THE PERMEABILITY OF GLOMERULAR CAPILLARIES OF AMINONUCLEOSIDE NEPHROTIC RATS TO GRADED DEXTRANS*

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We have recently used graded dextrans as tracers to investigate glomerular permeability in normal rats (1) in order to determine which structure in the glomerular capillary wall, the basement membrane or the epithelial slits, represents the primary filtration barrier to macromolecules. Three dextran fractions of specified mol wt (40,000, 62,000, and 125,000) were prepared which bracket the size of albumin, the molecule effectively retained by the normal glomerulus. When injected intravenously into rats, the dextran fractions behaved as predicted in that they were extensively filtered, filtered in small amounts, and extensively retained, respectively. In all three cases dextran particles were found initially in high concentration in the capillary lumen and in the subendothelial portions of the basement membrane but there was a sharp drop in their concentration at this level, i.e., between the inner looser portions of the basement membrane and its outer more compact portions. With the two largest fractions accumulation of particles occurred against the basement membrane in mesangial regions with time, but no accumulation was ever seen with any of the fractions in the epithelial slits or against the slit membranes. Therefore, we conclude that the results obtained with dextrans, like those obtained previously using ferritin as a tracer (2, 3), identify the glomerular basement membrane as the main barrier to the passage of macromolecules of the size of albumin in the normal glomerulus.

In order to extend these observations we have carried out similar studies on animals with experimental nephrosis (induced with an aminonucleoside of puromycin). Nephrotic animals represent an interesting situation in which to study glomerular permeability since they show albuminuria due to increased leakage of albumin by the glomerulus, accompanied by profound changes in the glomerular capillary wall (cf. 4): the usual epithelial foot process organization is lost with a concomitant reduction in the number of epithelial filtration slits, the epithelial cells become filled with protein absorption droplets (i.e., phagosomes and lysosomes), and the basement membrane appears thinner and less compact in some regions.

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We have previously noted (1) that this combination of changes which occurs in nephrotics, i.e., protein leakage accompanied by a reduction in the collective slit area, is difficult to reconcile with the model of glomerular filtration proposed by Karnovsky and his associates (5–8) which postulates that the critical filtration barrier resides at the level of the slits. The changes can be easily understood, however, if the critical filtration barrier is assumed to be the basement membrane. In this paper we report the results of experiments using dextrans as tracers to investigate glomerular permeability in nephrotic animals. In a separate paper (2) we report our detailed findings on the epithelial cell changes found in aminonucleoside-nephrotic animals.

Materials and Methods

**Dextran Fractions.** Two fractions of narrow molecular weight range dextrans were used in these experiments: a 62,000 mol wt fraction with an Einstein-Stokes radius (ESR) of 55 Å, and a 125,000 mol wt fraction with an ESR of 78 Å. They were provided by Dr. Erik Svensjö of Pharmacia AB, Uppsala, Sweden and were the same fractions used in our previous studies on normal animals. Other physical characteristics and details of the preparation of the fractions were given previously (1). For injection the dextrans were prepared as ~1 mM solutions by resuspension in normal saline. The 62,000 mol wt fraction was given as a 7% (wt/vol) solution, and the 125,000 mol wt fraction as a 10% solution. Before use they were heated to disperse aggregates.

**Animals.** A total of 18 young male Wistar-Furth rats with an initial body weight of 140–210 g were used in these experiments. Rats of this strain were used since it has been shown that their vascular permeability is not affected by dextrans (11).

**Production of Nephrosis.** Rats were made nephrotic by daily subcutaneous injections of a 0.5% solution of the aminonucleoside of puromycin at a dose of 1.67 mg/100 g body wt (4), rounded to the nearest 0.01 ml. Animals were reweighed every 3 or 4 days, and the dosage adjusted. Six animals received six daily injections and were used for tracer experiments on the 7th day; another 12 animals received nine daily injections and were sacrificed on the 10th day.

**Experimental procedures.** Rats were anesthetized with ether and the dextran solution (1 ml/100 g body wt) was infused into the saphenous vein over a 7–10 min period using a Harvard Infusion Pump (Harvard Apparatus Co., Inc., Millis, Mass.). At varying intervals from 8 min to 3 h, (Table I) the cortex of the left kidney was fixed in situ using the fixative mixture of Simionescu et al. (21). Pieces of the right kidney were fixed by immersing strips of cortex in the same fixative. Details of the procedure for fixation, subsequent processing of the tissue, as well as those for sectioning and microscopy were given previously (1).

Results

**Glomerular Morphology.** The course of the disease and the alterations which occur in the glomerular capillary wall in the aminonucleoside-nephrotic rat have been described before (4, 13–15) and were recently reviewed (reference 17 and footnote 2). Hence they will be considered only briefly here. It is important to

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1These findings were published previously in abstract form (9, 10).


Abbreviations used in this paper: ESR, Einstein-Stokes radius; HRP, horseradish peroxidase.

* Nephrosis has been induced either by administering daily subcutaneous injections (12–15) or a single larger (10 mg/100 g body wt) intravenous dose (16, 17). The development and course of the disease varies with the two different models. The daily injection protocol was chosen for these experiments because it is the one used in all previous work utilizing electron-opaque tracers to investigate glomerular permeability in the aminonucleoside nephrotic rat (3, 4, 18–20).
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Table I

Dextran Experiments

<table>
<thead>
<tr>
<th>Aminonucleoside treatment</th>
<th>Tracer given</th>
<th>Time interval*</th>
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<tbody>
<tr>
<td></td>
<td>mol wt</td>
<td>8-19 min</td>
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<tr>
<td>days</td>
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<tr>
<td>7</td>
<td>62,000</td>
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<td></td>
<td>125,000</td>
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<td>10</td>
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<td></td>
<td>125,000</td>
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* Timed from the start of tracer injection to initiation of fixation.
† Number of animals used.

stress that although a certain amount of variation is seen between individual animals and from glomerulus to glomerulus of the same animal, the findings are in general reproducible for a given period of treatment. On our regime of daily aminonucleoside injections, the main changes characteristic of the disease process can be detected after 6 days when increased numbers of absorption droplets are seen and some beginning loss of the regularity of foot process arrangement as well as a reduction in the number of epithelial slits is already evident. These changes become more pronounced with each day of treatment up to about 10 days, at which time the overall picture changes slightly in that the number of absorption droplets stabilizes or diminishes, huge intracytoplasmic vacuoles and extracellular pockets (where the epithelium has become detached from the basement membrane) are evident, and alterations are present in other cell layers. By 14 days, the frequency of epithelial absorption droplets is decidedly decreased, denuded areas of basement membrane are more frequent, and complete sloughing of an entire epithelial cell is occasionally noted. Many animals die by the 15th day.

For our studies we chose to use animals sacrificed after 7 and 10 days of treatment. 10-day animals were used because that is the time when the morphologic changes in the glomerulus and the nephrotic syndrome are full-blown and the stage investigated in all previous tracer experiments on aminonucleoside nephrosis (4, 18–20). In addition, 7-day animals were examined since they represent an earlier stage in the disease at which time glomerular changes are minimal or at least not so advanced, and yet proteinuria is present (13). Hence a detailed description is given on the morphology of the glomerulus of the 7- and 10-day animals used in this study.

7-DAY ANIMALS. At 7 days the endothelium, basement membrane, and mesangium appear similar to those in the normal animal. The only changes noted are in the epithelial cell (Fig. 1): first, there is evidence of increased heterophagy in that increased numbers of endocytic invaginations are seen along the plasma membrane, particularly those segments facing the basement membrane at the base of the foot processes, and increased numbers of phagosomes and protein absorption droplets (lysosomes) are seen within the cytoplasm. Second, the normal epithelial foot process organization is partially lost, and the slits are
correspondingly reduced in number. It is now clear, especially from work done
with the scanning electron microscope (22, 23), that this change is due to
withdrawal of the foot processes and their replacement by broad sheets of cyto-
plasm plastered along the outer aspect of the basement membrane, and that this
process is continuous and progressive in acute nephrosis. Third, some of the re-
mainingslits are altered to form occludingjunctions, i.e., the outer leaflets of the
plasma membrane of two epithelial cells sometimes appear fused in sectioned ma-
terial (Fig. 2). In freeze-etched preparations it is clear (references 9 and 10, and

![Image](https://example.com/image1)

**Fig. 2.** Field of a glomerular capillary from a 10 day nephrotic rat sacrificed 11 min after the
injection of 125,000 mol wt dextran. The tracer is seen in the capillary lumen (Cap), the
endothelial fenestrae (f), the urinary spaces (US), and between the endothelial cell (En) and
basement membrane (B). Note the variation in the width of the residual epithelial slits
(arrows). The two on the left show the normal ~250 Å intercellular gap, but the one on the
right is much narrower and is probably obliterated. No dextran is seen in any of the slits. Ep,
epithelium. × 53,000.

![Image](https://example.com/image2)

**Fig. 1.** Portions of glomerular capillaries from a 7 day nephrotic animal sacrificed 30 min
after the injection of 62,000 mol wt dextran. The basement membrane (B) and the endothelium
(En) (with its fenestrae (f)) appear unaltered, but changes are evident in the epithelium (Ep).
Distinct foot processes (fp) are recognizable only on the lower right. In other areas the
basement membrane is covered by broad sheets of epithelial cytoplasm (Ep) which are filled
with protein absorption droplets or lysosomes (ly). Three capillary lumina (Cap) filled with
particles of dextran are present in the field. Numerous dextran particles are present in the open
urinary spaces (US) where they are aggregated into coarse clumps. Dextran is also seen in two
of the smaller lysosomes (ly'). The amount of dextran seen in the urinary spaces and
epithelium is much greater than in normals. × 20,000.
footnote 2) that these junctions represent bands of membrane fusion or fasciae occludentes (occluding fascia) (cf. 24) which are not continuous around the entire perimeter of the epithelial cell. Fourth, and last, occasionally areas of beginning separation of the epithelial cell from the basement membrane occur, usually at the juncture of two capillary loops where there is a sharp inflection of the basement membrane and epithelium. These areas of separation are very small and infrequent at 7 days.

10-DAY ANIMALS. By 10 days some changes are evident in other layers besides the epithelium: the number of endothelial fenestrae is reduced, the number of mesangial cells appears increased, and the basement membrane shows areas of thinning (Figs. 8 and 9) and in some regions has a looser texture than is normally the case. As far as the epithelial cell is concerned, the loss of the foot process organization and reduction in slit number has become more pronounced, and the occluding junctions more extensive. The frequency of endocytic vesicles, phagosomes, and protein absorption droplets is just as prominent as at 7 days, but the relative numbers of the last two structures is quite variable from one cell or glomerulus to another. Some cells contain multiple large absorption droplets whereas others contain mainly larger vacuoles, with a content of low density, which range in size up to several micrometers (Fig. 9). In micrographs it is not always clear in a given plane of section whether the latter represent intracellular vacuoles or complicated, dilated extracellular spaces (sinuses) whose connections to the cell surface and/or residual slits is not evident in the plane of section. In the tracer studies the two can be distinguished at early time points because the contorted extracellular spaces contain tracer particles whereas the intracellular vacuoles do not. The areas of separation of the epithelial cell from the basement membrane are more common and more extensive at 10 days. As in the 7-day animals these are most often found at the juncture of two capillary loops (Fig. 12), but they are also found along more peripheral areas of the loops where they appear as pockets formed by detachment and invagination of the epithelial cell membrane.

7-Day Animals: 62,000 and 125,000 mol wt Dextrans. Except for the presence of somewhat greater numbers of particles of the smaller molecular weight fraction in the urinary spaces, the results obtained with these two fractions were similar and hence will be reported together. In many respects the findings are comparable to those in normal animals (Fig. 1): at short intervals particles are seen in high concentration throughout the capillary lumen, within the endothelial fenestrae, and in the subendothelial areas between the endothelium and basement membrane; particles are not usually seen in the basement membrane or in the epithelial slits, but some are seen in the urinary spaces beyond the level of the residual slits; with increasing time after injection (1–4 h) there is increasing accumulation of dextran along the luminal side of the basement membrane in the mesangial regions, but no accumulation is seen in the residual epithelial slits, of either the normal or tight variety, or piled against the slit membranes. Also, up to 3 h after injection (the longest interval studied) the particles are still retained in the capillary lumen at high concentration approximating that injected initially, indicating that relatively little of the
total dextran given has been lost from the circulation by filtration. As in the normal, at all intervals there is a sharp drop in the concentration of dextran between the inner, less compact portions of the basement membrane (lamina rara interna) and its denser portions (lamina densa), and at the longer time points some dextran can be seen within lysosomes in mesangial cells. The latter probably results from endocytosis of dextran residues (which accumulate against the basement membrane in mesangial regions) by mesangial cells comparable to that seen previously in the case of ferritin and other particulate tracers (4, 25).

In other respects the findings differ from those in the normal in that: (a) more dextran particles were found at all time points in the open urinary spaces, and (b) there was much more accumulation of dextran within protein absorption droplets in epithelial cells. From work with other tracers (4, 26, 27) such protein absorption droplets are known to correspond to lysosomes. By 3 h after injection, roughly half the lysosomes contain dextran as part of their content, with the dextran typically being segregated by itself into one or several regions of the lysosomes (see Figs. 10–11). Presumably the incorporation of dextran into lysosomes occurs in the same manner as shown previously for ferritin (3, 4, 26) by pinocytosis of dextran particles occurring primarily along the base of the epithelial cell foot processes, followed by fusion of vesicles with phagosomes (phagocytic vacuoles) which subsequently fuse with lysosomes (Fig. 10).

In summary, the only difference which can be noted between the findings in the 7-day nephrotic and the normal are the increase in the amount of dextran particles found in the urinary spaces and in protein absorption droplets in the epithelium. As far as the location of particles in the urinary spaces is concerned, they were found mostly lying free in the urinary spaces. At early intervals (before significant endocytic uptake and intracellular segregation of dextran had occurred) the presence of dextran particles served as a useful marker of the urinary spaces (Fig. 1).

10-Day Animals; 62,000 and 125,000 mol wt Dextrans. As in the case of the 7-day animals, the only difference between the findings with the two different fractions was in the number of particles present in the urinary spaces, i.e., greater numbers of particles were found in the urinary spaces with the small molecular weight fraction. Hence the results will be described together. The main findings at 10 days were the same as at 7 days: (a) most of the dextran particles are retained in the lumen (Figs. 3–4); (b) there is a sharp drop-off in the concentration of particles between the subendothelial portions of the basement membrane and the dense portions of the basement membrane (Figs. 3–6); (c) no accumulation is seen at any time point against the slits; but (d) many dextran particles are seen in the open urinary spaces beyond (Figs. 3 and 11); and (e) significant accumulation of dextran is found within absorption droplets in the epithelium after longer time intervals (Figs. 10–11). An additional finding in the 10-day animals is that more dextran is present within subepithelial areas (Figs. 6–8) where the outer, lighter layer of the basement membrane (lamina rara externa) appears widened (Figs. 8 and 9), possibly due to loosening of the attachment between the epithelium and the basement membrane.
membrane and epithelium (Fig. 12). These pockets have not been seen to form channels across the epithelial cell. At early intervals the concentration of dextran in the pockets is low, but at later intervals it is higher, approximating that found in the lumen. This suggests that the pockets may be mostly "blind" in the sense

**Fig. 3.** Portion of the glomerular capillary from a 10 day nephrotic rat sacrificed 3 h after the injection of 62,000 mol wt dextran. The concentration of dextran in the capillary lumen (Cap) is quite high. Numerous dextran particles can also be seen between the endothelium (En) and the basement membrane (B). No dextran is seen in the residual slits (arrows) but some is seen in the urinary spaces (US). The retention of dextran in the circulation plus the sharp drop in its concentration which occurs at the level of the dense portions of the basement membrane indicates that the latter is still retaining most of the dextran. × 55,000.

**Fig. 4.** Portion of a glomerular capillary from a 10 day nephrotic animal sacrificed 3 h after the injection of 125,000 mol wt dextran. The field is from the edge of a mesangial area (Me). Note the high concentration of dextran in the capillary lumen (Cap) and between the endothelium (En) and the basement membrane (B). No dextran is seen in the lamina densa (B), the subepithelial portion of the basement membrane or piled against the slit (arrow). A few particles are seen in the urinary space (US) beyond the slit. × 58,000.

**Fig. 5.** Mesangial area from a 10 day nephrotic animal 3 h after the injection of 125,000 mol wt dextran. Dextran is seen in the intercellular spaces between mesangial cell processes (long arrow) and between the mesangial cell (Me) and the basement membrane (B) (short arrow). Dextran is also present within a lysosome (ly') in the mesangial cell. Many pinocytic invaginations are present along the plasma membrane of the epithelial cell (Ep) where it faces the basement membrane, and numerous protein absorption droplets or lysosomes (ly) are seen in the epithelial cytoplasm. × 23,400.
FIG. 9. Portion of glomerular capillary from the same animals as in Fig. 8. The dense portion (lamina densa) of the basement membrane (B) appears thinner than usual, especially to the right; the subepithelial, outer, lighter layer of the basement membrane (lamina rara externa) appears correspondingly wider (→). Note also the presence of a large phagocytic vacuole (vac) and a protein absorption droplet or lysosome (ly) in the epithelial cell (Ep). The phagosome contains some dextran particles. Cap, capillary lumen; US, urinary spaces; Me, mesangial cell; and En, endothelial cell. x 32,000.

FIG. 6. Portion of glomerular capillary from a 10 day nephrotic animal sacrificed 11 min after the injection of 125,000 mol wt dextran. Dextran particles are present at high concentration in the capillary lumen (Cap) and at low concentration in the urinary spaces (US). A few particles are seen in the basement membrane (B), primarily in its subepithelial regions (long arrow). Note the presence of numerous pinocytic invaginations (short arrows) along the plasma membrane of the epithelial cell (Ep) where it faces the basement membrane (B). One of these invaginations is seen as a fully or almost fully internalized vesicle (ve) containing dextran. RBC, red blood cell. x 42,000.

FIG. 7. Glomerular capillary from a 10 day nephrotic animal 2 h and 20 min after the injection of 125,000 mol wt dextran. Dextran particles are seen on the epithelial side (Ep) of the basement membrane (B) located between the lamina densa and the epithelial cell membrane (long arrows) and in pinocytic invaginations of the epithelial cell membranes (short arrows). Tracer is also present between the endothelium (En) and basement membrane. x 67,000.

FIG. 8. Glomerular capillary from a 10 day nephrotic animal 17 min after the injection of 125,000 mol wt dextran showing an area in which the lamina densa (B) appears thin and dextran particles are present between the epithelial cell (Ep) and the basement membrane (B) (arrow). Cap, capillary lumen; and En, endothelium. x 126,000.
Fig. 10. Glomerular capillary from a 10 day nephrotic animal sacrificed 3 h after the injection of 125,000 mol wt dextran. The figure shows the segregation and concentration of dextran in protein absorption droplets of lysosomes (ly₁–ly₇). Dextran is also present in the capillary lumen (Cap) and in the intercellular areas of the mesangium (arrow). Fields such as this suggest that the dextran which is picked up by pinocytosis along the epithelial cell membrane facing the basement membrane (B) is ferried via pinocytic vesicles to phagocytic vacuoles or phagosomes (ly₁) which undergo condensation (ly₄) and fuse with other dense protein absorption droplets (ly₁–ly₄). The segregation of dextrans in specific areas of the lysosomes
Dextran is also seen in the pockets or areas of separation between the basement that they have no direct connection with the urinary spaces, and since there is no possibility of drainage, the dextran concentration gradually equilibrates with the concentration in the lumen.

**Extraglomerular Location of Dextran.** As in the normal, dextran is found: (a) in the lumen of all segments of the nephron, (b) in apical vacuoles and in lysosomes of the proximal tubule cell where it is undergoing reabsorption, (with the amount present varying inversely with the mol wt $[62,000 > 125,000]$), (c) in the peritubular capillaries where its concentration approximates that in the glomerular capillaries, and (d) penetrating the lateral intercellular spaces up to the level of the occluding zonules of the tubule epithelial cells. The main difference from the findings in the normal is that, as expected, much more dextran is seen in the lumen and in proximal tubule cells in the nephrotic animal.

**Discussion**

*Permeability Data Obtained.* Based on work using dextrans as tracers, we have previously obtained evidence that the basement membrane acts as the main filtration barrier retaining plasma proteins in the normal glomerulus (1). The findings with dextrans, together with virtually all of the experimental evidence obtained using other electron-opaque particulate tracers, identify the basement membrane, rather than the epithelial slits, as the principal glomerular filter (cf. 28). The present study, using dextrans as tracers in the nephrotic glomerulus, was undertaken in order to investigate the behavior of the filter, i.e. the basement membrane, under pathologic conditions. The aminonucleoside nephrotic animal, a model for lipoid nephrosis in humans, represents a particularly interesting situation in which to examine glomerular permeability. In this condition there is increased leakage of plasma proteins, primarily albumin, through the glomerulus and, along with the permeability defect, there are profound morphologic changes in the glomerular capillary wall — particularly in the arrangement of the epithelial filtration slits which are drastically reduced in number and in some instances are replaced by occluding junctions (references 4, 9, and 10, and footnote 2).

Available physiologic data indicates that the permeability defect in pure nephrosis is fine and widespread. In both the aminonucleoside model (17) and the human prototype (29, 30) of the disease, the glomerulus still behaves as a sieve (as in the normal) in that there is increasing restriction to passage of macromolecules (proteins [17, 29] and dextrans [29, 30]) with increasing molecular weight. In the nephrotic glomerulus the "pores" of the glomerular filter are assumed to have a slightly larger diameter, allowing increased amounts of albumin to escape. Results of recent micropuncture studies on the aminonucleoside nephrotic rat (31) have shown that the glomerulus still retains most of the albumin, confirming that the permeability defect in the filter is subtle.

appears to result from fusion of a dextran-containing phagosome (such as ly₃) with a protein absorption droplet (such as ly₂), resulting in images such as ly₁. Concentration and compaction of the content of the dextran phagosome result in lysosomes with dense dextran caps (ly₁–ly₃). It is of interest that in old lysosomes, protein and dextran residues remain segregated and do not mix. *Me,* mesangial cell; and *En,* endothelial cell. × 38,000.
To investigate glomerular permeability in nephrosis we have used two dextran fractions, a 62,000 mol wt fraction which is close to the size of albumin and a 125,000 mol wt fraction which is considerably larger. From the permeability data reviewed above, both fractions would be expected: (a) to be mostly retained by the filter, i.e. the basement membrane, but (b) to leak through it in greater amounts than in the normal. The findings bore out these predictions. The fact that the dextrans remained in the circulation at high concentrations over long periods, the marked drop in the concentration of tracer at the level of the basement membrane, and the accumulation of tracer against it in mesangial regions, demonstrated that most of the dextran is retained by the basement membrane. Increased leakage of dextran through the basement membrane is indicated by the presence of many more dextran particles in the open urinary spaces, in the lamina rara externa between the epithelium and the basement membrane, and within epithelial cells. The presence of dextrans in a subepithelial location at the base of the residual foot processes is evidence of increased leakage on a short-term basis, whereas the accumulation within protein absorption droplets in epithelial cells amounts to a demonstration of continual, long-term leakage.

Further new information is provided by the findings on the probable pathway of the filtrate early in nephrosis. The presence of numerous dextran particles marking the urinary spaces at short time points (up to 19 min) suggests that the major portion of the filtrate flows through the residual epithelial slits, rather than being transported through the cells as had been suggested previously (4, 18, 19). Probably the amount of the filtrate that is directed through the cells by pinocytosis is relatively small. Filtration through residual slits can be assumed to apply early in the disease process (up to 10 days) but the pathway, although still extracellular, may become more complicated later on with the development of extensive pockets and especially with sloughing of the epithelium (cf. footnote 2).

**Basement Membrane Changes in Aminonucleoside Nephrosis.** Our observations have confirmed that very early in nephrosis (7 days) no obvious morphologic alterations are detectable in the basement membrane, but as the disease progresses, various changes — especially thinning of the central dense layer of the basement membrane (lamina densa) and widening of the outer lighter layer (lamina rara externa) — become increasingly frequent. In view of these morphologic changes, it would be of interest to know if there is any change in the biochemical composition of the basement membrane of nephrotics. The
composition of the normal glomerular basement membrane is now reasonably well understood (32, 33). It is known to consist of two distinct types of glycopeptides: (a) low molecular weight peptides which belong to the collagen family of proteins, with disaccharide units (consisting of glucose and galactose linked to hydroxylysine), and (b) larger noncollagenous peptides with heteropolysaccharide units (made up of sialic acid, fucose, galactose, glucosamine, and mannose linked to asparagine). However, the precise way that these glycoproteins are assembled in the three-dimensional structure of the filter is still unknown.

Several analyses have been made of the biochemical composition of the glomerular basement membrane in aminonucleoside nephrosis (34–37) and abnormalities in the composition (34, 35) and/or in the rate of synthesis (34–37) of basement membrane components have been reported in all these studies. However, the results obtained so far are somewhat contradictory, and the precise defect in the composition and/or turnover of glomerular constituents has not
yet been pinpointed. Hence at present our information is too limited to enable us to devise a model that explains in physical terms the normal permeability properties of the basement membrane or the increased permeability associated with the nephrotic syndrome.

**Work with Other Electron-Opaque Tracers.** There have been several previous studies in which electron dense particulate tracers (3, 4, 20) or histochemically demonstrable mass tracers (18, 19) have been used to investigate glomerular permeability in aminonucleoside nephrotic rats.

Results obtained by Farquhar and Palade (3, 4) using ferritin (mol wt, 480,000) are very similar to those reported here for dextrans: most of the tracer was retained in the lumen by the basement membrane, but increased numbers of tracer molecules leaked through the basement membrane and were picked up by the epithelium and segregated into protein absorption droplets. It was concluded that the basement membrane behaved as the main glomerular filter, but was defective and allowed increased quantities of proteins to escape.

Gang and Mautner (20) have utilized lanthanum hydroxide to examine the ultrastructure of the glomerular basement membrane in aminonucleoside nephrotic rats. When this solution was injected into the renal arteries of normal rats and the kidneys fixed simultaneously, a fine precipitate of lanthanum particles was found distributed uniformly throughout the glomerular basement membrane. In aminonucleoside nephrotic rats similarly prepared, there were focal dense concentrations of lanthanum in the basement membrane and a general increase in the size and number of the lanthanum aggregates throughout this structure. Thus, this work points to the existence of abnormalities in the basement membrane in this condition, but otherwise the findings are difficult to interpret since at present the precise mechanism of lanthanum precipitation is not understood.

Studies with mass tracers (proteins with peroxidatic activity) in this disease model have also been difficult to interpret and have been limited so far to horseradish peroxidase (HRP) (mol wt, 40,000), a relatively small tracer, and catalase (mol wt, 240,000) which is relatively large. Venkatachalam et al. (18), using HRP, obtained similar results in both the normal and nephrotic: the tracer rapidly (within 1 min) gained access to the basement membrane, epithelial slits, and urinary spaces. In the nephrotic it was also found within pockets and vacuoles in the epithelium. No defect in permeability could be pinpointed which is not surprising in view of the tracer's relatively small size and the fact that no barrier to its passage could be defined in the normal.

These same authors (19) showed that there is increased glomerular permeability to catalase since this tracer was found in tubular lumina of nephrotics as early as 3 min after injection, whereas no catalase was detected in the tubular lumina in normals. However, the site of catalase leakage was difficult to pinpoint since the main findings were rather similar in both normals and nephrotics: there was a concentration gradient across the basement membrane; usually there was no penetration of the tracer through the epithelial slits into the urinary spaces in the normal, and no catalase was found in the residual slits and in the “close junctions” in the nephrotic. As with all the other tracers studied (ferritin, HRP, and dextrans), catalase was seen in epithelial pockets and vacuoles in nephrotics.
From these findings the authors concluded that the increase in permeability was due to changes in both the basement membrane and the epithelium, the lesion in the epithelium presumably taking the form of large, complicated channels bypassing the slits. The evidence for increased basement membrane permeability is clear cut, but that for the channels is open to question (see below). Considered as a whole the explanation appears unnecessarily complicated. The findings could be accounted for simply by an increase in the permeability of the basement membrane (in fact accepted by the authors) without further elaborations.

Filtrate Pathway in Nephrosis. Previous tracer work has raised a question concerning the pathway of flow of the glomerular filtrate in nephrotics. The drastic reduction in the collective slit area (from 20 to 2% of the outer basement membrane surface [14]) which occurs in nephrosis led to the suggestion that a substantial part of the filtrate might be diverted through the epithelial cells. Results obtained with ferritin and catalase did not help solve the problem, since due to the large size of these tracer molecules, which are much larger than albumin, only small amounts are filtered.

Results with HRP, which is smaller than albumin, are potentially more helpful. Since this tracer was found in the urinary spaces and in vacuoles and pockets within epithelial cells (18), it was concluded that it crossed the glomerular epithelium through residual slits as well as through transcellular channels, formed by pockets at the cell surface and by vacuoles within the cells. These channels were assumed to connect pockets facing the basement membrane with the urinary spaces and thereby establish a pathway through which HRP and catalase (and by implication the glomerular filtrate in general) reach the urinary spaces. The existence of these channels and their role in control of glomerular capillary permeability is open to question for two reasons: First, examination of some of the micrographs which are supposed to demonstrate communications to the urinary spaces (see especially Fig. 9 in reference 18) indicates that they are images of lysosomes discharging their residues (see Figs. 7 and 8 in reference 4, and Figs. 10 and 11 in reference 26) rather than transcellular channels. Second, as recognized by the authors, such channels cannot explain the molecular sieving that still operates in nephrosis.

As already mentioned, the results with dextrans (especially the occurrence of tracer in the urinary spaces at early time points) suggest that most of the filtrate flows through the slits. This conclusion is also supported by results of our recent unpublished experiments in which cationic proteins of small molecular weight were used to mark the filtration pathway.

Visualization of Dextran. In normal animals we found that dextran is readily visualized in the capillary lumen, the urinary spaces, and within the inner, looser, subendothelial portions of the basement membrane where it occurred as aggregates of varying size. However, it was not often seen within the denser portions of the basement membrane (lamina densa). We reasoned that this failure to see many particles in this location was due to their low frequency (and therefore little opportunity for aggregation) and to the high background created by the density of the lamina densa which rendered their staining and therefore their visualization difficult. This conclusion is supported by the present findings.
on nephrotic animals since in this situation where many more dextran particles are traversing the basement membrane, dextran particles can sometimes be visualized in the lamina densa and are often seen in the subepithelial region (lamina rara externa) of the basement membrane.

Functional Model of the Glomerulus. In light of these tracer experiments in nephrotic animals it is of interest to re-examine the functional roles for the glomerular capillary components proposed by Farquhar et al. (2, 25) based on their experiments with ferritin. According to this model, the basement membrane functions as the main filter responsible for the retention and selective filtration of macromolecules, the endothelium acts as a valve before it, and the epithelium as a monitor behind it, recovering part of the protein that leaks through the filter. The mesangial cell serves to unplug and recondition the filter by phagocytizing filtration residues (25). This model was recently (1) put to the test in normal animals using dextrans as tracers and was found still to be valid.

According to the model, in nephrosis (4) the filter is leaky and allows increased passage of proteins, and as a result the monitoring activities of the epithelium are greatly enhanced. This conclusion is supported by all the tracer studies in nephrotic animals — those with ferritin (4) and catalase (19), as well as the dextran results reported here — since there is evidence (a) that the basement membrane is more permeable to these tracers than is normally the case, and (b) that uptake (by endocytosis) of all these tracers is increased. On the other hand, there is no evidence for increased leakage at the level of the filtration slits or slit membranes. In fact, the evidence appears quite to the contrary since the slits are not only reduced in number, but many of them are tighter than usual (cf. references 4, 18, and 19, and footnote 2).

In addition to its monitoring function, in both the normal and the nephrotic glomerulus the epithelium probably acts as a supporting grid for the basement membrane, with the slits allowing passage of the filtrate into the urinary spaces. The arrangement is analogous to that in an artificial ultrafiltration membrane (38, 39) composed of a thin membrane, which accounts for selective permeability, and a "porous support" on which the thin membrane rests. The porous support functions to strengthen the thinner membrane, to increase the effective path length across it, and to limit hydraulic fluxes across the system by either reducing the radius or the frequency of the pores in the support (38). By analogy with a porous support, the epithelium may limit hydraulic fluxes across the glomerular capillary wall as a result of the distribution and width of the filtration slits. Such effects could explain the accumulation of dextran particles in the subepithelial spaces in nephrotics in which the frequency of slits is reduced: the particles pass the filter but are retained by the body of the epithelial cells (acting as a barrier). This accumulation would be expected to facilitate pinocytosis. The relatively normal glomerular filtration rates seen in pure nephrosis and in aminonucleoside nephrosis in the face of a drastic reduction (from 20 to 2%) of the collective slit area could be explained either by the fact that the available slit area is more fully utilized in nephrotics than in normals, or by the fact that in nephrotics the hydraulic pressure in glomerular capillaries is increased (as has been recently demonstrated to be the case in Masugi nephritis [40]), or by a combination of these factors.
On the basis of our evidence and the preceding discussion, we can conclude that protein leakage in nephrosis occurs by virtue of a generalized defect in the glomerular basement membrane which is probably due to alterations in the biosynthesis of one or more of its components. The fact that the epithelium is known (41, 42) to make basement membrane components, suggests that the primary biosynthetic lesion may be in these cells. The defective basement membrane results in increased protein concentration in the glomerular filtrate which in turn somehow brings about modifications in the foot processes. At least the available evidence suggests that the latter is a secondary lesion since modifications in the foot processes can apparently be induced experimentally by protein infusion (references 41-45, and footnote 5) or by perfusion with polycations (46).

Summary

Graded dextrans were used as tracers to study glomerular permeability in nephrotic rats. Two narrow range fractions were used, one which was approximately the same size as albumin (62,000 mol wt) and one which was considerably larger (125,000 mol wt). Nephrosis was induced with daily injections of an aminonucleoside of puromycin, and the animals examined after 7 days, when proteinuria is minimal, or after 10 days, when proteinuria has almost reached a maximum.

At both stages and with both dextran fractions the following results were obtained: (a) dextran was retained for up to 3 h (the longest interval studied) in the plasma at high concentration; (b) there was a sharp drop in the concentration of tracer between the inner, looser portions of the basement membrane (lamina rara interna) and its outer denser portions (lamina densa), (c) accumulation of dextran was seen in the mesangial areas with time; and (d) no accumulation of dextran was seen in the slits at any time. These results are the same as those reported earlier in normal animals, and they demonstrate that in nephrotics the basement membrane still behaves as the main filtration barrier retaining most of the plasma proteins.

Certain differences from the findings in normals were also noted in that increased amounts of the tracer were present on the epithelial side of the basement membrane: (a) in the urinary spaces; (b) in the subepithelial portions of the basement membrane; and (c) within lysosomes (protein absorption droplets) in the epithelial cytoplasm. In addition areas of thinning of the dense portions of the basement membrane (lamina densa) were seen which were accompanied by a corresponding widening of the less dense, subendothelial and subepithelial layers (lamina rara interna and externa, respectively). The presence of increased quantities of dextran on the epithelial side of the basement membrane indicates that the filter, i.e. the basement membrane, is leaky and allows increased passage of dextrans and therefore plasma proteins.

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1 See, however, the work by Lannigan and McQueen (47).
References


22. Arakawa, M. 1970. A scanning electron microscopy of the glomerulus of normal and