SUBSTRUCTURE IN AN EPITHELIAL BASAL LAMINA (BASEMENT MEMBRANE)

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Ultrastructural studies of the basal lamina (basement membrane) of most tissues describe a felt or meshwork of fibrillar material embedded in a gel-like amorphous matrix (7, 22, 23, 36). In contrast, a regularly banded structure associated with the basal lamina or in connective tissue has been described in studies on a variety of tissues including Descemet's membrane (8, 12, 13, 14), the subcommissural organ of rat brain (20, 21, 34, 35), the trabecular meshwork of the eye (9–11, 29, 30, 32), Meissner's touch corpuscles (2), Schwann cell tumors of the VIIIth nerve (17, 18, 25), and in experimentally traumatized rat peripheral nerve (24). In all of these cases, it was inferred or directly stated that the banded structure was a form of collagen, probably the long-spacing type. Bertram and Bird (1) also noted banding in the basal lamina of *Aedes aegypti* midgut epithelium. Apparently, their observations were an example of longitudinal sectioning through this structure. The present work adds another dimension to these observations and provides a further demonstration of highly ordered substructure in a tissue noted for metabolic activity (1, 28).

MATERIALS AND METHODS

The midguts of *Aedes aegypti* mosquitoes infected with the avian malarial parasite, *Plasmodium gallinaceum*, were fixed in (i) 2.5% glutaraldehyde in 0.1 N phosphate buffer or (ii) 2.5% glutaraldehyde in 0.1 N sodium citrate. The tissue was postfixed in 1% OsO_4 in 0.1 N phosphate buffer or 1% OsO_4 in 0.1 N sodium citrate. Some tissue was fixed in phosphate-buffered OsO_4 alone. The tissue was dehydrated in graded alcohols and embedded in Epon. Sections were cut on a Porter-Blum MT-2 ultramicrotome and stained with lead citrate alone or with alcoholic uranyl acetate followed by lead citrate according to Reynolds (26). Sections were viewed in a Siemens-Elmiskop I electron microscope.

OBSERVATIONS

Sections of the mosquito midgut epithelial basal lamina parallel to the epithelial base reveal an extensive, well ordered grid structure (Fig. 1). The grid components are moderately dense, parallel lines intersecting one another approximately at right angles. Often, the lines intersect at an angle close to but different from 90°. The moderately dense lines are about 75 A wide and surround a roughly circular space of low density about 150 A in diameter. In longitudinal sections, the epithelial basal lamina consists of stacked lamellae, filamentous areas of moderate density, and irregular areas of low density. The stacked lamellae often follow an undulating course (Fig. 2), but can be roughly parallel to the epithelial base (Fig. 3). A single lamella contains the grid structure seen in sections parallel to the epithelial base. This is best illustrated when the orientation of a given lamella changes within the same section from perpendicular to tangential (Fig. 2). The lamellae have a minimum width of about 100 A and have a beaded appearance in longitudinal sections (Figs. 2, 3). The center-to-center distance between the dense beads in a longitudinally sectioned lamella is

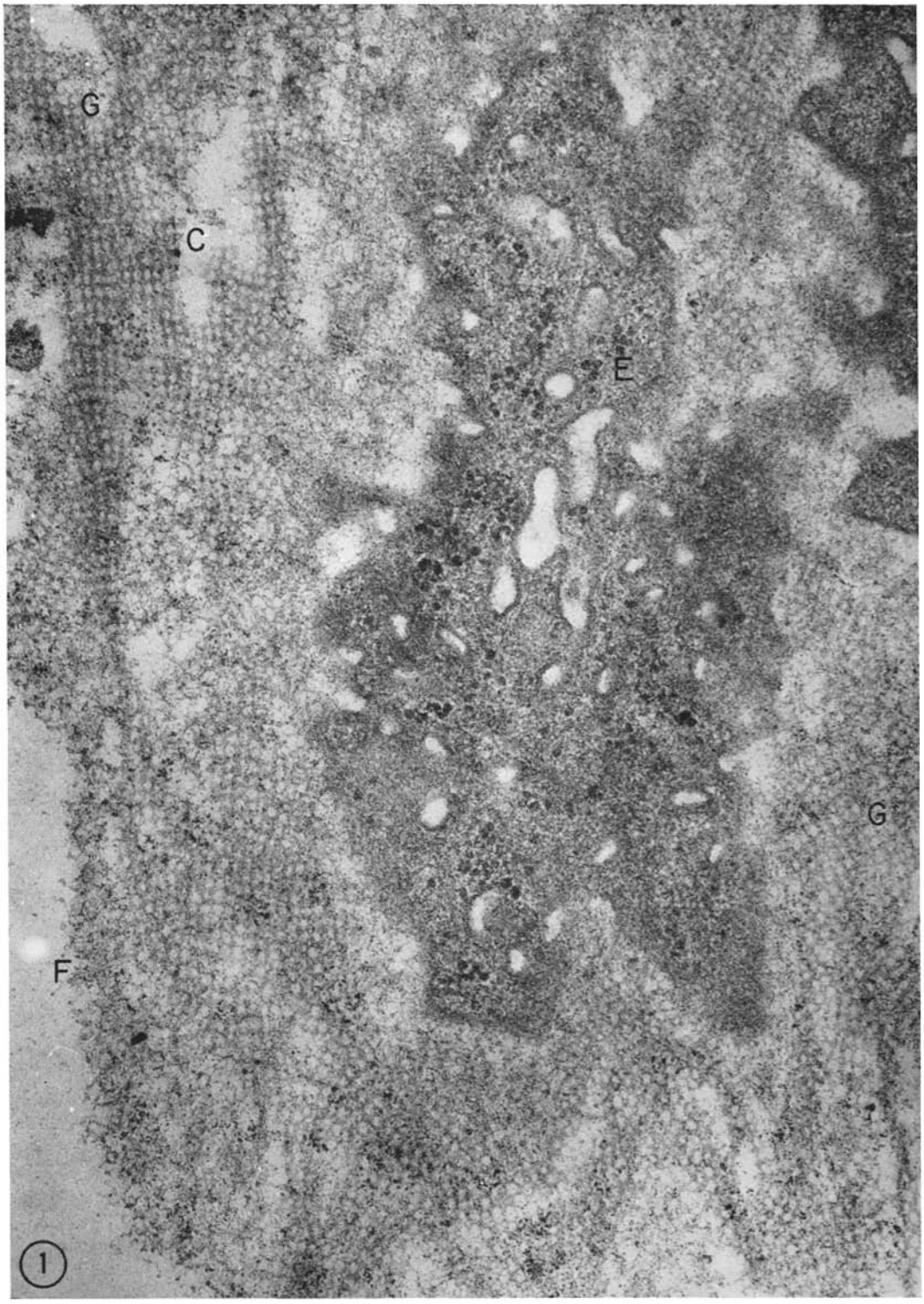

FIGURE 1 A section parallel to the base of *Aedes aegypti* midgut epithelium (*E*) within the substance of the basal lamina. The basal lamina consists of a well ordered grid structure (*G*), filamentous areas of moderate density (*F*), and irregular areas of low density (*C*). The grid is composed of dense lines intersecting at approximately right angles and enclosing a roughly circular area about 150 A in diameter. × 72,000.

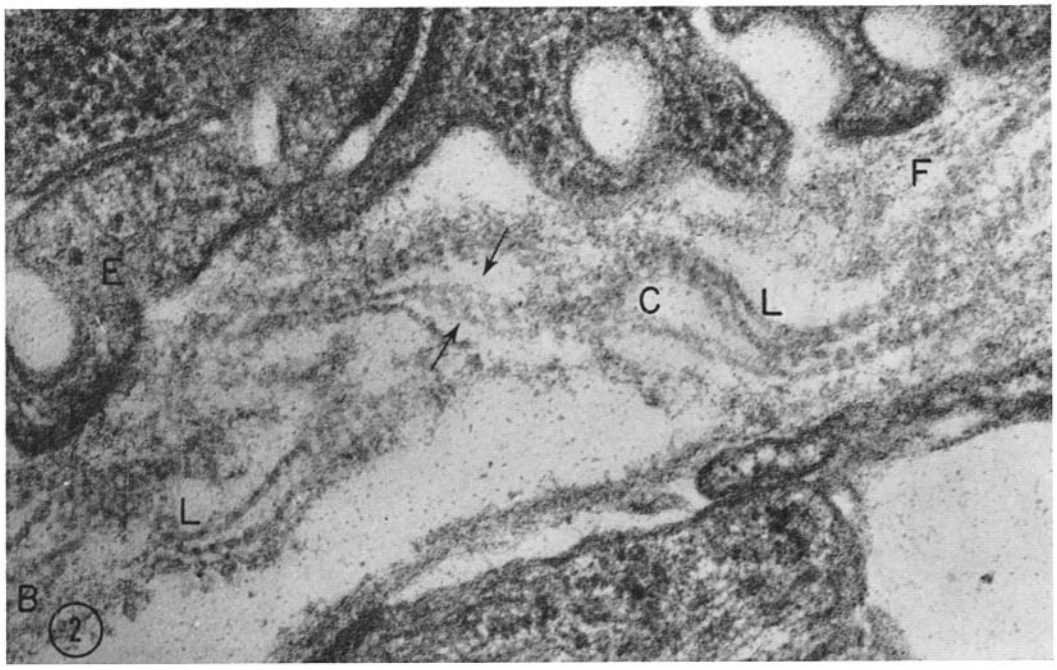

FIGURE 2 A longitudinal view of the midgut epithelium (*E*) and its basal lamina (*B*). The basal lamina consists of stacked lamellae (*L*) which may follow an undulating course, filamentous areas (*F*), and low density areas (*C*). A single lamella contains the grid structure seen in parallel sections (arrows). × 105,000.

about 245–290 A. The dense beads correspond to areas of intersection of the grid lines. Low density areas free of grid structure are usually located close to the epithelial base, but sometimes appear extensively throughout a given section parallel to the base (Fig. 1). These low density areas correspond to the uneven separations between the stacked lamellae occasionally seen in longitudinal sections (Fig. 2). The total thickness of the mosquito midgut epithelial basal lamina is about 0.2–0.5 μ. Surveys of numerous fields indicate that the grid structure is always present in the epithelial basal lamina. The grid structure appeared essentially the same with both fixative combinations. Staining with lead citrate alone did not appreciably change the morphology of the grid structure.

DISCUSSION

Basal laminae are noted for their amorphous composition (7). Specific exceptions to this generalization were noted above in which a structure thought to be fibrous long-spacing collagen was described within or associated with a basal lamina (8–14, 17, 18, 20, 21, 24, 25, 29, 30, 32, 34, 35). The basal lamina of the *Aedes aegypti* midgut epithelium is another exception to the generalization. Bertram and Bird (1) previously described a striated or banded appearance in the basal lamina of the midgut epithelium and the ovarian follicular tubes of *Aedes aegypti* mosquitoes. They felt that since the midgut epithelium and the ovarian tissue were both subject to considerable changes in volume, the banding represented a structural specialization related to the mechanical elasticity exhibited by these tissues.

Vanderberg et al. (33) noted a zipper-like structure in the midgut basal lamina of *Anopheles quadrimaculatus*. No functional interpretation was offered by the authors.

Roth and Porter (28) studied yolk protein uptake in the *Aedes aegypti* oocyte using ultrastructural and radioautographic techniques. They suggested that the midgut epithelial cells are at least one site of yolk synthesis. It follows from anatomical considerations that a product of the epithelium

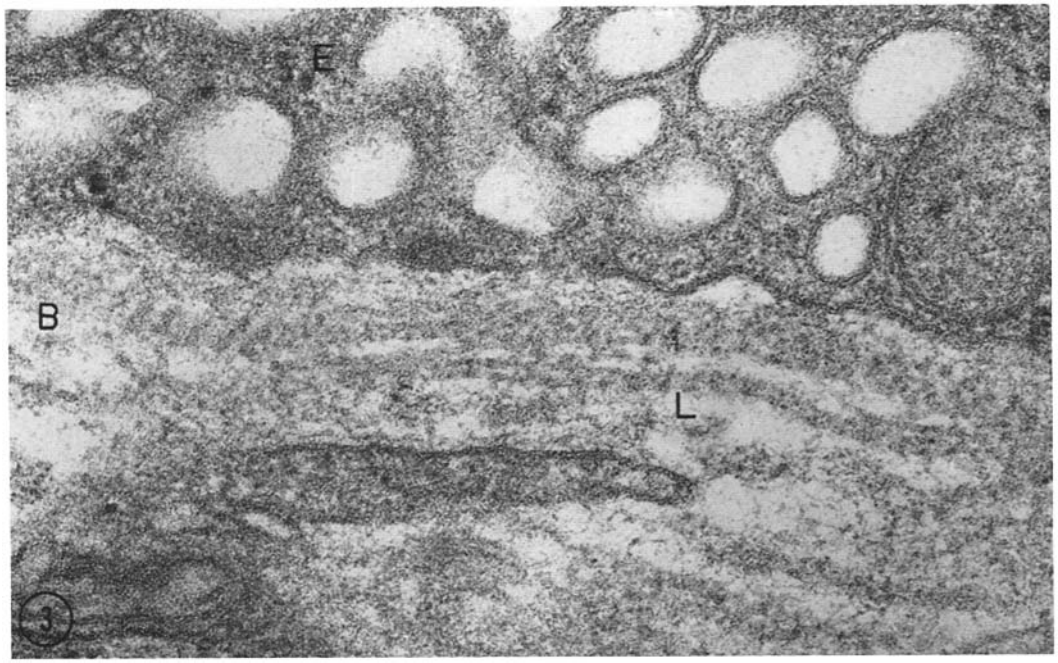

FIGURE 3 A longitudinal view of the basal lamina (*B*) of the midgut epithelium (*E*). The stacked lamellae (*L*) have a beaded appearance which corresponds to the intersection of grid lines seen in parallel sections. The lamellae may be roughly parallel to the epithelial base, with relatively little separation between adjacent lamellae. × 126,000.

destined for use in the mosquito must traverse the epithelial basal lamina into the hemocele. From the evidence presented in the present work, no definite statement can be made with regard to the functional significance of the grid structure. Of interest is the close similarity of appearance of the grid to that of a Millipore ultrafilter. A three-dimensional picture of the grid structure would be analogous to several Millipore filters stacked one above the other associated with an amorphous material. Although basal laminae, in general, are regarded as biological ultrafilters, a supportive function for the basal lamina grid structure cannot be ruled out at this time.

Some morphological studies indicate that the fibrillar component in basal laminae of various tissues may be collagen or reticular fibers (15, 27, 31). Dempsey (4) showed collagen fibrils leading into the connective tissue side of the basal lamina in chromium-shadowed visceral yolk-sac epithelium of the guinea pig. Chase (3) visualized collagen fibrils within the substance of the basal lamina of freeze-dried fat cells. Farquhar, Wissig, and Palade (6), studying the glomerular lamina densa, denied the existence of either collagen or reticular fibers in this location, but described two different and smaller fibrillar entities. Biochemical studies of glomerular basement membrane by Misra and Berman (19) indicate that 60% of the nitrogen analyzed was due to collagen while the remainder was probably associated with a glycoprotein species. Another biochemical study of the glomerular basement membrane by Lazarow and Speidel (16) showed that the basement membrane fraction is a complex glycoprotein whose major protein component is a type of collagen. In addition, these workers pointed out that the amino acid composition of the basement membrane fraction was similar to that of collagen and that the major protein portion of the fraction was solubilized by collagenase. In the present work, the periodicity of banding noted (245–290 A) in longitudinal sections of the midgut basal lamina is much smaller than that seen in the different forms of collagen. Studies are currently under way to

obtain a rough chemical classification of the grid structure by means of enzymatic digestion.

In a study thought to provide a model for the composition of basal laminae in general, Dische (5) emphasized the concept that these structures are an example of a low order of organization of collagen and ground substance. At this level of organization, the characteristic banding of collagen is not seen owing to the fact that the collagenous protein is not organized above the level of tropocollagen and is linked to one or more glycans. Higher levels of organization are represented by reticular fiber formations and the mature collagen seen in most connective tissues. These higher levels are characterized by a greater association of carbohydrate material with the ground substance and less with the collagenous proteins.

Jakus (13) noted that the ultrastructural picture of collagen is different in the sclera and cornea. Furthermore, the chemical composition of collagen from these two areas is identical, but the mucopolysaccharide contents differ quantitatively and qualitatively. She suggested that the mucopolysaccharides may be responsible for the morphological differences.

From the above discussion, it is suggested that the well ordered grid structure seen in the mosquito midgut basal lamina is a reflection of the association of carbohydrate to collagen in this location.

SUMMARY

A well ordered grid structure is described in the basal lamina of the *Aedes aegypti* midgut epithelium. In sections parallel to the epithelial base, the grid structure is a series of dense lines intersecting at approximately right angles to one another. The intersecting lines enclose a roughly circular area about 150 A in diameter. Longitudinal sections of the epithelial basal lamina reveal stacks of beaded lamellae following a course roughly parallel to the epithelial base. Each lamella contains the grid structure seen in the sections parallel to the epithelial base. The grid structure appears in all low-power survey micrographs. An analogy is drawn between the appearance of the basal lamina grid structure and Millipore ultrafilters. The chemical composition of basal laminae is discussed as well as the relation of the chemical constituents to the ultrastructural appearance. It is suggested that the basal lamina grid structure is a reflection of the association of carbohydrate to collagen in this location.

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BIBLIOGRAPHY

1. Bertram, D. S., and R. G. Bird. 1961. Studies on mosquito-borne viruses in their vectors. I. The normal fine structure of the mid-gut epithelium of the adult female *Aedes aegypti* (L) and the functional significance of its modification following a blood meal. *Trans. Roy. Soc. Trop. Med. Hyg.* **55**:404.
2. Cauna, N., and L. L. Ross. 1960. The fine structure of Meissner's touch corpuscles of human fingers. *J. Biophys. Biochem. Cytol.* **8**:467.
3. Chase, W. H. 1959. Structure of basement membrane of fat cells. *Arch. Pathol.* **67**:550.
4. Dempsey, E. W. 1953. Electron microscopy of the visceral yolk-sac epithelium of the guinea pig. *Am. J. Anat.* **93**:331.
5. Dische, Z. 1964. The glycans of the mammalian lens capsule—a model of basement membranes. *In* Small Blood Vessel Involvement in Diabetes Mellitus. M. D. Siperstein, et al., editors. American Institute of Biological Sciences, Washington, D. C. 201.
6. Farquhar, M. G., S. L. Wissig, and G. E. Palade. 1961. Glomerular permeability. I. Ferritin transfer across the normal glomerular capillary wall. *J. Exptl. Med.* **113**:47.
7. Fawcett, D. W. 1966. The Cell. An Atlas of Fine Structure. W. B. Saunders Co., Philadelphia. 353.
8. Feeney, M. L., and L. K. Garron. 1961. Descemet's membrane in the human peripheral cornea. *In* The Structure of the Eye. G. K. Smelser, editor. Academic Press Inc., New York. 367.
9. Feeney, L. 1962. Ultrastructure of the nerves in the human trabecular region. *Invest. Ophthalmol.* **1**:462.
10. Garron, L. K., M. L. Feeney, M. J. Hogan, and W. K. McEwen. 1958. Electron microscope studies of the human eye. I. Preliminary inves-

tigations of the trabeculae. *Am. J. Ophthalmol.* **46**:27.

11. GARRON, L. K., and M. L. FEENEY. 1959. Electron microscope studies of the human eye. II. A study of the trabeculae by light and electron microscopy. *A.M.A. Arch. Ophthalmol.* **62**:966.
12. JAKUS, M. A. 1956. Studies on the cornea. II. The fine structure of Descemet's membrane. *J. Biophys. Biochem. Cytol.* **2** (suppl.):243.
13. JAKUS, M. A. 1961. The fine structure of the human cornea. *In* The Structure of the Eye. G. K. Smelser, editor. Academic Press Inc., New York. 343.
14. JAKUS, M. A. 1962. Further observations on the fine structure of the cornea. *Invest. Ophthalmol.* **1**:202.
15. KARRER, H. E. 1956. The ultrastructure of mouse lung. *J. Biophys. Biochem. Cytol.* **2**:241.
16. LAZAROW, A., and E. SPEIDEL. 1964. The chemical composition of the glomerular basement membrane and its relationship to the production of diabetic complications. *In* Small Blood Vessel Involvement in Diabetes Mellitus. M. D. Siperstein et al., editors. American Institute of Biological Sciences, Washington, D. C. 127.
17. LUSE, S. A. 1960. Electron microscope studies of brain tumors. *Neurology.* **10**:881.
18. LUSE, S. A., D. ZOPF, and S. W. COX. 1963. An electron microscope study of in vitro and in vivo long-spacing collagen. *Anat. Record.* **145**: 254.
19. MISRA, R. P., and L. B. BERMAN. 1966. Studies on glomerular basement membrane. I. Isolation and chemical analysis of normal glomerular basement membrane. *Proc. Soc. Exptl. Biol. Med.* **122**:705.
20. NAUMANN, R. A. 1963. A unique intercellular material in the brain. *Anat. Record.* **145**:266.
21. NAUMANN, R. A., and D. E. WOLFE. 1963. A striated intercellular material in rat brain. *Nature.* **198**:701.
22. PALADE, G. E. 1953. Fine structure of blood capillaries. *J. Appl. Physics.* **24**:1424.
23. PALADE, G. E. 1961. Blood capillaries of the heart and other organs. *Circulation.* **24**:368.
24. PILLAI, P. A. 1964. A banded structure in the connective tissue of nerve. *J. Ultrastruct. Res.* **11**:455.
25. RAIMONDI, A. J., S. MULLAN, and J. P. EVANS. 1962. Human brain tumors: An electron microscope study. *J. Neurosurg.* **19**:731.
26. REYNOLDS, E. S. 1963. The use of lead citrate at high pH as an electron opaque stain in electron microscopy. *J. Cell Biol.* **17**:208.
27. RINEHART, J. F., and M. G. FARQUHAR. 1955. The fine vascular organization of the anterior pituitary gland. *Anat. Record.* **121**:207.
28. ROTH, T. F., and K. R. PORTER. 1964. Yolk protein uptake in the oocyte of the mosquito *Aedes aegypti* (L). *J. Cell Biol.* **20**:313.
29. ROWEN, J. W. 1962. Uber das ligamentum Pectinatum der Primaten. *Z. Zellforsch.* **58**:403.
30. ROWEN, J. W. 1963. Experimental studies on the trabecular meshwork in primates. *A.M.A. Arch. Ophthalmol.* **69**:335.
31. SHELDON, H. 1956. An electron microscope study of the epithelium in the normal mature and immature mouse cornea. *J. Biophys. Biochem. Cytol.* **2**:253.
32. SPEAKMAN, J. S. 1962. Nodular dystrophy of the trabecular meshwork. *Brit. J. Ophthalmol.* **46**:31.
33. VANDERBERG, J., J. RHODIN, and M. YOELI. 1967. Electron microscopic and histochemical studies of sporozoite formation in *Plasmodium berghei*. *J. Protozool.* **14**:82.
34. WETZSTEIN, R., A. SCHWINK, and P. STANKA. 1963. Pericapillar gelegene periodische Strukturen im Subcommissuralorgan bei Ratten. *Naturwissenschaften.* **50**:137.
35. WETZSTEIN, R., A. SCHWINK, and P. STANKA. 1963. Die periodische Strukturierten Korper im Subcommissuralorgan der Ratte. *Z. Zellforsch.* **61**:493.
36. YAMADA, E. 1955. The fine structure of the renal glomerulus of the mouse. *J. Biophys. Biochem. Cytol.* **1**:551.