THE NORMAL GLOMERULUS AND ITS BASIC REACTIONS IN DISEASE *

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In this report it is our desire to set forth in some detail the finer structural features of the normal glomerulus revealed by utilizing new techniques and to point out the basic alterations which characterize its major diseases.

The fine structure of the glomerulus has long been the subject of study. Recent advances in histologic technique have contributed much and offer further promise in elucidation of the intimate detail of this important functional unit. Thus the periodic acid-Schiff technique sharply delineates the basement membrane.[1] Colloidal iron preparations counterstained with cochineal and picrofuchsin, as previously described by us,[2] clearly differentiate the epithelial and endothelial elements. The cytoplasmic product of the epithelial cells, in common with acid mucopolysaccharides, has a strong affinity for colloidal iron, staining blue, while the cochineal stains the endothelial cytoplasm orange-yellow. Collagen assumes the red coloration of fuchsin. A combined iron and periodic acid-Schiff stain has been reported by Ritter and Oleson[3] as well as by us.[2] We believe that the method described by us delineates acid mucopolysaccharides somewhat more sharply and selectively, due chiefly to the higher acidity of the colloidal iron used. Finally, important structural detail has been revealed by electron microscopy.

This study is based upon a series of special histologic techniques including those noted. A consideration of certain pathologic alterations has also served to clarify structural detail and relationships.

THE GLOMERULUS

There is general agreement that the glomerular loops are composed of endothelial cells and a basement membrane which is surfaced by epithelial cells. The exact nature of the basement membrane has not been established. Our studies would indicate that the basement membrane of the glomerulus has both endothelial and epithelial compo-

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nents which are most intimately related. The major component of the basement membrane appears to be a differentiated cytoplasmic product of the endothelial cells which stains with cochineal and is Schiff positive. This membrane is surfaced by epithelial cells which elaborate a substance of a mucoid character (mucoprotein or mucopolysaccharide) which completely covers the delicate hyaline endothelial membrane. This relationship is shown in Figures 1 and 3. The Schiff-positive endothelial membrane stains reddish violet and appears to be a continuous structure which is quite delicate at the tips and lateral margins of the loops. The surfacing layer of mucoid material, which stains blue, obscures the more delicate peripheral segments of the endothelial basement membrane, but with critical illumination and focusing it can usually be seen. It is accentuated in certain pathologic states. The epithelial cells and their mucoid cytoplasmic secretion occupy the intercapillary spaces and cover the outer aspects of the loops as well. Collagen is not seen in the normal glomerulus and rarely in pathologic conditions. Of special interest and of potentially great functional significance is the finding that the endothelial membrane is crossed by delicate extensions of the mucoid substance derived from the epithelium. In fortunate preparations delicate strands of mucoid can be seen to traverse the basement membrane and minute amounts of the mucoid material may be seen on the luminal side of the membrane (Figs. 1 and 3). It would appear that there are in effect minute canaliculi in the basement membranes which are occupied by the mucoid material. These may represent specialized channels for selective filtration.

The fine details of this remarkable structural relationship are most clearly shown by electron micrographs. Reports on glomerular structure utilizing electron microscopy have been made by Pease and Baker[4] and by Dalton.[5] For preparation of electron micrographs the technical methods which we have used are essentially the same as those reported by Dalton. Our interpretations of structural relationships in the electron micrographs have been made in light of observations utilizing other techniques in both normal and pathologic material. Kidney tissue from the rat was prepared by perfusion with osmic acid. This evidently has affinity for the mucoid element of the epithelium and probably preserves it in the state existing during life. So fixed, the cytoplasmic secretion of the epithelial cells is rendered moderately opaque to the electron beam. Frond-like extensions of the mucoid material show multiple attachments to the basement membrane of the glomerular loop. There is, in fact, but little "space" between glomeru-

lar loops which is not occupied by the mucoid cytoplasm of the epithelial cells and its extensions. Pease and Baker considered the tongue-like projections to be integral parts of the basement membrane itself. Dalton, on the other hand, considered the ridges to be cytoplasmic processes of the epithelial cells applied to the basement membrane. On this point, our observations are in agreement with Dalton. Neither Pease and Baker nor Dalton relate the basement membrane proper to a cell. In fact, Dalton interprets his electron micrographs as showing a very delicate endothelial membrane internal to the basement membrane proper. This concept is difficult to reconcile with histochemical preparations which seem to indicate that the cochineal staining or Schiff-positive cytoplasm surrounding endothelial nuclei is the same as the finer extensions of the membrane. From the study of electron micrographs as well as histochemical preparations it appears that delicate cytoplasmic projections of the epithelial cells in fact cross the basement membrane. Figure 11 shows tongue-like processes of epithelial cells attached at many points to the endothelial membrane. From these points a mucoid substance appears to be thinly spread over the membrane and in fact to traverse this membrane at regular intervals in a manner analogous to the cement lines separating hexagonal tile. This is shown in Figure 11 and in the semi-diagrammatic representation in Figure 12.

Pathologic Changes in Glomeruli

The histochemical staining techniques employed in this study serve to delineate changes in glomerular structure somewhat better than older methods. Furthermore, study of abnormal glomeruli with these methods has aided interpretation of normal glomerular structure and of the histogenesis of the lesions.

In glomerulonephritis the pathologic changes are primarily of a proliferative character, involving both endothelial and epithelial cellular elements. Proliferation of endothelial cells is the major feature in most cases of glomerulonephritis. With endothelial cell proliferation there is reduplication of the endothelial cytoplasmic element of the glomerular loop. With the colloidal iron-cochineal technique this material stains yellow-orange (Fig. 4) and in the combined colloidal iron-Schiff technique it stains a crisp red (Fig. 2), which are the normal staining properties of the endothelial element of the capillary loop. The material is not collagenous. This is best seen in preparations stained with the colloidal iron and cochineal technique in which the basement membrane stains an orange-yellow, contrasting with the red-

staining collagen (Figs. 8 and 9). While the Schiff reaction stains the endothelial element of the basement membrane sharply, it does not differentiate it clearly from collagen. If the surface epithelium of the glomerular loop proliferates, it forms a variable amount of mucoid substance, but apparently not as much as the normal epithelial cell (Fig. 2). Proliferation of the endothelium in the glomerular loop cuts down the lumen and the normal endothelial-epithelial cell arrangement is distorted. Naturally both factors seriously impair the functional capacity of the glomerulus.

The glomerular lesion of disseminated lupus in its most characteristic form shows a striking hyaline thickening of the basement membrane, chiefly the endothelial element (Fig. 7). In addition the staining property of this membrane is somewhat altered. It stains rather brilliantly with eosin, and with the Schiff reaction the color may be in part orange rather than the simple red color. It seems likely that this color change is related to suffusion of the loop with some element of the plasma proteins, possibly fibrinogen. There may be also some granularity and swelling of the mucoid material of the epithelium. The appearances suggest that the material may be depolymerized and washed away with the glomerular filtrate. Casts with a blue mucoid staining reaction are commonly seen in tubules (Fig. 7). The basic pathologic reaction of lupus appears to involve a swelling of the connective tissue ground substance which becomes suffused with plasma protein,[6] and the deposition of plasma proteins including fibrin in the mucoid matrix is probably the basis of the characteristic "fibrinoid" degeneration. Not infrequently in lupus there is also a degree of proliferative change in the glomerular endothelium which is not fundamentally different from that occurring in proliferative glomerulonephritis.

In uncomplicated essential hypertension there is relatively little alteration of the glomeruli. They may show variable thickening of the endothelial element of the basement membrane. In the more severe cases this thickening may be considerable, and in the malignant form of essential hypertension the sclerotic arterioles also show necrosis with thrombosis causing varied degrees of injury to the glomeruli (Fig. 6).

The glomerular lesions of eclampsia bear some resemblance to the so-called membranous glomerulonephritis encountered in the nephrotic syndrome.[7] The endothelial element of the eclamptic glomerulus appears thickened and may show some longitudinal fibrillation. Most glomeruli are essentially devoid of blood (Fig. 5). In several cases

which we have had the opportunity to examine there were also alterations that are analogous to those of so-called lower nephron nephrosis, with partial disruption of the distal convoluted tubules and with the formation of granular hemoglobin-containing casts. There may be extensive accumulation of such hemoglobin casts in the loops of Henle and in the collecting tubules.

The glomerular alterations so frequently encountered in diabetes are of considerable interest and aid in the interpretation of the structural arrangement of the glomerulus. There is a striking thickening of the endothelial element of the basement membrane, particularly in the axial segments of the glomerular loops. The endothelial layer can usually be very clearly outlined in its entirety, which because of its delicacy is often difficult to demonstrate in the normal glomerulus. In the axial portions of the loops a broad layer of endothelial cytoplasm surrounds the nuclei, and as the cytoplasm extends away from the nuclei the endothelial layer becomes less prominent, but it can be outlined in its entirety including the thin segments of the peripheral loops. This endothelial cytoplasm is orange-yellow in preparations stained by colloidal iron and counterstained with cochineal. It is clear that this material is not collagen. The designation of "intercapillary glomerulosclerosis" appears to be a misnomer. In the first place collagen is not a component of the lesion and, in the second place, the lesion is, in our opinion, not in the "intercapillary" space but actually represents a basement membrane alteration.

Summary

Structural detail of the normal glomerulus has been studied by new histochemical techniques and electron micrographs. The basement membrane is considered to be primarily a differentiated cytoplasmic product of the endothelial cells. The surfacing epithelial cells elaborate a cytoplasmic secretion with properties of a mucopolysaccharide or mucoprotein. This substance is attached at many points to the basement membrane and is thinly spread over its entire surface. Evidence derived chiefly from electron micrographs is presented which indicates that the epithelial mucoid penetrates the endothelial basement membrane at regular intervals of approximately 0.12 μ.

Changes which are considered to be the basic alterations in structure occurring in the major glomerular diseases are presented briefly and illustrated.

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[*Illustrations follow*]

LEGENDS FOR FIGURES

FIG. 1. Distal segment of an essentially normal glomerulus stained by the combined colloidal iron and Schiff technique. The axial segments of the basement membrane stand out in red. The peripheral segments of the loops also stain red but cannot be seen clearly in this photograph. The deep blue-stained cytoplasm of the epithelial cells surfaces this Schiff-positive membrane. Careful inspection shows minute strands of bluish, presumably mucoid, material traversing the basement layer. A few red blood cells staining orange may be seen in the lumina of the capillary loops. × 600.

FIG. 2. Two glomeruli showing the changes of proliferative glomerulonephritis. Endothelial cell proliferation is evident with new formation of the Schiff-positive (red) endothelial cell cytoplasm or basement membrane. At the upper pole of the glomerulus shown above there is an epithelial crescent. It will be noted that the cytoplasmic extensions from these cells stain with colloidal iron. Colloidal iron-Schiff technique. × 130.

FIG. 3. Hilar segment of an essentially normal glomerulus stained by colloidal iron and cochineal. The primary element of the basement membrane here stains yellow. It will be noted that this yellow cytoplasm surrounds endothelial nuclei, indicating that this is a cytoplasmic product of the endothelial cell. A thin layer of similarly staining yellowish cytoplasm may be seen at the periphery of the capillary loops. This is not clearly shown here (see Fig. 1). The deep blue cytoplasm of the epithelial cells surfaces all of the glomerular loops. A few red blood cells are seen in the efferent arteriole. × 600.

FIG. 4. Glomerulus of proliferative glomerulonephritis stained by the colloidal iron-cochineal technique. The cells in excessive numbers are endothelial, and it will be seen that the surrounding cytoplasm stains yellow as does the normal basement membrane. The epithelial elements appear crowded out. A few epithelial cells surrounded by blue cytoplasm are seen, and some such bluish cytoplasm covers portions of the original outer aspects of glomerular loops. × 390.

(Legends continued on following page)

FIG. 5. Glomerulus and adjoining tubules from a case of eclampsia, stained by the colloidal iron-cochineal technique. The endothelial basement membrane is seen to be thickened and is clearly visible at the periphery of the glomerular loops as well as in the axial portions. The glomerulus is relatively avascular. The epithelial cells are inconspicuous, and their cytoplasm appears to be reduced and shows some granular dispersion. The adjoining distal convoluted tubules show degeneration of the lining cells and contain a granular hemoglobiniferous material analogous to that seen in so-called lower nephron nephrosis. × 300.

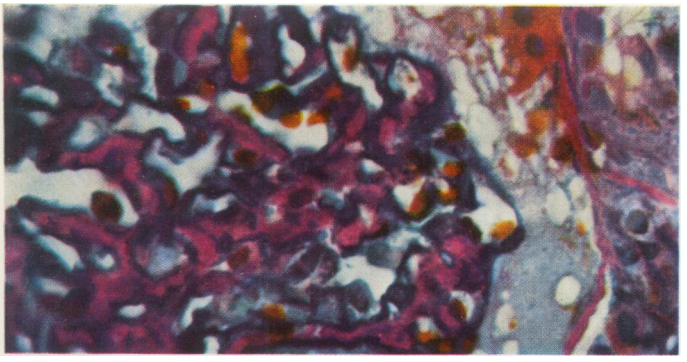
FIG. 6. Glomerulus and arteriole from a case of malignant hypertension. There is smudgy necrosis of a portion of the arteriolar wall as well as of some of the Schiff-positive endothelial cytoplasm at the hilus of the glomerulus. Epithelial cells are conspicuous, and some have desquamated. Colloidal iron-Schiff technique. × 130.

FIG. 7. Glomerulus and adjoining tubules from a case of acute disseminated lupus. The glomerular loops are bright red and clearly thickened. A few epithelial cells with blue cytoplasm are evident at the surfaces of the loops. Casts staining blue with colloidal iron are present in adjoining distal convoluted tubules. Colloidal iron-Schiff technique. × 65.

FIG. 8. Glomerulus from a case of so-called diabetic "glomerulosclerosis," stained by the colloidal iron-cochineal technique. It is evident that there is a marked thickening of the axial segments of the glomerular basement membranes. Endothelial cell nuclei are clearly seen, surrounded by an orange-yellow cytoplasm. The blue-staining epithelial cytoplasm can be seen surfacing glomerular loops and occupying the intercapillary spaces. The red-staining substance lying between some of the adjoining tubules is collagen. A zone of thickening of the parietal basement membrane is visible in the lower right-hand portion of the glomerular capsule. × 130.

FIG. 9. Detail of a segment of the glomerulus shown in Figure 8. Here the endothelial nuclei are seen to be surrounded by orange-yellow cytoplasm. There is pronounced hilar thickening of the axial segments of the endothelial basement membrane. The delicate but, in fact, thickened peripheral portions of this basement membrane may be seen. Some of the thickened endothelial membrane appears to be traversed by delicate strands of bluish material, presumably the cytoplasmic product of the epithelial cells. The capsular basement membrane, seen above, stains, as does that in the glomerular loops. The red-staining substance adjoining is collagen. × 390.

FIG. 10. Another glomerulus from a case of diabetic "glomerulosclerosis," with the formation of a distinctive, hyaline, ball-like structure. This is evidently a swelling or excess deposition of the endothelial cytoplasm. The blue-staining substance lying between the tubules is the connective tissue ground substance. The red-staining material shown in the lower left of the photograph is collagen. The absence of collagen in the glomerulus may be noted. × 130.



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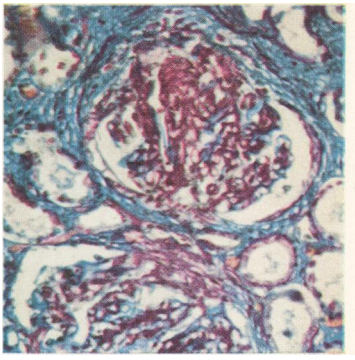

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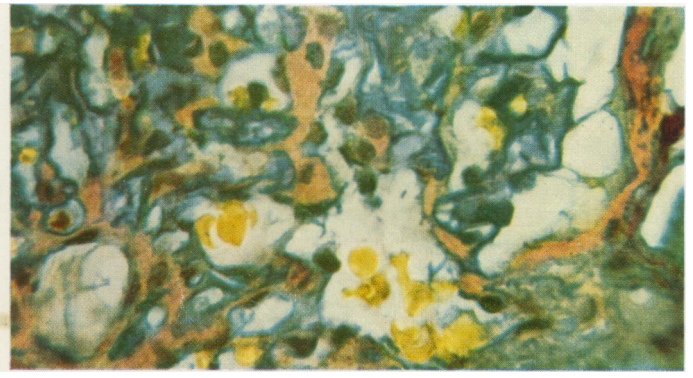

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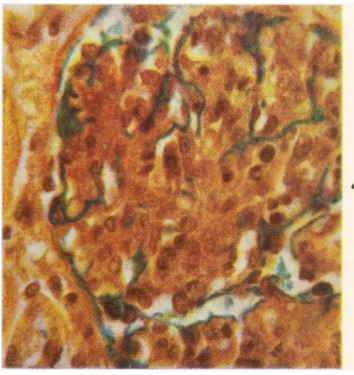

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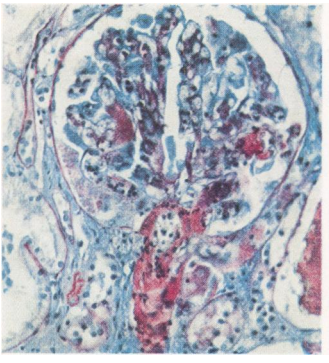

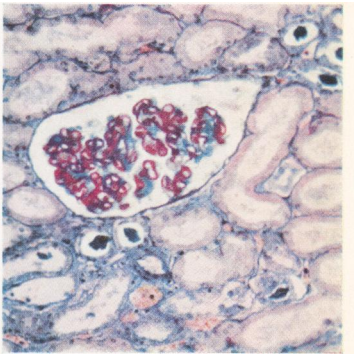

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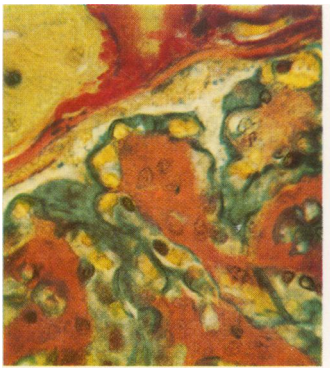

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FIGS. 11 and 12. An electron micrograph of a segment of rat glomerulus perfused with osmic acid (Fig. 11). Below (Fig. 12) is a semi-diagrammatic representation of the same field. Endothelial cell nuclei (EN) lie inside the capillary loops. Cytoplasm of the endothelial cell is believed to form the primary layer of the basement membrane. An epithelial cell (EP) is shown at the lower right. The cytoplasm of such cells gives rise to frond-like extensions of mucoid character (MC) which are applied to the surface of the endothelial membrane. This substance appears to penetrate the endothelial membrane at regular intervals (area A). A tangential section (T) shows a periodic penetration of the endothelial membrane by the epithelial mucoid cytoplasm, resembling cement lines in tile. × 5900.

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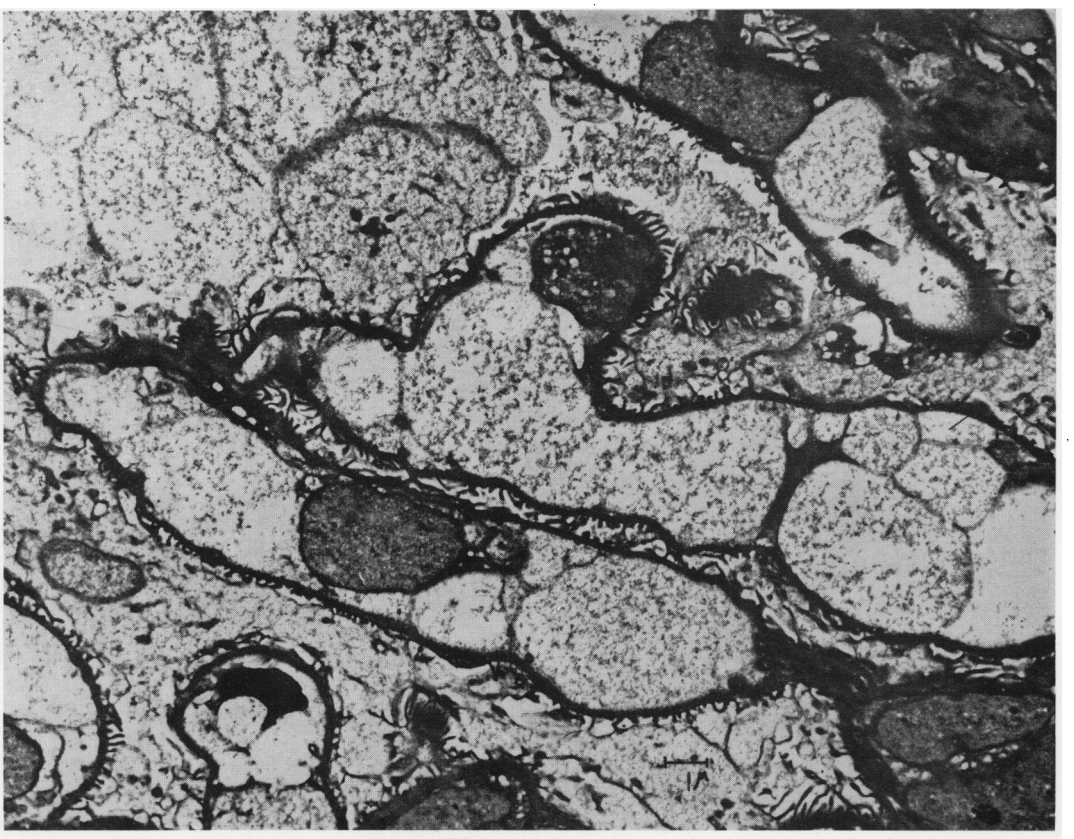

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