Glomerular Basement Membrane Damage in Aminonucleoside Nephrosis as Visualized by Lanthanum[1] (36477)

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(Introduced by J. L. Brandt)

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The administration of puromycin aminonucleoside to rats has been widely used experimentally for the induction of proteinuria (1). It is believed that the proteinuria is a consequence of an increase in the permeability of the glomerular basement membrane (GBM or basement lamina) to macromolecules (1). Studies on the morphologic alterations in the glomerulus, however, did not substantiate the foregoing assumption, because attempts with conventional electron microscopic techniques have not been successful in the demonstration of changes in the molecular structure of the GBM, even though increased movement of large tracers have been noted (2). Gang (3) has recently described the use of lanthanum hydroxide tracers in the study of GBM ultrastructure. Lanthanum hydroxide is an uncharged electron-dense colloidal particle, less than 20 Å in diameter, and has been employed to visualize spaces which under physiologic conditions are occupied by interstitial fluid, or water (4, 5). Further, the use of lanthanum tracers in our previous study on the glomerular changes in nephrotoxic serum nephritis permitted the visualization of two distinct types of changes in the electron microscopic appearance of the GBM (6). The purpose of the present study was, therefore, to determine whether the lanthanum-method could be used also in aminonucleoside nephrosis to detect an alteration in the ultrastructure of the basement membrane.

Materials and Methods. A total of 16 male albino rats of the Holtzman strain, with an average body weight of 150 ± 10 g were used. Eight of the animals were injected subcutaneously with aminonucleoside (1.5 mg/100 g body wt/day) for 7 days. The controls received pyrogen-free distilled water at the same dose, and by the same route. All of the rats were killed 5 days after the last injection, when the mean proteinuria in the experimental animals was greater than 250 mg/day. Before killing each animal was anesthetized with Nembutal (30 mg/kg), and the abdominal cavity was exposed. Alternately, in one animal from the right and in another from the left kidney cortical biopsies were taken for electron microscopy. The specimens were fixed with 3% gluteraldehyde and 1% OsO_4 in cacodylate buffer, were dehydrated in graded alcohols and embedded in Epon. After sectioning, the tissue was stained with uranyl acetate, and examined in Hitachi HU-8 and HU-12 electron microscopes. Three glomeruli from each rat were examined by electron microscopy.

After taking biopsies from a kidney, the renal artery of the other kidney was cannulated with a 26-gauge needle connected with a plastic tube to a syringe, containing a 1% suspension of lanthanum hydroxide in phosphate-buffered saline. The kidney was infused with the lanthanum suspension at the rate of 0.3 ml/min. At the same time the renal cortex was fixed by subcapsular perfusion with 3% gluteraldehyde in cacodylate

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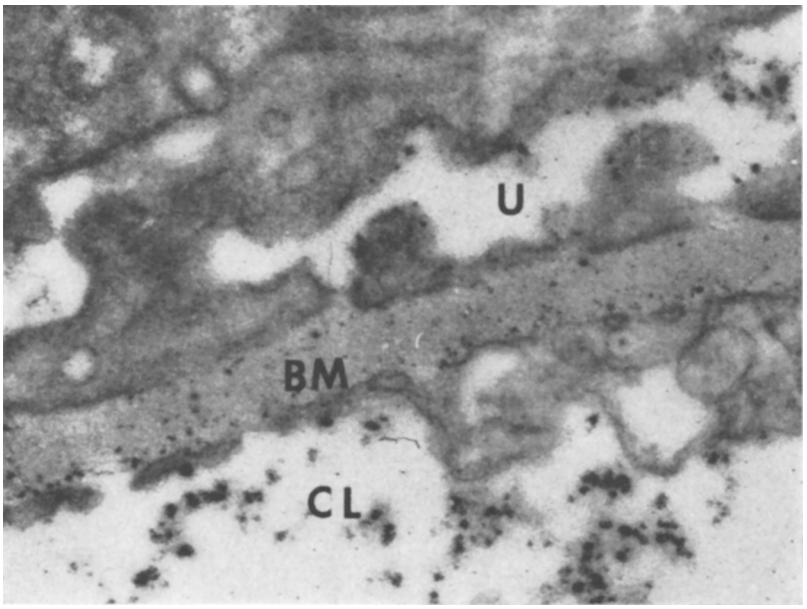

FIG. 1. Glomerular capillary wall of a control rat. Lanthanum is seen in aggregates in the capillary lumen (CL), in the urinary spaces (U), and is randomly distributed in the basement membrane (BM). Unstained; ×70,000.

buffer for a period of 10 min. Biopsies were then taken and processed for electron microscopy.

Results. By conventional electron microscopy, the general ultrastructural alterations of the glomerular capillary wall of the experi-

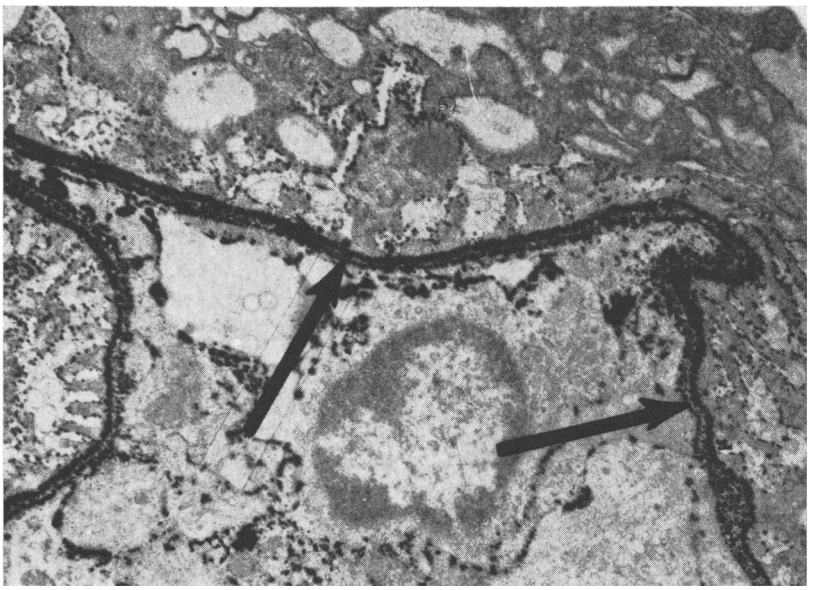

FIG. 2. Lower power view of part of a glomerulus from a rat with aminonucleoside nephrosis. There is an increase in the number and size of lanthanum aggregates in the basement membrane (arrows). Unstained; ×14,000.

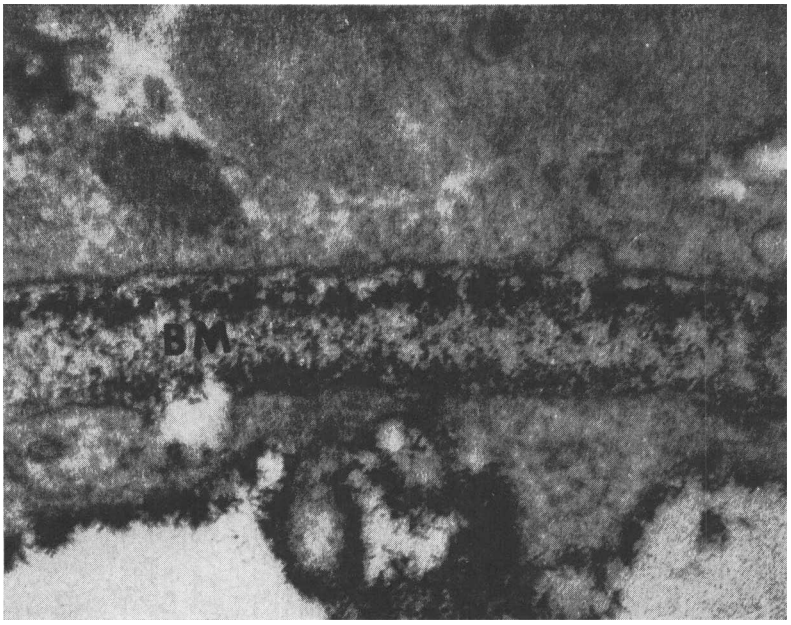

FIG. 3. Same as Fig. 2 at a higher magnification. Basement membrane (BM). Unstained; ×70,000.

mental rats were similar to those observed by others (1, 2). The endothelium and the basement membrane showed no alterations. In several capillary loops focal fusion of the foot processes of the visceral epithelial cells were visible.

As illustrated in Fig. 1, the glomeruli of control rats 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 molecules were seen as large aggregates sometimes several hundred angstroms in diameter. In the basement membrane the tracer molecules were visualized as small aggregates with varying diameters (20–150 Å). As was previously observed (3) in the GBM of control rats, spaces occupied by lanthanum with diameters less than 100 Å were restricted, in general, to the area of the lamina densa.

In the aminonucleoside treated rats, on the other hand, two distinct types of changes in the distribution of lanthanum in the GBM were observed. As illustrated in Figs. 2 and 3, in some of the capillary loops there was a generalized increase in the number, and size of aggregates of the tracer with an average diameter greater than 100 Å. This type of change occurred in about 60% of the glomeruli examined. In other capillary loops, there were segments of the basement membrane, measuring in length 600–1000 Å, in which the entire thickness of the membrane appeared to be filled with lanthanum (Figs. 4 and 5). This type of change in the distribution of lanthanum aggregates in the basement membrane was observed in 10% of the glomeruli studied, with a frequency of 1 to 2 per capillary loop. These changes in the distribution of lanthanum in the GBM were always accompanied by a focal fusion of the foot processes of the podocytes.

Discussion. In the present study, the infusion of lanthanum hydroxide into a renal artery together with subcapsular perfusion–fixation of the renal cortex enabled us to demonstrate two distinct types of alterations in the distribution of lanthanum in the GBM of aminonucleoside nephrotic rats. In some of the capillary loops a generalized increase in the size and number of lanthanum ag-

gregates was observed, in others focal-dense concentration of the tracer was noted. In a preliminary study we have reported (7), that the urine of these animals contained albumin as well as all classes of serum globulins. Since in nephrotoxic serum nephritis we have previously found the generalized increase in lanthanum aggregates in the GBM to coincide with albuminuria, and the segmental type with globulinuria, it is reasonable to as-

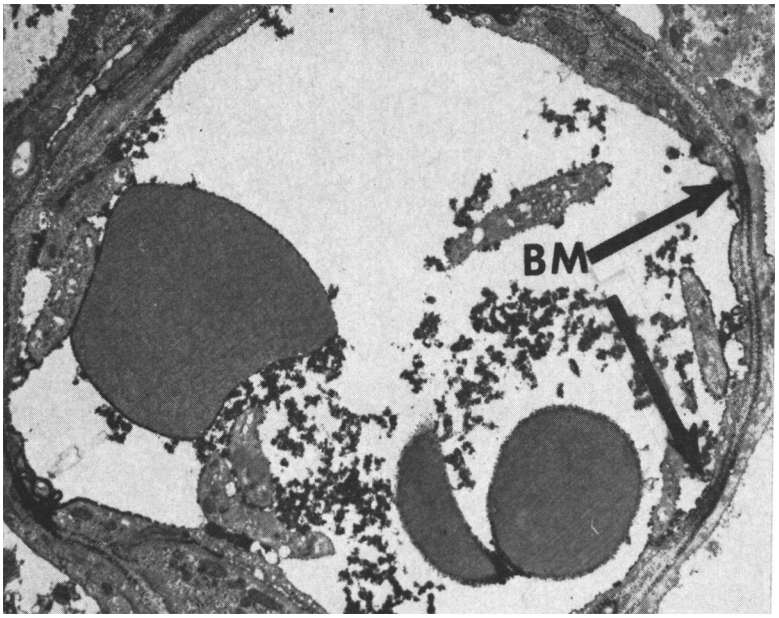

FIG. 4. Glomerular capillary wall of a rat with aminonucleoside nephrosis. Note focal-dense concentration of lanthanum hydroxide in the basement membrane (BM). Unstained; ×14,000.

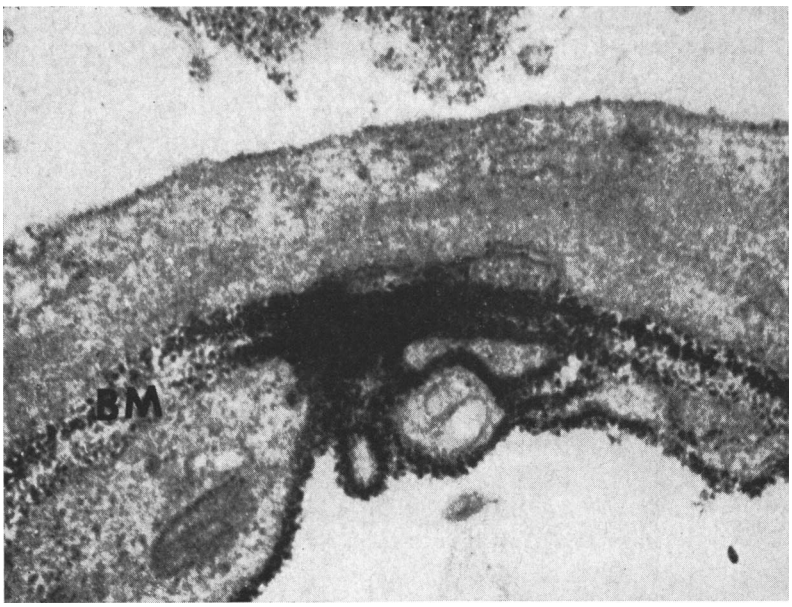

FIG. 5. Same as Fig. 4 at higher magnification. Basement membrane (BM). Unstained; ×70,000.

sume that the comparable changes in lanthanum distribution also reflect ultrastructural damage with altered permeability. This assumption is strengthened by the corresponding fusion of the foot processes of the podocytes.

Although under physiologic conditions the spaces in which lanthanum hydroxide particles are visualized are thought to be occupied by interstitial fluid, chemical binding of lanthanum to some component of the basement membrane remains a possibility. Nevertheless, the utilization of this tracer has made possible the demonstration of ultrastructural alterations in the GBM in two experimental diseases, both associated with proteinuria. Changes of the GBM with respect to chemical composition are different in aminonucleoside nephrosis (8) from those accompanying nephrotoxic serum nephritis (9, 10), while the ultrastructural changes as visualized with lanthanum are similar. Taken together, these observations strongly suggest that the ultrastructural alterations demonstrable with lanthanum correspond to conformational changes of GBM proteins. On the basis of these experiments it is suggested that the lanthanum method is a useful approach for the demonstration of ultrastructural alterations in the GBM.

Summary. The use of lanthanum hydroxide tracers permitted the demonstration of two distinct types of alterations in the ultrastructural appearance of the glomerular basement membrane in aminonucleoside nephrosis. In some capillary loops an increase in the number and size of lanthanum aggregates were observed, in others portions of the basement membrane measuring in length 600–1000 Å the entire thickness of the membrane appeared to be filled with tracer molecules. It is concluded that the lanthanum method is a useful approach for the demonstration of ultrastructural changes in the basement membrane in instances where the use of conventional electron microscopic techniques fail to do so.

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