

GAP JUNCTIONS IN MESANGIAL AND LACIS CELLS

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Since the initial description of Zimmermann (24, 25) who introduced the concept of mesangium, many authors have described characteristic features of the third cell of the mammalian glomerulus (2-5, 7, 9, 11-13, 18, 20-23). Situated between the basement membrane and the endothelial cells, mesangial cells are stellate cells embedded in the intercellular matrix. They send cytoplasmic projections toward the endothelium but do not participate properly in the constitution of the capillary lining. These cells are associated with the production of collagen fibrils (2, 11, 20, 22) and show phagocytic properties (4, 5, 12). Like the juxtaglomerular cells, they show marked resemblance to smooth muscle cells (3, 7, 12, 21, 23). We report here an additional morphological specialization of mesangial as well as lacis cells which consists of the presence of gap junctions in both cell types. Gap junctions appear to bridge not only different cells but also individual processes of the same cell. These junctions could provide the mesangium with the characteristics of a functional syncytium.

MATERIAL AND METHODS

Kidneys of Wistar rats having free access to food and water were used for this study. Pieces of kidney tissue

were fixed either by immersion in a 4% glutaraldehyde solution in 0.1 M phosphate buffer, or by perfusion of the whole animal with the same fixative at a lower concentration (2% glutaraldehyde). Before being frozen, the pieces of tissue were soaked in a 30% phosphate-buffered glycerol solution for at least 2 h. They were freeze etched according to Moor et al. (15) in a Balzers BAF 301 unit (Balzers High Vacuum Corp., Santa Ana, Calif.). The etching time was about 2 min. Replicas were examined with a Philips 300 electron microscope. Tissue fixed in the same way as for freeze etching was processed for conventional thin-section electron microscopy. Before dehydration and Epon embedding, the osmicated blocks were stained with uranyl acetate.

RESULTS

In freeze-etch replicas of rat kidneys, glomeruli are easily recognized as patchy areas showing numerous empty spaces lined by foot processes of epithelial cells and by a fenestrated endothelium (Fig. 1). The processes of mesangial cells can be identified mainly by their specific location between the basement membrane and the fenestrated endothelium (Fig. 2), whereas those of lacis cells are characteristically situated close to the macula densa, but in direct continuity with the mesangium.

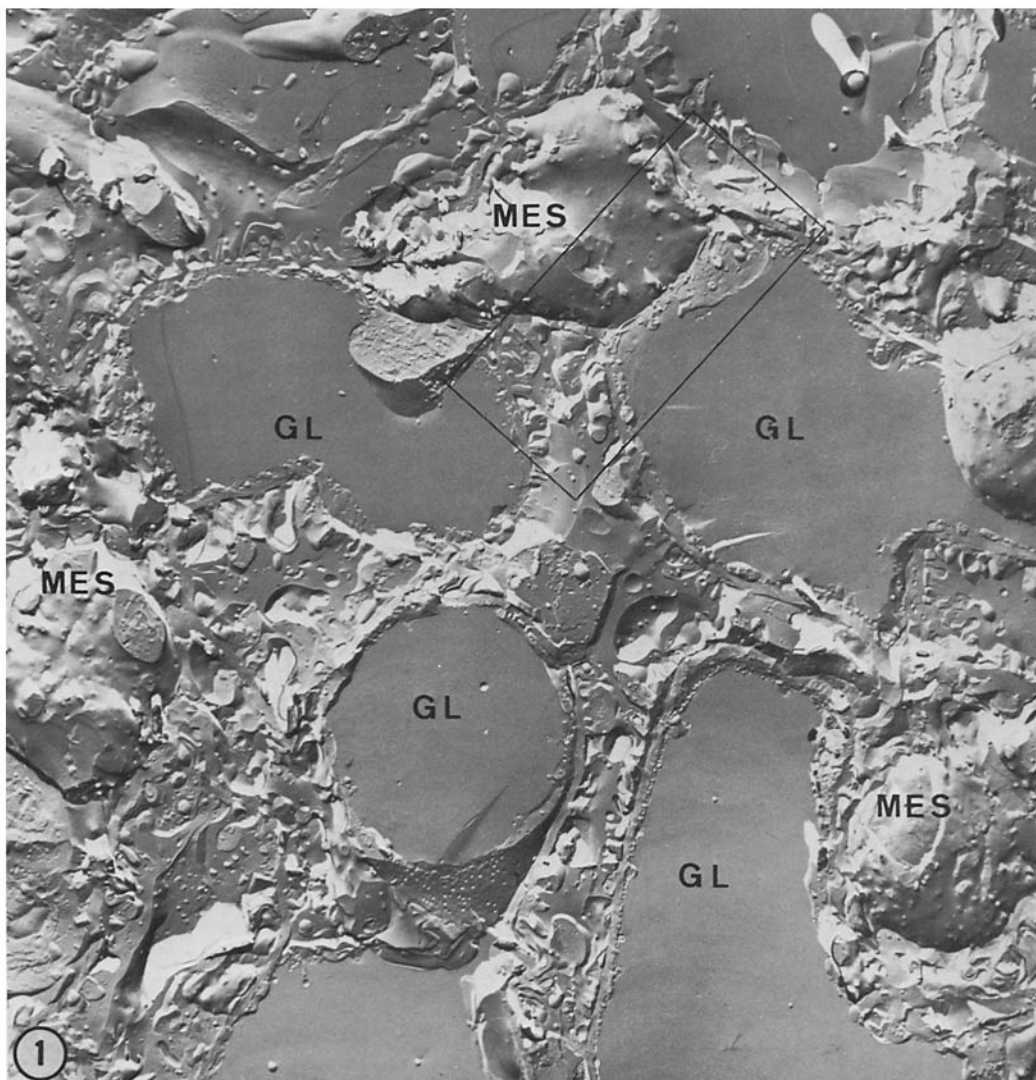


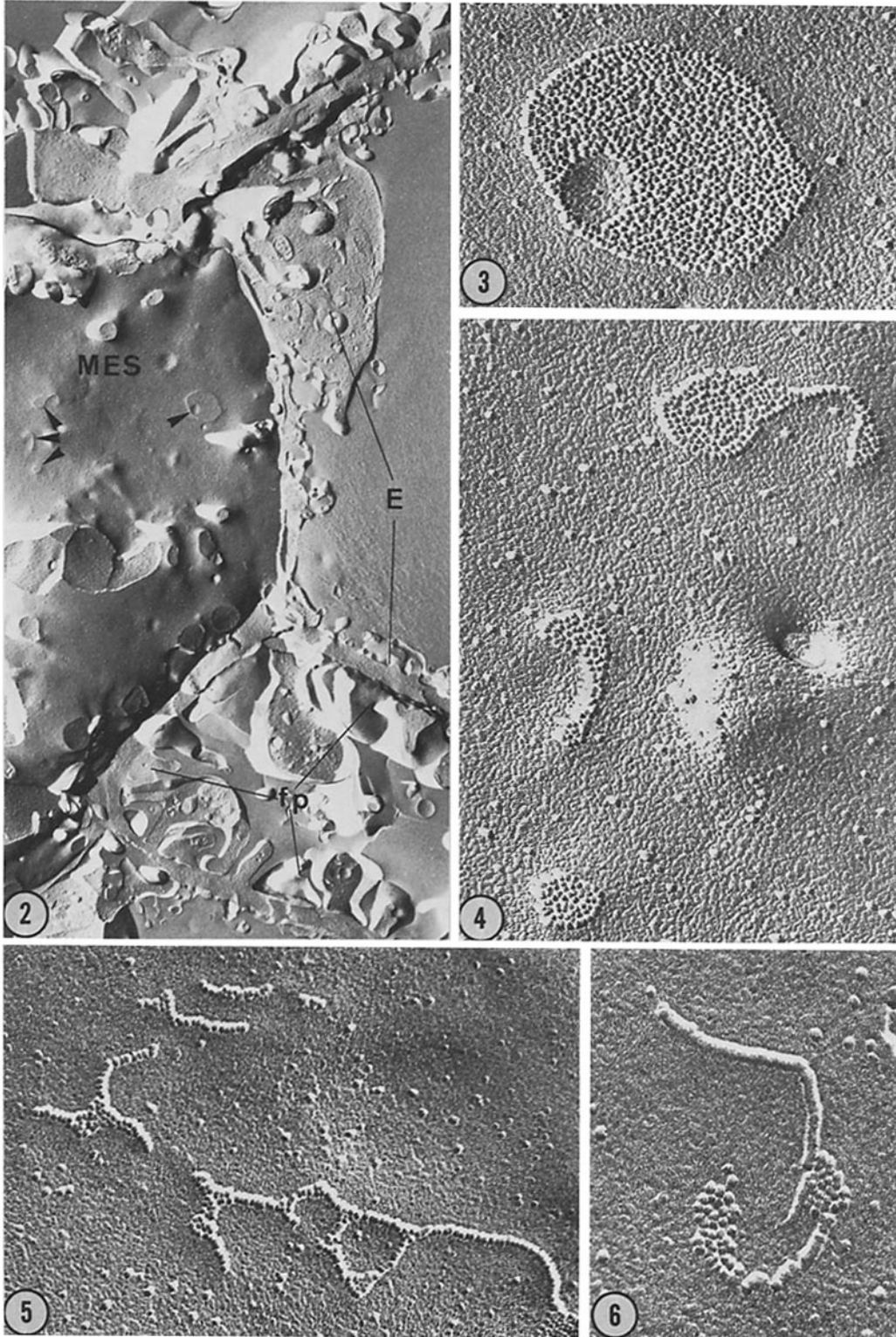
FIGURE 1 Low power magnification of a portion of a rat kidney glomerulus showing several glomerular loops (*GL*). The fracture plane has exposed membrane faces belonging to mesangial cells (*MES*) in their characteristic intercapillary position. $\times 4,000$.

FIGURE 2 Higher magnification of the part of a mesangial cell (*MES*) outlined by the rectangle in Fig. 1. On the fracture face A of the plasma membrane, arrowheads indicate gap junctions which are displayed at high magnification in Fig. 3 and 4. *E*, endothelium; *fp*, foot processes. $\times 11,000$.

FIGURES 3 and 4 Gap junctions are formed by the characteristic arrays of closely packed membrane-associated particles and they display various shapes. $\times 90,000$.

FIGURE 5 Linear and branching gap junctions in mesangial cells; these are formed by single chains of membrane-associated particles. $\times 80,000$.

FIGURE 6 Gap junction associated with a short continuous ridge as seen in tight junctions. $\times 130,000$.



When plasma membranes of mesangial cells were suitably exposed (Fig. 2), freeze-etch differentiations characteristic of gap junctions were found in both fracture faces, namely arrays of closely packed particles on A faces and closely spaced pits on B faces (10). While most of the gap junctions were macular in shape (Figs. 3, 4), some could vary so as to appear linear, formed of a single strand of membrane-associated particles (Fig. 5). In addition, short ridges as seen in tight junctions (6, 10) were sometimes associated with gap junctions (Fig. 6). In lacin cells as well, gap junctions could be recognized on fractured plasma membranes (Fig. 13). They appeared more numerous in these latter cells and showed also a greater variety of shapes. In accordance with the findings of freeze etching, zones of close apposition between adjacent plasma membranes from mesangial as well as lacin cells

were recognized in conventional thin sections (Figs. 7-12). The "in block" staining allowed a clear identification of the gap separating the external leaflets of the two plasma membranes (Inset, Fig. 8). In addition, thin sectioned material revealed a finding that was not detectable on freeze-etch replicas, namely the presence of gap junctions between two processes of the same cell (Figs. 9, 10). Although it is difficult to estimate the frequency of gap junctions between different cells and those occurring between the processes of the same cell, the latter appeared to be rather numerous.

DISCUSSION

Data presented above indicate that gap junctions occur frequently in mesangial as well as in lacin cells of the rat kidney glomerulus. In the kidney,

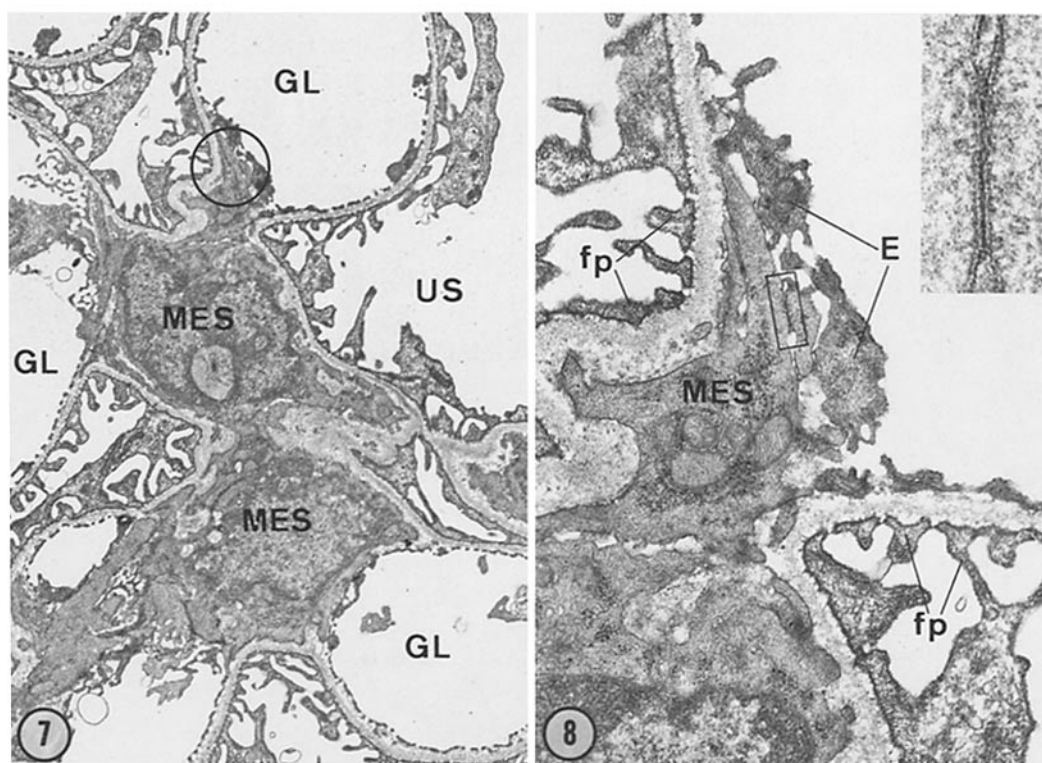


FIGURE 7 Low power magnification of a thin section of a glomerulus showing two mesangial cells (*MES*). The encircled area is displayed at higher magnification in Fig. 8. *GL*, glomerular loops; *US*, urinary space. $\times 6,000$.

FIGURE 8 Part of mesangial cells outlined in Fig. 7. In this figure, the rectangle delimits a zone of close apposition between two adjacent mesangial cell (*MES*) processes. *Inset*: same zone at high magnification. The two plasma membranes of mesangial cells can be seen separated by a dense line measuring about 50 Å in width and representing the gap filled by the uranyl stain. *E*, endothelium; *fp*, foot processes. $\times 19,000$; *Inset*, $\times 130,000$.

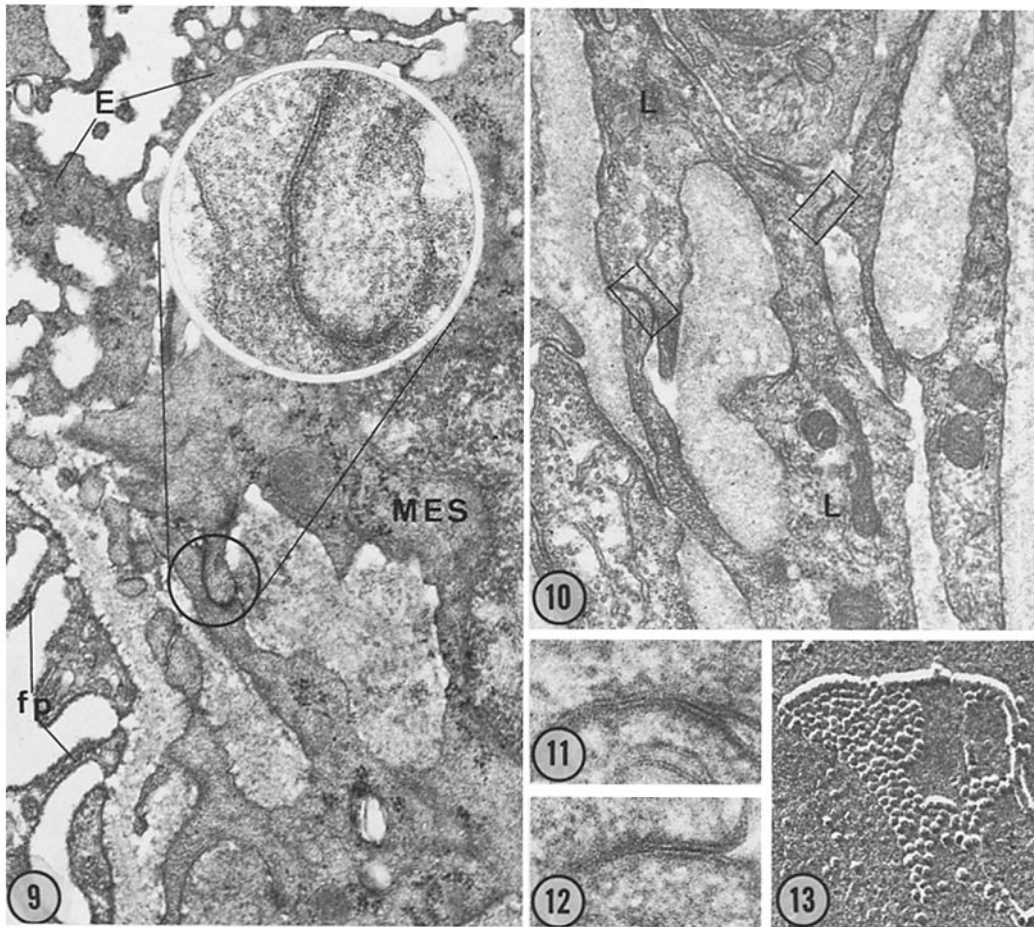


FIGURE 9 Thin section in the mesangial region. The encircled area, magnified in the *inset*, shows a gap junction occurring between two processes of the same mesangial cell (*MES*). *E*, endothelium; *fp*, foot processes. $\times 25,000$; *Inset*, $\times 120,000$.

FIGURES 10–12 Thin section in the lakis region. Cell processes from lakis cells (*L*) are shown to make close contacts outlined in the rectangles. Left-hand rectangle: close apposition between processes of the same cell. This zone is shown at high magnification in Fig. 11. Fig. 12 is the high magnification of the close apposition zone outlined in the right-hand rectangle. Both pictures show gap junctions in transverse section. Fig. 10, $\times 28,000$; Fig. 11, $\times 115,000$; Fig. 12, $115,000$.

FIGURE 13 Freeze-etch replica showing the typical appearance of a gap junction as it appears on membrane faces of the lakis cells. This gap junction is also associated with short ridges as seen in tight junctions. $\times 99,000$.

gap junctions have been described so far only in the proximal tubule (16, 19) by thin-section electron microscopy. As far as their freeze-etch appearance is concerned, gap junctions between mesangial or lakis cells are similar to the many others described in various epithelia (6, 10, 17). In contradistinction to other tissues, however, where gap junctions usually bridge two different cells, many of those present in mesangial and lakis cells occur between

processes of the same cell. Although it remains unexplained so far, this peculiarity is shared, to our knowledge, only with the vascular smooth muscle cells where such “auto” gap junctions have been described (8). Another point of similarity between these two cell types is represented by the fact that mesangial cells are rich in microfilaments and that they contain a protein with antigenic properties similar to those of the actin of the uterine smooth

muscle (1). These data, together with those reported here, seem therefore to support the hypothesis that mesangial and lakis cells are modified smooth muscle cells (1, 3, 7, 12, 23) and that they could perform a contractile function within the glomerulus (1, 7, 23). In this respect, one of the possible functions of the gap junctions, besides their role in cell adhesion and cell communication (14), would be to represent the anatomical site of electrical coupling between mesangial or lakis cells. Such a coupling would allow them to behave as a functional syncytium. Although we do not know so far whether mesangial cells share gap junctions with lakis cells, nor whether the latter are coupled to smooth muscle cells of the glomerular arterioles, it is tempting, on the basis of the available data, to imagine that all these cell types are involved in a coordinated contractile function regulating the blood flow in the glomerulus.

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REFERENCES

1. BECKER, C. G. 1972. Demonstration of actomyosin in mesangial cells of the renal glomerulus. *Am. J. Pathol.* **66**:97.
2. BENCOSME, S. A., R. S. STONE, H. LATTA, and S. C. MADDEN. 1959. Acute reactions with collagen production in renal glomeruli of rats as studied electron microscopically. *J. Ultrastruct. Res.* **3**:171.
3. DUNIHUE, F. W., and W. G. BOLDOSSER. 1963. Observations on the similarity of mesangial to juxtaglomerular cells. *Lab. Invest.* **12**:1228.
4. FARQUHAR, M. G., and G. E. PALADE. 1962. Functional evidence for the existence of a third cell type in the renal glomerulus: Phagocytosis of filtration residues by a distinctive "third" cell. *J. Cell Biol.* **13**:55.
5. FARQUHAR, M. G., S. L. WISSIG, and G. E. PALADE. 1961. Glomerular permeability. I. Ferritin transfer across the normal glomerular capillary wall. *J. Exp. Med.* **113**:47.
6. FRIEND, D. S., and N. B. GILULA. 1972. Variations in tight and gap junctions in mammalian tissues. *J. Cell Biol.* **53**:758.
7. HUHN, D., J. W. STEINER, and H. Z. MOVAT. 1962. Die Feinstruktur des Mesangiums im Nierenglomerulum von Hund und Maus. *Z. Zellforsch. Mikrosk. Anat.* **56**:213.
8. IWAYAMA, T. 1971. Nexuses between areas of the surface membrane of the same arterial smooth muscle cell. *J. Cell Biol.* **49**:521.
9. JONES, D. B., C. B. MUELLER, and M. MENEFFEE. 1962. The cellular and extracellular morphology of the glomerular stalk. *Am. J. Pathol.* **41**:373.
10. KREUTZIGER, G. O. 1968. Freeze-etching of intercellular junctions of mouse liver. Proceedings of the 26th Meeting of the Electron Microscope Society of America. Claitor's Publishing Division, Baton Rouge, La. 234.
11. LATTA, H. 1961. Collagen in normal rat glomeruli. *J. Ultrastruct. Res.* **5**:364.
12. LATTA, H., and A. B. MAUNSBACH. 1962. Relations of the centrolobular region of the glomerulus to the juxtaglomerular apparatus. *J. Ultrastruct. Res.* **6**:562.
13. LATTA, H., A. B. MAUNSBACH, and S. C. MADDEN. 1960. The centrolobular region of the renal glomerulus studied by electron microscopy. *J. Ultrastruct. Res.* **4**:455.
14. MCNUTT, N. S., and R. S. WEINSTEIN. 1973. Membrane ultrastructure at mammalian intercellular junctions. *Prog. Biophys. Mol. Biol.* **46**:47.
15. MOOR, H., K. MUHLETHALER, H. WALDNER, and A. FREY-WYSSLING. 1961. A new freezing ultramicrotome. *J. Biophys. Biochem. Cytol.* **10**:1.
16. SILVERBLATT, F. J., and R. E. BULGER. 1970. Gap junctions occur in vertebrate renal proximal tubule cells. *J. Cell Biol.* **47**:513.
17. STAHELIN, L. A. 1972. Three types of gap junctions interconnecting intestinal epithelial cells visualized by freeze-etching. *Proc. Natl. Acad. Sci. U. S. A.* **69**:1318.
18. SUZUKI, Y., J. CHURG, E. GRISHMAN, W. MAUTNER, and S. DACHS. 1963. The mesangium of the renal glomerulus. Electron microscopic studies of pathologic alterations. *Am. J. Pathol.* **43**:555.
19. TRUMP, B. F., and R. E. BULGER. 1971. Experimental modification of lateral and basilar plasma membranes and extracellular compartments in the flounder nephron. *Fed. Proc.* **30**:22.
20. VASSALLI, P., G. SIMON, and C. ROULLIER. 1963. Electron microscopic study of glomerular lesions resulting from intravascular fibrin formation. *Am. J. Pathol.* **43**:579.
21. YAMADA, E. 1955. The fine structure of the renal glomerulus in the mouse. *J. Biophys. Biochem. Cytol.* **1**:551.
22. YAMADA, E. 1960. Collagen fibrils within the renal glomerulus. *J. Biophys. Biochem. Cytol.* **7**:407.
23. ZAMBONI, L., and C. DE MARTINO. 1968. A reevaluation of the mesangial cells of the renal glomerulus. *Z. Zellforsch. Mikrosk. Anat.* **86**:364.
24. ZIMMERMANN, K. W. 1929. Ueber den Bau des Glomerulus der Menschlichen Niere. *Z. Mikrosk.-Anat. Forsch. (Leipz.)* **18**:520.
25. ZIMMERMANN, K. W. 1933. Ueber den Bau des Glomerulus der Säugerniere. *Z. Mikrosk.-Anat. Forsch. (Leipz.)* **32**:176.