a relatively unstirred zone beneath the endothelium. This would produce a "concentration-polarization" layer, such as is known to limit albumin penetration through coarse-pored membranes in artificial ultrafiltration systems [21]. According to this hypothesis, molecules composing the concentration-polarization barrier would tend to become dispersed as slowing of flow occurred and ultrafiltration stopped. Albumin molecules could then gain access to the coarse pores in the GBM and diffuse across the capillary wall to teach the urinary space. Deen, Robertson and Brenner [22] analyzed the possible effects of concentration-polarization upon hydraulic permeability across the glomerular capillary wall; they calculated that such effects are likely to be substantial in the absence of erythrocytes (which enhance plasma-mixing), and may persist, although to a lesser extent, in the presence of erythrocytes. In favor of the concentration-polarization hypothesis is the finding by Westberg and Michael [23] that collagenase extracts of human GBM contain 1.5 to 3.1 μg/mg of albumin, 2.0 to 7.5 μg/mg of IgG and 10.8 to 26.6 μg/mg of fibrinogen. These surprizingly high concentrations of IgG and particularly

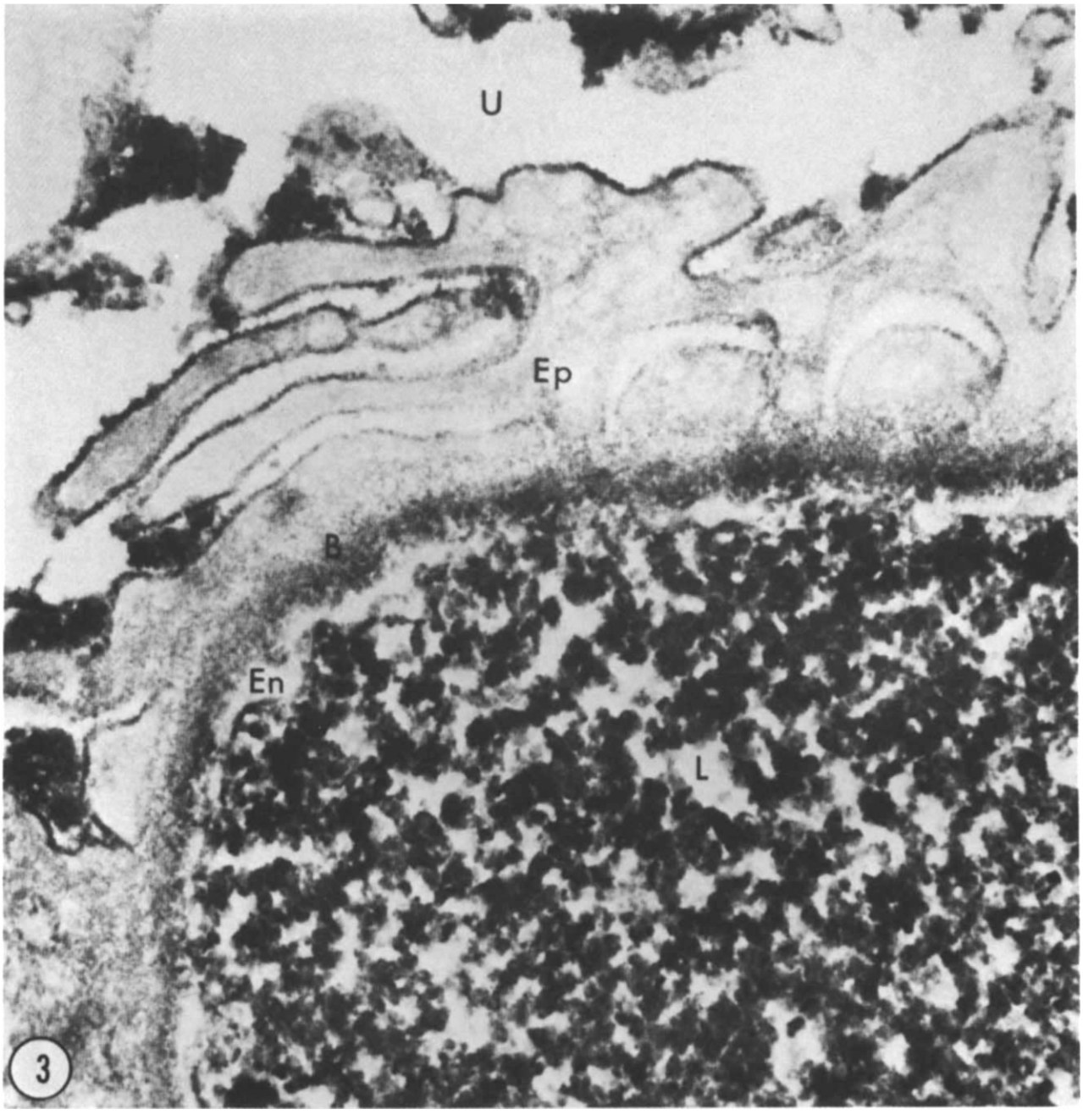

Fig. 3. *Electron micrograph of superficial glomerulus subjected to routine immersion fixation (cut up in fixative), and then treated with anti-albumin Fab-HRP conjugate (×43,000).* Clumped reaction product is present in the capillary lumen (L), in the basement membrane (B) and in the urinary space (U). En, endothelium; Ep, epithelium.

fibrinogen in the GBM may reflect the selective trapping of large plasma proteins, such as might be involved in concentration-polarization. It is possible that several molecular species (including plasma glycoproteins) may act in combination to set up such a concentration-polarization barrier. If such molecules contained significant numbers of anionic groups, they could also restrict the passage of polyanions (including albumin) because of charge effects [24–27]. For charge to be important in glomerular barrier function (as is suggested by studies showing increased GBM penetration by cationized ferritin molecules [26] and diminished fractional clearance values for sulfated dextrans [27]), it appears necessary to invoke the presence of such a layer, between the *lamina densa* and the endothelium, because isolated GBM contains

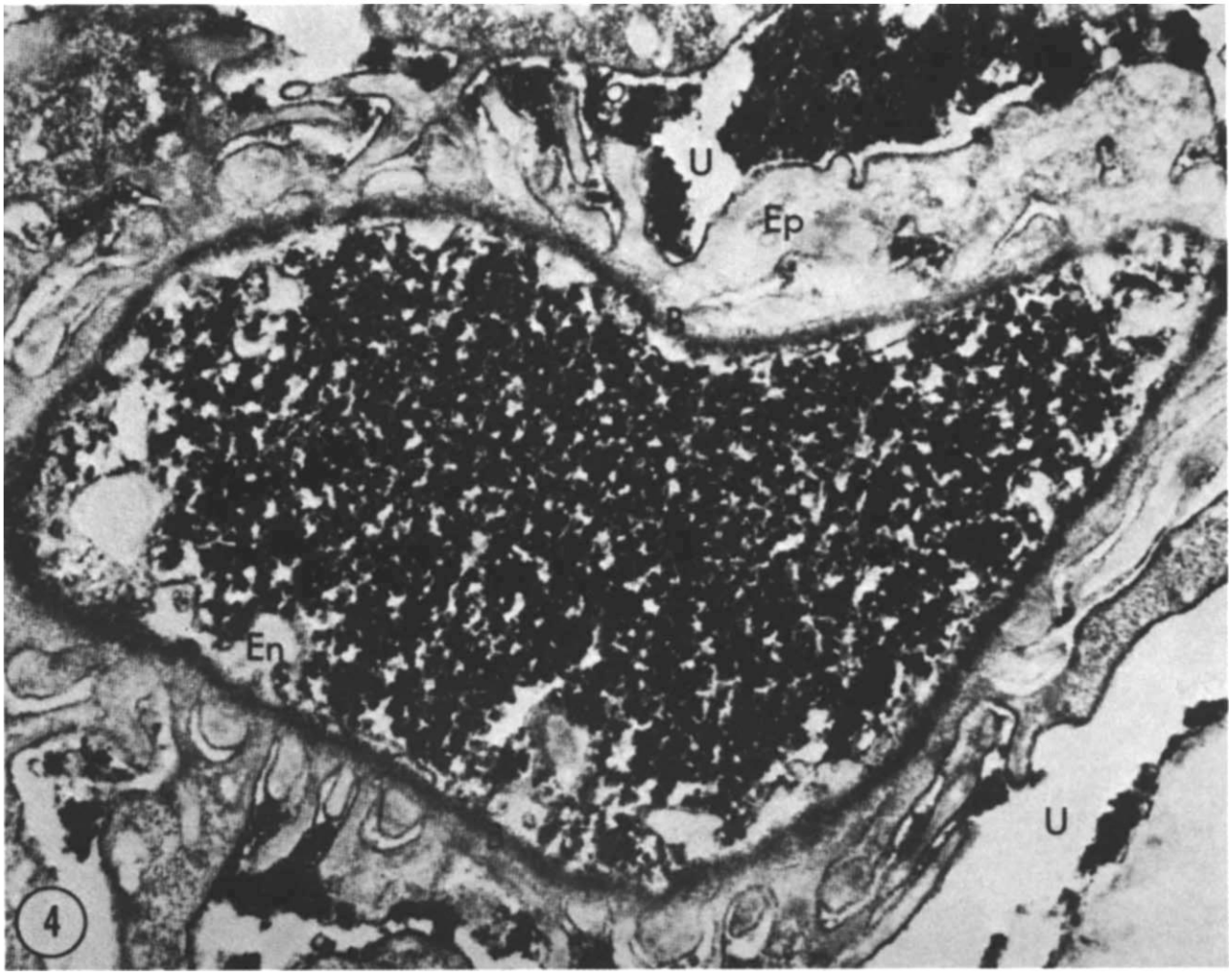

Fig. 4. *Electron micrograph of superficial glomerulus fixed* in situ *after ligation of the renal artery and vein, and then treated with anti-albumin Fab-HRP conjugate (×18,000).* Clumped reaction product is present in the capillary lumen, basement membrane (B) and, in very large amounts, in the urinary space (U). En, endothelium; Ep, epithelium.

low levels of sialic acid and does not stain with colloidal iron [23]. Indeed, from their results, Westberg and Michael predicted that sialic acid-containing proteins might be concentrated "as a thin coating on the surface of the membrane" [23].

After simultaneous ligation of the renal artery and vein, much larger amounts of albumin penetrated to the urinary space than after ligation of the artery alone. It is possible that, during ligation of the pedicle *in toto,* the renal vein was compressed fractionally before complete occlusion of the artery. This could cause vascular engorgement and an increase in intraglomerular pressure as blood flow stopped; it has been found that the pressure in the renal vein rises in such circumstances [28]. It is unlikely that an increase in intraglomerular pressure is responsible for the penetration of albumin into the urinary space after ligation of the renal artery alone (or during immersion fixation), because the glomerular vessels in such animals were always collapsed rather than distended; furthermore, after ligation of the renal artery alone, there is no increase in renal vein pressure [28].

Our findings indicate that the results of previous experiments, using exogenous tracers, must be reassessed, particularly with regard to the conditions of fixation. Farquhar, Wissig and Palade [1] clamped the "renal pedicle" (presumably including the vein) simultaneously with injecting fixative into the substance of the kidney; they found that i.v. administered ferritin (mol wt, 480,000, radius = 61 Å) progressively accumulated in the subendothelial region (i.e., the *lamina rara interna*), although they also noted a few ferritin molecules embedded throughout the entire depth of the GBM. We have recently ob-

served a similar distribution of ferritin molecules in glomeruli fixed immediately after ligation of the renal artery and vein [29]. We found less penetration of ferritin into the GBM in superficial glomeruli of Munich-Wistar rats, fixed *in situ* during normal blood flow [29]. This indicates that even the distribution of a molecule as large as ferritin can vary with different methods of fixation. Recently, Caulfield and Farquhar [2] have examined the glomerular distribution of several graded fractions of dextrans: (*a*) mol wt, 32,000; effective radius (a_e) = 38 Å; (*b*) mol wt, 62,000; a_e = 55 Å; and (*c*) mol wt, 125,000; a_e = 78 Å. Although physiological studies indicate that dextrans larger than mol wt 60,000 are effectively excluded from filtration [30–33], Caulfield and Farquhar found dextran clumps in the urinary space with each of the fractions examined. The present study indicates that this may be attributable to the technique of fixation: the renal artery, vein and ureter were clamped simultaneously with the injection of fixative into the cortex. In addition, the authors noted that, although dextran could be seen in the capillary lumen and the urinary space, it could not be detected within the intervening GBM. They proposed that this favored the GBM as the main permeability barrier in the glomerular capillary wall. However, as mentioned by the authors, the dextran particles seen electron microscopically were considerably larger than expected on the basis of molecular weight determinations. Thus, aggregation apparently occurred during fixation. It is possible that dextran was, in fact, present in the GBM (probably in an unfolded state [32, 33]) but

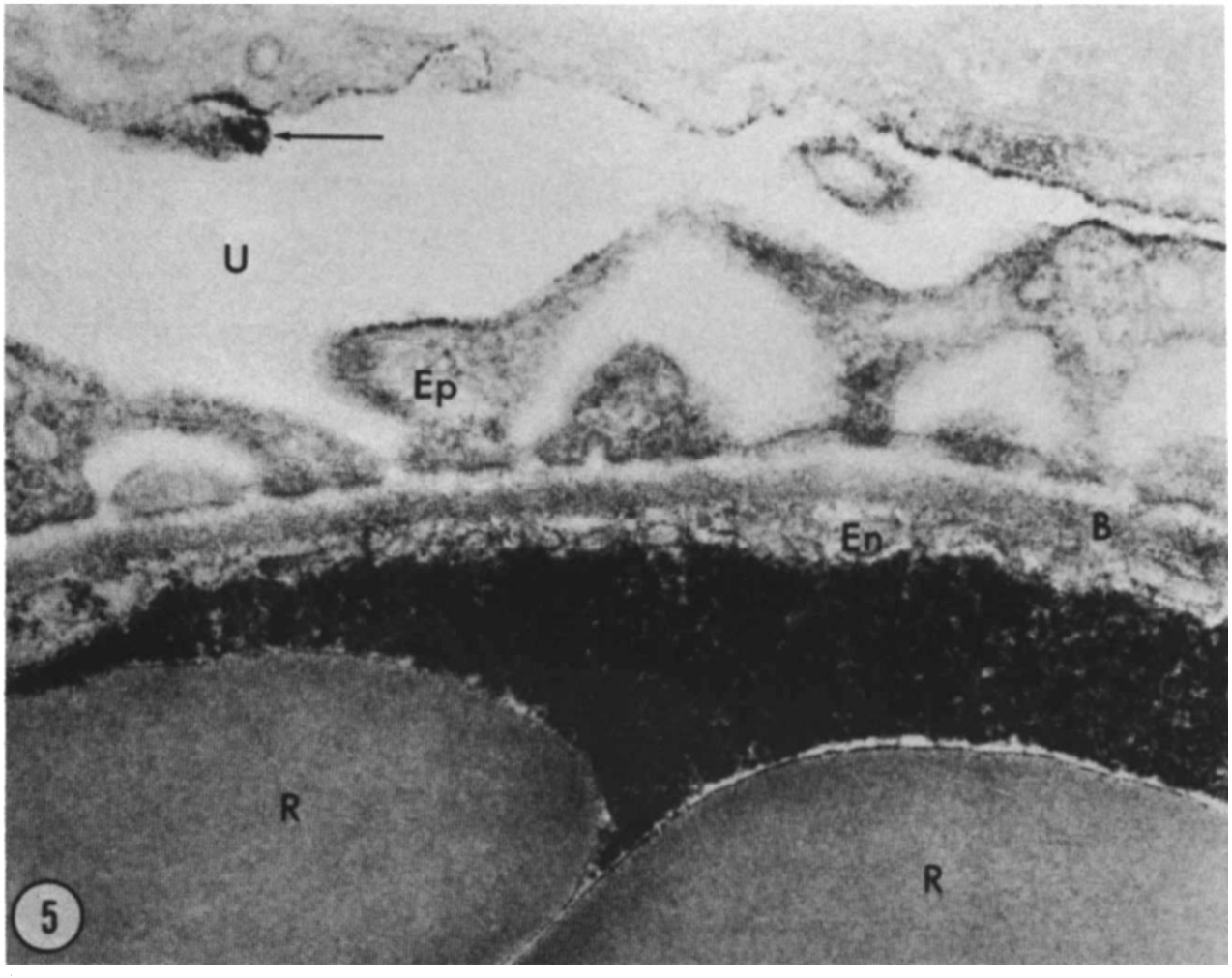

Fig. 5. *Electron micrograph of superficial glomerulus fixed* in situ *under "reflow" conditions (after releasing ligature on renal artery and vein), and then treated with anti-albumin Fab-HRP conjugate (×36,000).* The distribution of reaction product returns to that seen during normal blood flow (Fig. 1), with little penetration evident beyond the endothelial fenestrae. Note residual staining on epithelial cell surfaces (*arrow*). R, erythrocyte in capillary lumen; En, endothelium; B, basement membrane; Ep, epithelium; U, urinary space.

was not seen by the methods used because it did not aggregate into stainable clumps. This failure of aggregation could simply be due to the presence of GBM matrix around individual dextran molecules.

Venkatachalam et al [5] examined the distribution of i.v. injected catalase (mol wt, 240,000; a_e = 52 Å) after immersion fixation of the kidney (without prior ligation of the renal pedicle). Using an ultrastructural cytochemical technique, they found that catalase penetrated the GBM, but did not enter the urinary space [5]; they concluded that proteins the size of catalase, or smaller, were held up mainly by the slit diaphragm. Because this conclusion does not accord with the results of the present paper, we have recently repeated these catalase studies in Munich-Wistar rats. We have found that, in superficial glomeruli fixed *in situ* during normal blood flow, the distribution of catalase resembles that of endogenous albumin—i.e., most reaction product is held up at, or just beyond, the endothelial fenestrae [34]. Thus, although studies using immersion fixation indicate that the slit diaphragm has pores of a size which can significantly restrict the passage of catalase, the experiments with superficial glomeruli indicate that catalase does not reach this level during normal blood flow conditions. What, then, is the role of the epithelial layer in glomerular ultrafiltration? That it may be involved in limiting hydrodynamic flux across the glomerular wall is supported by the following: (*a*) Catalase can pass through the GBM but not the slit diaphragm under diffusion conditions (i.e., during immersion fixation), suggesting that pores in the slit diaphragm are smaller than those in the GBM. (*b*) For the GBM, the effective area available for water flux has been estimated to be 80% of the total capillary surface area [13], whereas the slit diaphragm pores constitute only 2 to 3% of the total capillary area [7]; the latter figure correlates more closely with estimates based on hydraulic conductivity data [13, 17].

In conclusion, we propose that glomerular barrier function cannot be attributed solely to structural elements in the glomerular capillary wall [1–7]. Such structural elements certainly contribute to barrier function during ultrafiltration, but our data indicate that normal hemodynamic conditions are also required for optimal retention of albumin molecules. As suggested by Farquhar, Wissig and Palade [1], it is likely that the GBM plays the most significant structural role in limiting the passage of macromolecules to the urinary space. However, the pores in the GBM appear to be too large to hold up albumin molecules without the superimposition of a functionally dependent restriction. Such restriction may be mediated by molecular sieving phenomena [16, 17] (possibly in association with concentration-polarization [21] and charge effects [26, 27]). Whatever the mechanisms involved, the present study provides ultrastructural confirmation of physiological data indicating that the passage of solutes across the glomerular wall is affected by glomerular hemodynamics [16, 35]. Accordingly, proteinuria could result not only from increased porosity of the GBM, but also from changes affecting normal ultrafiltration flux.

Acknowledgments

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References

1. Farquhar MG, Wissig SL, Palade GE: Glomerular permeability: I. Ferritin transfer across the normal glomerular capillary wall. *J Exp Med* 113:47–66, 1961
2. Caulfield JP, Farquhar MG: The permeability of glomerular capillaries to graded dextrans: Indentification of the basement membrane as the primary filtration barrier. *J Cell Biol* 63:883–903, 1974.
3. Hall BV: The protoplasmic basis of glomerular ultrafiltration. *Am Heart J* 54:1–9, 1957
4. Graham RC, Karnovsky MJ: Glomerular permeability: Ultrastructural cytochemical studies using peroxidases as protein tracers. *J Exp Med* 124:1123–1134, 1966
5. Venkatachalam MA, Karnovsky MJ, Fahimi HD, Cotran RS: An ultrastructural study of glomerular permeability using catalase and peroxidase as tracer proteins. *J Exp Med* 132:1153–1167, 1970
6 Karnovsky MJ, Ainsworth SK: The structural basis of glomerular filtration. *Adv Nephrol* 2:35–60, 1973
7. Rodewald R, Karnovsky MJ: Porous substructure of the glomerular slit diaphragm in the rat and mouse. *J Cell Biol* 60:423–433, 1974
8. Porter RR: The hydrolysis of rabbit γ-globulin and antibodies with crystalline papain. *Biochem J* 73:119–126, 1959
9. Nakane PK, Kawaoi A: Peroxidase-labeled antibody: A new method of conjugation. *J Histochem Cytochem* 22:1084–1091, 1974
10. Graham RC, Karnovsky MJ: The early stage of absorption of injected horseradish peroxidase in the proximal tubule of the mouse kidney: Ultrastructural cytochemistry by a new technique. *J Histochem Cytochem* 14:291–302, 1966
11. Avrameas S, Ternynck T: The cross-linking of proteins with

glutaraldehyde and its use for the preparation of immunoadsorbents. *Immunochemistry* 6:53–66, 1969

12. BRENNER BM, TROY JL, DAUGHARTY TM: The dynamics of glomerular ultrafiltration in the rat. *J Clin Invest* 50:1776–1780, 1971
13. RENKIN EM, GILMORE JP: Glomerular filtration. *Handb Physiol* 8:185–248, 1974
14. COTRAN RS, KARNOVSKY MJ: Vascular leakage induced by horseradish peroxidase in the rat. *Proc Soc Exp Biol Med* 126:557–561, 1967
15. SIMIONESCU N, PALADE GE: Dextrans and glycogens as particulate tracers for studying capillary permeability. *J Cell Biol* 50:616–624, 1971
16. PAPPENHEIMER JR: Passage of molecules through capillary walls. *Physiol Rev* 33:387–423, 1953
17. LANDIS EM, PAPPENHEIMER JR: Exchange of substances through the capillary walls. *Handb Physiol* 2(2):961–1034, 1963
18. LAMBERT PP, BRAUMAN H, VANDERVEIKEN F, MALMENDIER C, DE KOSTER JP, DE MYTTENAERE M: Application de la technique du "stop-flow" à l'étude de l'excrétion des substances protidiques: I. Excrétion de l'hémoglobine. *Bull Acad R Med Belg* 24:777–799, 1959
19. MALMENDIER CL, DE KOSTER JP, VANDERVEIKEN F, BRAUMAN H, DE MYTTENAERE M, LAMBERT PP: Stop-flow analysis applied to the excretion of hemoglobin. *Am J Physiol* 199:292–294, 1960
20. BRAUMAN H, VANDERVEIKEN F, DE KOSTER JP, DE MYTTENAERE M, GREGOIRE F, LAMBERT PP: L'excrétion de l'ovalbumine étudiée par la technique de la "diurèse interrompue". *Rev Fr Clin Biol* 6:232–238, 1961
21. BLATT WF, DRAVID A, MICHAELS AS, NELSEN L: Solute polarization and cake formation in membrane ultrafiltration: Causes, consequences, and control techniques, in *Membrane Science and Technology. Industrial, Biological, and Waste Treatment Processes,* edited by FLINN JE, New York, Plenum Press, 1970, pp. 47–97
22. DEEN WM, ROBERTSON CR, BRENNER BM: Concentration polarization in an ultrafiltering capillary. *Biophys J* 14:412–431, 1974
23. WESTBERG NG, MICHAEL AF: Human glomerular basement membrane: Preparation and composition. *Biochemistry* 9: 3837–3846, 1970
24. AREEKUL SL: Reflection coefficients of neutral and sulphate-substituted dextran molecules in the isolated perfused rabbit ear. *Acta Soc Med Upsal* 74:129–138, 1969
25. GARBY L, AREEKUL S: Reflection coefficients of neutral and sulphate-substituted dextran molecules in the capillaries of the isolated rabbit ear, in *Capillary Permeability: The Transfer of Molecules and Ions between Capillary Blood and Tissue,* edited by CRONE C, LASSEN NA, Copenhagen, Munksgaard, 1970, pp. 560–562
26. RENNKE HG, COTRAN RS, VENKATACHALAM MA: Glomerular permeability to ferritin: Effect of molecular charge. *J Cell Biol,* in press
27. CHANG RLS, DEEN WM, ROBERTSON CR, BRENNER BM: Permselectivity of the glomerular capillary wall to macromolecules: III. Evidence for electrostatic repulsion of polyanions. *Kidney Int* 8:212–218, 1975
28. HONDA N, AIZAWA C, YOSHITOSHI Y: Postocclusive reactive hyperemia in the rabbit kidney. *Am J Physiol* 215:190–196, 1968
29. RYAN GB, KARNOVSKY MJ: An ultrastructural study of the mechanisms of proteinuria in aminonucleoside nephrosis. *Kidney Int* 8:219–232, 1975
30. WALLENIUS G: Renal clearance of dextran as a measure of glomerular permeability. *Acta Soc Med Upsal* 59 (suppl 4): 1–91, 1954
31. ARTURSON G: Glomerular permeability to dextrans, in *Capillary Permeability: The Transfer of Molecules and Ions between Capillary Blood and Tissue,* edited by CRONE C, LASSEN NA, Copenhagen, Munksgaard, 1970, pp. 520–530
32. MOGENSEN CE: The permeability of the glomerular capillaries as studied by renal dextran clearance in normal and diabetic subjects, in *Capillary Permeability: The Transfer of Molecules and Ions between Capillary Blood and Tissue,* edited by CRONE C, LASSEN NA, Copenhagen, Munksgaard, 1970, pp. 531–543
33. RENKIN EM: Permeability and molecular size in "peripheral" and glomerular capillaries, in *Capillary Permeability: The Transfer of Molecules and Ions between Capillary Blood and Tissue,* edited by CRONE C, LASSEN NA, Copenhagen, Munksgaard, 1970, pp. 544–547
34. RYAN GB, KARNOVSKY MJ: Morphological aspects of glomerular permeability to proteins, in *Abs of Symposia of the 6th Int Cong of Nephrol,* Florence, Italy, 1975, p. 69
35. LAMBERT PP, GREGOIRE F, DE BRAUCOURT CDH: Hémodynamique glomérulaire et excrétion de l'hémoglobin, *Arch Int Physiol* 60:506–534, 1952
36. RYAN GB, KARNOVSKY MJ: The distribution of endogenous albumin in the rat glomerulus (abstract). *Fed Proc* 34:877, 1975