Ultrastructure of the Glomerular Basement Membrane, as Visualized by Lanthanum (35024)

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(Introduced by J. Leonard Brandt)
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The glomerular capillary wall consists of three layers: endothelium, basement membrane, and the foot processes of the visceral epithelium. The endothelium is separated from the foot processes by the basement membrane (1–3). The basement membrane itself, as revealed by the electron microscope, is a triple-layered structure: a central dense zone (lamina densa) with a less dense zone on each side (lamina rara interna and lamina rara externa) (2, 4–9). The less dense zones are thought to represent a greater fluid content (10).

Studies on the transport of injected electron dense substances such as ferritin (2, 11), Thorotrast (12), Imferon (13), dextrans (14), and peroxidases (15) of different sizes have indicated that molecules having a diameter of 150 Å, or aggregates of smaller tracers, are able to pass through the basement membrane fairly rapidly. However, the exact route or mode of transport for these macromolecules, or for small molecules and water, is not yet known since no morphologic data are available on the size or distribution of spaces or channels in the basement membrane.

The purpose of the present study was to elucidate this problem by attempting to visualize intermolecular spaces in the basement membrane with the use of lanthanum hydroxide, an electron dense colloidal particle with a diameter less than 20 Å. When tissues are infused with, or placed in glutaraldehyde and/or osmium tetroxide containing lanthanum hydroxide, the particles settle into fine tissue spaces during fixation and are visualized as electron dense areas with the electron microscope (16, 17). It is assumed that the electron opaque aggregates of lanthanum hydroxide fill the spaces which under physiological conditions are occupied by interstitial fluid, or water (17).

Materials and Methods. A total of 15 male Sprague-Dawley rats, weighing 150–175 g, were used. The rats were anesthetized with sodium pentobarbital (30 mg/kg) and the abdominal cavity was exposed.

In 10 of the animals the kidneys were decapsulated, cortical biopsies were taken and the specimens were placed in a drop of ice-cold 3% glutaraldehyde in *s*-collidine buffer (pH 7.45) containing 1% lanthanum hydroxide (17). The remaining kidney tissue was then perfused with the glutaraldehyde fixative containing lanthanum, and biopsies were then taken. The tissue fragments were then cut into minute pieces (less than 1 mm) and were fixed for an additional 16 hr at 4°. They were then washed 4 times in a cold suspension of 1% lanthanum hydroxide in *s*-collidine buffer (pH 7.45) by the dropwise replacement method. The tissues were postfixed for 2 hr in 1% OsO_4 in *s*-collidine buffer (pH 7.4) also containing 1% lanthanum. After dehydration in graded alcohols, the tissues were treated with propylene oxide and embedded in Epon (18).

In the remaining 5 animals the renal artery was exposed and the kidneys were infused with a 1% suspension of lanthanum hydroxide in *s*-collidine buffer. At the same time the renal cortex was fixed by subcapsular perfusion with 3% gluteraldehyde in the same buffer. The duration of infusion and simultaneous fixation was 10 min. Biopsies

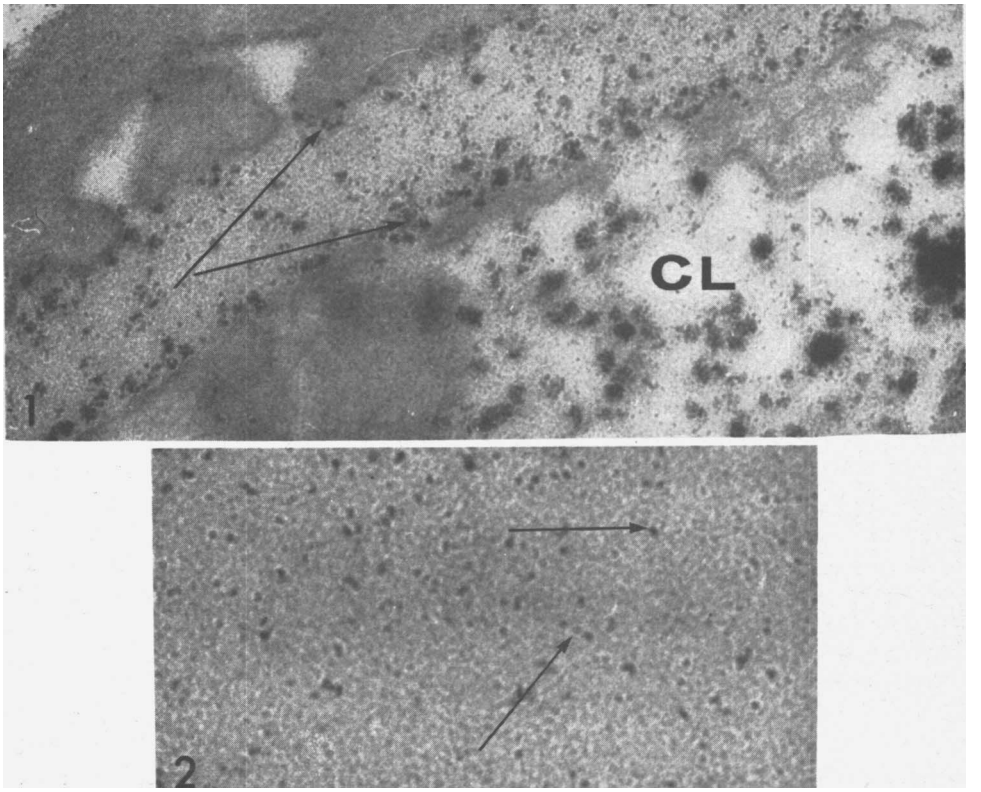

FIG. 1. Segment of a glomerular capillary wall showing lanthanum hydroxide particles in the capillary lumen (CL); as large aggregates in the lamina rara interna and externa (arrows) and randomly distributed particles in the region of the lamina densa; unstained; ×150,000.

FIG. 2. Part of the lamina densa from Fig. 1, at a higher magnification. Lanthanum is deposited as aggregates of varying sizes (arrows); unstained; ×500,000.

were then taken and processed for electron microscopy as described in the foregoing paragraph, but without added lanthanum.

Silver to gold colored sections were cut on an LKB Ultratome II with diamond knives, placed on uncoated copper grids, and examined in an RCA EMU-2E and Hitachi 7S electron microscopes.

Electron micrographs were taken at primary magnifications from ×3625 to 100,000. All measurements of aggregate size were performed on electron photomicrographs taken at × 100,000 and enlarged five times. Measurements were taken only at the periphery of the capillary loops, and in areas where the opposing plasma membranes of the endothelial cells and the epithelial foot processes were sharply contoured, *i.e.*, areas of cross section. All measurements were made to the nearest millimeter (20 Å).

Results. The glomeruli of rat kidney fixed by infusion or by immersion in 3% glutaraldehyde and postfixed in 1% OsO_4, both containing 1% lanthanum hydroxide, as well as those infused with the tracer but fixed in the absence of lanthanum showed lanthanum hydroxide particles in the capillary lumen, in the basement membrane, and in the urinary spaces. In the capillary lumen and in the urinary spaces the tracer was seen as large aggregates several 100 Å in diameter (Fig. 1). In the basement membrane, lanthanum was visualized as small aggregates, the diameter of which varied between 20 and 200 Å (Fig. 1). In general, spaces occupied by lanthanum with diameters less than 100 Å were

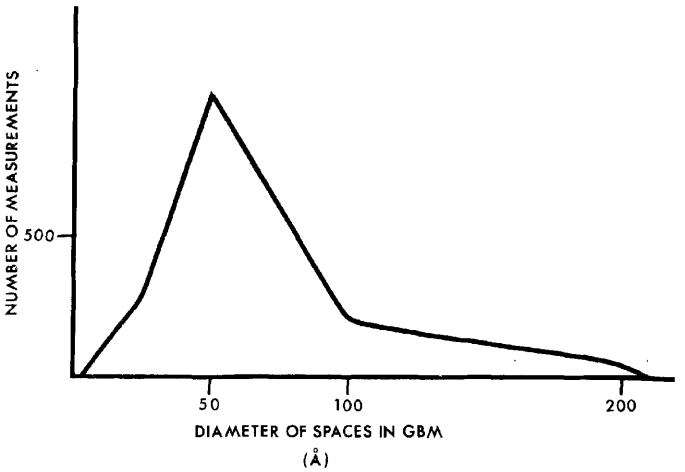

FIG. 3. Distribution curve of aggregate size measurements. The majority of the aggregates in the glomerular basement membrane have diameters ranging from 40 to 75 Å. Most of the aggregates with diameters greater than 100 Å were located in the laminae rarae (see Fig. 1).

restricted to the area of the lamina densa and were distributed in a random fashion (Figs. 1 and 2). Occasionally larger aggregates were also present. The lamina rara interna and externa contained larger aggregates with an average diameter of approximately 150 Å. The number of these aggregates was more numerous in the lamina rara interna than in the lamina rara externa.

The distribution curve of aggregate size showed that aggregates ranging in diameter from 20 to 125 Å have a "normal" distribution in the glomerular basement membrane (Fig. 3). The peak of the curve was at 50 Å. The majority of the aggregates had diameters in the range of 40 to 75 Å.

It must be pointed out, that in the biopsies processed by immersion fixation in the presence of lanthanum, only those glomeruli in which Bowman's capsule had been cut during preparation of the tissues showed penetration of the particles.

Discussion. As described by Revel and Karnovsky (16) and by Schatzki (17), we also observed no penetration of the plasma membranes by lanthanum. In the interstitial spaces lanthanum hydroxide is assumed to fill intermolecular or large intramolecular spaces of the structural macromolecules which under physiological conditions are occupied by water (16, 17). It is reasonable to assume that lanthanum hydroxide particles were present in such spaces normally existing in the glomerular basement membrane and not in artifactual spaces created by fixation, since no difference in the distribution of the tracer was seen between tissues fixed in the presence of lanthanum or those which were injected with the colloidal particles. However, chemical binding of the trivalent lanthanum cations with acidic groups in the basement membrane cannot be ruled out, if dissociation of the colloid occurred.

The failure of lanthanum to penetrate glomeruli with an intact Bowman's capsule after immersion fixation was the most important limitation of the immersion-type of approach for the study of space distribution within the glomerulus.

In the area of the lamina densa the lanthanum particles ranged in size from 20 to 100 Å. This observation indicated that molecules up to 100 Å in diameter could pass through the membrane. However, the distribution curve of aggregate-size showed that the available space for the passage of larger molecules, with diameters greater than 75 Å, is substantially less than for smaller ones and thus suggested that the lamina densa might be the rate limiting structure in the glomerular filtration of macromolecules. The above suggestion is in agreement with the present

concept of glomerular filtration of macromolecules based on physiological and morphologic studies (2, 12–15, 19).

Pappenheimer (20), assuming the hydrostatic pressure of the blood to be the filtration force, calculated that the capillary wall consists of pores with an effective radius of 30–40 Å. The majority of the spaces delineated by lanthanum did in fact have a radius comparable to that suggested by Pappenheimer.

Huang *et al.* (21), on the basis of filtration studies using isolated rat glomerular basement membrane, suggested that the basement membrane has a structure analogous to a cross-linked dextran, and that the mechanism of filtration is similar to gel filtration. The random distribution of the pores in the lamina densa supports this hypothesis.

As compared to the lamina densa the laminae rarae showed numerous large spaces occupied by lanthanum, averaging 150 Å in diameter. Consequently, the spaces constitute a substantially great portion of the laminae rarae. Since lanthanum is assumed to permit the visualization of spaces occupied under physiological conditions by extracellular fluid or water, the electron microscopic appearance of these regions of the basement membrane might indicate a greater fluid content, as was previously suggested (10).

It is concluded that the glomerular basement membrane consists of three components differing in molecular ultrastructure. The laminae rarae are made up of loosely connected network of protein, and therefore would retard the passage of large molecules only. The lamina densa is a random porous molecular filter with intermolecular spaces varying in diameter from 20 to 100 Å, acts as a coarse filter, and is the rate limiting structure in the glomerular filtration of macromolecules. It allows the rapid passage of water and small molecules through all of the spaces, but will retard the movement of molecules with diameters greater than 75 Å.

The supposition that the spaces in the basement membrane outlined by lanthanum are representative of the permeability characteristics of the membrane is further strengthened by the close correlation between the size and number of spaces, and the degree and selectivity of the proteinuria in rats with nephrotoxic serum nephritis (22).

Summary. Cortical tissues from the kidneys of rats were infused with a suspension of lanthanum hydroxide, or were placed in fixatives containing lanthanum to visualize intermolecular spaces in the glomerular basement membrane. Lanthanum particles were seen in aggregates throughout the basement membrane. The diameter of the aggregates varied between 20 and 200 Å. The diameter of the majority of aggregates ranged from 40 to 75 Å. Aggregates with diameters greater than 150 Å were located mainly in the lamina rara interna, less in the lamina rara externa.

It is concluded that the basement membrane is a random porous molecular filter and the lamina densa is the rate limiting structure in the glomerular filtration of macromolecules.

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