Electron Microscopy Studies of the Human Iris Muscles

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INTRODUCTION

The existence of the iris muscles, according to Duke-Elder and Wybar[1] the sphincter muscle of the eye was first noted by Meckel in 1820, and the dilatator muscle was first found by Bruch in 1844. The first person to separate and to describe both of the muscles was Joseph Lister in 1853, but the characteristics of these muscles was not yet certain. Recently, Tousimis and Fine (1959)[2] first used the electron microscope to study the monkey iris, and later, Ueno (1962)[3] also observed one eye of an aged woman with the electron microscope. The characteristics of the dilatator muscle, however, still has not yet been clearly pointed out.

According to Duke-Elder and Cook[4] the sphincter muscle differentiated from the anterior pigment epithelial layer at 65 mm. stage, and detached from its origin at the 6th month stage. The dilatator muscle also differentiated from the anterior pigment epithelial layer at 6th month, and is not fully formed until after birth.

This study intends to make clearer the relationship between the dilatator muscle and the pigment epithelial cells, the differences of the dilatator muscle in various part of the iris, the differences in the muscle between the adult and infant, and lastly, to compare the dilatator muscle and the sphincter muscle histologically.

MATERIAL AND METHOD

Three human eyes enucleated by operation were used in this study.

Two eyes, one from a 66 years old man and the second from a 46 years old woman, and one eye from a 19 months old infant were used in this study. These eyes were enucleated because of orbital malignant tumour in former cases and in later case because of retino-blastoma.

All three eyes were dissected into two halves and preserved immediately after enucleation in sucrose P.G. for 1 to 2 days, these irides were removed and

further dissected into small pieces and washed with Millong's solution, and fixed in 1 % osmium tetroxide for two hours at 4 C temperature. Dehydration was done 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 citrate and obsedved with a J.E.M.-7 electron microscope.

RESULTS

(1) Dilatator Muscle :

a) The dilatator muscle is situated closely in front of the pigment epithelial cells of the iris, and arranged radially to the pupil as the spokes of a wheel, and almost parallel to the adjacent pigment epithelial layer. The muscle cell is spindle-shaped and has a rather smooth outline. A number of processes of the dilatator muscle which protrude into the stroma from either the anterior pigment epithelia or the dilatator muscle cells, which makes it appear that the dilatator muscle consists of layers. these processes are arranged parallel to the axis of the dilatator muscle. The dilatator muscle is situated close to the pigment epithelial cells, and there are spaces of variable width between the processes of the muscle, so, the invading of the processes of the chromatophores or collagen fibres in these spaces can always be seen.

The dilatator muscle is ordinarily surrounded by a thin layer of basement membrane, which shows as a homogeneous, electron dense, thread-like material, and its density similar to the basement membrane that found in the iris vessels. The thickness varies but it is generally about 400 A. These basement membranes surround most parts of the dilatator muscle cells and their processes, but sometimes, there is an absence of the basement membrane between the muscle cell and the adjacent pigment epithelium. There is a narrow space between the basement membrane and the cell-membrane of the dilatator muscle. Which is about 200 A in width. Attachment of the collagen fibres to the basement membrane is always found.

b) The fine structure of the dilatator muscle :

The cell-membrane shows as a single, electron dense line limiting the cytoplasm of the dilatator muscle. In higher magnification the cell-membrane is found to be a double membrane with a narrow space between the two membranes.

Nuclei of the dilatator muscle are oval or spindle-shaped with a rather smooth outline, and sometimes deeply infolded nuclei can be seen. The nuclei of the dilatator muscle cells are situated in the center of the cell and in the lateral view nearer the adjacent pigment epithelia. The axes of the nuclei are parallel to the longitudinal axis of the muscle. The nucleus is bounded by a continuous

nuclear membrane.

Golgi complex are occasionally found as electron dense anastomosing cords and vacuoles near the nucleus.

Centrioles of the dilatator muscle cells are frequently present and are always seen close to the nucleus and near the geometric center of the dilatator muscle cell, However, as the nucleus of the dilatator muscle cell is ordinarily situated close to the adjacent pigment epithelia, in the longitudinal section of the dilatator muscle cell, the centrioles are always found superior to the nucleus of the dilatator muscle cell. Each centriole consists of nine cylinders. Two centrioles nearly perpendicular to each other form a diplosome, and one of these two centrioles is perpendicular to the longitudinal axis of the muscle. The diameter of the centriole measures about 4000 A.

Mitochondria exist in large number in the cytoplasm of the dilatator muscle cell, they are situated deep in the cytoplasm and surround the nucleus. They are oval or ovoid in shape with well developed cristae.

Endoplasmic reticulum are found as an intracytoplasmic membrane system. There is an abundance of electron dence granules attached to these membrane systems causing the endoplasmic reticulum to be rough-surfaced.

Ribosome granules are found in abundance and show as electron dense granules distributed in the cytoplasm of the dilatator muscle. They show no morphological differences with the granules attached to the endoplasmic reticulum.

Glycogen granules show as numerous small electron dense granules, they congregrate to form star-shapes or cloud-like-shapes and are distributed in the cytoplasm of the dilatator muscle.

Pinocytotic vesicles exist immediately beneath the cell-membrane, and a portion of them have openings into the cell-membrane.

Pigment granules are distributed in the cytoplasm of the dilatator muscle, and often congregrate near the adjacent pigment epithelium, in some sections they also appear in the processes of the dilatator muscle. These pigment granules are relatively average in size. The amount of the pigment granules in the various dilatator muscle cells are considerable different, from few amount to seems abundant as the pigment epithelial cells.

Myofilaments : An abundance of electron dence myofilaments can be seen in the cytoplasm of the dilatator muscle. They are mainly parallel to the axis of the muscle, and distributed throughout the cell. The myofilaments are more prominent in the peripheral part of the cytoplasm of the dilatator muscle cell, and some of the peripheral myofilaments become attached the cell-membrane to form dence spots beneath the cell-membrane.

c) Near the pupillary area the dilatator muscle usually disappears and numerous microvilli of the anterior pigment epithelia protrude into the stroma of the iris, filaments can be seen within these microvilli and there is no morphological

difference between these filaments and the myofilaments of the dilatator muscle. Farther from the pupil these microvilli become larger and subsequently develop into the processes.

The dilatator muscle is situated in front of the pigment epithelia, and the dilatator muscle appears irregularly. Either transformation occurs from microvilli to the processes and subsequently to the muscle cell or appears suddenly among the epithelia in the pupillary area.

In the case of the infant, the cross section of the dilatator muscle is finer than the adult and has more infoldings. The number of myofilaments shows no difference between the adult and the infant. The shape and size of the pigment granules of the dilatator muscle of the infant are very different, the number of the pigment granules in the dilatator muscle of the infant being less than for the adult.

(2) Sphincter muscle :

a) These muscle cells are situated in the stroma of the iris near the pupil, They are arranged circularly to the pupil. The sphincter muscle is always elongated or sea-slug shaped with numerous short, dull round processes protruding into the stroma in different directions.

The sphincter muscle is surrounded by a thin layer of basement membrane, with a narrow space between the basement membrane and the cell-membrane. External to the basement membrane, the sphincter muscle is always surrounded by an additional net-work, which is identified as the processes of either the chromatophores or the nonpigment cells of the stroma. An invasion of collagen fibres between the processes of the stromal cell and the basement membrane of the sphincter muscle is always seen. In some places the sphincter muscle is found to have a connection with either the anterior pigment epithelia or the dilatator muscle.

b) The fine structure of the sphincter muscle :

The cell-membrane of the sphincter muscle shows as a electron dense, thin line, and in higher magnification is found to consist of double membrane, to limit the cytoplasm of the sphincter muscle.

Nuclei of the sphincter muscle ordinarily are oval-shaped and have a rather smooth outline. They are situated in the center of the muscle cells, and parallel to the longitudinal axes of muscle cells. Deep folding of the nucleus can be seen.

Endoplasmic reticulum found in the cytoplasm of the sphincter muscle are the rough-faced type.

Mitochondria : there are numerous mitochondria in the cytoplasm of the sphincter muscle. They are oval or elongated in shape with well developed cristae.

Ribosome granules are distributed in an average way in the cytoplasm of the

sphincter muscle.

Glycogen granules can be seen in the cytoplasm of the sphincter muscle cells, they tend to aggregate to form glycogen star or small cloud-like pieces.

Pinocytotic vescles are arranged mainly immediately beneath the cell-membrane.

Myofilaments in the sphincter muscle are very prominent, they always congregate to form myofilament bundles. The orientation of these bundles is usually parallel to the longitudinal axis of the sphincter muscle. The distribution of the myofilaments is not only prominent in the peripheral part of the cell but also in the center of the cell. The peripheral part of bundles of the myofilaments are also found to have attachment to the cell-membrane to form electron dense spots immediately beneath the cell-membrane.

Pigment granules are not found in the sphincter muscle.

DISCUSSION

The sphincter muscle cell and the dilatator muscle cell of the iris are morphologically different. Generally the shape of the muscle is different in contraction or relaxation. (1) The sphincter muscle has a more irregular outline, so in both cross and longitudinal sections, the sphincter muscle cell always has a sea-slug shape with many short, dull round processes. The dilatator muscle cell is spindle shaped and sometimes has an irregular outline. The amount of myofilaments in each muscle is also different, with the sphincter muscle cell having more myofilaments and more tendency towards aggregation to form the bundles of myofilament. The dilatator muscle contains pigment granules but the sphincter muscle does not. The sphincter muscle has more glycogen granules in the cytoplasm than the dilatator muscle.

The condition of the dilatator muscle is more complex. The dilatator muscle is situated in front of either the single or double pigment epithelial cells. The dilatator muscle either transforms from microvilli to the processes and subsequently to the muscle or appears suddenly among the pigment epithelia in the pupillary area.

Tousimis and Fine (1959) in their paper of Ultrastructure of the Iris simply described the dilatator muscle of the monkey iris as consisting of a few layers of elongated smooth muscle cells. Ueno (1962) described the dilatator muscle as situated in front of the double pigment epithelial cells and consisting of a few layers of elongated cells, and they were the processes of the pigment epithelial cells. In the optical microscopic period, the dilatator muscle even was described as is formed of fibers entirely similar to smooth muscle cells without nuclei. (5)

The so-called dilatator muscle is a group of cellular elements coming from the anterior pigment epithelia at different stages of development. Near the

pupillary area, there are many microvilli containing fine filaments which protrude into the stroma from the anterior pigment epithelia. These cellular elements, perhaps, represent the primary development stage of the dilatator muscle. The processes which protrude into the stroma from anterior pigment epithelial cells contain numerous and easily seen myofilaments. The anterior pigment epithelia from which these processes come contain fewer pigment granules, These processes, perhaps, represent a more advanced stage of development. The cells situated in front of the pigment epithelia, contain less pigment granules and have more numerous and easily seen myofilaments and pigment granules are found to distribute principally on the side adjacent to the pigment epithelia. These cells have apparent shape and outline as pointed out above, perhaps, these cells represent the most advanced stage of development of the dilatator muscle. It is these cells which are the so-called dilatator muscle cells.

The dilatator muscle cells which are situated in front of the double pigment epithelia, perhaps, represent cells which have differentiated from the anterior pigment epithelia. The dilatator muscle cells situated in front of the single pigment epithelium, may represent cells transformed from the anterior pigment epithelial cell.

The dilatator muscle cells of the human iris, they contain more or less pigment granules in the cytoplasm, even in their most matured form. It is the reason what it was termed as myo-epithelial cell (4).

The facts of finer and more infoldings of the dilatator muscle and lower frequency of more mature muscle cells in the infant and because of dilatator muscle of adults is larger and more developed, perhaps, indicate the posibility of the development of the dilatator muscle after birth. Also, this may be one of the reasons a new born infant is not as sensative as an adult to mydriatic.

CONCLUSION

Three eye obtained from two adults and an infant were studied. The dilatator muscle and the sphincter muscle of the iris were observed and have been described.

The dilatator muscle of the human iris has been found to consist of a group of cellular elements which transformed from the anterior pigment epithelia. These cellular elements be classified into three different stages of development: (1) The microvilli containing fine filaments which protrude into the stroma from the anterior pigment epithelia. These elements, perhaps, represent the primary stage of the development of the dilatator muscle. (2) The processes which protrude into the stroma from anterior pigment epithelia contain numerous and easily seen myofilaments. The anterior pigment epithelia from which these processes

come contain fewer pigment granules. These cellular elements may represent a more advanced stage of development of the dilatator muscle. (3) The cells situated in front of the pigment epithelia, contain fewer pigment granules and have more numerous and easily seen myofilaments and have a spindle-shape. These cells may represent the most mature stage of the development of the dilatator muscle. It is these cells which are the so-called dilatator muscle cells.

The dilatator muscle cell is spindle shape and contains myofilaments which are not congregated but distributed throughout the cell, in positions generally parallel to the longitudinal axis of the cell and the cytoplasm is pigmented. The sphincter muscle cell of the iris is sea-slug shaped and has more myofilaments than the dilatator muscle cell, usually these myofilaments congregate to form bundles which are generally more or less parallel to the longitudinal axis of the cell and the cytoplasm is non-pigmented.

The dilatator muscle cell of the infant is finer and has more infoldings. The more mature muscle cells appear considerablely less frequency than in adult dilatator muscle.

ACKNOWLEDGEMENT

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EXPLANATION OF ABBREVIATION

ant : anterior
bm : basement membrane
cent : centrioles
col : collagen fibril
dil. m : dilatator muscle
inf : infolding
mit : mitochondria
mv : microvilli
myf : myofilaments
N : nucleus
n : nucleole
pig : pigment granules
pig. ep : pigment epithelium
post : posterior
proc : cytoplasm process
pv : pinocytotic vesicles
sph. m : sphincter muscle

EXPLANATION OF PHOTOGRAPHYS

Fig. 1. A longitudinal section of the iris. ×3500

In the lower right a dilatator muscle cell has appeared among the pigment epithelia in the pupillary area. In the upper right is a group of sphincter muscle cells.

Fig. 2. A longitudinal section of the iris. ×4500

On the left side a portion of the pigment epithelium is seen. The dilatator muscle has not yet appeared. In the center and lower right sphincter muscle cells are seen. The sphincter muscle cell in the center distinctly shows a connection to the anterior pigment epithelium in the upper left. In the upper right an unmyelinated nerve fiber can be seen.

Fig. 3. A longitudinal section. ×5200

This photography shows a dilatator muscle cell with few processes, in front of the single pigment epithelium. The myofilaments which run continuously from the muscle cell to the processes can be clearly seen. The oval-shaped nucleus is situated close to the adjacent pigment epithelium. Immediately above the nucleus is a diplosome. Few pigment granules can be seen, and those that can be seen are mainly distributed on the side adjacent to the pigment epithelium.

Fig. 4. A horizontal section of dilatator muscle cells. ×10000

In the lower Left, an abundance of mitochondria can be seen. In the center is a portion of a nucleus is seen. The myofilaments of the cell are distributedmainly immediately beneath the cell membrane and the pinocytotic vescles. The basement membrane can also be seen.

Fig. 5. A horizontal section of a dilatator muscle layer. ×10000

The scattered distribution of the myofilaments can be seen. In some places the myofilaments are joined together. The attachment of some of the myofilaments to the cell-membrane can be seen as electron dense spots. The relatively tight arrangement of the dilatator muscle cells can be seen.

Fig. 6. Cross section near the pupillary area. ×4500

In the upper left hand corner, a layer of dilatator muscle cells is seen. This layer is in front of a double layer of epithelia. The posterior pigment epithelium shown in the lower right hand half contains numerous pigment granules. In the anterior pigment epithelium, the nucleus of the cell can be seen. In the dilatator musele layer a nucleus and a few pigment granules on the side adjacent to the anterior pigment epithelia can be seen.

Fig. 7. A cross section of the dilatator muscle. ×10000

The dilatator muscle cells are in front of the pigment epithelia. These muscle cells are surrounded by a thin, homogeneous layer of basement membrane. The absence of the basement membrane in certain places is indicated by arrows (↓). A small number of pigment granules are seen in the cytoplasm of the muscle cells. On the right side a nucleus with a nucleolus can be seen. There is an abundance of glycogen granules which have congregrated just to the left of the nucleus.

Fig. 8. A cross section of the dilatator muscle of the infant eye. ×12000

The muscle is small with numerous infoldings and a thin, homogeneous basement membrane. A few pigment granules can be seen on the side adjacent to the anterior pigment epithelia.

Fig. 9. A longitudinal section of the dilatator muscle of an infant eye. ×22000

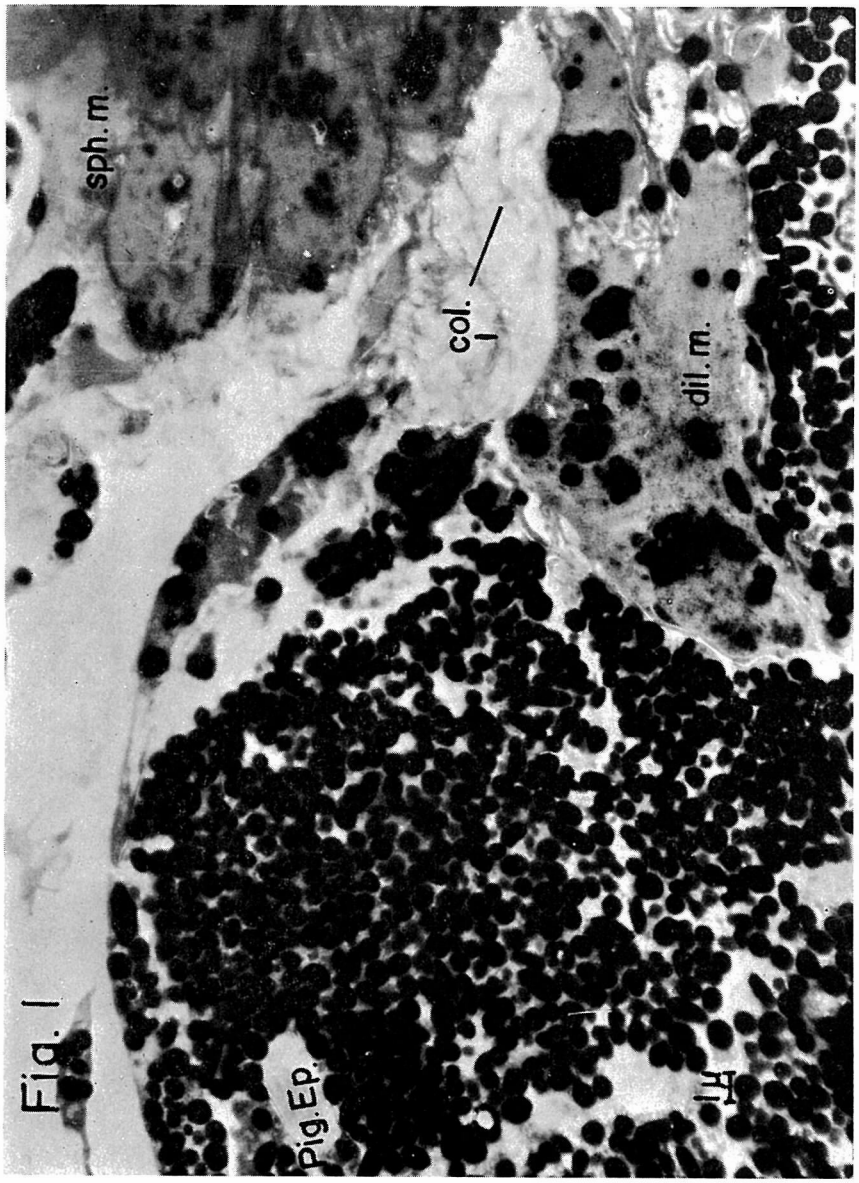
The anterior pigment epithelium shows in the lower right, with three processes eXtending from it. The myofilaments can be distinctly seen in the pigment epithelium and the continuity of these myofilaments from the epithelium to the processes can also be seen. Note the scattered distribution of the myofilaments.

Fig. 10. A longitudinal section of the sphincter muscle cell. ×6000

Numerous bundles of myofilaments can be seen. Note that generally the position of the bundles is parallel to the longitudinal axis of the cell.

Fig. 11. A oblique section of a sphincter muscle cell. ×15000

The myofilament bundles can be seen distinctly. Noice that the bundles have varioue positions in relation to the longitudinal axis of the cell.



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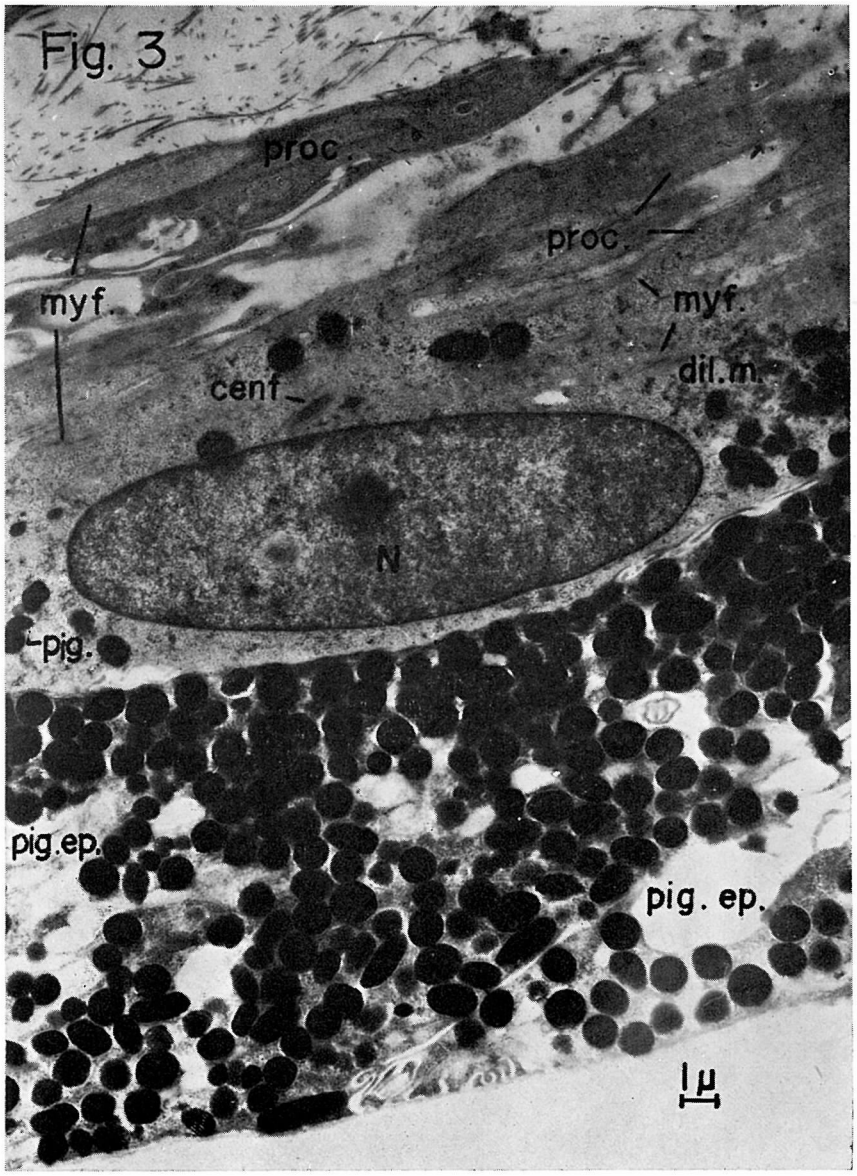
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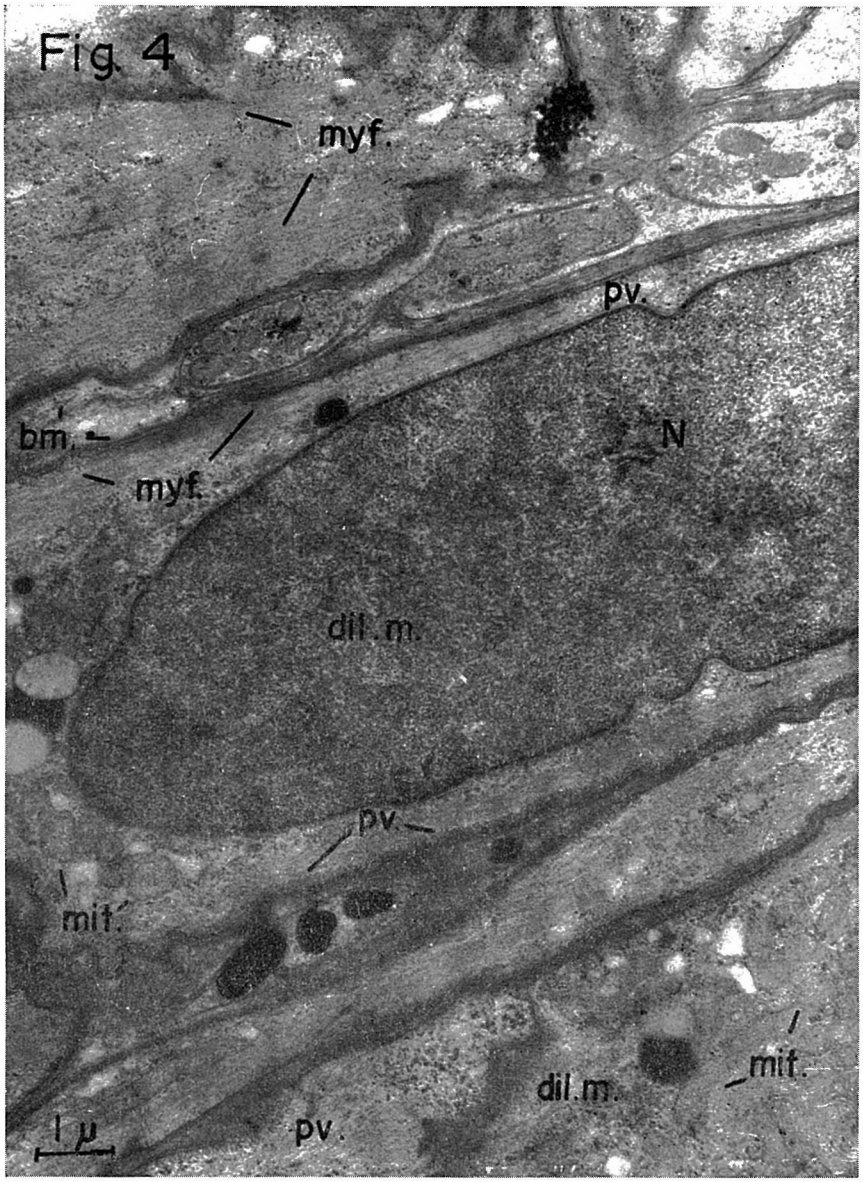
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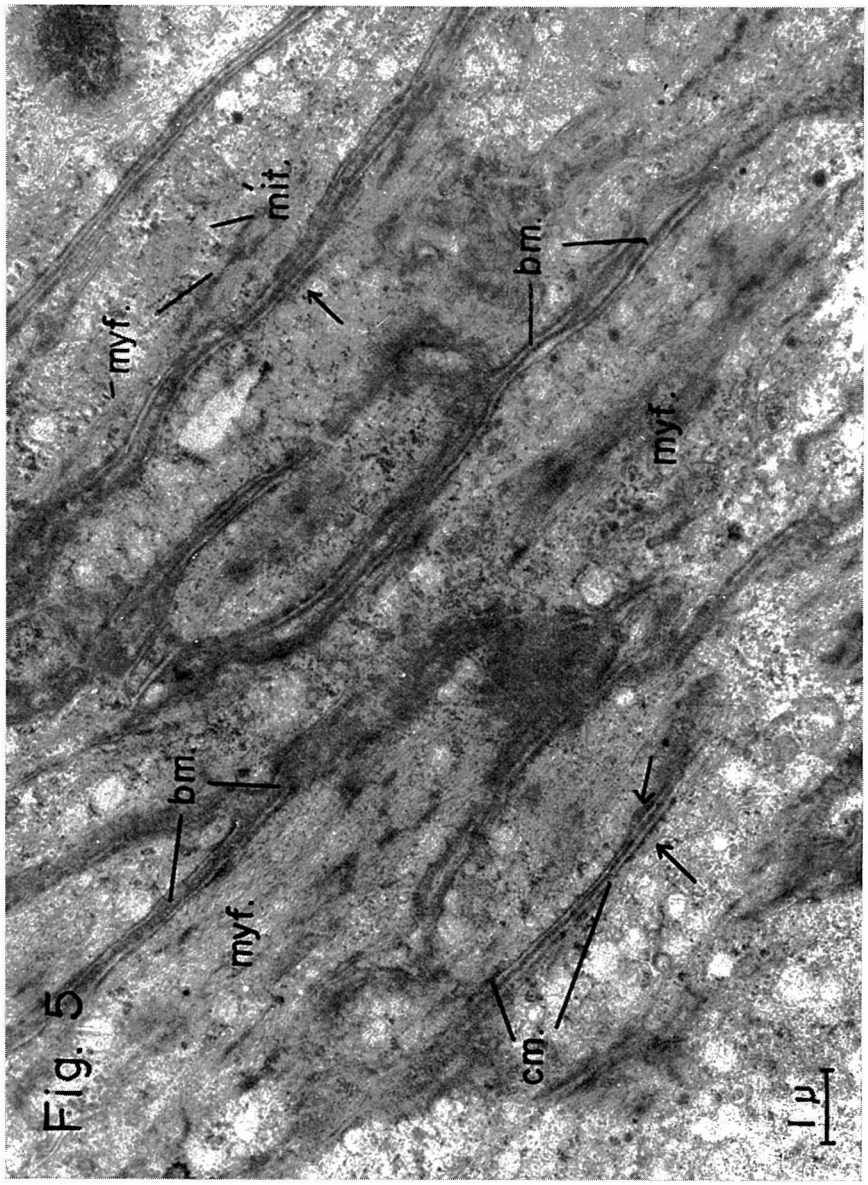
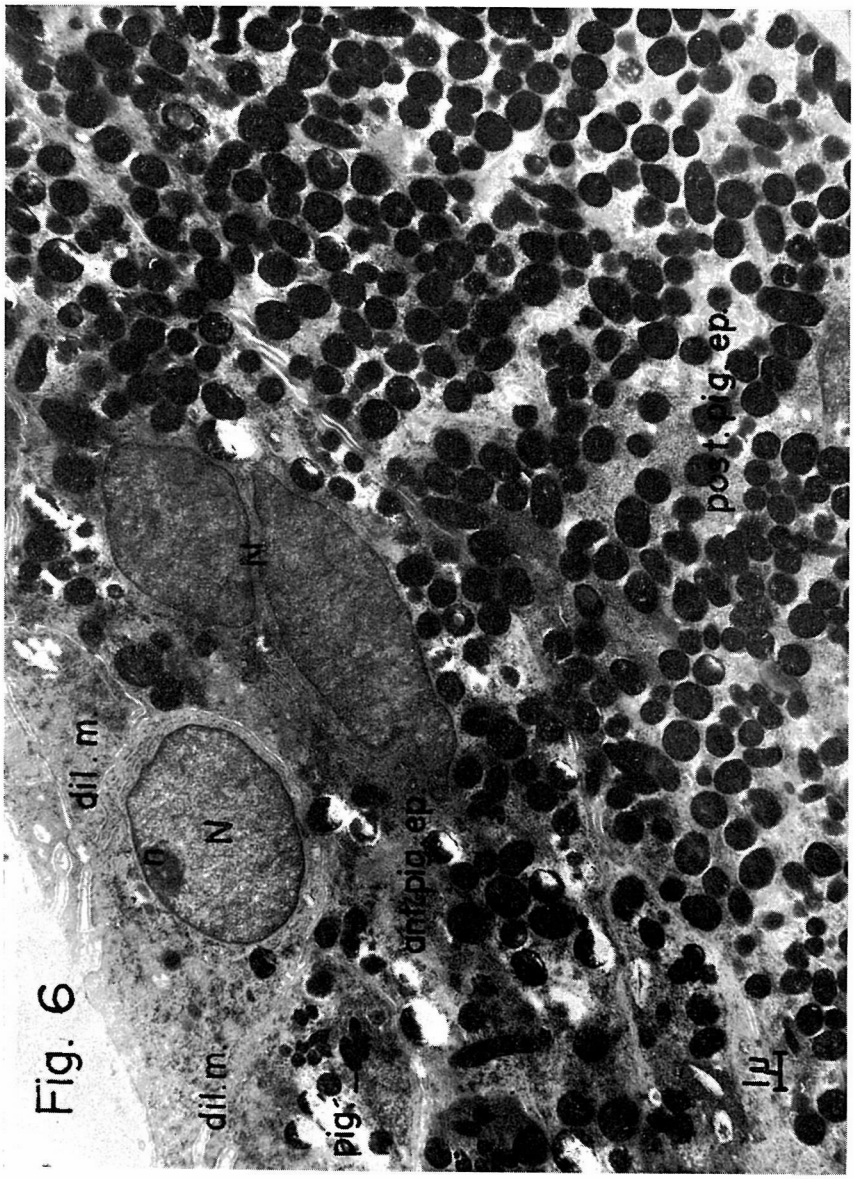


Fig. 5



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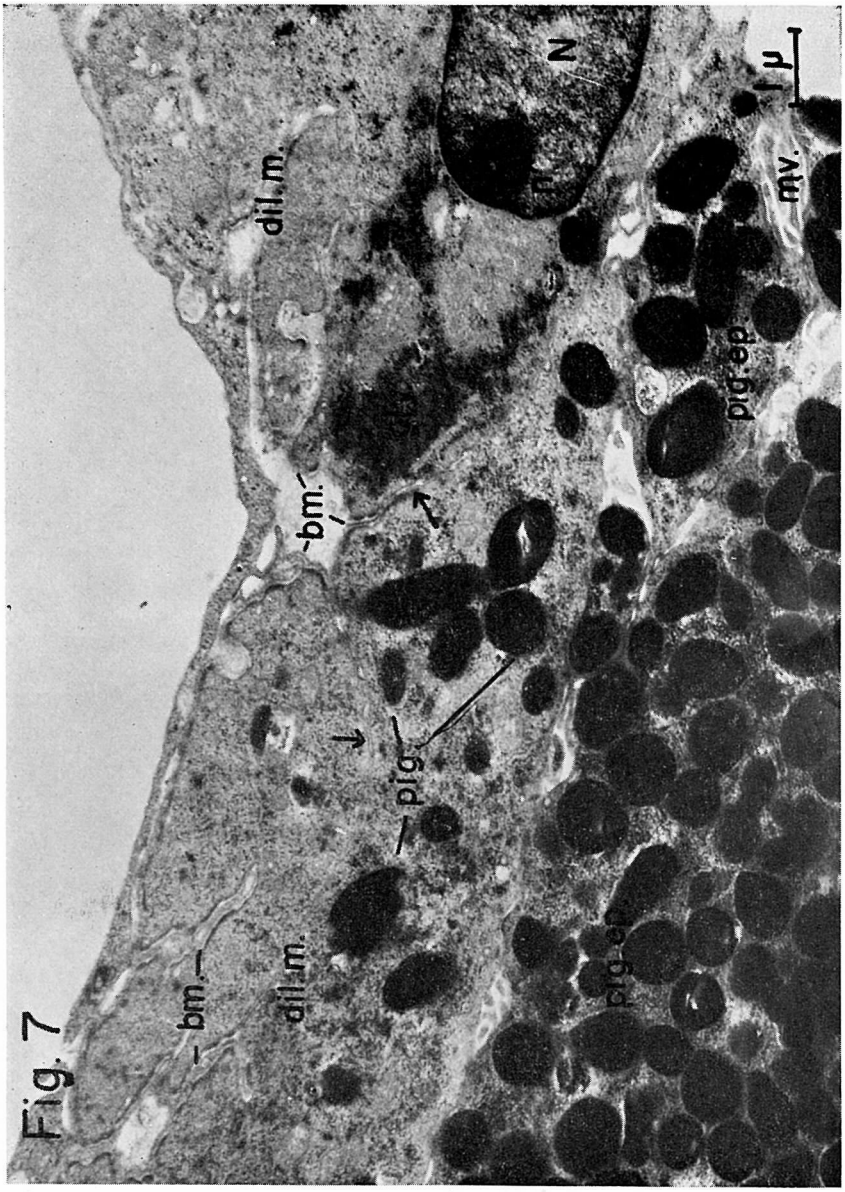
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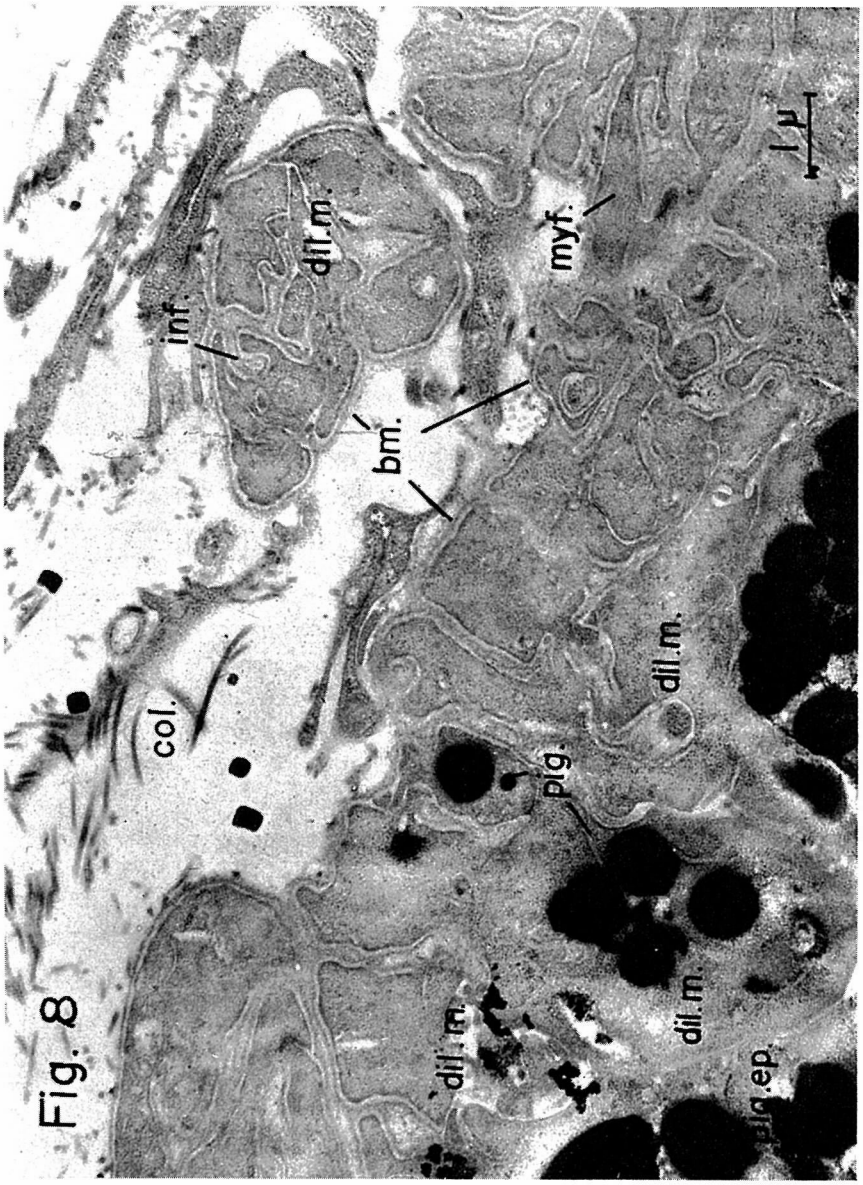
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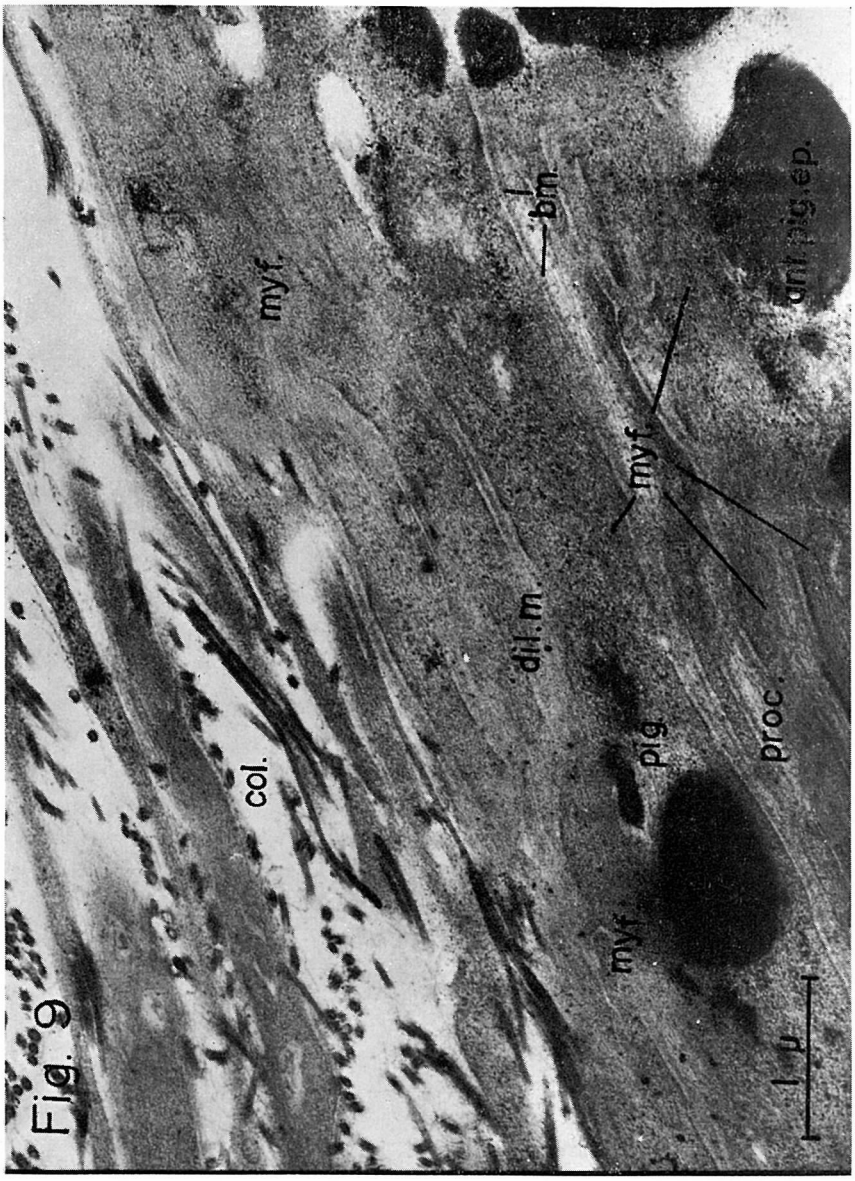
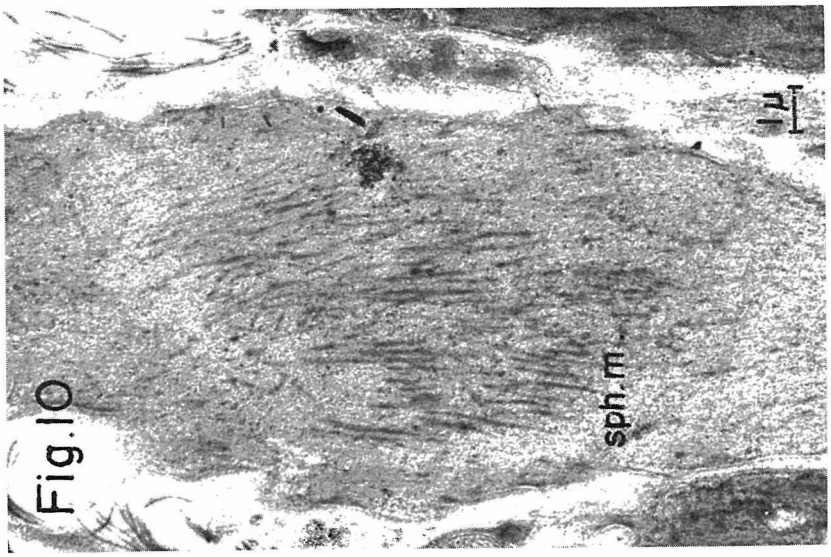


Fig. 9



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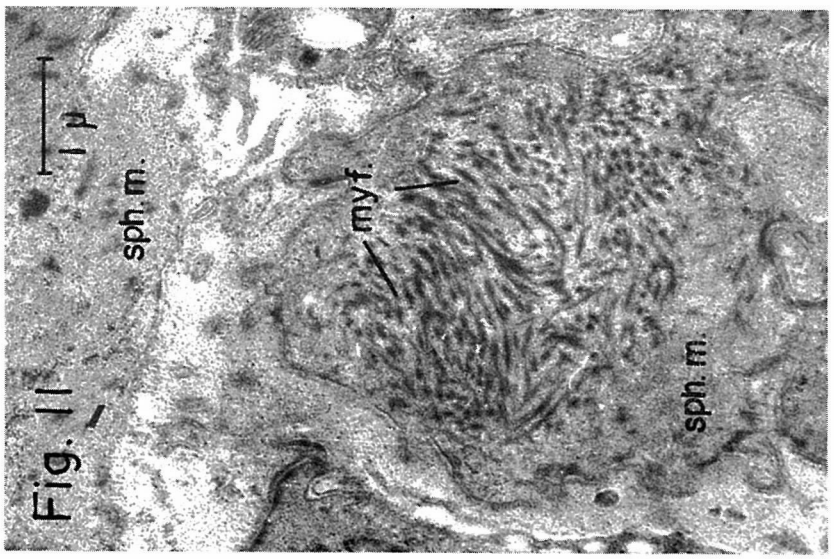

Fig. 11

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