Fine Structure of Teleost Kidney as Revealed by Electron Microscopy

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INTRODUCTION

Since Pease and Baker started observing the kidney of a rat by an electron microscope in 1954, a great number of investigators have followed suit in examining those organs of men, rats, mice, rabbits, dogs, marsupealia, frogs, turtles and insects. The following are among them:

Bargmann, W., Knoop, A., Schiebler, Th. H. (1955), Bergstrand, A., Bucht, H. (1957), Dalton, A. J. (1951), Fawcett, D. W. (1954), Hall, B. V., Roth, E., Johnson, V. (1953, 1954, 1956), Meyer, G. F. (1957), Mueller, C. B., Mason, Jr., Stout, D. G. (1955), Oberling, Ch., Gautier, A., Bernhard, W. (1951), Pak Poy (1957, 1958), Pease, D. C., Baker, R. F. (1950, 1954, 1955), Picle, C. F., Dong, L. (1955), Reid, R. T. W. (1954), Rhodin, J. (1955), Rinehart, J. F. (1955), Sakaguchi, H. (1955), Sakaguchi, H. & Suzuki, A. (1956, 1957), Takaki, B. (1956), Thiel, A. (1958), and Yamada, E. (1955).

MATERIAL AND METHOD

In this study, kidneys from *Cyprinus carpio*, *Hepatus aliala* and *Acanthogobius flavimanus* have been used. All specimens were obtained immediately postmortem. These slices were cut with a razor blade into cubes about 1 mm in size and immediately dropped into a solution of 2 per cent osmium tetroxide buffered to p.H 7.4 with veronal buffer. After fixation in the osmic acid solution for 30 minutes, the blocks were dehydrated by successive transfers of 20 minutes each, through 70, 90 and 100 per cent alcohol, filtrated with one-half methacrylate and one-half 100 per cent alcohol and then two changes of methacrylate (3 : 7 methyl : buthyl methacrylate). Embedding was performed in a No. 1 gelatin capsule with methacrylate to which 2, 4-dichlorobenzol peroxide 1 mg per ml. was added to catalyze the hardening process. After drying for twenty-four hours as 45°C., the capsules were dissolved from the plastic block which was then ready for sectioning. Thin sections were cut on an ultramicrotome with a glass knife and stained with Lawn's method. These were then examined by an electron microscope.

And we used another embedding medium: after dehydration by passing through alcohol of ascending concentrations successively and finally by pure alcohol, spec-

imens were immersed in 50/50 mixture of alcohol and styrene for 1 hour, and then immersed into cold (4°C) pure styrene overnight.

Embedding was accomplished in gelatin capsule in which styrene monomer containing 2, 4-dichlorobenzol 1 mg per ml. as an initiator was present. Polymerization was completed within 2 days by ultraviolet irradiation at 60°C.

The capsules were dissolved from the plastic block which was then ready for sectioning. Thin sections were cut on an ultramicrotome with a glass knife and stained with Lawn's method. These were then examined by an electron microscope.

RESULT

1) Fine structure of the uriniferous tubules

The teleost kidney pertaining to the mesonephros, instead of being devided into the medullary substance and cortical one like the mammalian kidney, consisted of nothing but the collections of irregular uriniferous tubules. In the kidneys of most of the mammalia, the descending and ascending limbs of the loop of Henle and the collecting tubules were each arranged regularly, whereas in the teleost kidney, nothing was arranged so regularly as those; their arrangement was exceedingly irregular.

The epithelial cells of the uriniferous tubule of the teleost kidney were composed of cilia, microvilli, terminal bars, basal intussusception, mitochondria, endoplasmic reticulum, and Golgi's apparatus.

a) Cilia

In the uriniferous tubule of the teleost kidney were observed cilia (Fig. 1). In the cross section, the electron dense ciliary membrane looked circular in shape. In the central part of it there were two central filaments arranged in pairs side by side. They were surrounded by 9 peripheral filaments arranged in a circle (Fig. 2). The diameter of the external circle formed by the peripheral filaments showed in any of fishes almost a certain definite value. For instance, in a carp (*Cyprinus capio*) it proved to be 160 mμ, and in a *Acanthogobius flavimanus* 153 mμ. In the border between the cilia and the basal body two electron dense transverse partitions were observed. The upper partition was a septum limiting the lower end of the cilia while the lower one the upper end of the basal body (Fig. 3).

There was single rootlet fiber which at the basal body ran at a definite angle and in a certain direction deep into the cytoplasm. And this rootlet fiber had a periodicity of appearance with a definite intervals of time and iteration. *Acanthogobius flavimanus*, for example, had a periodicity of 770 Å, showing regular lateral stripes (Fig. 4).

b) Microvilli

On the free surface of the epithelial cell of most of the uriniferous tubules of the teleost kidney, there grew thick fine microvilli as large and pretty long (Fig. 5). In

Hepatus aliala the tips of the microvilli were round (Fig. 6, 7) and in *Cyprinus capio* in some cases there were some microvilli which were observed to have the tubules closed (Fig. 8).

c) Terminal bar

In the border of the cells lining the cavities of the uriniferous tubules of the teleost kidney, terminal bars, the thickned cell membrane and desmosomes were observed (Fig. 9, 10, 11).

d) Basal intussusception

In the epithelial cells of the uriniferous tubules of the teleost kidney, the basal intussusception was observed (Fig. 12).

e) Vacuole-like structure

Toward the border of the cytoplasm where microvilli attached short rods or vacuole-like structures in shape were observed, but neither mitochondria nor any basal intussusception (Fig. 13).

f) Aspect of the intercellular contact

The aspects of the intercellular contact of the epithelial cells of the uriniferous tubule of the teleost kidney were observed slightly curved, but smooth (Fig. 13), containing endoplasmic reticulum, mitochondria (Fig. 13) and Golgi's apparatus (Fig. 14).

2) Fine structure of glomerulus

The structures found in the glomerulus are: basement membrane of Bowman's capsule, epithelium of Bowman's capsule, epithelium of the glomerulus, basement membrane of the glomerulus, endothelial cells, mesangium, capillary lumen, and erythrocyte.

a) Epithelial cells

Epithelial cells are astrocyte-like in shape and possess many long protoplasmic arms (trabeculae) which extend in all directions. The trabeculae end in foot processes which lie on the extracapillary surface of the basement membrane. Cross sections of these foot processes are irregular form, each process is 2500 Å in width at its base, and is apart from adjacent one 400 Å. The epithelial cells have large, spherical nuclei and fairly large cell bodies (Fig. 15).

b) The basement membrane of the glomerulus

We have found the basement membrane of the fish glomerulus to be single homogeneous membrane 550–600 Å thick. There is a clear of about 275–300 Å between the capillary basement membrane and the foot processes (lamina rara externa) and also there is a clear space of about 240–280 Å between the capillary basement membrane and the endothelium (lamina rara interna) (Fig. 15, 16).

c) The glomerular capillary endothelium

The endothelial cytoplasm lines the inner aspect of the basement membrane. The

endothelial cytoplasm is of variable and irregular thickness. In some places this layer is quite thin (450–500 Å). In its thinner segments it contains a series of small vesicle in the range of 450 Å to 500 Å in diameter (Fig. 16).

d) The glomerular stalk

Zimmermann, in 1933, claimed that 25 to 35 per cent of the endothelial cells were in reality fibrocytes and that they formed a supporting stalk which stemed from the hilus of the glomerulus. He labeled the stalk as the mesangium and the fibrocytes mesangial cells. The mesangial cell bodies, or portions containing the nucleus, lie in the central portion of the stalk. The mesangial cells looked dark. In the cytoplasm of the mesangial cell we recognized mitochondria, vesicles and endoplasmic reticulum (Fig. 17, 18).

CONSIDERATION

The width of the epithelial cell foot processes which lie on the extracapillary surface of the basement membrane, Hall (1953, 1954) reports, is 1000–2000 Å in the cases of a rat and a rabbit, Rhodin (1955) 500–1000 Å in a mouse, Pease (1955) 1000–1500 Å in a rat, Yamada (1955) 1000–1500 Å in a mouse, Mueller (1955) 2200 Å in a dog and a man, Sakaguchi and Suzuki 1500–2500 Å in a dog and we found it 2500 Å in a carp.

As to the process interstice, Pease (1955) says it is 400 Å in a rat, Yamada (1955) 200–300 Å in a mouse, Sakaguchi and Suzuki (1958, 1959) 200–600 Å in a man, Kinoshita and Hirokawa (1958) 150–600 Å in a man and we found it 400 Å in a carp.

As to the lamina densa, Dalton (1951) reports it is 1000 Å in a mouse, Hall (1953, 1954) 500–1000 Å in a rat and a rabbit, Reid (1954) 1000 Å in a mouse, Rhodin (1955) 650 ± 13 Å in a mouse and we found it 550–600 Å in a carp.

Referring to the lamina rara externa, Rhodin (1955) says it is 300 Å in a mouse and Pease 300 Å in a rat while we found it 275–300 Å in a carp.

The diameter of a small vesicle of the endothelial cytoplasm, Rinehart (1955) reports it 200–400 Å in a rat, Mueller (1955) 450 Å in a dog and a man, and we found it 450 Å in a carp.

CONCLUSION

1) In the uriniferous tubule of the teleost kidney, cilia are observed. In the border of the cilia and the basal body, the electron dense transverse partition is observed.

2) There is a single rootlet fiber observed. *Acanthogobius flavimanus* has the periodicity of 770 Å, showing regular lateral stripes.

3) The diameter of the circle formed by the peripheral filaments is 160 mμ in a carp.

4) On the surface of the epithelial cell of most of the uriniferous tubule of teleost kidney there grow thick fine microvilli as large and pretty long. In *Hepatus aliala* the tips of the microvilli are round. And in *Cyprinus capio* in some cases some microvilli are observed to have tubules closed.

5) Besides, the terminal bar, basal intussusception, vacuole-like structures, endoplasmic reticulum, mitochondria and Golgi's apparatus are observed.

6) The spaces of the intercellular contact are smooth, having little interdigitation.

7) The inner aspect of the capillary loop in the glomerulus are composed of epithelium, basement membrane, endothelial cells and mesangium.

8) The epithelial cells possess many long protoplasmic arms (trabeculae) which extend in all directions. The trabeculae end in foot processes which lie on the extracapillary surface of the basement membrane. Each process is 2500 Å wide at its base, and is 400 Å apart from the adjacent one in a carp.

9) The lamina densa looks dark, having no structure 550–600 Å wide. The width of the lamina rara externa is 275–300 Å while that of the lamina rara interna is 240–280 Å in a carp.

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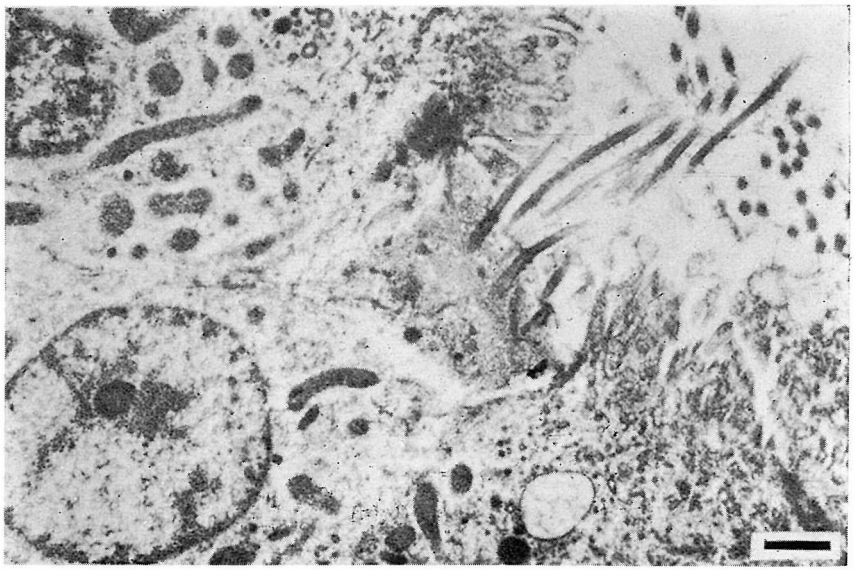
EXPLANATION OF FIGURES

Fig. 1. Cilia were observed in the uriniferous tubule.
Fig. 2. The cross section of cilia.
Fig. 3. The cilia, the basal body and a rootlet fiber.
Fig. 4. The rootlet fiber.
Fig. 5. The microvilli in a carp.
Fig. 6. The microvilli in a *Hepatus aliala.*
Fig. 7. The microvilli in a *Hepatus aliala.*
Fig. 8. Some microvilli are observed to have tubule closed in some cases in a carp.
Fig. 9. The terminal bar.
Fig. 10. The terminal bar.
Fig. 11. The terminal bar.
Fig. 12. The basal intussusception.
Fig. 13. The intercellular contact of the epithelial cells of the uriniferous tubule.
Fig. 14. Golgi's apparatus.
Fig. 15. The epithelium of the glomerulus.
Fig. 16. The basement membrane of the glomerulus.
Fig. 17. The mesangial cell.
Fig. 18. The mesangial cell.



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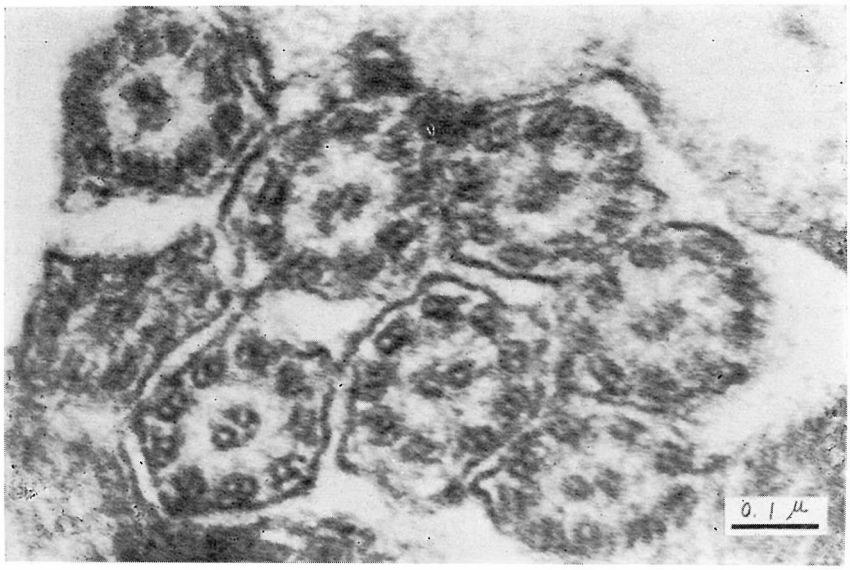

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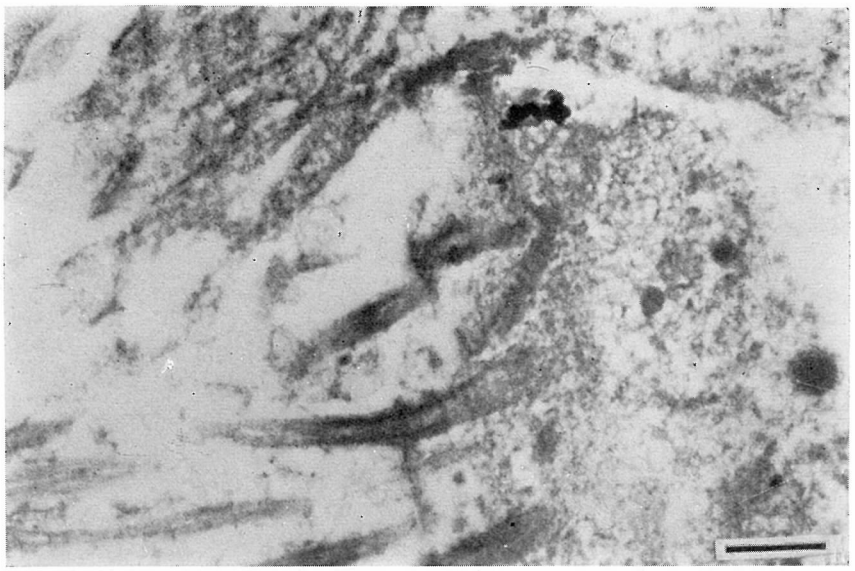

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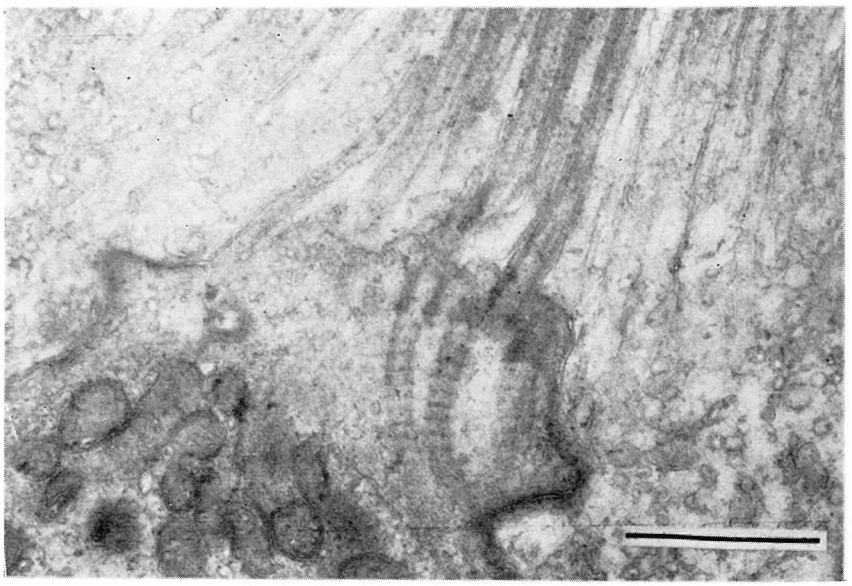

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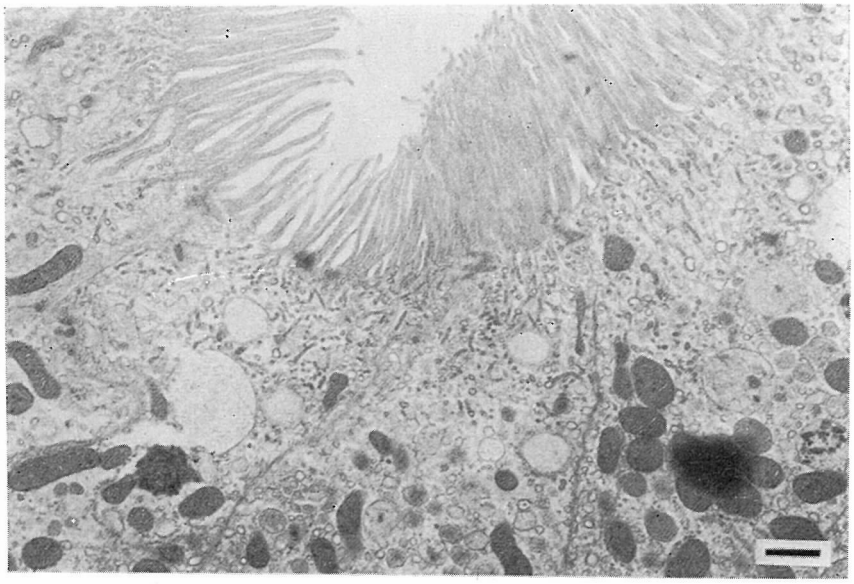

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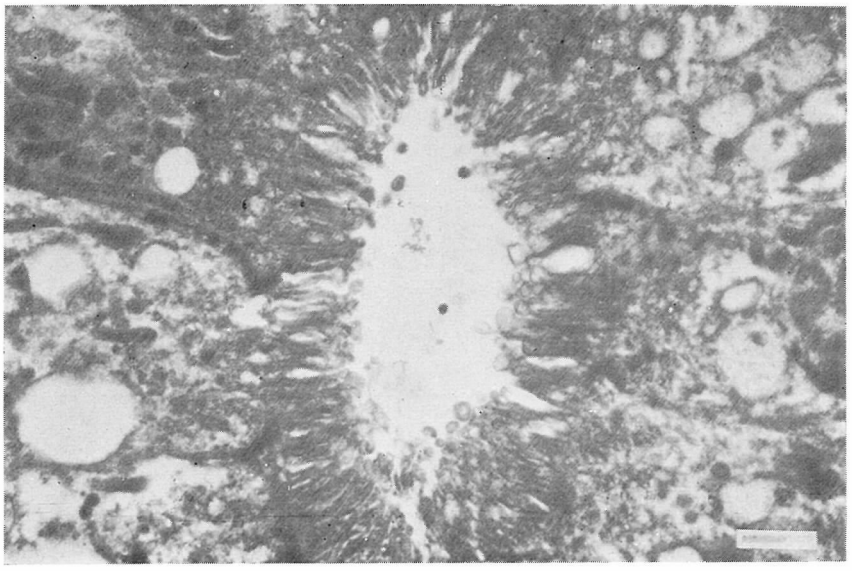

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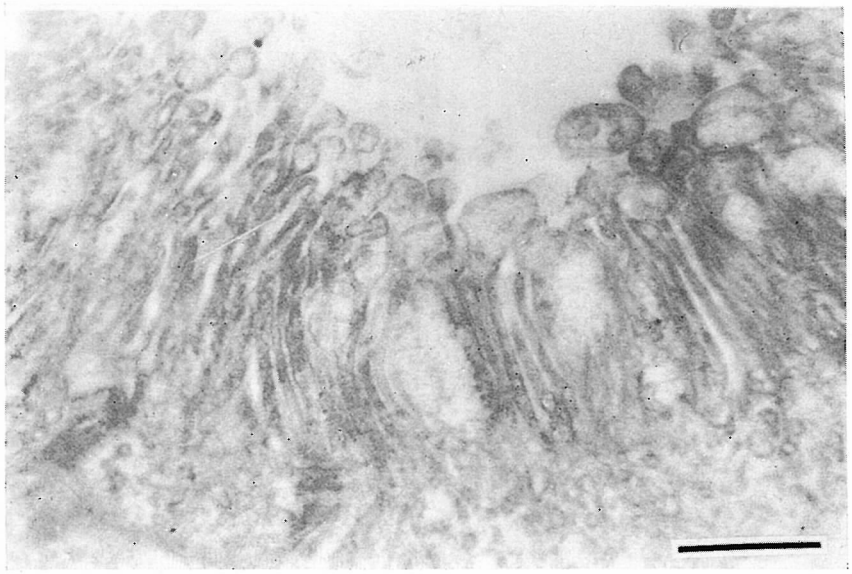

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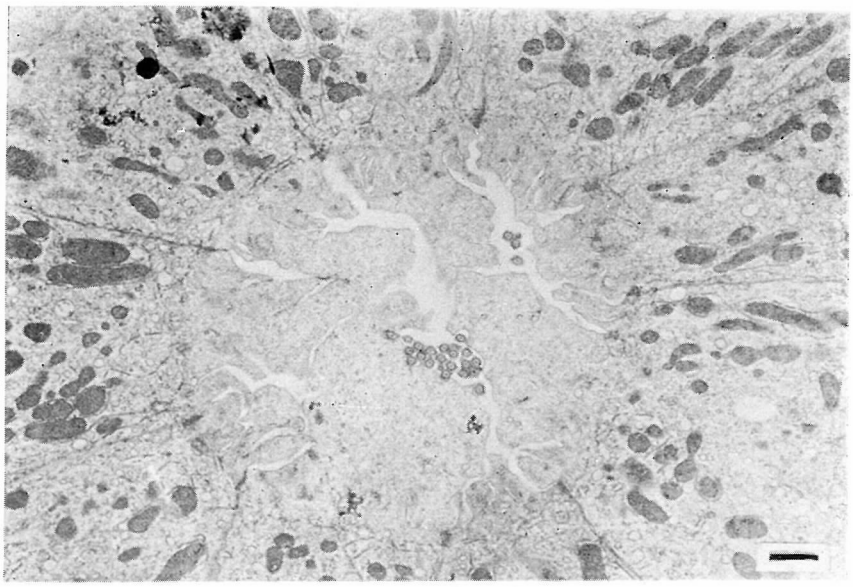

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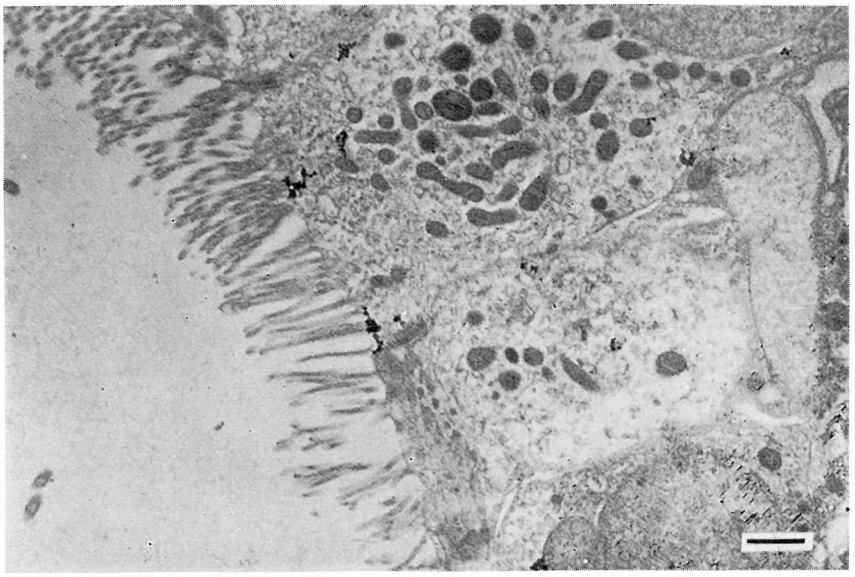

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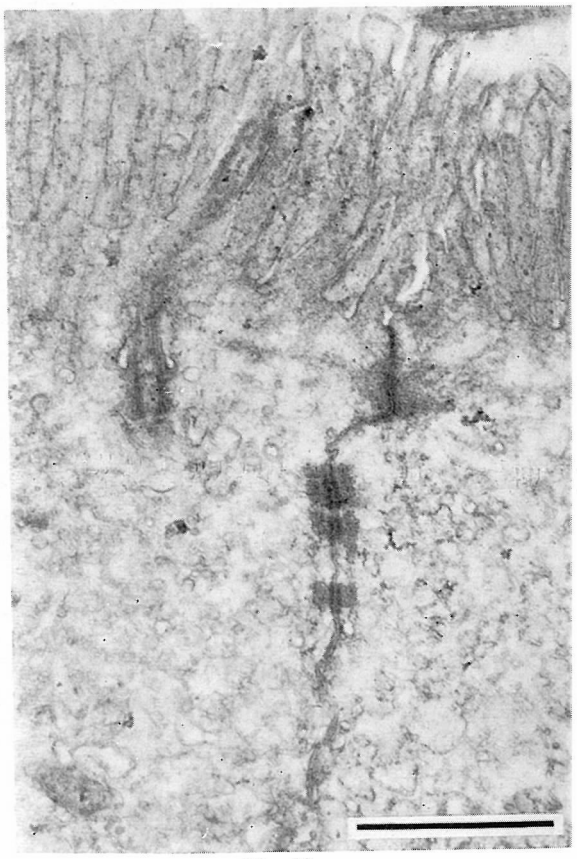

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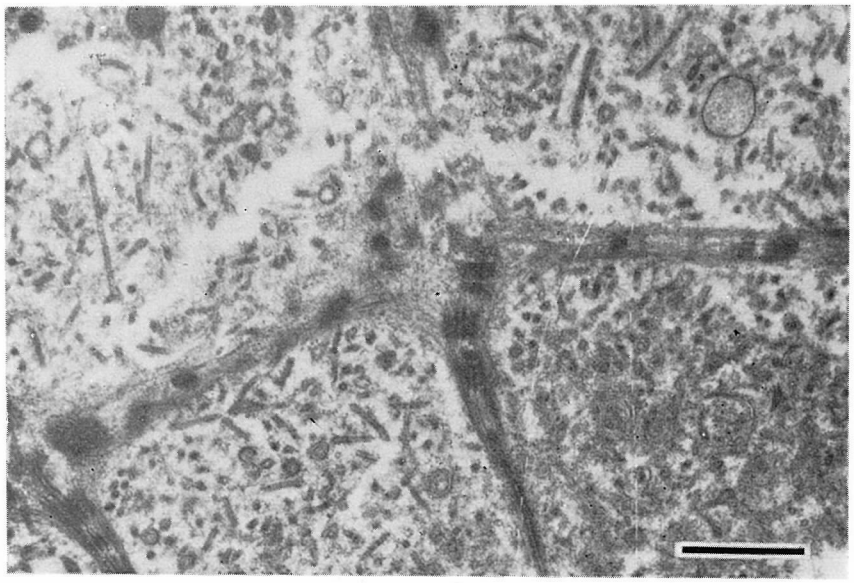

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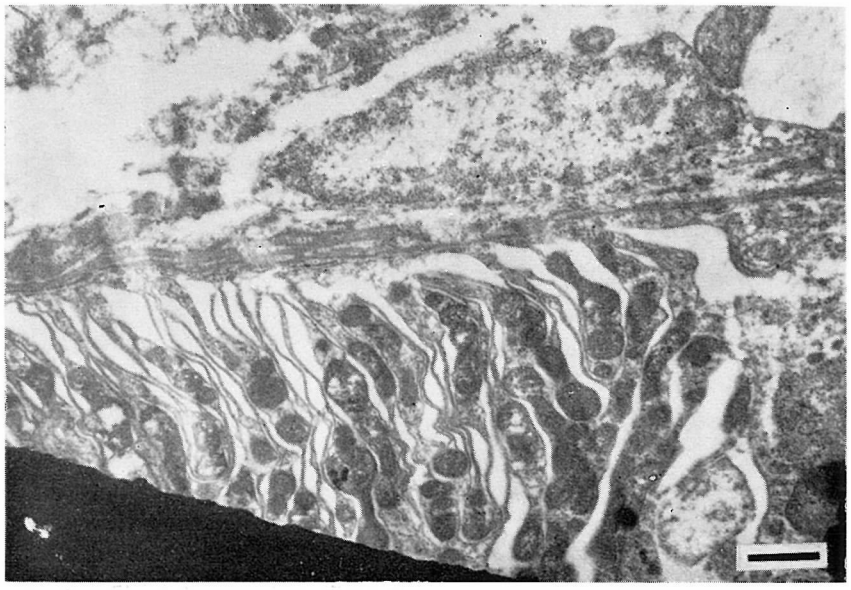

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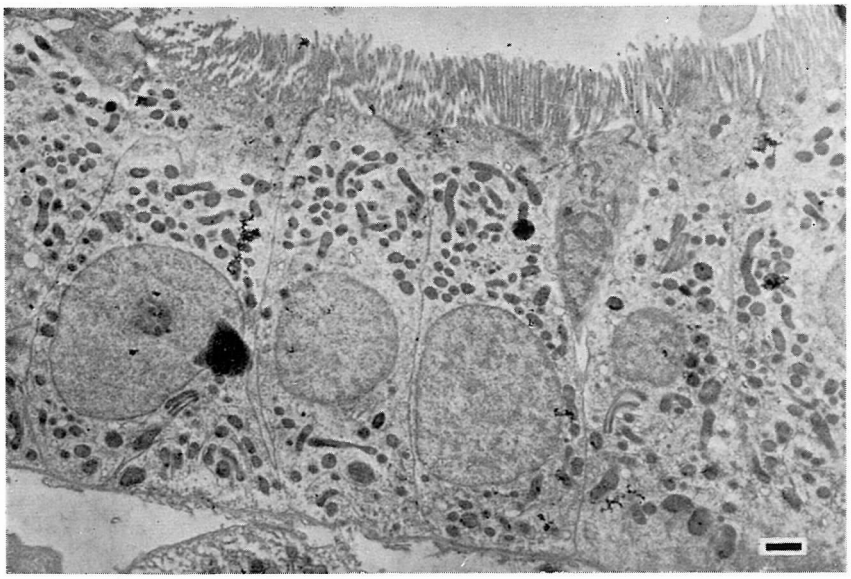

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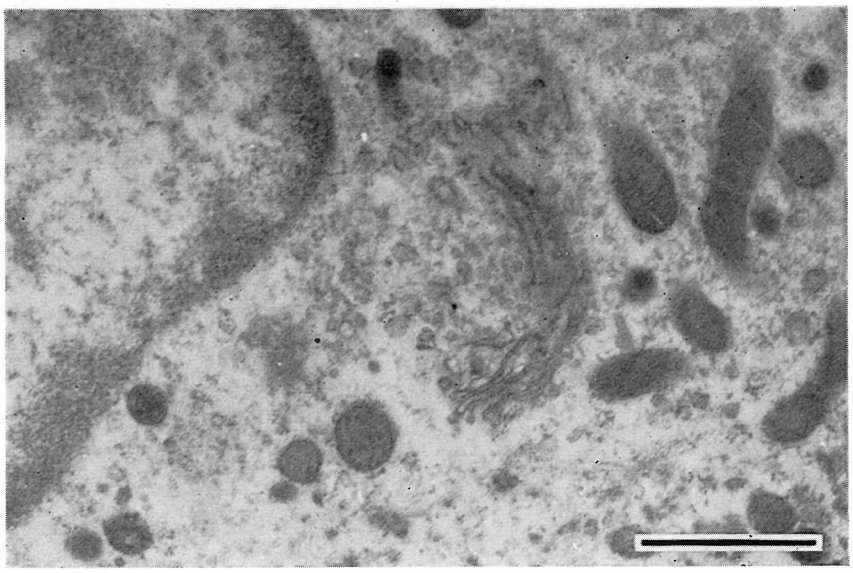

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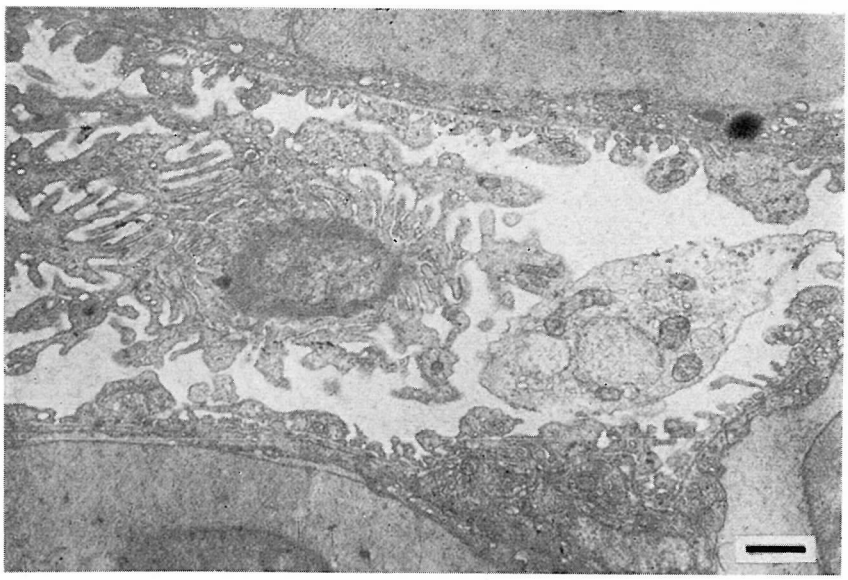

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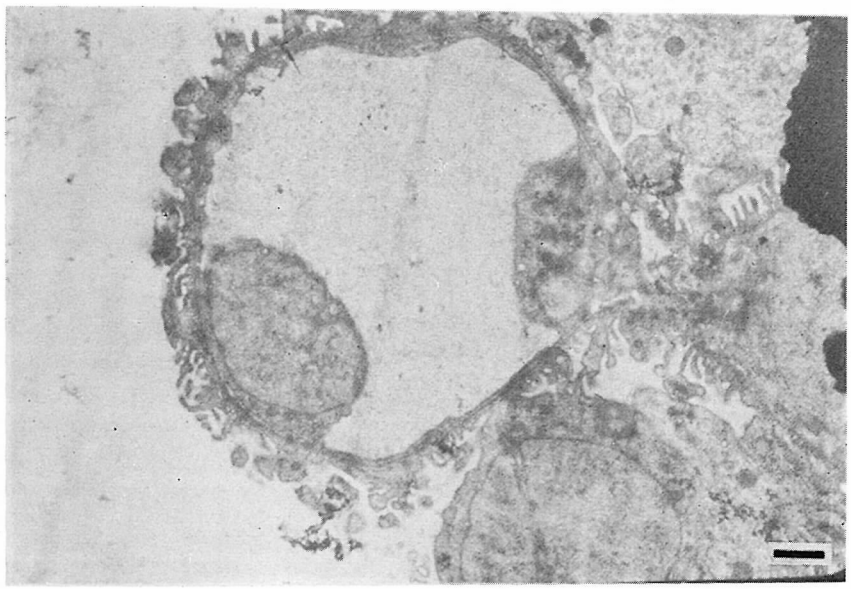

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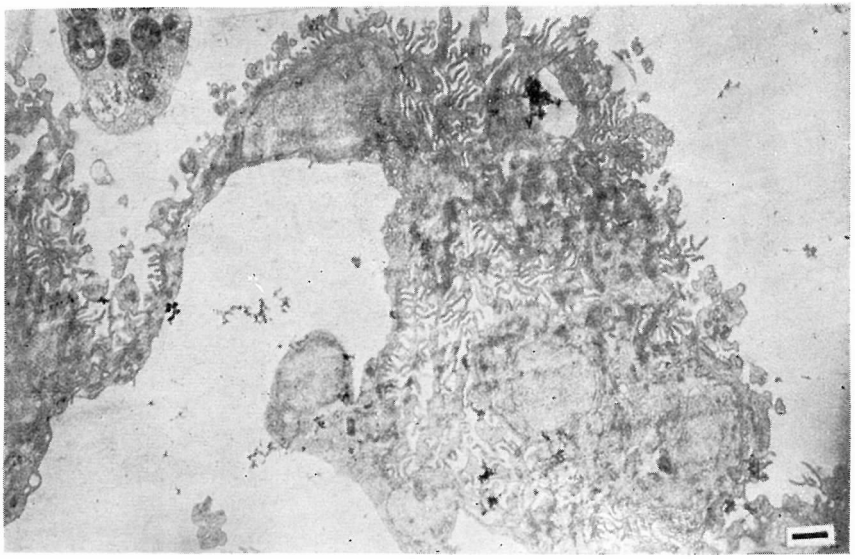

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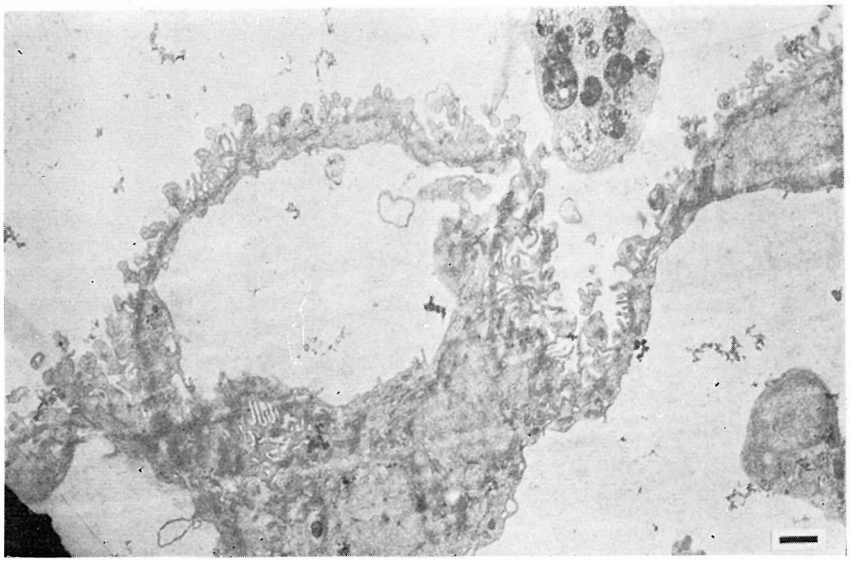

Fig. 18.