The Electron Microscopic Studies on Human Iris Vessels.

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INTRODUCTION

The iris is a part of uveal tract. On its embryological aspect, the iris stroma and vessels constitute part of the mesoderm, and share the different origin from the retina, and shares the same origin with the choroid. The posterior layer of the iris, however, is the continuous part of the retina-the neural ectoderm.

The circulation of the iris is mainly supplied by the anterior ciliary arteries, and partly by the long posterior ciliary arteries, and the anastomosis occur among them to form the major arterial circle in the root of the iris. The arteries run like spokes in a wheel toward the pupillary margin and form a minor circle around the pupil. Radially arranged veins drain the minor circle to the periphery of the iris into a venous circle and thence into the vorticose veins.

The muscles of the iris, in fact, are the only tissue requiring anything more than a minimal blood supply.

It is generally believed that a part of the metabolism of the aqueous humor is participated by the iris. In fact, the iris considerably lacks of secretory organelles. It has been generally supposed that the exchange of the water and non-electrolytes between the iridic blood stream and the aqueous humor depend on the permeative mechanism of the iris vessels, therefore, the fine structure of the iris vessels might have special importance to find out the mechanism of the aqueous metabolism. On the other hand, the iris vessels seem to have its characteristics to conduce the bleeding, e. g. the hemorrhage seldom occur in peripheral iridectomy, hence, the knowledge of the fine structure of the iris vessels might help to reveal this secret.

The purpose of this study is to find out the special anatomic structure of the human iris vessels under the electron microscope. and tried to understand the relationship between the solutes transfer and the anatomic structure and which mechanism involves the less frequence of the bleeding in iris surgery.

MATERIAL AND METHOD

Five human irides out of three patients obtained when the peripheral iridectomy

were performed in senile cataract surgery, these included a man and two women, their ages are 58, 63, 57, years old.

The iris specimens were fixed with 1 % Osmium Tetroxide immediately after removal for two hours at 4 C and were dehydrated in graded concentrations of alcohol. Thin sections were made with a Porter-Blum Ultramicrotome. Staining was carried out with uranyl acetate in distilled water, followed by lead citrated and observed by J.E.M.T-5 electron microscope.

RESULTS

Under the electron microscope, the iris vessels were differentiated into capillary, arteriole and venule by their fine structures :

1 Capillary.

As the Fig. 1-2 show, that the lumen of the iris capillary is enclosed completely by a single layer of endothelial cells and the arrangement of adjacent endothelial cells overlap each other slightly, linking, to form the terminal bars (intercellular junctions) (Fig. 1). The opposing membranes of these bars ordinary show the increased electron density, and sometimes it seems fused together to form a firm terminal barrier (Fig. 3). Occasionally, it involved the subjacent cytoplasm of the neighbour cells. In this case, the subjacent cytoplasm shows an increase of electron density (Fig. 4). On the other hand, however, part of the intercellular junctions, varied wide spaces existed, and it shows no changes of electron density, either in apposing membranes or subjacent cytoplasm (Fig. 5-10).

The endothelial cells are flat-bodied and varied in thickness, consisting of a thin cell membrane, with a nucleus situated ordinary in the center of the cell (Fig. 1-2). In some sections, it protrudes into the lumen and causes the thickening of the cell. The endothelial cells does not show any fenestration. The nucleolus were seen as drift-like clouds, increasing of electron density, and its margin is not always distinguishable. The nucleus of the endothelium is usually surrounded by double nuclear membranes, external to the inner membrane there is prominent perinuclear space (nuclear envelope) (Fig. 1). This space extended and has the connection with the other cavities that were enclosed by the same membrane.

The abundance of fine fibrils in the cytoplasm of the endothelium can be seen, they are distributed scatteringly mainly parallel to the vessel longitude, these fine fibrils in higher magnification, were found consisting of many small spots in bead-like arrangement (Fig. 11-13).

As the Fig. 4 shows, there is an abundance of pinocytosis vesicles in the cytoplasm of the endothelial cells. These vesicles usually tend toward at either surfaces of the cell, and part of them have the opening in the cell membranes (either the inner surface or the outer surface), In addition to these pinocytosis

vesicles, there are many large vesicles found in the cytoplasm of the endothelial cells (Fig. 1). Fawcett[32] (1965) termed the former as micro-pinocytosis vesicles and the latter as pinocytosis vesicles.

In the cytoplasm, there are also Numerous free granules, which have higher electron density, round or spindle in shape, it was generally supposed as lysosome. Mitochondria and Golgi apparatus also are seen.

The free surface of the endothelial cells are rough and irregular, the cells always have the microvilli (protruding cell border) that protrude into lumen, these microvilli often appear near the intercellular junctions of the endothelia, when the cells overlap the others and also appear in anywhere of the free margin of the endothelia (Fig. 1-2).

The basement membrane is varied in thickness, and situated external to the endothelial layer, It surrounded and separated the endothelial cells from pericytes, and look like bands, increased electron density, sometimes, it appears as fibrillar structure in higher magnification (Fig. 15). The basement membrane of the endothelial layer occasionally was penetrated by endothelium and then it contacted directly to the pericyte, or sometimes, the reverse took place. The basement membrane also shows that small holes (rodent phenomenont) exist, it appears as lower electron density, but it was never identified as fenestration through the inside to the outside due to the holes, except it was penetrated by cells, as mentioned (Fig. 14-15). In some sections, there are potential spaces existing between the basement membrane and the neighbour cells, it is outside to the endothelia on the one hand and by the pericytes on the other hand, it measured about 350 Å in width (Fig. 14).

The capillary is also surrounded by incomplete intramural pericytes layer, that is situated external to the endothelial layer and its basement membrane. The pericytes have its fine structures similar to the endothelia, but have more mitochondria in its cytoplasm (Fig. 2), it also is embedded by the basement membranes, these basement membranes vary greatly in thickness, sometimes, it appears that there is no basement membrane in some places (Fig. 2).

External to these pericytes, there is a thickening sheath of collagen fibrils, The diameter of the fibrils are different, they are between 330Å-1600Å the diameter becomes larger as it is arranged toward the outside, the fine collegan fibrils site in the inner thence part and ordinarily arranged parallel to the longitude of the vessels tightly, while the larger collegan fibrils occupied outer thicker part and arranged in varied directions loosely (Fig. 2).

Sometimes, the site of the melanophores appear near the capillary wall and protrude into the collegan fibrils sheath and almost has contact with the outermost basement membrane.

2 Arteriole.

The arteriole is also consisting of endothelial layer and basement membrane

as the capillary, but external to these, are the smooth muscle cells. The fine structure of these smooth muscle cells are similar to the endothelial cells and pericytes, however, it has much more prominent myofibrils in their cytoplasm, it also shows many granular dense areas adhere to the cell membrane. These cells also are usually surrounded by the basement memdrane, however, neither collegan fibrils nor elastic fibrils be seen between cells (Fig. 17).

3 Venule.

As it is shown in Fig. 18, the lumen of the venule is enclosed by a single layer of endothelial cells and the basement membrane, the endothelial layer share the same characteristics with the capillary are non-fenestration. External to these two layers, there are pericytes distributed circularly. Outermost is the collegan fibrils sheath, and as pointed out previously, the venule wall is notably thinner than the iris arterioles, and there are more melanophores near by the venules wall (Fig. 18).

DISCUSSION

In this study, the specimens obtained from the senile cataract patients, and the limited locally in periphery of the iris, so, it is impossible to avoid the effects of the unknown cataract inducing factors and the aged change.

As Kinney[1)2)] in his investigations of blood-aqueous barrier pointed out, "that the transfer mechanism of the aqueous humor in adult and young animals is different, and the barrier properties of the iris and / or ciliary body are also changing during time interval under consideration, it is possible also that simultaneous variation may occur in the secretory rate and perhaps in rate of leakage".

The basal structure of the iris vessels are similar, innermost is a thin and non-fenestrated endothelial layer, external to it is a basement membrane and either the pericytes or smooth muscle cells with their circular basement membrane. The outermost part surrounded by a thickening collegan fibrils sheath.

Tousimis and Fine[21)22)] (1959) observed the iris vessels first with electron microscope, later, Garron and co-workers[20)] (1959), Ikui. Tomida and Maeda[35)36)] (1960) also studied the iris vessels with electron microscope.

The pinocytosis vesicles were first electron microscopically observed by Palade[3)] (1953), and Moore[11)] (1957) studied vesiculation in endothelial cells and described it as "It seems likely that small identation develope at the interior or exterior plasma membrane, then pinch off to form vesicles, move across and join with the opposite plasma membrane to releave their content". Palade[4)] (1960) further studied the vesiculation and concluded it as constituting a means of active and selective transmission through capillaries wall, this might be designated as cyto pemsis (transmission by cell). Zeveifach[26)] (1961) share the same opinion with

them on vesiculation of the endothelium.

Buck[12] (1958) described the fine structure of endothelium of the large arteries in animals. He found pinocytosis vesicles along the opposing membranes of adjacent endothelia, and the large invagination of the surface plasma membrane. He also noted that vesicles were arising from it. In this study, however, the pinocytosis vesicles appear only in both surfaces, either the free surface, that which is toward the lumen or the outer surface which contacts with the basement membrane. The large invagination also was noticed in this observation, however, the arising of the vesicle from the large invagination was never found. These pinocytosis vesicles were not only found in the endothelia but also in pericytes. The pinocytosis vesicles also reported in retinal (Maeda),[19] choroidal (Hogan and Feeney),[27] ciliarial (Holmberg)[16] vessels of the human eyes. Holmberg,[16] however, shares the contrary opinion, He believes "The small vesicles seen in the cytoplasm probably without much importance in this respect (ciliary vessels), at least, in capillaries provided with pores.

Variable wide perinucleus spaces were seen in either endothelia or pericytes. Watson[6] (1955) noticed the fusion of the vesicles and endoplasmic reticulum, then he assumed the function of these perinucleus cisterna would serve to supply these systems of the cells with fluid as well as with membrane substance.

Fine fibrils were seen in the endothelial cytoplasm of the human iris vessels. This was previously noted by Hogan[27] in the endothelia of the human choroidal vessels.

There are lipid droplet vesicles existing in the cytoplasm of the endothelia of the iris vessels, they were previously described by Maeda[19] in retinal vessels.

The endothelial layer of the iris vessels are continuous and nonfenestrated. It is different with the choroidal and ciliarial endothelial layers. It is also different with the retinal vessels, owing to iris vessels which sometimes show variable wide intercellular spaces, even in relative narrow intercellular space, neither the apposing membranes nor subjacent cytoplasm show increasing electron density.

The basement membrane occasionally was penetrated by endothelial protuson, then the endothelium contacted directly the pericyte. Many holes existed in the basement membrane, it shows lower electron density, various in shape and largeness, it ordinarily is limited in the external edge and never penetrating the basement membrane due to holes as identified in this observation.

In some sections, there are potential spaces existing on either side of the basement membrane, it measures as about 350Å in width. Its existence has been denied by Tomida in his study on iris vessels. Holmberg discovered it also as existing in ciliarial vessels.

In this observation, neither collegan fibrils nor elastic fibrils was found between the endothelia and their pericytes.

The retinal vessels are surrounded by glia, while the choroidal vessels are

enclosed by both collagen and elastic fibrils, and ciliarial vessels are embedded in connective tissue, the iris vessels, however, have its prominent collegan fibrils sheath.

The fine structure of the iris vessels have been described and compared, then consider its function and connection with the vessels structure perhaps is an advantage.

Kinney[1)2)] pointed out the iris involved the exchange of the water and non-electrolytes of the aqueous humor. Gregeason[13)] (1958) in his investigation of the tissue space of the human iris revealed that the iridic crypts was not covered with endothelia, so he concluded that the iris vessels have direct contact with the aqueous humor and the vessel wall is the only bar for the exchange between the blood stream and aqueous humor. Davson[29)] (1962) in his text book Physiology of the Eye, says the eye concerned with the iris as a tissue that comes into close relationship with aqueous humor. Moore and Ruska[11)] (1957) in their investigation of the capillaries and arteries pointed out two ways for blood containing releave from vessels. In the kidney glomerulus, fluid is releaved from the capillaries bed through the pores in the capillaries endothelia across the basement membrane into the Bowman space. The basement membrane as an ultrafilter, retains the blood proteins but is penetrated by glucose and other dissolved material. In most other capillaries, fluid and dissolved substance have to pass endothelial cytoplasm to leave the blood stream, there by, active transmission and selective mechanism other than ultrafiltration cross the basement membrane. In this observation, the intercellular junction of the iris vessel as a bar is imperfect, in some sections, it shows variable wide intercellular spaces, and the hasement membrane is rich with holes and protential space exists, so the structure of the iris vessel wall would permit the solutes through it. Cunha-vaz and Ashton[33)] (1966) in their studies identified that the trypan blue can penetrated the iris vessel wall and colloidal carbon retained by the iris vessel wall. Majno and Palade[25)] (1961) in their investigation have proved that the basement membrane as a filter allowing fluid to escape but retaining and concentrating suspended particulate matter of the size used. Moore and Ruska[11)] (1957) concluded vesiculation as a means of exchange between the blood stream and peripheral tissues. In this study, the author agrees that the following two possibilities, perhaps involve the exchange of the fluid through the iris vessel wall. The first posibility is; by means of the pinocytosis vesiculation. The second is, through the variable wide intercellular spaces and the leakable basement membrane. This study also revealed that the endothelium of the iris vessel have the abundance of fine fibrils in its cytoplasm, probably this involves the less bleeding frequence of the iris in surgery, owing to the fine fibrils of the cytoplasm ordinarily means the contractile function.

CONCLUSION

Five human iriss pecimens out of three patients obtained during the peripheral iridectomy were performed in senile cataract.

The iris vessels show similar structures, consisting of endothelial layer, basement membrane, and either pericytes or smooth muscle cells,

The characteristics of the iris vessels are as following:

1. The endothelial cell is the non-fenestration, the abundance of pinocytosis vesicles and rich with fine fibrils in its cytoplasm. The variable wide intercellular spaces could be seen and without changing of electron density in these places.

2. The basement membrane varied in thickness and showed many holes, but fenestration was never found due to holes, sometimes, it was penetrated by endothelium or pericyte. On the either side of the innermost hasement membrane, the narrowing potential spaces in some sections could clearly be distinguished.

The mechanism of the exchange between the blood stream of the iris vessels and the aqueous humor is perhaps by means of the pinocytosis vesiculation and through the varlable wide intercellular spaces and the leakable basement membrane.

The fact that the fine fibrils in the endothelia exists perhaps involves the less frequence of bleeding in iris surgery.

ACKNOWLEDGEMENT

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EXPLANATION OF FIGURES

Fig. 1. this is a cross section of the capillary.
The upper part of this figure, a typical endothelium was sectioned, the nucleus and its envelope in the central superior, there are two large vesicles in the right side near the intercellular junction, the abundance of the fine fibrils are sectioned crossly and obliquely in the superior left, there are many micro-pinocytosis vesicles arranged along the cytoplasmic membranes, two micro-villi can be seen on either side, parts of the pericytes showed in the lower and right. × 7200

Fig. 2. It is a cross section of the capillary.
The pericyte with its nucleus site in the left side, some mitochondria can be seen in its cytoplasm around the nucleus, The narrowing peribasement membrane spaces show between the pericyte and endothelium. Three intercellular junctions existing in this figure, two of them show increasing electron density, and seem fused together to form firm terminal bars, and the middle one shows a narrowing intercellular space, it shows no increasing of electron density, either in the opposing membranes or subjacent cytoplasm,

Outermost is the collegan fibrils sheath, the fine collegan fibrils site near the lumen, arranged tightly and parallel to the longitude of the vessel, while the larger collegan fibrils arranged loosely and outer to the former in different orientation. × 5900

Fig. 3. It is a intercellular junction of the capillary.
The junction shows prominent increasing of electron density, the apposing membranes seem fused together to form firm terminal bar, a micro-villi also seen protudes into the lumen. × 10000

Fig. 4. There is a narrowing intercellular space shown in the upper right, innermost of it shows increasing of electron density, it not only occur in the apposing membranes but also in subjacent cytoplasm. Either endothelial cells show the abundance of micro-pinocytosis vesicles. × 10000

Fig. 5-6. There are two relatively wide intercellular spaces shown in figures, neither the apposing membranes nor subjacent cytoplasm show increasing of electron density.
Fig. 5. × 7600 Fig. 16. × 12000

Fig. 7, 8, 9, 10. There are four narrowing intercellular spaces, they all show no increasing of electron density in apposing membranes or subjacent cytoplasm.
Fig. 7. × 12000 Fig. 8. × 15000 Fig. 9. × 5200 Fig. 10. × 6300

Fig. 11, 12, 13. It is a longitudinal section of the capillary.
The abundance of fine fibrils can be seen clearly in the cytoplasm of either endothelia, Fig. 12, 13, show in higher magnification.
Fig. 11. × 13600 Fig. 12. × 10000 Fig. 13. × 50000

Fig. 14. There are two prominent peri-basement membrane spaces shown in the lower left. The pericyte was found to penetrated the basement membrane then contact directly with the endothelium in the lower right of this figure. × 15000

Fig. 15. This basement membrane shows the fibrillar structure in superior, and the endothelium penetrates the basemen membrane and contacts with the pericyte directly in the lower left. × 9400

Fig. 16. There are many holes existing in the basement membranes, which look like as rodent phenomenon, but never basement membrane fenestration due to holes as identified.
Upper left shows a basement membrane absent area, then the either cells contact directly. × 6300

Fig. 17. It is a longitudinal section of the arterioles.
These cells show the abundance of fine fibrils, these fibrils arranged mainly parallel to the vessel longitude, and also partly arranged vertical to it. There are many granular electron dense areas, they appear adhere to the cell membrane, these cells perhaps are the smooth muscle cells of the arterioles. × 11000

Fig. 18. This is a part of the cross section of the venoles.
Its is composed of a single layer of endothelial cells, external to it is basement membrane and few pericytes, outermost part is the collegan fibrils sheath, the venolus wall is notably thinner than the arterioles do. × 4500

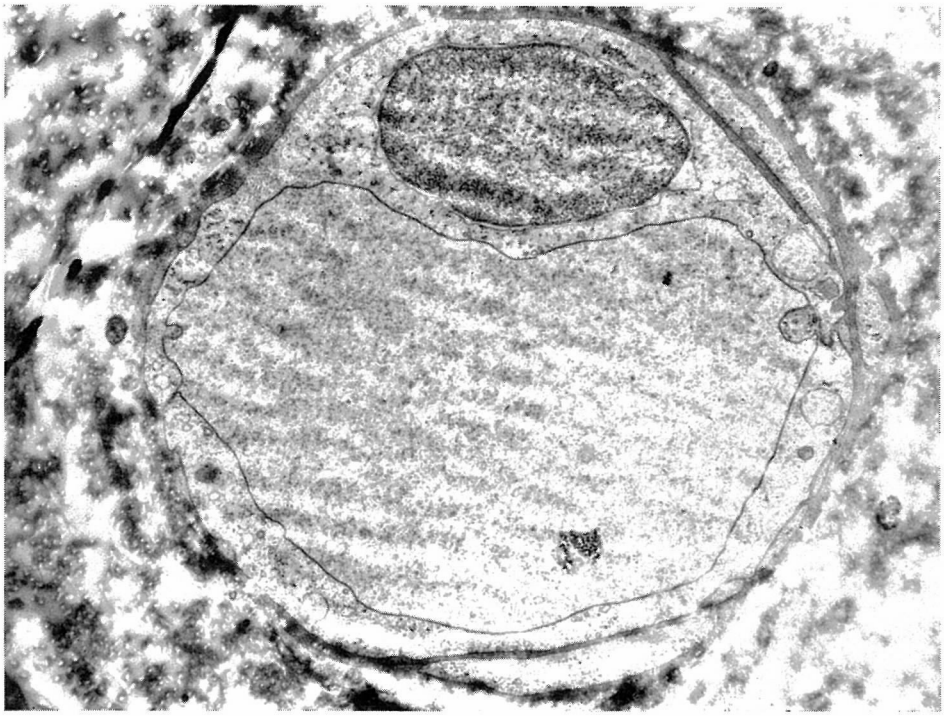


Fig. 1. × 7200

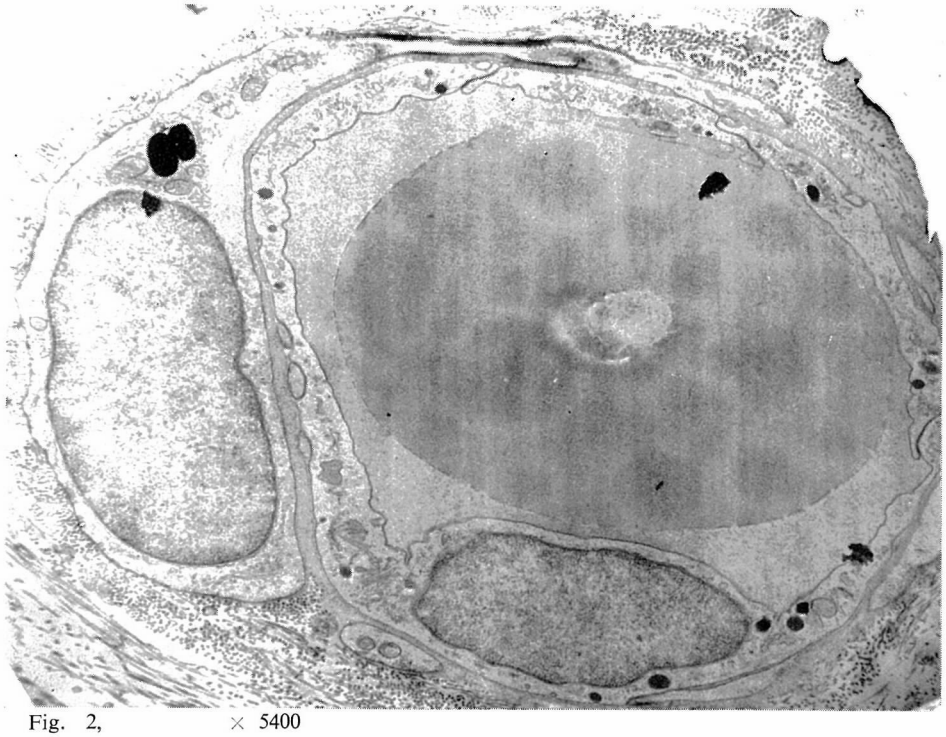


Fig. 2, × 5400

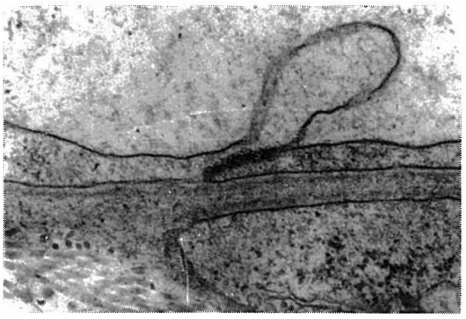


Fig. 3. × 10000

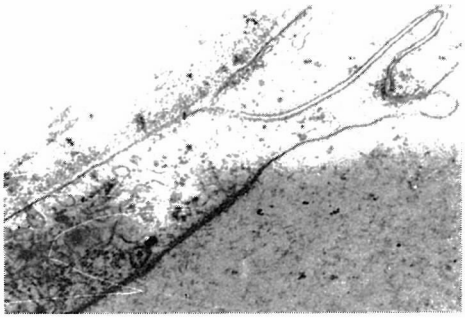


Fig. 4. × 10000

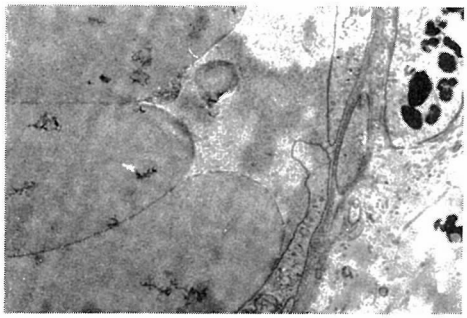


Fig. 5. × 7600

Fig. 6. × 12000

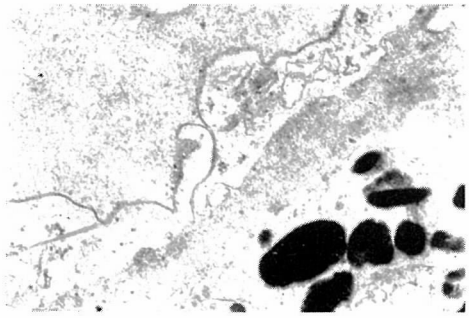


Fig. 7. × 12000

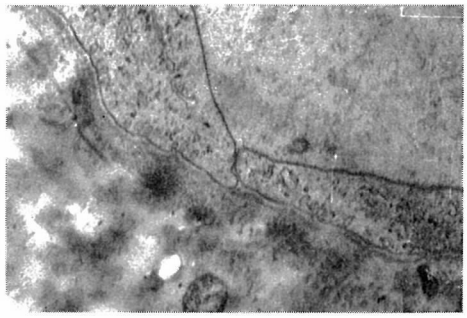


Fig. 8. × 15000

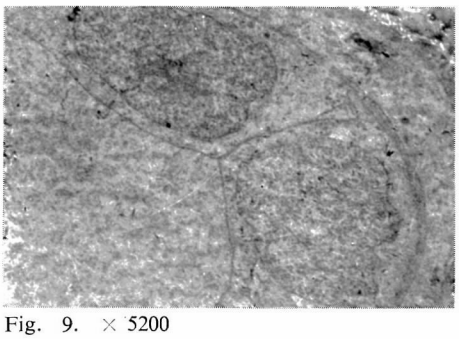


Fig. 9. × 5200

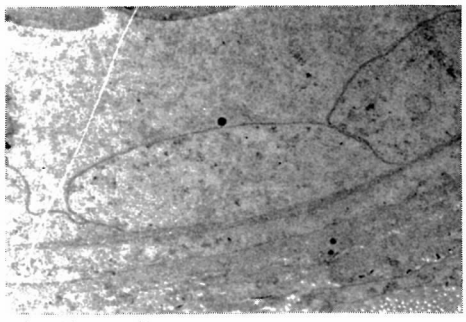
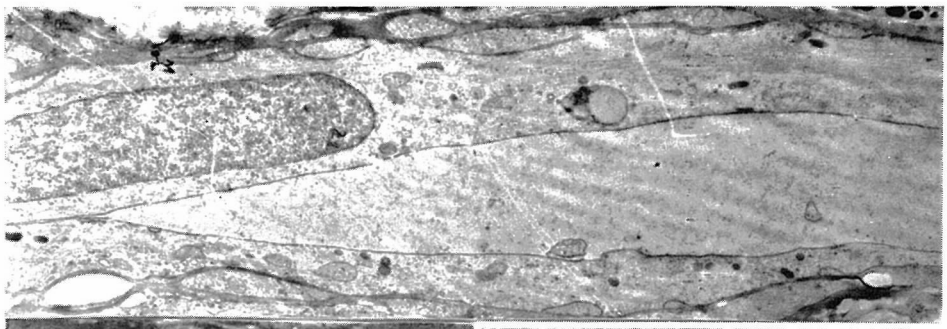


Fig. 10. × 6300

Fig. 11. × 3600



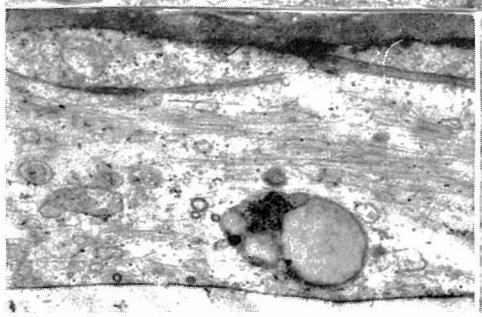


Fig. 12. × 10000

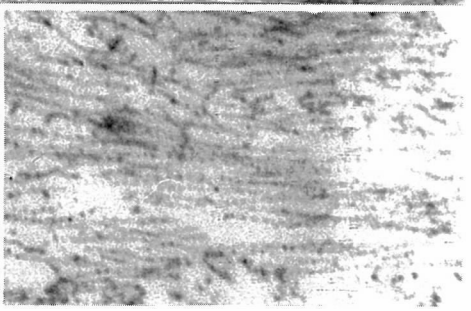


Fig. 13. × 50000

Fig. 14. × 15000

Fig. 15. × 9400

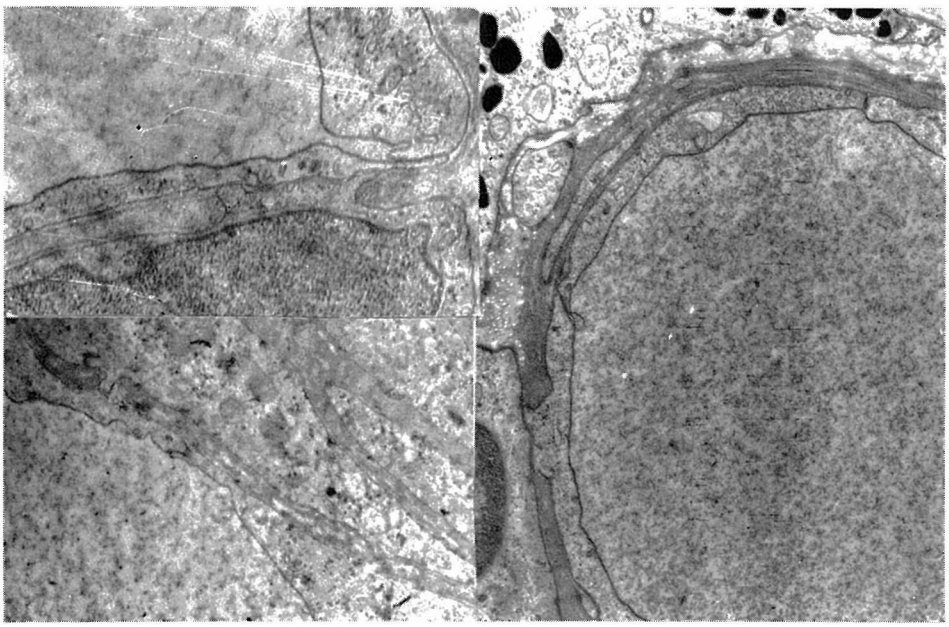


Fig. 16. × 6300

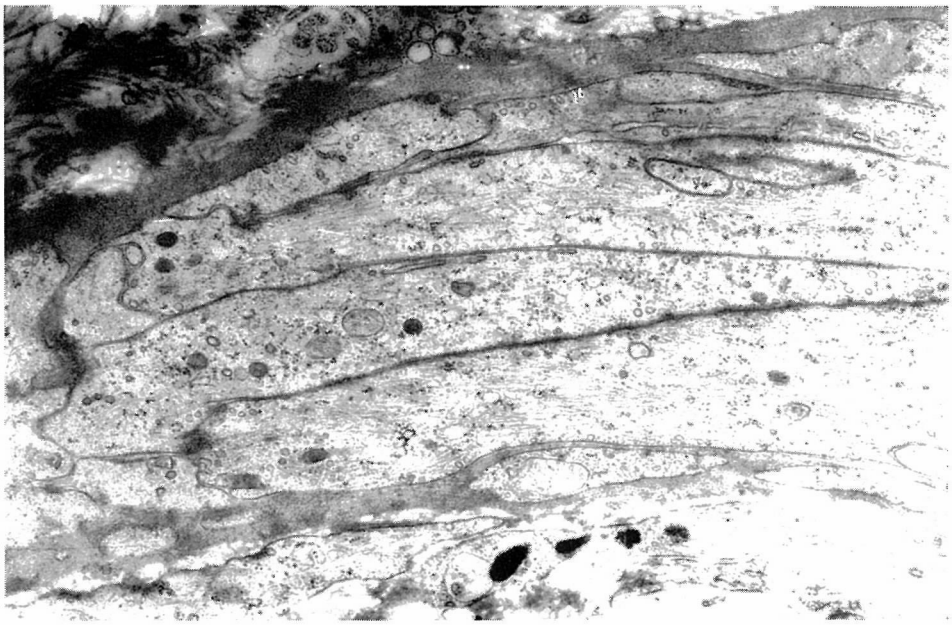


Fig. 17. × 11000

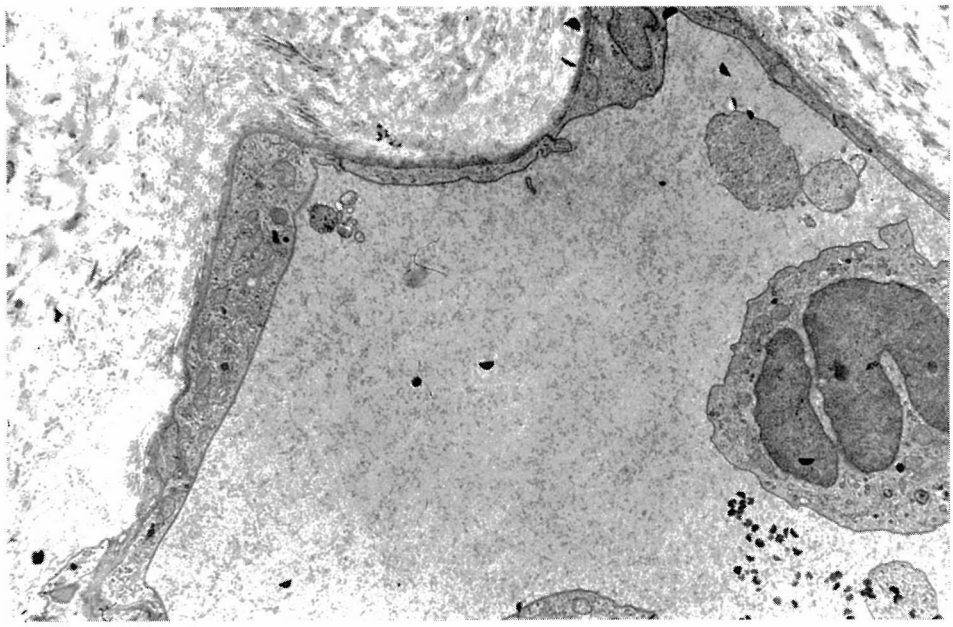


Fig. 18. × 4500

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