

# THE FINE STRUCTURE OF THE RENAL GLOMERULUS OF THE MOUSE\*·‡

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PLATES 134 TO 140

(Received for publication, September 15, 1955)

## INTRODUCTION

Present concepts of glomerular structure stem from the classical paper of Bowman (7) who described in 1842 the vascular pattern of the kidney and the connection between the glomerulus and the renal tubule. Details of the large literature dealing with the structure of the renal glomerulus up to the year 1930 can be found in the reports of McGregor (27) and von Möllendorff (32). Outstanding is the description of Zimmermann (54) who, in 1933, presented many details of the fine structure of the mammalian glomerulus as seen with the light microscope. The glomerulus has also been studied recently with the electron microscope (Pease and Baker (41), Dalton (9), Oberling *et al.* (34), Rinehart *et al.* (46, 47), Reid (44), Hall *et al.* (15-18), Fawcett (13), Mueller *et al.* (33), Pease (39, 40), Rhodin (45), Policard *et al.* (42)). Especially noteworthy have been the studies of Hall, who first recognized that the endothelial lining of the glomerular capillary is fenestrated or perforated with comparatively large pores several hundred Angstrom units in diameter, and who noted that the urinary filtration surface was covered with elongated cellular processes of cells which he termed "podocytes," but which have been known to histologists since Gerlach (14) as epithelial cells—a term used in the present study, in honor of its long historical usage. Hall (15) noted slits between the long processes of his "podocytes," or epithelial cells, and postulated that urinary filtration took place through the large pores of the endothelial cells and the slits between the processes of the epithelial cells. Between these two cells Hall reports a structure which he called "lamina densa," which appears to be nothing but a dense central layer of the basement membrane of the light microscopists such as McGregor (27), von Möllendorff (30-32), Bargmann (1-3), Borst (6), Zimmermann (53, 54), McManus (28, 29), Bensley and Bensley (4), and Ohmori (35) have also described. Through this structure, Hall postulated, filtration must occur. These important observations on the urinary

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\* This work was supported in part through grants from the Washington State Division, American Cancer Society, Inc., the United States Public Health Service, Department of Health, Education, and Welfare (Grant H-1309), and the Biological and Medical Research Fund of the State of Washington.

‡ A preliminary report of some aspects of this study was presented before the Histochemical Society on April 5, 1955. An abstract was published in *J. Histochem. and Cytochem.*, 1955, **3**, 309.

filtration structure have since been confirmed by Rhodin (45) and Pease (39, 40), and are here confirmed again. Moreover, this paper extends the observations of these authors on the urinary filtration surface, and adds new details of glomerular structure not recognized by previous workers using the electron microscope.

#### *Materials and Methods*

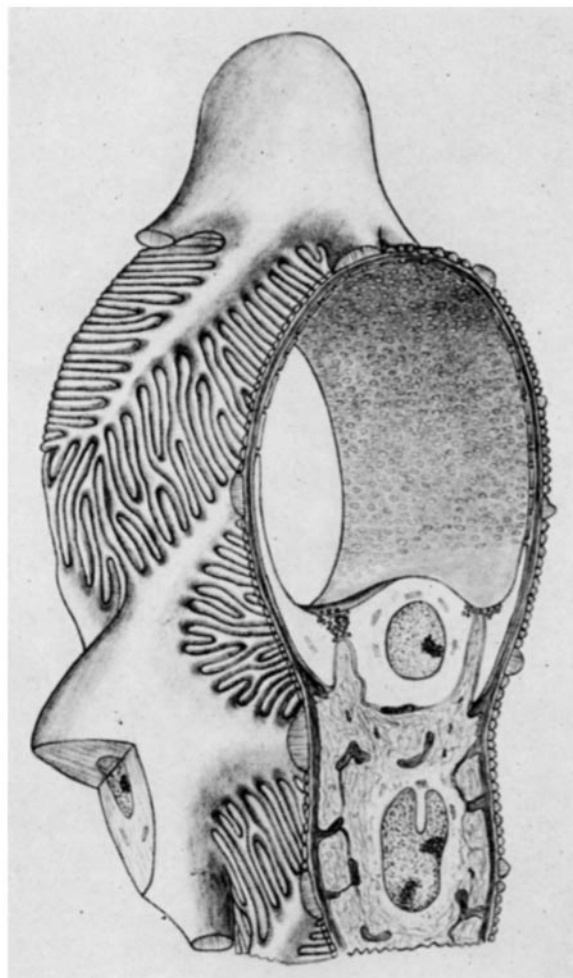
All observations are based on sections of mouse kidney, prepared and studied as described in a previous paper (Yamada (51)) on the gall bladder epithelium.

#### OBSERVATIONS

*General Features.*—Details of our present knowledge of renal glomerular structure are based on the light microscopic studies of Zimmermann (53, 54), von Möllendorff (31, 32), Bargmann (2), and others, who recognized four components in the glomerulus: epithelial cells, endothelial cells, basement membrane, and intercapillary tissue (or mesangium of Zimmermann). Text-fig. 1 represents the relationships of these components, as conceived from the evidence presented in this study and in the studies of Zimmermann and others. Figs. 1 and 2 present a general survey of the glomerular structure. Fig. 1 represents a portion of the vascular pole of the renal corpuscle, showing Bowman's capsule (*B*), a portion of the renal tubule (*T*), and an arteriole (*A*), probably afferent, with its endothelium (*E*) and its so called "epithelioid cell" coating. The endothelial cell of the arteriole to the right is seen forming a drop-like profile, as described by Zimmermann (54). The basement membrane (*M*) is seen to comprise a framework for the glomerulus, and is continuous with that of the renal tubules, Bowman's capsule, and arterioles with their epithelioid cell investment. The epithelial lining of the urinary space (*S*) forms a continuous layer covering Bowman's capsule and the vascular glomerular tuft. This observation confirms the findings with the light microscope of McGregor (27), Clara (8), and others. Although this epithelial lining is indeed continuous, the character of the epithelial cells lining the parietal portions of Bowman's capsule is very different from that of the cells overlying the visceral glomerular tuft. The parietal epithelial cells are simpler in structure, and are not described in detail in this paper.

In the lower left a glomerular capillary (*C*) appears in cross-section, showing its endothelial cell with conspicuous nucleus (*Ne*), its basement membrane (*M*), and epithelial cell. To the right below one can see a cell (*X*) embracing a portion of the arteriole. This cell presents many characteristics of a smooth muscle fiber. The similarity of the smooth muscle fiber shown in this figure to the intercapillary cells will be discussed later in this paper. Zimmermann (54) described a transition from smooth muscle fibers to the so called epithelioid cells. A similar transition is here thought to exist between the intercapillary cells and smooth muscle fibers.

Fig. 2 shows a general view of a portion of a glomerular capillary tuft not close to the vascular pole. Here one can see two sections of capillaries (*C*) and inter-



TEXT-FIG. 1. Schematic diagram showing the concept of a portion of a glomerular tuft as developed in this paper. Two epithelial cells are shown with their trabeculae and numerous interdigitating pedicels covering almost completely the filtration and intercapillary tissue of the tuft. Between the pedicels are narrow filtration slits. The sheet-like extension of the endothelial cell displays numerous filtration pores which endow this portion of the endothelial cell with a colander-like appearance. The basement membrane is represented as an intermediate layer in the filtration surface which is continuous with a coarse spongework in the intercapillary tissue where it enmeshes intercapillary or mesangium cells. The intercapillary cell is represented with a fibrillar matrix. It sends round processes through the basement membrane into or even through the endothelial cell, forming intracapillary colliculi. Near the colliculi are seen endothelial juxtacollicular vesicles. Details of the filtration surface are shown in TEXT-fig. 2.

capillary or mesangium tissue between them. This tissue shows intercapillary cells enmeshed within the sponge-like network of the basement membrane. Two nuclei of such cells are represented (*Ni*). Here it is clearly shown that the intercapillary cells make contact with both epithelial and endothelial cells, with only basement membrane between. However, in certain restricted areas of contact with endothelial cells, the interposed basement membrane is lacking and the intercapillary cell membrane is in direct contact with that of the endothelial cell. In such restricted areas the intercapillary cell may send blunt or rounded processes penetrating into the endothelial cell, or even perforating the latter entirely so that the end of the intercapillary cell process lies in direct contact with blood plasma (Fig. 2). Such penetrating processes were also observed by Zimmermann (54) with the light microscope and by Policard *et al.* (42) with the electron microscope. Zimmermann called them "*Intracapillarhöckerchen*." Zimmermann's term is used in this paper in a translated form, *intracapillary colliculi* (*colliculus intracapillaris*). They can be seen in Figs. 2, 8-13 (*Q*). Two intracapillary colliculi are represented in Text-fig. 1.

The capillary tuft wall constituting the filtration surface appears to have three main components: the epithelial cell processes, the basement membrane, and the endothelial cell. The endothelial cell shows moderate thickness at the proximal part, but is extremely thin over the filtration surface. The general relationships are portrayed in Text-fig. 1.

Further details of the structural components of the renal glomerulus will be now described.

*Epithelium of the Glomerulus (Visceral Epithelial Cell of Bowman's Capsule).*—This epithelium is continuous with the parietal epithelial cells of Bowman's capsule at the vascular pole of the renal corpuscle (Fig. 1). Numerous branching interdigitating processes of epithelial cells (*K*) coat the glomerular tuft on the outside completely except for very narrow slit-like spaces between the processes themselves (Figs. 2-10). This epithelial cell, first described by Gerlach (14) in 1845 and confirmed by many later authors, has been called "*Deckzelle*" (von Möllendorff, Zimmermann), "pericyte" (von Möllendorff, Bargmann), "epicyte" (Clara, Kulenkampff), and "podocyte" (Hall). Bowman (7), however, thought the glomerular capillary to be naked and "uncovered by any structure."

A characteristic feature of the epithelial cell is its general shape. The nuclear region of the epithelial cell is thick and round, and protrudes into the intracapsular urinary space from the capillary tuft (Text-fig. 1, Figs. 3, 5, 6). The nuclear region of the cell body stands up from the basement membrane as if supported by main processes or trabeculae which extend out over the capillary surface, branching into many smaller long, slender, ridge-like processes which Hall called pedicels. These pedicels interdigitate with each other and apply themselves to the basement membrane of the glomerular tuft. The grosser char-

acteristic features of the epithelial cell were first described by Zimmermann (52), who used silver impregnation and the light microscope. Similar findings were also demonstrated later by von Möllendorff (30-32), Bargmann (1-3), Zimmermann (53, 54), Clara (8), and Kulenkampff (24, 25). However, the electron micrographs show that the interdigitation of the process of the epithelial cells is more intimate and extensive than one might gather from light microscope studies. Fig. 4 shows a plan view of pedicellar processes (*K*) cut tangentially to the urinary surface of the capillary. Between the pedicels can be recognized narrow slit-like interspaces (*L*) about 200 to 300 Å wide (in some cases wider), through which the glomerular filtrate probably flows. These slit-like interspaces are designated as *epithelial filtration slits*. Each individual terminal pedicel is about 0.1 to 0.15  $\mu$  wide. Cross-sections of such pedicels and slits are shown in Figs. 1-3, 5-9. Their dimensions and spacings are below the limits of resolution of the light microscope. Although Zimmermann (54) classified epithelial cell processes into four types, it has not been possible to correlate his classification with the present electron microscope findings. On the other hand, Hall's (15) classification of these processes into "trabeculae" and "pedicels" has been found to be convenient and has been adopted in this study.

As is shown in Figs. 2 and 9, the epithelial cell covers not only the glomerular capillary wall but also the intercapillary tissue. This finding was also mentioned by Zimmermann (54) and Policard *et al.* (42). Thus Zimmermann disagreed with von Möllendorff's concept that the epithelial cell is a kind of pericyte.

The characteristic processes of the epithelial cells have been observed in several previous electronmicroscope studies (Pease and Baker (41), Oberling *et al.* (34), Dalton (9), Reid (44), Hall *et al.* (15-18), Rinehart *et al.* (46, 47), Fawcett (13), Pease (39, 40), Rhodin (45), Policard *et al.* (42), and Mueller *et al.* (33)). However, the several authors mentioned above are not agreed in the interpretation of their findings. The grosser topography of these processes has been adequately described by Hall (15, 16), Pease (39, 40) and Oberling *et al.* (34).

In the cytoplasm of the epithelial cell, usually in the perinuclear region, one can recognize submicroscopic cytoplasmic components such as the endoplasmic reticulum (*R*) (Figs. 3, 5, 6), Golgi bodies (*G*) (Fig. 3), and mitochondria (*D*) (Figs. 3, 5, 6). The structure of these components is essentially as has been described in other cells (Palade (36), Porter (43), Dalton and Felix (10)). Large cytoplasmic inclusion bodies described by Hall (15) as Golgi bodies (Hirsh-Baker type) were not encountered in this present study. However, one finds vesicles and granules which are difficult to characterize. Some appear to correspond to the so-called "small Golgi vesicles" described previously in gall bladder epithelial cells by Yamada (51), in nerve cells by De Robertis and

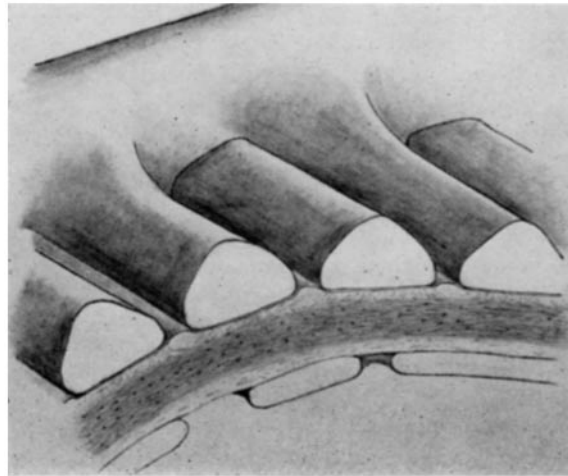
Bennett (11), and in pancreatic acinar cells by Sjöstrand and Hanzon (48). Other vesicles, apparently characteristic of these cells, are found in conglomerate clusters surrounded by a dense membrane or capsule which is often incomplete in the section (Fig. 11, insert (*H*)). The capsule is seen as an oval profile 0.15 to 0.25  $\mu$  in diameter. In any given section five to ten small vesicles about 400 A in diameter may appear within each capsule. These encapsulated accumulations of vesicles are termed the *glomerular epithelial vesicular conglomerates* (*H*).

The cytoplasm in the epithelial cell processes displays a matrix similar to that of the cytoplasm close to the nuclear region, but does not reveal mitochondria, Golgi bodies, endoplasmic reticulum, or vesicular conglomerates (Figs. 4, 7).

The cytoplasm of the terminal processes or pedicels of the epithelial cell in contact with the basement membrane is somewhat denser than that in the cell body proper. The cell membrane over the pedicel does not seem to be specialized. Fig. 7 shows that very delicate filaments extend from the cell membrane of the pedicels across to the dense central layer of the basement membrane (lamina densa of Hall (15)). These filaments are termed the *radiating pedicellar filaments* (*filamenta radiata pedicelaris*). They appear to traverse a peripheral layer of the basement membrane (*lamina rara externa*), which will be described later in this paper. Policard *et al.* (42) described similar filaments about 100 A in diameter running relatively regularly across to the dense portion of the basement membrane from the pedicels. Fig. 7 also shows that there is a delicate membrane about 30 A thick bridging the epithelial filtration slits, separating the urinary space from the basement membrane. This gossamer-like membrane is here called the *filtration slit membrane* (*OM*). In some places this membrane appears to extend continuously as an envelopment or canopy over the processes of the epithelial cells, between the cell membrane and the basement membrane.

This filtration slit membrane appears in Rhodin's (45) and Policard's (42) pictures, but is not mentioned in the texts. However Rhodin does state that the pedicels (or processes of the epithelial cell, as he calls them) are surrounded by a double cell membrane. Close inspection of his published pictures (Figs. 2 and 3) does not reveal clear evidence that a double membrane exists on the sides of the pedicellar processes. Although two membranes can indeed be distinguished over the surface of the pedicels applied to the basement membrane, the relationships between these two membranes are not clearly revealed at the sites where the cell membrane curves away from the basement membrane and is reflected along the side of the pedicellar processes. Neither Rhodin's Fig. 3, nor Policard's Figs. 4-6, nor the Fig. 7 of this paper shows the topography of these membranes sufficiently clearly to resolve all doubt as to their relationship. However, one is led to lean towards the impression that the geometry of this second membrane may be essentially analogous to that of the "limiting membrane" described by Yamada (51) in connection with the gall bladder epithelium, and that it does

not constitute an outer component of a double cell membrane, as Rhodin postulates. This impression is diagrammed in Text-fig. 2, which resembles Fig. 7 of Policard *et al.*, but which represents a concept of the positions of these membranes alternative to that presented by Rhodin. Further work will be necessary before an adequately documented concept can be established. The concept presented in Text-fig. 2 represents the filtration slit membrane as a continuous structure overlying the basement membrane, interposed between it and the



TEXT-FIG. 2. A model of a portion of filtration surface of the glomerular capillary as derived from this and other studies. Three components of the basement membrane are shown: as lamina rara externa, lamina densa, and lamina rara interna. Two endothelial filtration pores and five epithelial pedicels are diagrammed. Between each pedicel is a filtration slit bridged by the filtration slit membrane. This membrane is represented as a part of a limiting membrane interposed between the basement membrane and pedicels.

pedicels, and bridging the filtration slits, in this respect coinciding with Policard's model.

The nucleus of the epithelial cell is bounded by a perforated double nuclear membrane resembling that already described in other cells (Watson (49, 50), Hartman (20)) (Figs. 3, 5, 6). In the present series the nuclear profile is always rounded, without complicated folds as described by Hall (15).

*Basement Membrane.*—Although Bowman (7) denied the existence of a glomerular basement membrane continuous with that of the capsule, many later observers demonstrated that a basement membrane can be revealed with staining procedures such as Masson, silver, or periodate-Schiff. (The literature has been reviewed by McGregor (27). See also McManus *et al.* (28, 29).)

A continuous basement membrane in the glomerulus can also be recognized

clearly in electron micrographs (*M*) (Figs. 1, 2, 5-10, 12, 13). It forms the principal framework of the glomerulus, enclosing the glomerular capillaries almost completely except at the inner side of the capillary loop where the nucleus of the endothelial cell is usually located. Here in this restricted area the basement membrane is lacking or incomplete (Fig. 2, 8, 9, 11) and the endothelial cell is in direct contact with the intercapillary cell. This feature was also recognized by Zimmermann (54) and Policard *et al.* (42).

From the capillary wall, the basement membrane continues to the intercapillary tissue (or Zimmermann's mesangium) where it forms a coarse sponge-like network (Figs. 2, 8, 9). Within this network are enmeshed the intercapillary or mesangial cells, which are described later in this paper. The basement membrane in this portion varies in thickness from about 800 to 1400 Å (Figs. 2, 8, 9). In Fig. 13, one can observe the intricate basement membrane framework of the intercapillary tissue in a portion of the glomerulus close to the vascular pole. Thus the thickness and configuration of the basement membrane vary with location in the glomerulus.

However, the constituent structure of the basement membrane, as it appears in electron micrographs, does not vary significantly from place to place within the glomerulus. Fig. 7 shows at high magnification a portion of the filtration surface of the glomerulus, including the basement membrane. In confirmation of Rhodin (45), three layers can be distinguished in the basement membrane. There is a middle dense layer about 500 to 600 Å thick, appearing to be composed of a dense feltwork of fine filaments about 30 Å in diameter, amongst which are scattered, apparently at random, numerous dense particles about 40 Å in diameter. These are called the *filtration membrane particles*. On either side of this dense layer are thinner less dense laminae, each about 100 to 200 Å thick. The three layers are called the *lamina densa*, the *lamina rara externa* (lying adjacent to the pedicels of the epithelial cells), and the *lamina rara interna*, (adjacent to the endothelial cell) respectively. Pease (39, 40) also recognized three layers of the basement membrane and spoke of the less dense layers as the cement layers. However, since no cementing function has been demonstrated for these layers, a purely descriptive term such as lamina rara would seem to be preferable. Policard *et al.* (42) observed the structures here termed lamina densa and lamina rara externa but conceived of the former as the entire basement membrane, and of the latter as an intermediate space. Hall apparently recognized only the dense layer of this structure, which he called the lamina densa, a term which is here retained for the central main portion of the basement membrane. However, Hall chose to include cytoplasmic areas of the endothelium and epithelium in the structure he called "the basement membrane" (16, p. 13). In another paper, Hall (15, pp. 5 and 6) states that the three components he mentions—the endothelium, the "podocytes" or epithelium, and the "lamina densa," cannot be separately resolved with the



light microscope. Yet it is clear that the essential relationships were understood by Zimmermann and others. Many workers with the electron microscope forget that very delicate structures placed close together—separated in the normal state by distances below the limits of resolution of the light microscope—can yet be distinguished by the light microscope as separate elements if parted from each other by resolvable distances, as by swelling or teasing. Thus in frayed out specimens the myofilaments were distinguished by Kölliker in 1888 (23), and the individual filaments of the sperm tail were pictured by Ballowitz in 1890 (5), though normally these filaments are not separated by resolvable distances. Hence there is no reason why, in principle, the two cellular and the intermediate connective tissue components of the glomerular filtration surface could not be recognized as separate entities with the light microscope. Since the best concepts of the classical light microscopists are confirmed with the electron microscope, their terminology can still be used, and there is no necessity to redefine the concept of the “basement membrane,” as attempted by Hall (15). Although Hall (15) described relatively regular pores in the basement membrane, such findings are not encountered in this study, though there may well be very much smaller interstices between the meshes of the fine filaments felted together in the membrane, as suggested by Fig. 7.

*Endothelium of the Glomerular Capillary.*—The inner surface of the glomerular capillary is covered with endothelium. The thickest portion of the endothelial cell is in the nuclear region, usually adjacent to the inner side of the capillary loop and to the intercapillary tissue (Figs. 1, 2, 8, 10–12). Except for this portion, the cell body is represented as an extremely thin sheet-like extension which embraces the capillary lumen and is applied directly to the capillary surface of the basement membrane (Figs. 2, 5–7). This extension is about 300 to 400 Å thick. Hence it has been difficult to discern this structure with the light microscope, although it was recognized by Zimmermann (54) who used the Golgi-Kopsch method. Bensley and Bensley (4) stated that the endothelium is continuous without defect and may be selectively impregnated by silver. However, some earlier electron microscope studies included reports that the endothelium was absent in the glomerular tuft (Pease and Baker (41)).

The thin pericapillary extensions of the glomerular endothelial cells are characterized by numerous rounded perforations between 500 and 1000 Å in diameter (*O*). In Fig. 6 one can observe such perforations of the endothelial cell both in plan view and in cross-section. These perforations are designated as the *endothelial filtration pores*. They endow these portions of the endothelial cell with a coarse sieve-like or colander-like appearance (Fig. 6). Fig. 7 represents the filtration surface of the capillary at high magnification and shows the complete nature of the perforations comprising the endothelial filtration pores. These characteristic perforations of the endothelial cell extensions were first noted by Oberling *et al.* (34). Rinehart and Farquhar (46) and Reid (44) con-

sidered them to be vesicles. Hall (15) first recognized them as true perforations and called this portion of the endothelial cell the "lamina fenestrata." Pease (39, 40) has also confirmed these as true pores. Fawcett (13) described occasional discontinuities in the frog's glomerular endothelium, but denied regular fenestration. Mueller *et al.* (33) were reluctant to regard these as definitive complete pores. The fact that the capillary endothelium of certain other tissues taken from the same animal at the same time does not show such a fenestrated appearance suggests that the glomerular endothelial cell is specialized for its function. Palay (38) has reported that the posterior pituitary has fenestrated capillary endothelium similar to that of the glomerulus, and Pease (39, 40) has found similar apertures in the peritubular capillaries of the kidney.

The cytoplasm of the nuclear region of the endothelial cell presents mitochondria (*D*), Golgi bodies (*G*), and endoplasmic reticulum (*R*) (Figs. 8, 10, 13). The fine structure of these cytoplasmic components does not differ essentially from that of other cells (Palade (36), Dalton and Felix (10), Porter (43)).

In addition, a characteristic specialized structural feature can be observed in the endothelial cell. This is a collection of numerous densely packed vesicles and caveolae<sup>1</sup> about 500 Å in diameter, seen usually close to intracapillary colliculi (*Q*) (Figs. 2, 8-11). Hence these structures are designated as *endothelial juxtacollicular vesicles* and *juxtacollicular caveolae* respectively.

The cytoplasmic matrix of the endothelial cell, apart from the above mentioned organelles, is very clear in appearance, and is rather easily distinguished from that of the intercapillary cell (Figs. 2, 8-11, 13), from which it is separated by well defined cell membranes.

Fig. 12 represents a tangential section through the endothelium at a point at which two adjacent cells come into contact. Along the line of contact one sees thickened cell membranes with associated dense material, characteristic of the endothelial intercellular borders in this tissue. This structure reminds one of the so called "terminal bars" of simple columnar epithelium, as recently described in the gall bladder (Yamada (51)). This finding demonstrates that the endothelium of the glomerular capillary is not syncytial, although many attempts to demonstrate endothelial cell boundaries in the glomerulus with the light microscope using silver methods have failed. (See McGregor (27), von Möllendorff (32).)

*Intercapillary Tissue or Mesangium of the Glomerulus.*—The question of the presence of intercapillary or mesangial tissue in the glomerulus has been a topic of controversy for nearly a century. (Literature reviewed by McGregor

<sup>1</sup> The term "caveolae intracellularis" or "intracellular pit" or "cave" has been introduced (Yamada (51)) to refer to small pockets, pits, vesicles, caves, or recesses lined with cell membrane and communicating with the outside of the cell, extending inward and indenting the cytoplasm. Such caves or vesicles were first described by Palade (37) in capillary endothelium.

(27).) Zimmermann (53, 54) demonstrated clearly the existence of mesangium in all the mammals which he studied. He also noted the fibrous structure in the matrix of this tissue. He thought that the mesangium, together with the basement membrane, played an important role in supporting the glomerular tuft. Bensley and Bensley (4), Bargmann (2), von Möllendorff (31, 32), and Borst (6) also described the intercapillary tissue. Bargmann (2) failed to find this tissue in amphibia. McManus *et al.* (29) demonstrated intercapillary tissue by means of the PAS<sup>2</sup> method. Yet many electron microscopists have failed to recognize this tissue. Although Oberling *et al.* (34) suggested the presence of the so called mesangium cell, Hall (15, 16) has denied its existence. Rinehart *et al.* (46, 47), Reid (44), Pease (39, 40), and Dalton (9), did not mention it. Fawcett (13) stated that there are no collagenous fibers in the glomerulus of frog. Mueller *et al.* (33) recently thought the endothelium of the glomerular capillary to be syncytial in nature and the mesangium to be nothing but a continuation of the endothelium. This concept is almost identical with Hall's interpretation. Policard *et al.* (42) stated that the existence of the mesangium is now unquestionable, and described the mesangium cell as being located inside the basement membrane, and as possessing a dense cytoplasm. However, their pictures do not show all these details clearly.

This paper confirms the existence of the intercapillary tissue of the glomerulus, as postulated by numerous light microscopists and by Policard *et al.*, and adds new details to our knowledge of its fine structure.

In the intercapillary tissue or mesangium one can recognize two elements. The first is the framework of the basement membrane, which is continuous from the capillary wall to the kidney parenchyma. The second is the intercapillary cell (mesangium cell), enmeshed within the network of the basement membrane. Although these cells were described as "fibroblasts" by von Möllendorff, Zimmermann, Bargmann, and others, their fibroblast nature has not been established, and their properties remain obscure. Hence it is deemed wise to refer to them tentatively as "intercapillary cells," until their nature can be determined.

Fig. 13 represents a portion of the glomerulus close to the vascular pole and shows a relatively large amount of intercapillary tissue. The cell bodies and the cytoplasmic processes of the intercapillary cells are enmeshed within the complicated network of the dense basement membrane, the profiles of which present a mosaic pattern. In the cytoplasm of the intercapillary cells one can recognize slender mitochondria, endoplasmic reticulum, minute vesicles, and numerous delicate filaments about 60 Å in diameter. These filaments tend to arrange themselves along the axes of the cell processes, and suggest a similarity of the intercapillary cell to smooth muscle cells (Figs. 1 and 2).

<sup>2</sup> PAS refers to the periodate-Schiff method for staining  $\alpha$ - $\beta$  glycols and substituted glycols.

In the peripheral portion of the glomerular tuft, the structural pattern is almost the same, though the amount of intercapillary tissue is much less (Figs. 2, 8-11).

The characteristic fibrillar cytoplasmic structure of the intercapillary cells permits them to be distinguished easily from the endothelial and epithelial cells. Moreover, confusion with epithelial cells is rendered unlikely by virtue of the interposed basement membrane and the characteristic topography and processes of the epithelial cells (Figs. 8, 9).

Processes of intercapillary cells invaginating or even perforating entirely the endothelial cell have been mentioned earlier in this paper. Zimmermann (54) supposed that these "*Intracapillarhöckerchen*" or intracapillary colliculi supply nourishment to the intercapillary cell. One sometimes encounters a cluster of vesicles in these colliculi. These are called the *endocollicular vesicles* (Fig. 9, *Qv*), and are close to the juxtacollicular vesicles and caveolae in the endothelial cell.

The nucleus of the intercapillary cell (*Ni*) is oval, usually showing a fold or recess (Figs. 2, 13).

#### DISCUSSION

These findings confirm those of Hall, Rhodin, and Pease regarding the nature of the filtration surface of the glomerulus, and, although presented with a somewhat different terminology, appear to place on a firm basis our concepts of the structure of this important barrier. Basically, all four studies agree on the existence of three components in the glomerular filter. One is endothelial, is about 300 to 400 A thick, and is perforated with numerous pores, like a colander, the pores being 500 to 1000 A in diameter—far larger than plasma protein molecular dimensions, but small enough to retain cells and platelets. On the urinary surface is a second cellular layer, the cells of which are called variously epithelial cells, podocytes, *Deckzellen*, or pericytes. These cells have branching interdigitating processes or pedicels applied to the filtration surface. Between the pedicels are long narrow slits about 200 to 300 A wide, through which the filtrate presumably passes. Between these two cellular layers is a third extracellular component, presumably connective tissue in nature, about 800 A thick, called by Hall the "lamina densa" and by Pease and Rhodin the basement membrane, a term used also in this paper. This appears to be the definitive filter of the glomerulus, as the meshes between its fine fibrillar structural elements are far smaller than the relatively coarse slits or fenestrations in the two cellular layers. If the concept developed by these four writers be correct, the glomerular filtrate need not pass through any cell membrane or cytoplasm in its passage from plasma to tubule. The endothelial and epithelial cell components might well be able to change in such a way as to regulate the available filtration area in the basement membrane exposed to plasma or filtrate.

The fourth component of the glomerulus—the intercapillary cell—was not recognized by Hall, Pease, or Mueller *et al.*, though it is abundantly described in the light microscope literature. The presence in these cells of myofilament-like threads suggests a contractile function similar to that of smooth muscle. The intracapillary colliculi, the processes of the intercapillary cells invaginating the endothelial cells or perforating the endothelial cells to the plasma space, suggest that a part of the intercapillary cell may thus be brought under the direct chemical influence of components of the blood plasma, perhaps to respond with some regulatory physiological action. The presence of the endothelial juxtacollicular vesicles and caveolae in endothelial cells close to the invaginating colliculi suggests that these portions of the endothelial cells may have a specialized physiological function, perhaps imbibing or sampling the plasma by a process of micro-pinocytosis (Lewis (26)), translating the imbibed material to the vicinity of the intruding colliculus, where characteristic endocollicular vesicles are also found.

Although nerve fibers (Knoche (21, 22), Harman and Davis (19)) and reticular or argentophil fibers (Bensley and Bensley (4), Clara (8), and Ehrlich and Piel (12)) have been described in the glomerulus, nerves have not been recognized in this study, nor have any fibers with the periodicity of collagen been encountered. However, it is realized that the basement membrane seems to be made up of fine connective tissue filaments, that these may be related in some way to collagen or to reticulin, and may show similar staining reactions. Moreover, it is possible that some of the structures thought to be processes of intercapillary cells might in reality be nerve fibers.

The concept of the structural organization of a glomerular capillary tuft as derived from this and other studies with the light and electron microscope is presented in Text-figs. 1 and 2.

#### SUMMARY

Sections of mouse renal glomerulus fixed by perfusion with buffered osmium tetroxide solution have been studied with the electron microscope.

Four components are recognized in the mouse glomerulus: epithelium, basement membrane, endothelium, and intercapillary cell. The three cellular components all display in their cytoplasm mitochondria, Golgi bodies, endoplasmic reticulum, and uncharacterized vesicles.

The concepts of Hall, of Pease, and of Rhodin regarding the glomerular filtration surface are confirmed.

The epithelial cells are characterized by intricate, branching, interdigitating ridge-like processes or pedicels, the summits of which press against the urinary surface of the basement membrane, covering the glomerular capillary tuft almost completely except for narrow spaces about 200 to 300 Å wide between the processes. These spaces are termed the epithelial filtration slits, and are bridged

by a very delicate gossamer-like membrane about 30 A thick,—the filtration slit membrane.

The basement membrane is interposed everywhere between epithelial processes and endothelium, and between epithelial and intercapillary cells. The basement membrane of the filtration surface of the glomerular capillary has smooth surfaces and is about 800 A thick. It consists of three layers—a thick central lamina densa, appearing to have a very delicate felt-like structure, flanked on each side by thinner lamina rara externa and lamina rara interna. This membrane continues to the intercapillary space and makes a complicated sponge-work of varying thickness in which the intercapillary cells are enmeshed.

The endothelial cells are of moderate thickness in the nuclear region, but send out thin sheet-like extensions over the filtration surface. These extensions are about 300 to 400 A thick and are characterized by numerous round endothelial filtration pores about 500 to 1000 A in diameter.

The intercapillary tissue or mesangium is composed of the network of the basement membrane and the intercapillary cells.

The intercapillary cells, with characteristic fine fibrillar cytoplasm, make contact with epithelial and endothelial cells and are enmeshed within the network of the basement membrane. Rounded processes of intercapillary cells penetrate into the endothelial cell through the basement membrane, and may even perforate entirely through the endothelium. Such processes are called (after Zimmermann) the intracapillary colliculi.

In the endothelial cytoplasm close to the intracapillary colliculi are many dense endothelial juxtacollicular vesicles and caveolae.

The cell boundaries of the endothelial cell resemble terminal bars.

Some physiological speculations relating to glomerular structure are advanced.

The description of Zimmermann (54), based on a light microscope study, is confirmed in many respects.

The author is greatly indebted to Dr. H. Stanley Bennett who gave him the opportunity of doing this work and also invaluable kind advice during the course of this study.

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## EXPLANATION OF FIGURES

All figures present prints of electron micrographs of sections of renal glomerulus of the mouse, fixed by perfusion with Palade's buffered osmium tetroxide solution. The bar in each picture represents the distance of 1 micron except when otherwise indicated.

<i>A</i> , arteriole (probably afferent).	<i>Np</i> , nucleus of epithelial cell.
<i>B</i> , Bowman's capsule.	<i>Ni</i> , nucleus of intercapillary cell.
<i>C</i> , glomerular capillary lumen.	<i>O</i> , endothelial filtration pores.
<i>D</i> , mitochondria.	<i>Om</i> , filtration slit membrane.
<i>E</i> , endothelial cell of glomerulus.	<i>P</i> , epithelial cell.
<i>F</i> , so called epithelioid cell.	<i>Pt</i> , filtration membrane particles.
<i>Fr</i> , radiating pedicellar filaments.	<i>Q</i> , intracapillary colliculus.
<i>G</i> , Golgi bodies.	<i>Qv</i> , endocollicular vesicles.
<i>H</i> , epithelial vesicular conglomerate.	<i>R</i> , endoplasmic reticulum.
<i>I</i> , intercapillary cell or mesangium cell.	<i>S</i> , intercapsular space or urinary space of the renal corpuscle.
<i>J</i> , endothelial juxtacollicular vesicles.	<i>T</i> , renal tubule.
<i>K</i> , pedicel or process of epithelial cell.	<i>U</i> , endothelial cell border.
<i>L</i> , epithelial filtration slits.	<i>V</i> , caveola intracellularis.
<i>M</i> , basement membrane.	<i>W</i> , cell membrane.
<i>Md</i> , lamina densa.	<i>X</i> , smooth muscle fiber.
<i>Me</i> , lamina rara externa.	<i>Y</i> , red blood cell.
<i>Mi</i> , lamina rara interna.	<i>Z</i> , pores of nuclear membrane.
<i>Ne</i> , nucleus of endothelial cell.	

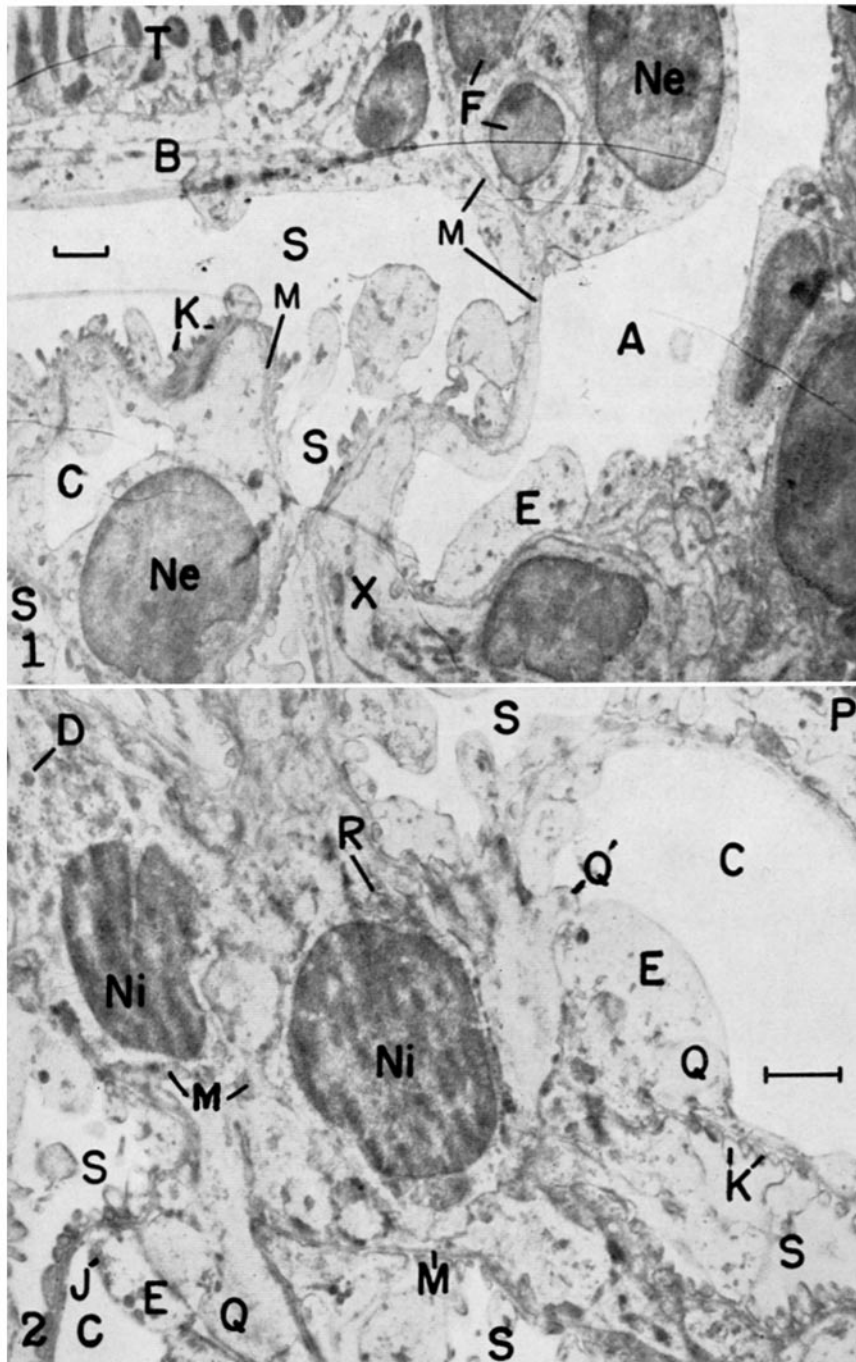
## EXPLANATION OF PLATES

### PLATE 134

FIG. 1. A section through the vascular pole showing a portion of the renal tubule (*T*), arteriole (*A*) (probably afferent), Bowman's capsule (*B*) with its epithelium and basement membrane (*M*), and glomerular capillary tuft. The continuity of the epithelium of Bowman's capsule with that of the glomerular tuft is shown. Also the basement membrane of the glomerular tuft is continuous with that of Bowman's capsule, arteriole, and the surroundings of the "epithelioid cells" (*F*) around the arteriole. To the left one can observe a cross-section of a glomerular capillary (*C*) with its conspicuous endothelial nucleus (*Ne*), basement membrane, and epithelial cell process coating (*K*). To the right below is the edge of the intercapillary tissue, containing intercapillary cells resembling smooth muscle fibers.  $\times 7,000$ .

FIG. 2. A peripheral portion of the glomerular tuft. The structure of the intercapillary tissue with two nuclei of intercapillary cells (*Ni*) is evident. To the left below and upper right, one can see sections of capillaries (*C*) which present endothelium (*E*), basement membrane (*B*), and epithelial cell (*P*). The endothelial cells are penetrated by intracapillary colliculi or rounded processes of intercapillary cells (*Q*), one of which perforates entirely and appears in the capillary lumen (*Q'*). In the cytoplasm of the intercapillary cells one can recognize endoplasmic reticulum (*R*), mitochondria (*D*), and numerous vesicles. Also one can detect characteristic fibrillar structures resembling the myofilaments of smooth muscle which are not found in the cytoplasm of the endothelial cells. The intercapillary cells have complicated shapes and are enmeshed within a spongework formed of basement membrane sheets of varying thickness. In some places, one can observe intercapillary cell boundaries without intervening basement membrane.  $\times 10,000$ .





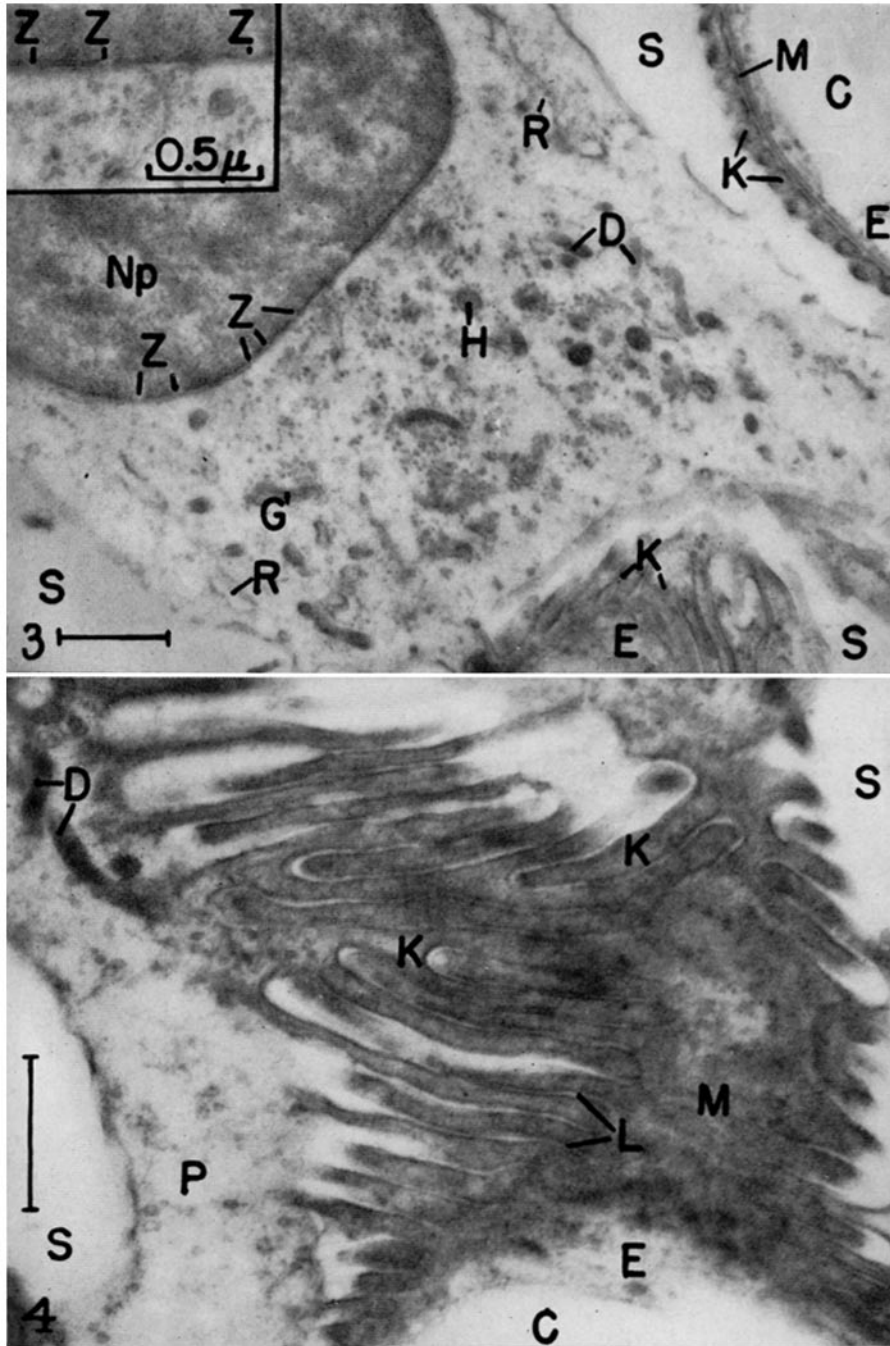
(Yamada: Fine structure of mouse renal glomerulus)

PLATE 135

FIG. 3. The nuclear region of an epithelial cell and a portion of two capillaries (*C*). The capillary in the upper right corner shows the epithelial cell processes in cross-section. The capillary in the lower portion is cut in tangential section. In the cytoplasm of the epithelial cell one can recognize endoplasmic reticulum (*R*), mitochondria (*D*), Golgi bodies (*G*), vesicular conglomerate (*H*), and many minute vesicles. The nuclear membrane displays several pores (*Z*).  $\times 15,000$ .

Insert represents at higher magnification a portion of the nuclear membrane with nuclear pore (*Z*). Note that the outer nuclear membrane is continuous with the cytoplasmic membranes of the endoplasmic reticulum, as described by Watson (50).  $\times 34,000$ .

FIG. 4. A tangential section through a capillary tuft. To the right one can observe a portion of the basement membrane (*M*) cut tangentially. This picture reveals the intimate interdigitation of the long slender branching epithelial cell processes (*K*). Between the processes or pedicels are the epithelial filtration slits (*L*).  $\times 25,000$ .

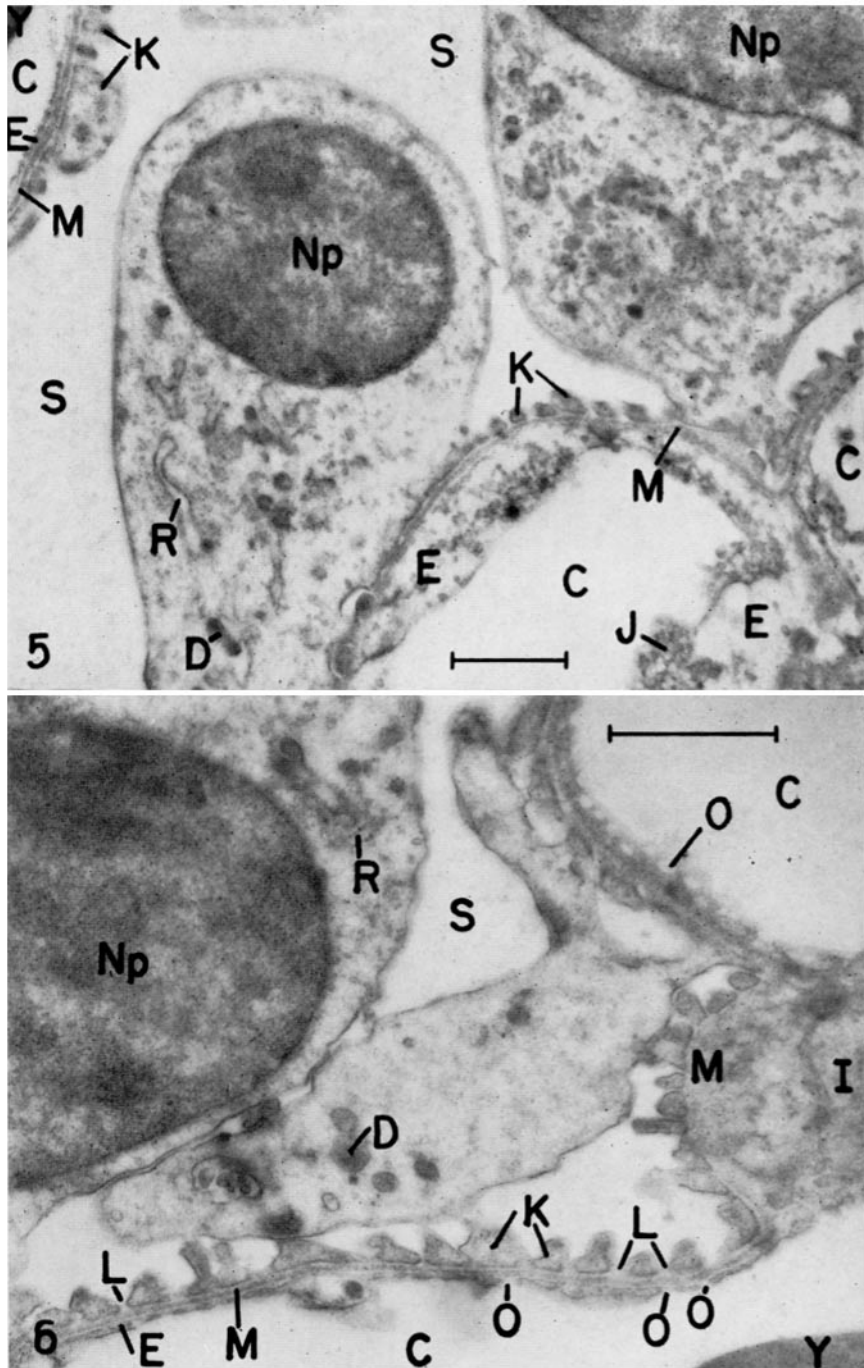


(Yamada: Fine structure of mouse renal glomerulus)

PLATE 136

FIG. 5. Two epithelial cells with nuclei (*Np*) and a portion of the filtration surface of a capillary tuft. The cell body of the epithelial cell protrudes from the capillary tuft into the capsular space. In its cytoplasm are mitochondria (*D*), endoplasmic reticulum (*R*), and numerous minute vesicles. At the capillary wall one can observe three components, the endothelial cell (*E*), the basement membrane (*M*), and the epithelial cell processes or pedicels (*K*). The profiles of endothelial cells in the upper left corner and along the right border are extremely thin and show discontinuities representing the endothelial filtration pores. In the endothelial cytoplasm is an accumulation of dense vesicles, the endothelial juxtacolicular vesicles (*J*).  $\times 15,000$ .

FIG. 6. An epithelial cell and a portion of the filtration surface of the glomerular capillary showing clearly the three basic components. In the cytoplasm of the epithelial cell one can observe endoplasmic reticulum (*R*), mitochondria (*D*), and vesicles. The capillary wall in the upper right corner is cut in tangential section and shows its endothelial filtration pores in plan view, revealing a colander-like appearance. Below, the filtration surface is cut in cross-section, showing the epithelial cell processes or pedicels (*K*), epithelial filtration slits (*L*), basement membrane (*M*), and endothelial filtration pores (*O*).  $\times 22,000$ .

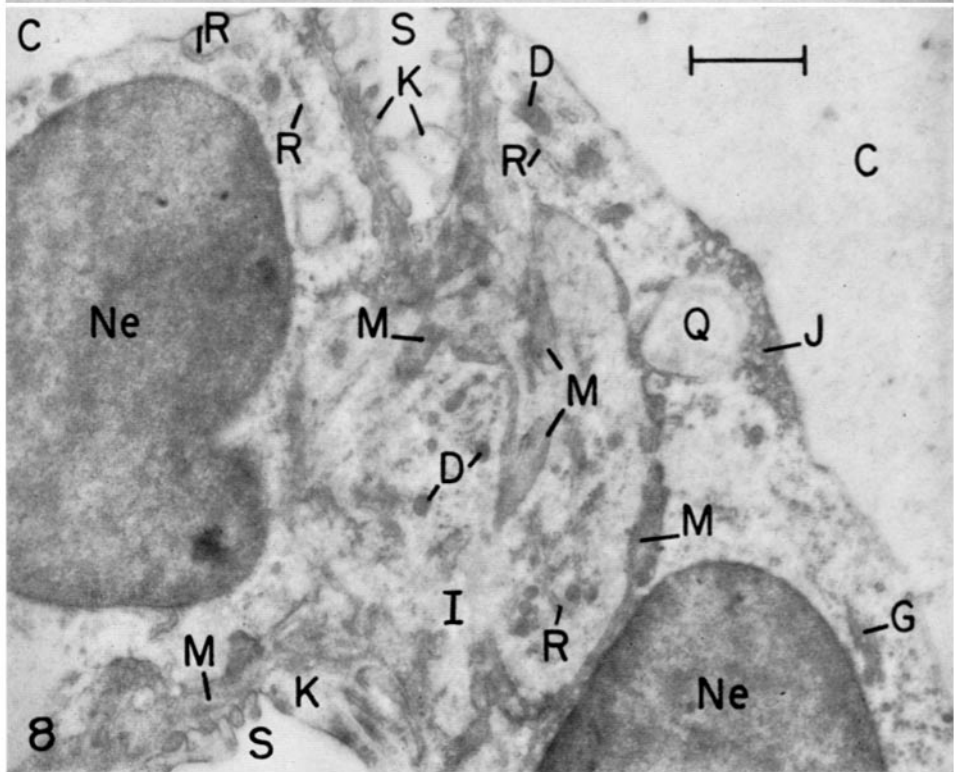
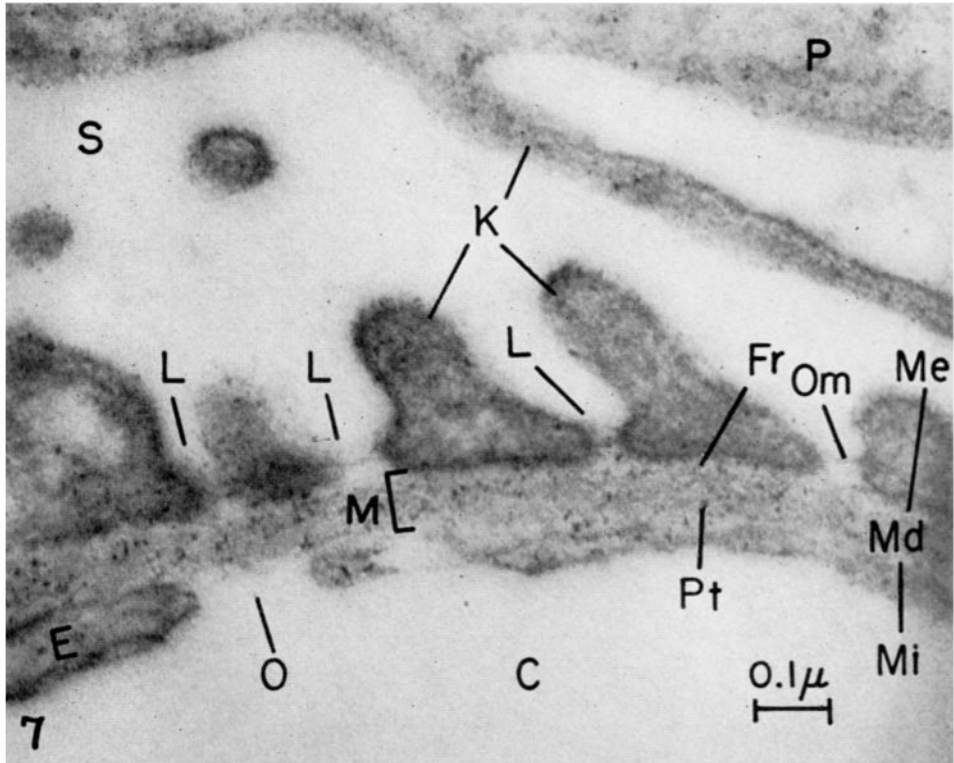


(Yamada: Fine structure of mouse renal glomerulus)

PLATE 137

FIG. 7. A high power picture of the filtration surface of a glomerular capillary showing the epithelial cell processes or pedicels (*K*), epithelial filtration slits (*P*), the filtration slit membrane (*Om*), basement membrane (*M*), endothelial cell (*E*), and endothelial filtration pores (*O*). Lamina densa (*Md*) of the basement membrane shows a fine felt-like fibrillar structure with dense filtration membrane particles (*Pt*) scattered through it. Between the lamina densa and the cell components are narrow layers of lesser density, the lamina rara externa (*Me*) and lamina rara interna (*Mi*). The former is crossed by fine filaments, the radiating filaments (*Fr*), which extend out from the pedicels.  $\times 95,000$ .

FIG. 8. A portion of a glomerular loop cut so as to show two nuclei (*Ne*) of endothelial cells with mesangium (*I*) between them. In the middle above and below, one can see urinary space (*S*), distinguishable from the capillary lumen by its lining of epithelial cell processes. Note the sponge-like framework of the basement membrane (*M*) in the intercapillary tissue. In the cytoplasm of the endothelial cell one can recognize mitochondria (*D*), endoplasmic reticulum (*R*), Golgi bodies (*G*), and many vesicles. A characteristic accumulation of endothelial juxtacolicular vesicles (*J*) is observed close to a penetrating intracapillary colliculus (*Q*) from a neighboring intercapillary cell. Mitochondria and endoplasmic reticulum are also recognized in the cytoplasm of the intercapillary cell.  $\times 15,000$ .



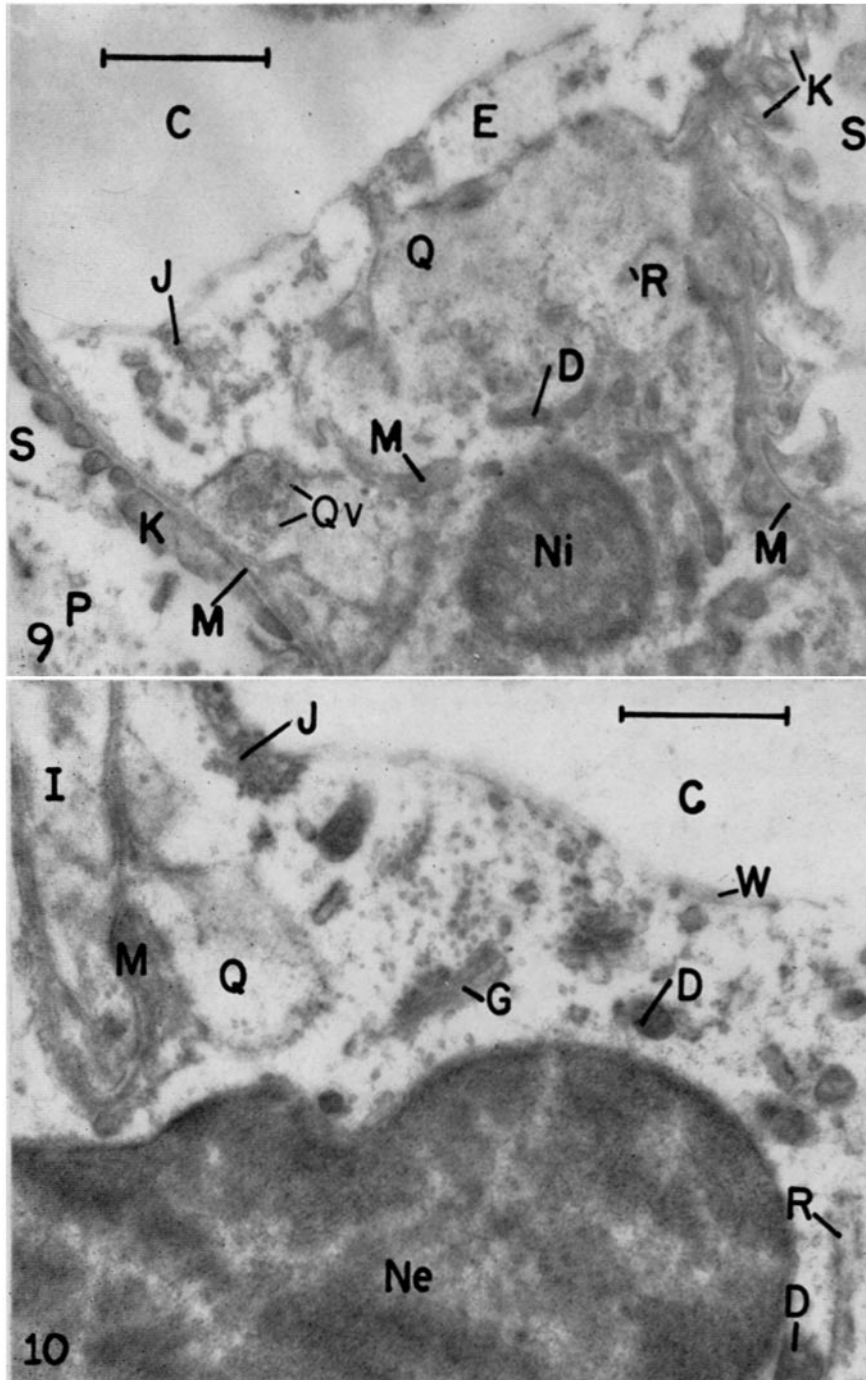
(Yamada: Fine structure of mouse renal glomerulus)

PLATE 138

FIG. 9. A portion of the proximal part of a capillary loop showing capsular space (*S*) to the right and left. The endothelial cell (*E*) of this picture shows moderate thickness and dense juxtacolicular vesicles (*J*). An intercapillary cell with its nucleus (*Ni*) contacts both endothelial cell and epithelial cells. One of the processes of the intercapillary cell penetrates almost to the capillary lumen (*C*) as an intracapillary colliculus (*Q*). The fibrillar structure of the intercapillary cell cytoplasm is evident, and is in contrast to the pale, almost structureless ground matrix of the endothelial cell. In this section most areas of contact between endothelial and intercapillary cell are direct, without interposed basement membrane. In the cytoplasm of the intracapillary colliculus to the left are seen endocollicular vesicles (*Qv*).  $\times 22,000$ .

FIG. 10. The nuclear region of an endothelial cell. An intercapillary cell on the left sends a penetrating process or intracapillary colliculus (*Q*) into the endothelial cell. Close by, the endothelial cytoplasm shows juxtacolicular vesicles (*J*). Mitochondria (*D*), Golgi bodies (*G*), and endoplasmic reticulum (*R*) are also evident.  $\times 22,000$ .





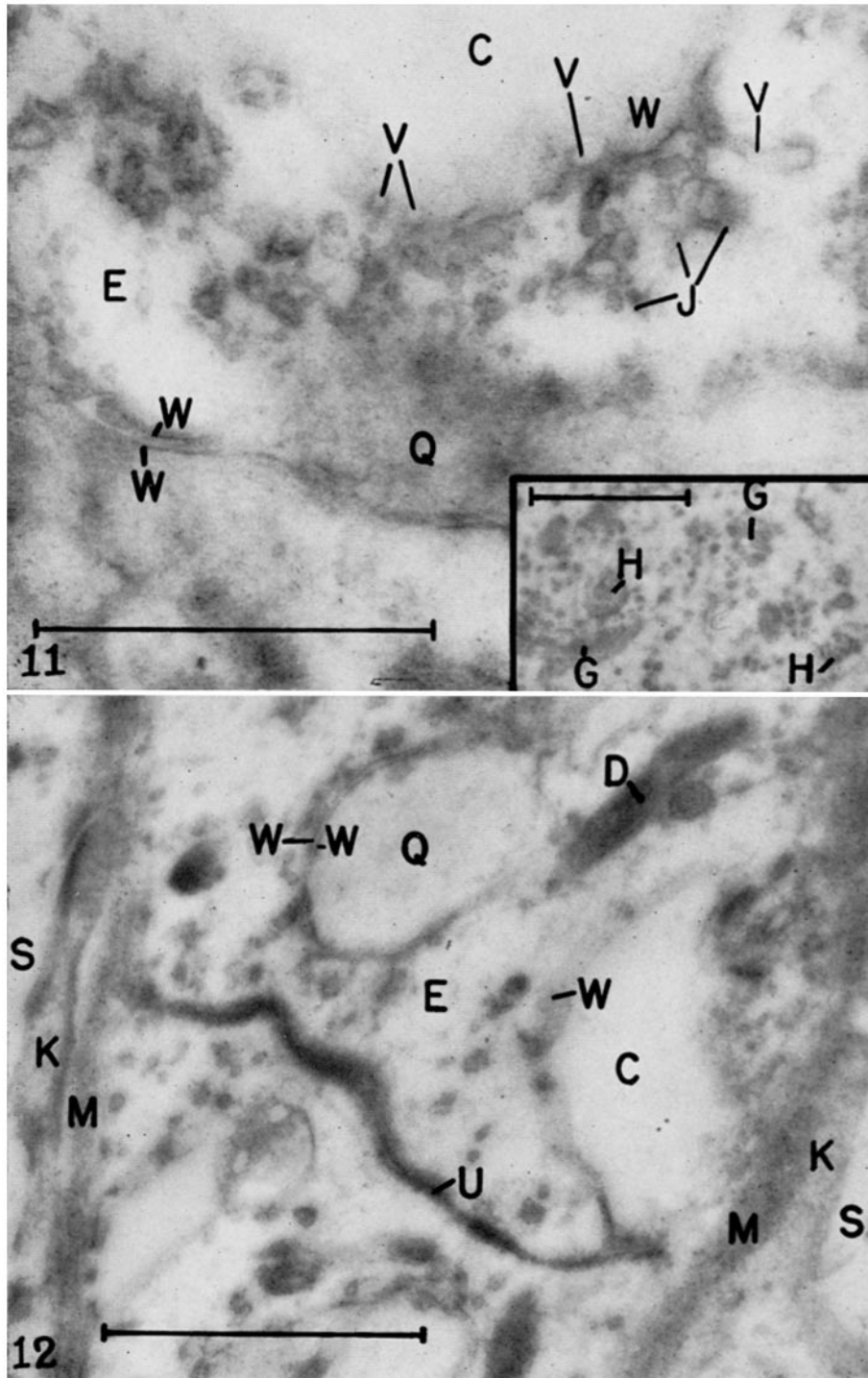
(Yamada: Fine structure of mouse renal glomerulus)

PLATE 139

FIG. 11. An oblique section through a proximal portion of a capillary tuft, showing a characteristic display of juxtacollicular vesicles. One can observe long cave-like or tunnel-like canals or pits opening into the capillary lumen (*C*) through round stomata in the cell membrane. These are called caveolae intracellulares (*V*), or intracellular pits. An intercapillary cell process or colliculus (*Q*) protrudes into the endothelial cell.  $\times 55,000$ .

The insert represents cytoplasm of an epithelial cell showing Golgi bodies (*G*) and epithelial vesicular conglomerate (*H*).  $\times 22,000$ .

FIG. 12. A tangential section through the proximal part of a capillary showing the boundary between two adjacent endothelial cells. The cell boundary is shown as a dark line (*U*) composed of the thickened cell membranes of each cell, with associated dense material. About the middle of the line, between the two membranes one can recognize a narrow intercellular space. A cross-section of a process or colliculus of an intercapillary cell is shown as a round area in the endothelial cytoplasm (*Q*). To the left and right can be recognized capsular space (*S*) with epithelial cell processes or pedicels.  $\times 44,000$ .

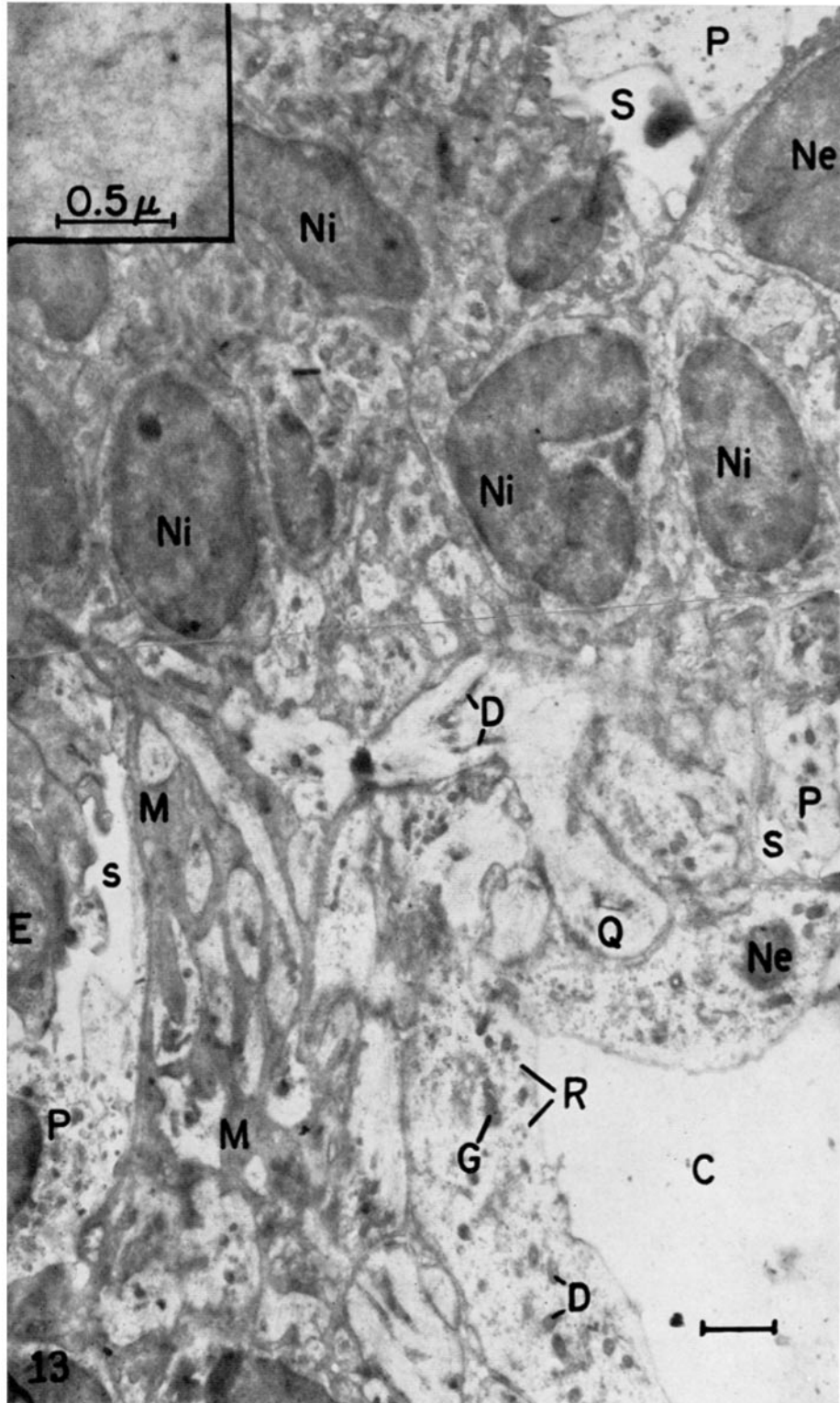


(Yamada: Fine structure of mouse renal glomerulus)

PLATE 140

FIG. 13. A section through the glomerular tuft close to the vascular pole, displaying a relatively large amount of intercapillary tissue. Many processes of intercapillary cells are embedded within the meshes of the basement membrane. Some of the processes invaginate neighboring endothelial cells as intracapillary colliculi (*Q*). In the cytoplasm of intercapillary cells one can recognize mitochondria, endoplasmic reticulum, vesicles, and characteristic fine filaments which seem to arrange themselves along the axis of the process like the myofilaments of smooth muscle. The cell body of the endothelial cell shows the tip of a nucleus (*Ne*), mitochondria (*D*), Golgi bodies (*G*), and endoplasmic reticulum (*R*).  $\times 10,000$ .

The insert represents at higher magnification a portion of an intercapillary cell, showing its fibrillar cytoplasm.  $\times 34,000$ .



(Yamada: Fine structure of mouse renal glomerulus)