GLOMERULAR PERMEABILITY IN THE BULLFROG

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ABSTRACT

Filtration studies suggest similar size pores in the glomerular filters of mammals and amphibians. However, the glomerular wall in the bullfrog exhibits several structural features not found in mammals. The subendothelial space of the basement membrane is often greatly enlarged and infiltrated by cellular elements. The lamina densa of the basement membrane shows extensive variation in thickness and packing of its filaments. On the other hand, the epithelial slits in the bullfrog are closed by a slit diaphragm which appears similar in size and structure to the slit diaphragm in mammals. Horse spleen ferritin, a protein with a hydrodynamic radius of 61 Å, was used as an ultrastructural tracer to determine whether the highly variable structure of the basement membrane renders this layer more permeable than its mammalian counterpart. Within 10 min after intravenous injection, ferritin was found throughout the basement membrane and often in clusters within the subepithelial layer adjacent to the slit diaphragm. Virtually no ferritin was found within the urinary space, podocytes, or cells of the proximal tubule. Ferritin distribution was the same in both superficial glomeruli and more deeply lying glomeruli regardless of the method of fixation. These results indicate that in the bullfrog the slit diaphragm is a principal filtration barrier to ferritin and thus to smaller plasma proteins.

KEY WORDS glomerulus filtration Rana catesbeiana ferritin slit diaphragm basement membrane

The renal glomerulus functions as a passive filter which prevents passage into the urinary space of albumin and larger macromolecules. In recent years the structure of the mammalian glomerulus has been studied extensively in attempts to define the morphological site of restriction within the glomerular capillary wall. However, results of experiments with macromolecular tracers for electron microscopy have been equivocal. In the rat, large tracer molecules such as horse spleen ferritin and high molecular weight dextrans appear to be restricted primarily by the basement membrane (4, 9). On the other hand, certain peroxidase tracers apparently cross the basement membrane in greater quantities and are restricted to different extents at the level of the epithelial slits (13, 14, 43, 44). The structure which would most likely serve as a filtration barrier within the epithelial layer is the slit diaphragm, a thin, ribbonlike structure which bridges the extracellular channel between interdigitating foot processes. Fixation with tannic acid has revealed that the mammalian slit diaphragm is perforated by small, rectangular pores of a size (40 × 140 Å) which would be compatible with restriction of proteins near the size of albumin (36). Nevertheless, recent experiments (38) in which endogenous albumin has been visualized immunocytochemically within the rat
glomerulus support the earlier notion that in this species the basement membrane is the principal filtration barrier. These experiments further suggest that the observed permeability of the basement membrane is critically dependent on the maintenance of renal blood flow during initial tissue fixation.

Renkin and Gilmore (30) have noted that mammalian and amphibian glomeruli behave similarly in their filtering properties and have calculated that the filtration barriers in both species have similar theoretical pore sizes. In addition, both classes of animals have serum albumins that are nearly identical in size, isoelectric points and other physical-chemical properties (25-27, 41, 45, 46). In each species, albumin represents the lowest molecular weight protein retained by the glomerular filter (2, 30). Because of these similarities, we thought that the structure of the filtration barriers within the glomeruli of these two vertebrate classes would also be very similar. To assess this possibility, we studied the ultrastructure of the glomerular wall in an amphibian, *Rana catesbeiana*. We report that although the bullfrog has a slit diaphragm similar to that found in mammals, the morphology of the basement membrane in this species differs significantly from that of the mammalian structure. Furthermore, the basement membrane in the bullfrog appears readily permeable to horse spleen ferritin, regardless of the method of fixation. This finding suggests to us that in the bullfrog the slit diaphragm is important in determining the overall permeability of the glomerular wall to plasma proteins.

MATERIALS AND METHODS

Animals

Bullfrogs (*Rana catesbeiana*), ~200-400 g, were obtained from Southern Biological Supply Company (McKenzie, Tennessee). Before experiments, animals were kept in a large aquarium with access to both land and running tap water maintained at 25°C. Animals were anesthetized by either double pithing or submergence in a 2% solution of urethane in tap water (2, 5) for 20-30 min.

Morphological Studies

Three primary fixatives were used in these studies: (a) 1% formaldehyde and 1% glutaraldehyde (FG) (19), (b) 2% acrolein, 1% formaldehyde, and 1% glutaraldehyde (AFG) (15), and (c) 1% tannic acid and 1% glutaraldehyde (TAG) (11, 36), each in 0.1 M phosphate buffer, pH 7.3. Tannic acid was obtained from Fisher Scientific Co. (Pittsburgh, Pa.) (lot 700339), and J. T. Baker Chemical Co. (Phillipsburg, N. J.) (lot 43554). The following three methods of fixation were employed:

**Perfusion fixation:** A midventral incision was made in the abdomen of the anesthetized animal to expose the kidneys and major blood vessels. The dorsal aorta was cannulated with a 27-gauge needle at a point just anterior to the renal arteries. Perfusion with amphibian Ringer's solution (18) was begun, and, immediately thereafter, the vena cava was cut to allow venous drainage. Blanching of the kidneys from their normal reddish brown color to a pale tan indicated a successful perfusion. The perfusion fluid was then changed to one of the primary fixatives. Gravity feed pressure was maintained at 32 cm water throughout the perfusion. After 15-20 min, small pieces of kidney were removed, cut into small blocks, and placed in small vials for the remaining fixation and embedding steps.

**Immersion fixation:** The kidney surface was flooded with a large volume of fixative. Small quantities of the fixative were then immediately injected into the kidney cortex with a 30-gauge needle (9). After ~5 min, the tissue was removed from around the sites of injection, cut into small blocks, and treated in the same manner as perfusion-fixed tissue.

**Topical fixation:** Superficial glomeruli, present in large numbers on the kidney surface in the frog, were fixed without interruption of blood flow by slowly dropping fixative onto the kidney surface *in situ* from a 25-ml burette for 20 min. Blood flow through the superficial glomeruli and other surface vessels was monitored during this period with a dissecting microscope. At the end of 20 min, surface tissue was removed and cut into thin strips, ~0.5 x 1 x 2 mm, to preserve orientation of the superficial glomeruli.

In all fixation procedures, tissue blocks were fixed an additional 2 h at room temperature, rinsed in 2.5% sucrose in 0.1 m phosphate buffer at 0°-4°C for 30 min or overnight, and then postfixed in 2% OsO4 in phosphate buffer at 0°C for 1½-2 h. Fixed blocks were then dehydrated in a graded series of ethanols and embedded in Epon 812 (23).

Ferritin Studies

Ferritin (monomeric, 6 x recrystallized, cadmium-free) was obtained from Polyscience, Inc. (Warrington, Pa.). To insure against trace contamination from heavy metal, the ferritin was dialyzed against 0.1 M EDTA, pH 7.4, for 24 h at 4°C (9) and then against several changes of amphibian Ringer's solution. Dialyzed ferritin was concentrated to 100 or 200 mg/ml by ultrafiltration. Osmolarity of the final ferritin solutions as measured with a freezing-point osmometer (Precision Systems, Inc., Sudbury, Mass.) was 225 mosmol, virtually identical to that of the Ringer's solution.

Three ferritin doses were used in these tracer studies: 50 mg (0.25 ml) per 100 g body weight ("low dose"),...
100 mg (0.5 ml) per 100 g body weight (“intermediate dose”), and 250 mg (2.5 ml) per 100 g body weight (“high dose”). An abdominal incision was made in each anesthetized frog, and a sample of urine, when present in sufficient quantity, was removed from the bladder. A 27-gauge catheter was inserted into the abdominal vein, and the selected dose of ferritin was injected over a period of 4-5 min with an infusion pump (Sage Instruments Div., Orion Research Inc., Cambridge, Mass.) for low and intermediate doses, or by hand for high-dose experiments. The ferritin was allowed to circulate an additional 5, 15, or 55 min, giving total circulation times of 10, 20, and 60 min. At the end of the circulation period, a second urine sample was taken. FG or AFG fixative was then applied topically to the left kidney to fix superficial glomeruli. 5 min later, after blood flow had ceased in the superficial glomeruli, immersion fixation of the right kidney was commenced by injection of fixative into the kidney cortex. After ~2-3 min, tissue was removed from the right kidney while topical fixation of the left kidney was continued for an additional 10-15 min. Excised tissue from each kidney was then processed in the manner used for morphological studies. Total protein in urine samples was measured colorimetrically with a sulfosalicylic acid assay (6).

A total of 15 frogs, five for each time period, were used for the tracer experiments. The experimental conditions for each frog are summarized in Table I.

Microscopy

Sections of embedded tissue were cut on a Porter-Blum MT-2 ultramicrotome (DuPont Instruments-Sorvall, DuPont Co., Wilmington, Del.). For light microscopy, 0.5-μm thick sections were cut with glass knives, stained with azure II-methylene blue (35), and photographed with a Zeiss WL photomicroscope (Carl Zeiss, Inc., New York). For electron microscopy, silver to pale gold thin sections were cut with diamond knives and stained either for 5 min in uranyl acetate (48) and 5 min in lead citrate (32) (morphological studies), or for 3-5 min in lead citrate alone (ferritin experiments). Stained sections were examined at 60 kV in a Phillips 200 electron microscope with magnifications calibrated by means of a diffraction grating replica with 21,600 lines per cm. Micrograph measurements were made from negatives on a Nikon model 6C profile projector (Ehrenreich Photo-Optical Industries, Inc., Garden City, N. J.).

RESULTS

Morphology

The kidney of the bullfrog contains numerous glomeruli, ~150 μm in average diameter, many of which lie close to the outer surface of the cortex and are clearly visible through the attenuated capsule of the intact organ. The capillary wall in the frog glomerulus has been described briefly by Pak Poy (28) and, as in the mammal, consists of three basic structural layers across which substances in the capillary lumen must pass to gain entry to the urinary space. These layers are the endothelial cell layer, a trilaminar basement membrane, and the epithelial cell layer. Remarkable, however, is the extreme variability in overall thickness of the capillary wall (Fig. 1), a thickness which can range between 1 and 8 μm even within a single glomerulus. Several other morphological features of the individual layers distinguish the bullfrog glomerulus from that in mammals.

ENDOTHELIAL LAYER: Much of the endothelial cell cytoplasm is flattened to form a thin wall around the capillary lumen and is perforated by numerous fenestrations (Fig. 2). However, these fenestrations, 100 to over 300 nm in diameter, are often larger and not so uniformly spaced as in the mammal (9, 21). In many cases, the fenestrations appear to be bridged by thin diaphragms similar to those found within endothelial fenestrations of several mammalian tissues (8, 24).

BASEMENT MEMBRANE: Between the lamina densa of the basement membrane and the endothelial cell lining is a greatly enlarged subendothelial space. This space, composed largely of loosely packed filaments and a small number of cellular elements, varies widely in thickness from 100 nm to over 1 μm (Figs. 2 and 3). This layer in the bullfrog is considerably more extensive than

| Animal  | Anesthesia | Ferritin dosage | Circulation time | Primary fixative |
|---------|------------|-----------------|-----------------|-----------------|
| 1       | pithed     | low             | 10              | FG              |
| 2       | pithed     | low             | 20              | FG              |
| 3       | pithed     | low             | 60              | FG              |
| 4       | urethane   | low             | 10              | FG              |
| 5       | urethane   | low             | 10              | AFG             |
| 6       | urethane   | low             | 20              | FG              |
| 7       | urethane   | low             | 20              | AFG             |
| 8       | urethane   | low             | 60              | FG              |
| 9       | urethane   | low             | 60              | AFG             |
| 10      | pithed     | intermediate    | 10              | FG              |
| 11      | pithed     | intermediate    | 20              | FG              |
| 12      | pithed     | intermediate    | 60              | FG              |
| 13      | pithed     | high            | 10              | FG              |
| 14      | pithed     | high            | 20              | FG              |
| 15      | pithed     | high            | 60              | FG              |
FIGURE 1 Light micrograph of a TAG-perfused glomerulus in the bullfrog *Rana catesbeiana*. Evident is the large variation in thickness of the capillary wall. Several subendothelial cells are indicated (arrowheads). × 550.

the subendothelial space in mammals in which it exhibits a more uniform thickness reported to be between 10 and 60 nm (21, 22, 50). In addition to thin filaments 3–8 nm in diameter which are also seen in the mammal (21, 22), the subendothelial space contains numerous thicker filaments (Fig. 4), ~13–14 nm in diameter, apparent only infrequently in the mammal (9, 21, 22). In addition, cross-banded collagen fibrils, 55–80 nm in diameter, are frequently seen in this area. The degree of packing of all three filament types varies greatly from region to region within the subendothelial space, with some areas nearly devoid of all filaments (Figs. 4 and 5).

The subendothelial space in many areas is infiltrated by numerous cells remarkable for their many slender projections which often extend for considerable distances into the surrounding spaces (Figs. 1 and 3). Unlike the underlying endothelial cells, these cells and their processes do not form a continuous cellular layer and in many regions are even absent (Fig. 2). These cells have been referred to variously as pericapillary tissue (28) or mesangium (7). However, their widespread distribution throughout extensive regions of the capillary wall appears to differ from the more confined, axial position of mesangial cells in mammals. We prefer to classify these cells in the bullfrog as subendothelial cells, to emphasize their position within the subendothelial layer of the basement membrane.

As in the case of the subendothelial space, the lamina densa also varies widely in thickness from 30 to over 400 nm (Fig. 4). This variation is apparently much greater in the bullfrog than in the rat in which it has been measured as only 50–80 nm (21, 22). The lamina densa also exhibits wide variations in the degree of packing of the 3- to 8-nm diameter filaments of which it is composed. In general, this layer is less compact and less uniform in appearance than the basement membrane in mammals, regardless of the method of fixation (Figs. 4 and 5).

EPITHELIAL LAYER: As in the mammal,
Figure 2. Glomerular wall perfusion-fixed with formaldehyde-glutaraldehyde. The three main structural layers of the capillary wall are evident. These include the endothelial cell layer (En), the basement membrane (BM), and the epithelial cell layer (Ep). Numerous fenestrations are seen in the endothelium (arrows). Asterisks mark the subendothelial layer. L, capillary lumen; US, urinary space. × 21,000.
Figure 3 Glomerular wall perfusion-fixed with formaldehyde-glutaraldehyde. This micrograph illustrates the endothelial (En), subendothelial (SEn), and epithelial (Ep) cell types found within the glomerular wall. Also evident is the greater overall thickness of the subendothelial layer (asterisks) as compared to that seen in Fig. 2. L, capillary lumen; US, urinary space. × 11,000.
the epithelial podocytes send out a series of interdigitating foot processes which line the outer aspect of the basement membrane. The foot processes are less numerous than those described in mammalian glomeruli (21, 28) although the intercellular spaces between adjacent processes appear...
to be similar in width, ~40-50 nm at their narrowest point, to the mammalian filtration slits (36). The plasma membrane of the podocytes is covered by a glycocalyx, up to 25 nm thick, which is particularly prominent after TAG fixation (Fig. 6).

Although the basement membranes in the frog and the rat differ dramatically, the slit diaphragm in the frog, as visualized after TAG fixation, appears very similar in basic structure to the mammalian diaphragm (36). The slit diaphragm consists of a central filament and periodic cross bridges. In cross section, as shown in Fig. 6, the central filament appears as an electron-opaque region connected by less dense cross bridges to the membranes of the adjacent epithelial foot processes. Fig. 6 (inset) shows a face view of the diaphragm and illustrates the porous substructure which results from the periodic spacing of the cross bridges. In most face views, however, the substructure of the diaphragm is obscured, an observation which we believe is due to the superimposed image of the adjacent glycocalyx present on the surface of the podocytes (Fig. 6). Dimensions for the substructure of the slit diaphragm appear in Table II.

**Ferritin Permeability**

Because of the high degree of variability in the structural organization of the basement membrane in the bullfrog, ferritin was used as an ultrastructural tracer to determine whether this layer is more permeable than its mammalian counterpart.

**Urinary Protein:** Urine samples from eight of the 15 animals used for ferritin studies were assayed for total protein to screen for possible proteinuria. All samples, whether taken before or after circulation of tracer, revealed no detectable protein (<10 µg/ml).

**Tracer Distribution:** The distribution

![Figure 6](image)

**Figure 6** Epithelial layer after TAG fixation. The slit diaphragm, evident between foot processes, has been cut in cross section. The prominent glycocalyx which covers the foot processes can be seen overlying the slit diaphragm (arrows). × 93,000. **Inset:** A face view of the slit diaphragm is seen with a region indicated where the periodicity of the cross bridges is evident (arrows). × 93,000.
TABLE II
Dimensions of the Bullfrog Slit Diaphragm

| No. of measurement | Mean ± SE       |
|--------------------|----------------|
| a. Overall width   | 38             |
| b. Diameter of central filament | 16         |
| c. Cross-bridge periodicity | 37        |
| d. Cross-bridge diameter | 19        |
| e. Space between adjacent cross bridges (cross-bridge periodicity minus diameter) | 45         |

of ferritin within glomeruli prepared by conventional immersion fixation appeared similar after 10-, 20-, and 60-min total circulation times regardless of the method of anesthesia, the ferritin dose, or the fixation procedure (Figs. 7-11). At a given dose, the observed concentration of ferritin within the capillary lumen varied considerably from glomerulus to glomerulus and even among different capillary loops within a single glomerulus. In general, more capillaries had high concentrations of tracer at the higher ferritin doses. At all time points, ferritin had crossed the endothelial fenestrations and was seen in numerous locations throughout the width of the basement membrane, often in clusters immediately adjacent to the region of the slit diaphragm within the subepithelial space (Figs. 7, 8, 10, and 11). Ferritin did not penetrate the basement membrane uniformly, however. In many localized regions the concentrations within the subendothelial and subepithelial layers appeared similar to but rarely exceeded the concentration in the adjacent capillary lumen. In other regions, particularly within areas of the lamina densa, there appeared to be a lower tracer concentration than in the capillary lumen. The overall amount of ferritin within the subepithelial layer and lamina densa in a given region did not appear dependent on the thickness of the lamina densa or basement membrane as a whole but was roughly proportional to the ferritin concentration in the capillary lumen. The overall amount of ferritin within the subepithelial layer and lamina densa in a given region did not appear dependent on the thickness of the lamina densa or basement membrane as a whole but was roughly proportional to the ferritin concentration in the capillary lumen.

Tracer was occasionally identified within vesicles of the subendothelial cells. However, no ferritin was found at any time within either foot processes or cell bodies of epithelial cells. Individual ferritin particles were only infrequently identified within the urinary space, and virtually no tracer was seen within cells of the proximal tubule, even after 60-min circulation time with the highest ferritin dose.

To determine whether maintenance of blood flow during fixation might affect the observed location of tracer within the capillary wall (37, 38), ferritin distribution in glomeruli prepared by immersion fixation was compared with the distribution in superficial glomeruli fixed topically in situ without intentional interruption of blood flow. When topical fixation was commenced, the rate of blood flow within superficial glomeruli immediately began to decrease and gradually stopped within 4 min. Ferritin distribution in these glomeruli was identical after each of the three circulation times, and in all other respects was similar to the results obtained with immersion fixation (Figs. 9 and 11).

DISCUSSION
Our results indicate that in the bullfrog, ferritin can readily cross the entire width of the glomerular basement membrane but is prevented from entering the urinary space by the slit diaphragm. Ferritin, with a mol wt of 480,000 daltons (17) and a hydrodynamic radius (a_e) of 61 Å (40), is a significantly larger molecule than plasma albumin (69,000 daltons; a_e, 35.5 Å) (27, 30). Both proteins are highly anionic with isoelectric points of 4.6 for ferritin (31) and 5.2-5.6 for albumin (25). We reason, therefore, that serum albumin can leak across the basement membrane in the bullfrog, and that the slit diaphragm, in addition to the basement membrane, is an important filtration barrier to this smaller protein as well. These results differ significantly from what has been found with the rat in which, under normal conditions, neither ferritin nor apparently albumin crosses the basement membrane in appreciable quantities (9, 38).

Our conclusion assumes that the frogs used for tracer experiments were normal and not proteinuric. In support of this, we could not detect urinary protein in the frogs we used, whether urine samples were collected before or after circulation of tracer. In addition, we found virtually no tracer within the proximal tubules, the site where, presumably, protein would be rapidly reabsorbed if glomerular leakage had occurred (12). We nevertheless considered several experimental factors which might have altered the normal distribution of ferritin across the glomerular wall without necessarily causing detectable proteinuria.

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Our initial experiments were performed on double-pithed frogs, a method of anesthesia which conceivably could have altered glomerular filtration. Therefore we also performed experiments on animals anesthetized with urethane. Ferritin penetration of the basement membrane was identified...
tical in each case. We further note that these two methods of anesthesia were those employed in numerous physiological studies (2, 5, 16, 20, 34, 49), the results of which have been used to define the normal filtration properties of the frog glomerulus (2, 30, 33).

Injection of the ferritin tracer could also have altered glomerular permeability by raising either the total plasma volume or the colloid osmotic pressure of the plasma (3, 30). To minimize these effects, we performed several experiments with small volume doses of ferritin. As calculated from published estimates of 3.7-8 ml per 100 g body weight for the plasma volume in the bullfrog (29, 42), the low and intermediate ferritin doses used in our studies would have increased the plasma volume a maximum of 3-7% and 7-14%, respectively. These ferritin doses are comparable on a weight basis to, and two-to fourfold smaller on a volume basis than, the doses used in the experiments of Farquhar et al. on ferritin permeability in the rat (9), an animal with a plasma volume of 4.0 ml per 100 g body weight (47). We found no difference in relative ferritin distribution in glomeruli exposed to either of these two doses. Furthermore, results were identical even with the high ferritin dose which, by similar calculations, could have caused an increase in plasma volume of 31-67%. This ability of the animals to tolerate large volume doses may be due in part to the rapid equilibration between the plasma compartment and large lymph sinuses in the frog (5). On the basis of these considerations, we do not believe that plasma expansion or increased colloid osmotic pressure significantly influenced the results of the tracer experiments.

We have compared the distribution of ferritin in the bullfrog under different conditions of fixation analogous to those used by Ryan et al. (37) and Ryan and Karnovsky (38). These investigators have shown that the penetration of plasma proteins through the glomerular wall in the rat is critically dependent on the maintenance of blood flow through the glomerulus during initial fixation. They observed that endogenous albumin and immunoglobulin G, as demonstrated by specific staining with peroxidase-conjugated antibody fragments, are largely confined to the capillary lumen and endothelial fenestrae when superficial glomeruli are fixed topically under conditions of normal blood flow. However, if glomeruli were immersion-fixed, or if blood flow was interrupted...
before fixation, the proteins were found throughout the basement membrane and, in the case of albumin, within the urinary space. Our results contrast sharply with these results. We have found that in the frog the penetration of ferritin is identical in both superficial glomeruli topically fixed and more deeply lying glomeruli prepared by immersion fixation. The lack of dependence on blood flow is perhaps not surprising because, in the bullfrog, flow through individual glomeruli is normally intermittent (1, 10, 34), whereas no such intermittency has been clearly demonstrated in the rat (30).

We have observed, in all of our tracer experiments, clustering of ferritin within subepithelial regions beneath some slit diaphragms. Accumulation of ferritin molecules as a filtration residue within these regions might be expected if the slit diaphragms were acting as a sieve to the tracer. However, we rarely found the ferritin concentration higher in the subepithelial layer than in the adjacent capillary lumen. Filtration residues within the glomerular wall have been reported in tracer studies on mammals. Farquhar et al. (9) and Caufield and Farquhar (4) have claimed that accumulations of ferritin and dextrans occur along the endothelial side of the basement membrane in rats, although their micrographs did not show tracer accumulations greater than luminal concentrations except within axial regions. A similar situation has been reported for catalase (44). Apparent pooling of myeloperoxidase (13) and cationic derivatives of ferritin (31) within the subendothelial and subepithelial spaces of the mouse basement membrane has been observed. However, this pooling has been interpreted to result, at least in part, from binding of these cationic proteins to negatively charged sites either within the basement membrane or on the surface coat of the foot processes (4, 31). In contrast, Ryan et al. (37) and Ryan and Karnovsky (38) perceived apparently lower concentrations of albumin and immunoglobulin G within the subendothelial space as compared to within the capillary lumen in rats. They believed that these findings were due to the preferential concentration of other, larger plasma proteins in the subendothelial space.

Several reasons may exist for our failure to observe well-defined filtration residues in the bullfrog. Filtration residues, when they do exist, may be simply difficult to preserve, even with the best available techniques of fixation. In addition, the net filtration pressure necessary for formation of the residues probably varies widely from region to region within glomeruli, due both to increasing colloid osmotic pressure as filtration proceeds along the length of functional capillaries (3) and, in the case of the frog, to glomerular intermittency. It is also possible that a high density of fixed negative charges in the subepithelial layer, as is known to be present in the mammal (31), may help to prevent accumulation of anionic ferritin in this region by charge repulsion.

We believe that the differences in ferritin penetration across the glomerular wall between the frog and rat can be most easily explained simply on the basis of the pronounced dissimilarities in the structural organization of the basement membranes of the two species. We have noted several distinguishing features of the frog basement membrane. However, the variable structure of the lamina densa appears most relevant because this layer is believed to be the major permeability barrier to macromolecules in mammals (4, 9, 38). Specifically, those numerous regions in the frog where the filaments of the lamina densa appear loosely packed could provide channels for rapid movement of ferritin into the subepithelial layer. Another factor which could influence the permeability of ferritin would be the presence of the negatively charged groups within the structural components of the basement membrane. Since cationic ferritin, but not the native protein with negative charge, is able to penetrate the mouse basement membrane, it is conceivable that the increased permeability to native ferritin in the bullfrog might be due to a lower charge density within the basement membrane of this species. Other factors, including significantly reduced glomerular capillary pressure (15 mm Hg) and filtration rate (12–100 nl/min·μm²) in the frog compared to the corresponding values in the rat (45 mm Hg and 90–100 nl/min·μm², respectively) (3, 16, 30), might also account for differences in ferritin penetration.

In contrast to the basement membrane, the substructure of the slit diaphragm in the bullfrog, although more difficult to resolve than in mammals, is compatible with the exclusion of ferritin. The approximate space (45 Å) between adjacent cross bridges within the slit diaphragm is significantly smaller than the diameter (122 Å) of the spherical ferritin molecule. It is intriguing, furthermore, that this width is similar to the 40-50 Å width of the rectangular pore within the slit dia-
phragm of the rat, mouse, and human (36, 39).

This suggests that the mammalian slit diaphragm may also be impermeable to ferritin, although under normal flow conditions the slit diaphragm would presumably serve only as a back-up filter to the less permeable basement membrane. Significant in this regard is the observation that cationic ferritin, similar in size to native ferritin, does not cross the slit diaphragm in the mouse although it penetrates the basement membrane in large quantities (31). It is also conceivable that the podocyte surface coat, rather than the slit diaphragm, may serve as a filtration barrier in the region of the slit (22). We have noted that the surface coat in the bullfrog is particularly prominent. However, we are inclined to favor the slit diaphragm as the barrier on the basis of its structure and the fact that, when we can visualize it in tracer experiments, little ferritin is ever found on its urinary side.

The importance of the permeability and structural disparities between the basement membranes in the bullfrog and mammals is not clear because the overall permeability of the glomerular wall to macromolecules is similar in each species (30). Nevertheless, it is possible that a shift in barrier function from the slit diaphragm to an increasingly less permeable basement membrane may have occurred during the evolution of a more efficient glomerular filter.

We sincerely thank Toby Zakin for her assistance during the course of these experiments and Ophelia Wells for her help in the preparation of the manuscript.

Received for publication 2 July 1976, and in revised form 30 June 1978.

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