# EDITORIAL

## BASEMENT MEMBRANE IN DIABETIC GLOMERULOSCLEROSIS

The concept of diabetic glomerulosclerosis, including its morphogenesis and its relation to the clinical syndrome of nephrosis, is under constant revision. A recent study[1] of basement membrane measurements in glomeruli of diabetics seems to contradict current concepts and may therefore deserve a comment. A brief historical note is helpful for an understanding of the problems.

The specific lesion was defined for some time as an intercapillary deposit with secondary encroachment upon peripheral glomerular capillaries. This concept was challenged by those investigators who did not accept the existence of intercapillary or mesangial tissue in the glomerulus and who conceived of diabetic glomerulosclerosis as a primary lesion of the peripheral capillaries diffusely distributed throughout the glomerular tufts. This premise led to the assumption that the nodule was merely a coalescence of thickened peripheral basement membranes. For a while this hypothesis seemed to be supported by electron microscopists who found all of the cells in the axial region of the glomerulus within the basement membrane and, hence, interpreted them as endothelial cells.

For a number of reasons, the tendency to regard diffuse glomerulosclerosis as a primary lesion, virtually pathognomonic for diabetes, has gained rather wide acceptance. In the first place, diffuse glomerulosclerosis and so-called mixed forms, i.e., mixed with nodules, are much more common in diabetes than the pure nodular form. Secondly, most recent investigators have noticed irregular thickening of the glomerular basement membrane in almost all glomeruli in any case, irrespective of the duration of overt carbohydrate intolerance.[2] They have, moreover, described these changes in so-called prediabetics.[3] Thirdly, the nodule in the axial position does not account for the nephrotic syndrome.

To assume that primary glomerular changes take place in the capillary basement membrane was all the more justified since it has become known that diabetes affects capillary basement membranes in many organs of the body. The term "microangiopathy" has been coined for this phenomenon, and the concept of glomerulosclerosis as a part of diabetic angiopathy is undoubtedly correct.[4]

The changing pattern of our concept of glomerulosclerosis deserves critical analysis.

At the outset, the term "mesangium" must be clarified. Many names have been given to a group of cells in the stalk of the glomerulus, which are situated between the capillaries, but covered by a basement membrane continuous with that of the capillaries proper. For the past few years, however, there has been agreement that this group of cells is morphologically and functionally different from endothelial cells and should be segregated as a structural entity which plays an important role in the morphogenesis of many glomerular diseases.[5]

*Diffuse and Nodular Glomerulosclerosis*

The diffuse type of glomerulosclerosis is doubtless far more common in diabetes than the nodular form, but it is incorrect to assume that nodules occur only in the presence of the diffuse form. When searched for by light microscopy, isolated nodules can be demonstrated without the diffuse form. This statement makes very unlikely the assumption that the nodule is a sequela of thickening of peripheral basement membrane, irrespective of frequency or infrequency with which isolated nodules are found.

Many electron microscopists, however, have reported an increased width of the peripheral basement membrane in diabetes not demonstrable by light microscopy. They have supported their impression by measurements and have concluded that irregular thickening exceeding the accepted normal width is invariably found in glomerular capillaries of diabetics.

In view of the importance of these observations, a statistically analyzed large series of measurements of glomerular basement membranes from normal subjects was compared with a comparable number of measurements in diabetics.[1] *This study showed that the mean width of peripheral basement membrane in glomeruli of diabetics was within normal limits except for those in which nodules were present.* Since these findings are in obvious contrast to the present consensus, an attempt should be made to explain this discrepancy.

In the first place, the study showed great variation in thickness of basement membranes of normal glo-

meruli, though the mean values were very close to those in previous reports.[6] To determine an increase in thickness, however, it is necessary not only to have an adequate number of measurements, but also the range and the calculated mean must be demonstrated to exceed the normal range and mean by appropriate statistical analysis. Statistically analyzed data, commonly accepted in other areas of medical research, have not been adequately applied in previous reports on diabetic glomerulosclerosis.

Secondly, the measurements in this study were confined strictly to those areas of the basement membrane where a perpendicular plane of section was assured.

Thirdly, areas were excluded from measurement where the basement membrane covered the mesangium. This last requirement is perhaps the most important. One must realize that in the area referred to as mesangium a spongious network of basement membrane-like trabeculae is found between the "deep cells." The trabeculae are in direct continuity with the overlying basement membrane and it is therefore difficult to determine an accurate delineation of the basement membrane proper toward the mesangium. It is not possible to obtain a sufficiently large number of measurements in this area to be significant. Moreover, there are no definite criteria to determine whether the plane of section in this area is perpendicular or oblique.

In glomeruli of diabetics, even without formation of nodules, there is an increase in the number and thickness of trabeculae in the mesangial matrix merging at the surface with the basement membrane. In this manner the mesangium encroaches upon the peripheral basement membrane, increasing its thickness from the axial region toward the periphery. As a consequence, the portions in which precise measurements of perpendicular sections of the basement membrane can be made become smaller as the mesangial deposits increase. It is likely that the site of measurements constitutes the major difference between this and previous studies.

When these requirements for number and sites of measurement are rigidly applied no increase in the mean width of the peripheral basement membrane is found in glomeruli *without nodules* from diabetics with overt disease of recent onset to ten years' duration. No change in the basement membrane was noted in one case of prediabetes. If irregular patchy thickening were significant in these cases it would be reflected in the mean width. There was marked thickening, however, in five of seven cases with *nodular* glomerulosclerosis.

It may be argued that only the thin portions of the basement membrane are measured in this study and the thicker portions simply excluded by restriction of sites of measurements. This criticism is valid, but the purpose of the study was the measurement of the basement membrane in peripheral portions of the capillary which can only be recognized by identifying the cytoplasm of endothelium lining it. It cannot be measured precisely when the basement membrane overlies the mesangium. Moreover, the determination of thickness requires a perpendicular plane of sectioning. It has been suggested to include both the oblique and perpendicular sections,[7] but such measurements could give no useful information for it is not possible to ascertain the various angles of obliqueness which determine such measurements.

By restricting the measurements to the peripheral portion of the capillary as defined above it became apparent that the thickening, for the most part, takes place in connection with the mesangial matrix and its trabeculae which progressively encroach upon the peripheral lining of the capillary, thus contributing to its width. The data seem to confirm the original contention, namely, that the involvement of peripheral capillaries is secondary to or independent of nodule formation. Only a few investigators have held consistently to this view. Recently measurements of peripheral basement membrane in glomeruli of juvenile diabetics did not show significant difference from normal values.[8]

This raises again the question of what is meant by the term "diffuse glomerulosclerosis." To us the term connotes the deposition of glycoprotein and other substances diffusely throughout the glomerular mesangium with an increase in number and thickness of basement membrane-like trabeculae. The peripheral capillary wall may or may not be involved.

Electron microscopy in recent years seemed to contradict this concept and supported Bell's contention of diffuse thickening of peripheral basement membrane. Our own earlier studies employing electron microscopy seemed to support this impression until extensive objective measurements were applied. In fact, one case of diabetes with only diffuse thickening of the basement membrane when submitted to critical measurement revealed a normal basement membrane thickness.

We therefore concluded that the mesangium is the structure primarily affected in the glomeruli of diabetics. It is likely that altered metabolism of these mesangial cells results in the development of larger masses of basement membrane-like bars which eventually fuse with the peripheral basement membrane. It is not cer-

tain whether the cells proliferate, whether they synthesize mucopolysaccharides, hydroxyproline and collagen, or whether they absorb glycoproteins from the blood stream by pinocytosis. At any rate, this process may take place in a diffuse, histologically nonspecific fashion, or in a nodular, histologically specific fashion. In either case, it is likely to extend gradually towards the periphery of the capillaries.

*Glomerulosclerosis and microangiopathy*

How do we relate this concept of glomerulosclerosis to that of the widely disseminated diabetic microangiopathy? A common denominator for changes in peripheral capillaries in various organs can be postulated if we assume that the perithelial cells of the capillaries, the mural cells in the retina and the mesangial cells of the glomeruli are functionally closely related. If we assume further that they participate in the maintenance of the basement membrane and that their metabolism is similarly changed in diabetes, the basement membrane, to which they are related would be affected in different ways depending on differences in structures of the capillaries in various organs. Whether such changes occur prior to the recognizable metabolic disorder of diabetes is controversial.

None of the changes in diabetic microangiopathy can be correlated with any degree of certainty with clinical symptomatology. Retinal aneurysms only exceptionally account for visual disturbances unless they accumulate in the macula densa or if they are the cause of vitreal hemorrhage. Glomerular changes are poorly related to the nephrotic syndrome. The variable changes in the basement membranes found by electron microscopy in the state of glomerulonephrosis are more likely the result rather than the cause of increased permeability to protein. Attempts have been made to explain diabetic neuropathy or gastroenteric disturbances, including diabetic diarrhea, by changes in small vessels. Most of these however, fall into the category of arteriolosclerosis rather than capillary basement membrane lesions. Their causative relation to the gastroenteric or neurologic damage is still in a state of speculative reasoning.

ACKNOWLEDGMENT

This study was supported by NIH Grant AM-06866-03.

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