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Electron Microscopic Studies on the Planarian Eye

I. Fine structures of the normal eye.

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Introduction

The fundamental and systematic investigation on the structure of the planarian eye was carried out by Hesse (1897). Even at the present time, his illustrations are cited as the demonstrative planarian eye in various text-books. He ranged the turbellarian eyes into three groups according to the grade of complexity of their structure; the first group was of the simplest eye which was composed of one or two pigment cells and visual cells, as represented in *Planaria torva.* The second group was of the eye in which more numerous pigment cells and visual cells were present than in the first group, as in *Dendrocoelum lacteum* and *Rhyncodemus.* The last group was of the eye which possessed the most complicated structure composed of numerous cells, as in *Planaria gonocephala* and *Dugesia lugubris.*

In the same year, Jähnichen (1897) observed more precisely the structure of the tip of the visual cell in *Planaria gonocephala* by sectioning, smear preparation and maceration methods. His description was so genuine that it may be the best as far as we can observe it with the light microscope. Especially, he illustrated well connection between the brain and the eye.

Jähnichen's description agreed substantially with Hesse's one, but their opinions were different from each other at the point of view whether a membrane to plug the "opening region" of the eye-cup is present or not. Namely, Hesse negrected this membrane, while Jähnichen insisted on its presence and even stated that the fibers of the visual cells probably pass away through it to the outside of the eye. Lang (1913) described also the membrane in front of the eye in *Planaria polychroa.*

Taliaferro (1920) described the histological configulation of the eye in *Planaria maculata* on the status to light reaction. He divided the visual cell into three parts; a rhabdome, a central highly refractive region and a nuclear portion.

Recently, electron microscope has been introduced in the field of biological investigation and various fine structures hitherto unknown of the cell became visible. In planarians, some investigators (Pedersen, 1959 a, b, 1961 a, b, 1963; Klima, 1959, 1961; Török and Röhlich, 1959; Skaer, 1961; Ishii, 1963, 1964, 1965 a, b, 1966; Mc Rae, 1963; Morita, 1965; Morita and Best, 1965; Oosaki and Ishii, 1965) have observed the characteristics in the fine structures of several tissue cells, but it may be still insufficient to furnish the definite idea because of delicate tissues of planarians. Especially, the electron microscopic observations on the planarian eye were scarce and fragmentary, if at all (Press, 1959; Röhlich und Török, 1961; McRae, 1964; Kishida, 1965, 1966).

On the other hand, the histological observations on the regenerating planarian eye were often perfomed by several investigators (Stevens, 1907; Steinmann, 1926; Keiller, 1911; Keil, 1924), but their studies could add nothing to the observation of the normal eye by the previous authors.

Under these considerations, several problems concerning the planarian eye still remain. The present paper is concerned with the ultra-fine structures of the pigment cell, the pluged membrane of the eye-cup and the visual cell of the normal planarian eye disclosed with the electron microscope.

Material and Methods

The material used was a sexual form of *Dugesia japonica* collected at the suburb of Kanazawa city. The worms were reared in decalcified tap water for ten days without food, and they were utilized for histo- and cytological preparations.

Light microscopic preparation. Paraffin and epon-embedded sections were prepared for preliminary studies prior to electron microscopic obrervation. Helly's solution was exclusively used as fixative for paraffin sectioning. The serial sections were cut in 5-7μ. The sections were stained with Delafield's haematoxylin-eosin, Mallory's triple stains, Heidenhain azan or Heidenhain iron haematoxylin-light green.

In order to make thinner sections, the osmium tetroxide-fixed specimens embedded in Epon 812 were cut in 0.5-1 μ with the ultramicrotome. The sections were sticked on the slide glass by heating, stained in 0.5% solution of phosphate buffered toluidine blue according to Yamamoto's method (1963).

Electron microscopic preparation. Planarian head pieces were fixed at 4°C for 2 hours in 1% solution of osmium tetroxide buffered with veronal-acetate or phosphate at pH 7.6. While, the intact worms were fixed for 3 hours in 5% cold glutaraldehyde buffered with phosphate at pH 7.6, before the heads were cut off at the level of their auricles. They were washed in phosphate buffer with the same pH, following the fixation of cold 1% osmium tetroxide for 2 hours. The head pieces with the eye were dehydrated in acetone of graded concentrations and embedded in Epon 812 (Luft, 1961). The orientation of the specimens was either transversely, holizontally or longitudinally taken and the sections were cut with a JUM-5A ultramicrotome. The sections were stained with uranyl acetate alone or counterstained with uranyl acetate and lead citrate according to Reynolds (1963). The preparations were observed with JEM-6A electron microscope and taken on photographs.

Observations

Light microscopic examination.

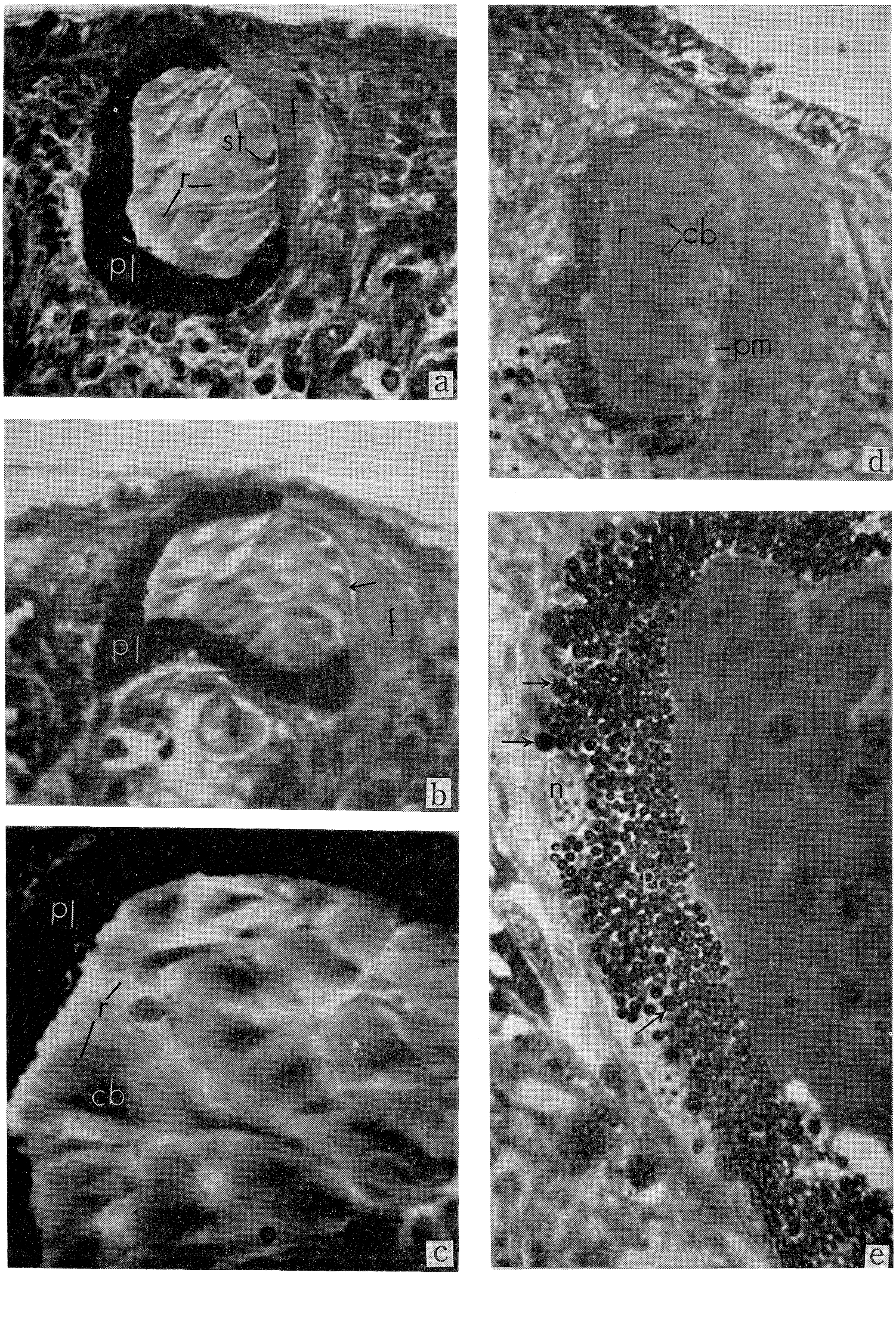
The eye of *Dugesia japonica* consists of the pigment layer and numerous visual cells. Since the cells of pigment layer in paraffin sections are closely packed with the black pigment granules, none of various cell components are almost recognizable, except for nucleus which is located in the basal part of the pigment cell (Fig. 1a). In epon-embedded 0.5-1μ sections, other larger granules darkly stainable with buffered toluidine blue are found (Fig. 1e). The pigment layer is generally designated as the eye-cup and appeared in a crescent form in section. Thickness of the pigment layer is 10-15μ and the outer diameter of the eye-cup is 70-90 μ.

On the other hand, a thin membrane of 1-2 μ in thickness is able to be found in the opening region of the eye-cup in epon-embedded sections (Fig. 1d) and even occasionally in paraffin sections (Figs. 1a and b). In the following description the membrane is referred to as a pluged membrane and a space which is enclosed by the pigment layer and the pluged membrane is designated as the eye-cavity. In epon-embedded sections, there are some vacuoles, large granules stained with toluidine blue and a few black pigment granules in the pluged membrane (Fig. 1d), and many tip portions (retinulae) of the visual cells in the eye-cavity. The retinulae in one eye are 150-200 in this species. The retinula of the visual cell is divided disto-proximally into three parts; rhabdome, conical body and stalk. The rhabdome is of a fan shape about 7 μ in length and about 10 μ in width. The conical body is about 2 μ in width, which gradually become thinner towards its proximal part. The stalk is of roughly 1 μ in thickness (Fig. 1c). As is shown in Table 1, the stainability of the three parts of the visual cell is different from each other in paraffin sections.

Especially, the rhabdome is clearly demonstrated by iron haematoxylin staining, i. e., it stains green against dark blue or black of the other parts of retinula. Moreover, it contains darkly stained fibrils running along a longitudinal axis (Fig. 1c). But the presence of the fibrils is not the decisive criterion of the rhabdome, because the fibrils are occasionally seen in the conical body, too.

In 0.5-1 μ sections, the conical body especially the axial part stains strongly dark, the stalk blue and the rhabdome pale blue with toluidine blue. However, it is difficult to describe the complete form of the rhabdome with this dye, because the boundary between the rhabdomes is not clearly defined and they appear to be homogeneous, any fibrils being not found in them (Figs. 1d and e).

In the peripheral part of the "opening region" of the eye-cup, that is, in the area transitional from the pigment layer to the pluged membrane, it can be ascertained by observations of both paraffin sections and epon-embedded 1 μ sections (Figs. 1a and d) that some stalks of the visual cells pass away from the eye-cavity,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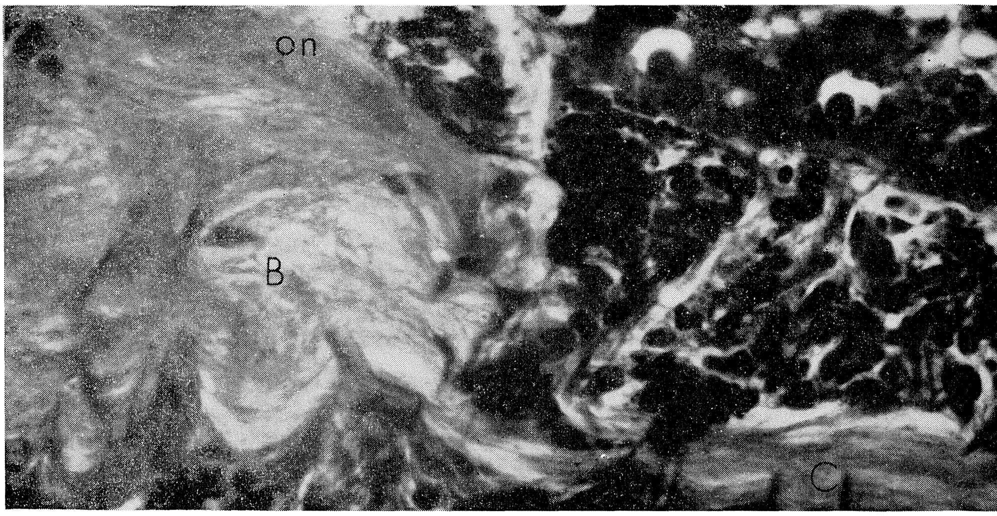


Fig. 2. Light photomicrograph showing connection between the optic nerve and the brain. Optic nerve (on) runs along the superficial area of the brain (B) and get to the commissure (c). ×720

but such a passage of the stalks is not found in the central part of the "opening region".

The stalks which are outside the eye-cavity are not so well stainable that it becomes impossible to trace each stalk even by critical examination of both paraffin and epon-embedded sections. Instead, a thick band of the fibrous structures can be remarkably observed in the area outside the pluged membrane, but it is difficult to say whether the band consists of nerve fibers of the visual cells, connective tissue fibers or both. Judged from stainability of the band, it seems to be similar to the fibrous tissue of the brain. Moreover, from serial transverse sections it is assumed that some of the fibrous strand in the band seem to fuse with the fibrous tissue of the brain. In other words, the fibrous strands may be the optic nerve. The optic

Fig. 1. Light photomicrographs of the planarian eyes.

a. Section through the eye showing very dark pigment layer (pl) in crescent form stained with Heidenhain iron-haematoxylin and light green. Thin pluged membrane is seen in so-called "opening region" of the eye, though indistinct due to conglutination with the fibrous tissue (f) in front of the eye. Fan-shaped rhabdomes (r) of the visual cells are less stainable than the stalk (st). Stdlks go through the pluged membrane. ×600

b. Section through the eye showing the pluged membrane, indicated by arrow, stained with Mallory's triple stains. ×600

c. Enlargement of the previous photomicrograph showing the tip ends of the visual cells. Rhabdome (r) has pallisade structure in which the fibrils are ranged longitudinally. Some fibrils appear to enter the conical body (cb). ×1800

d. Section through the eye showing the rhabdome (r), conical body (cb) and pluged membrane (pm) (epon-embedded 1 μ section and stained with buffered toluidine blue). Vacuoles and darkly stained granules are seen in the membrane. ×600

e. Enlargement of the photomicrograph of the pigment layer of the eye in Fig. 1d, showing pigment granule (P) and larger granules (indicated by arrows). ×1800

Table 1. Stainabilities of the parts of retinula in sections.

Methods		Result		
Embedding	Staining	Rhabdome	conical body	stalk
Paraffin	Delafield's haematoxylin-eosin	red	violet	dark blue
	Mallory's triple staining	sky blue	dark violet	dark violet
	Heidenhain azan	violet	orange or red	orange
	Heidenhain iron haematoxylin-light green	green	dark blue or black	dark blue
Epon	Toluidine blue	pale blue	dark blue	blue

nerve which gets to the beain proceeds medial in the superficial part of the fibrous tissue of the brain and reaches to the commissurae part of it (Fig. 2).

In the peripheral part of the fibrous band, there are some large vesicular nuclei which are similar to those in the brain cells with respect to their staining property. The cells with such nuclei are likely the visual cells, but it is difficult to identify them in the present preparations.

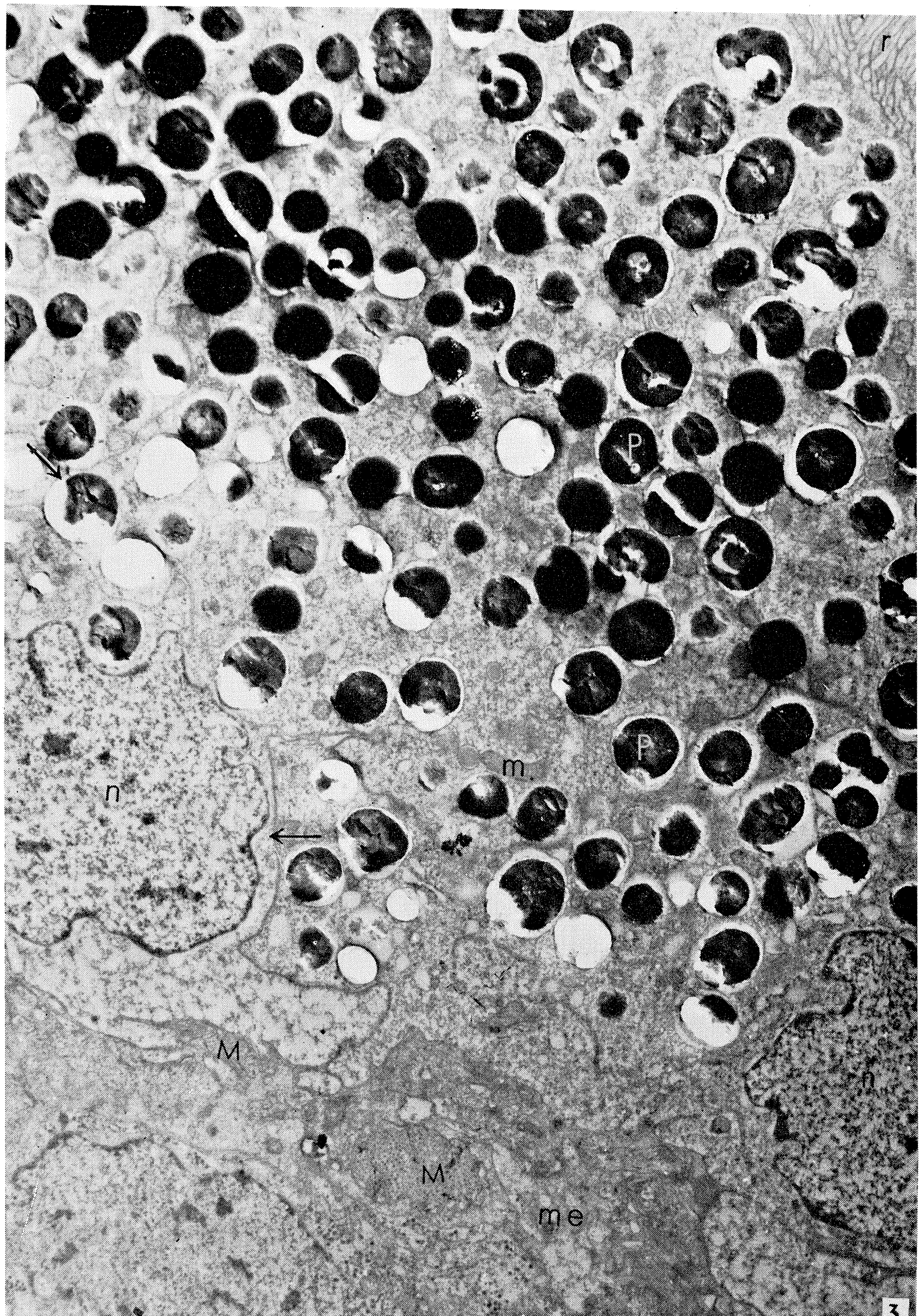
Electron microscopic examination.

Based on the observation described above with light microscope, further study of the planarian eye was performed with electron microscope. For the sake of convenience, the description will be given separately for the pigment cell layer and the visual cells which are now divided each into five parts, rhabdome, conical body, stalk, perikaryon and axon.

(a) *Pigment cell layer.* The inner surface of the pigment cell layer is in contact with the free tip ends and lateral sides of rhabdomes. The outer surface of the layer is attached directly to the muscular cells, mesenchymal cells and subepidermal pigment cells without the intercalary basement membrane (Fig. 3). Plasma membranes of the adjacent pigment cells are faced each other with the space of 200 Å, and presents interdigitation, especially in a complicated manner near both surfaces of the pigment cell layer (Figs. 4 and 5). The septate desmosomes are sometimes found between the plasma membranes in the region near the eye-cavity (inset of Fig. 4).

The nuclei of the pigment cells are found in the cell periphery distant to the

Fig. 3. Electron micrograph showing a part of the pigment cell layer. Innerside of the pigment cell layer (upper right) makes contact with rhabdome (r) of the visual cell, while its outerside (bottom) is contiguous to muscle cell (M) and mesenchymal cell (me) without intercalation of a basement membrane. Pigment granules (p) are distributed throughout cell and a few mitochondria (m) in slender or globular form are visible also. Nucleus (n) of the pigment cell is situated in area distant to the eye-cavity. Cell-boundary between two pigment cells is indicated by arrows. ×10000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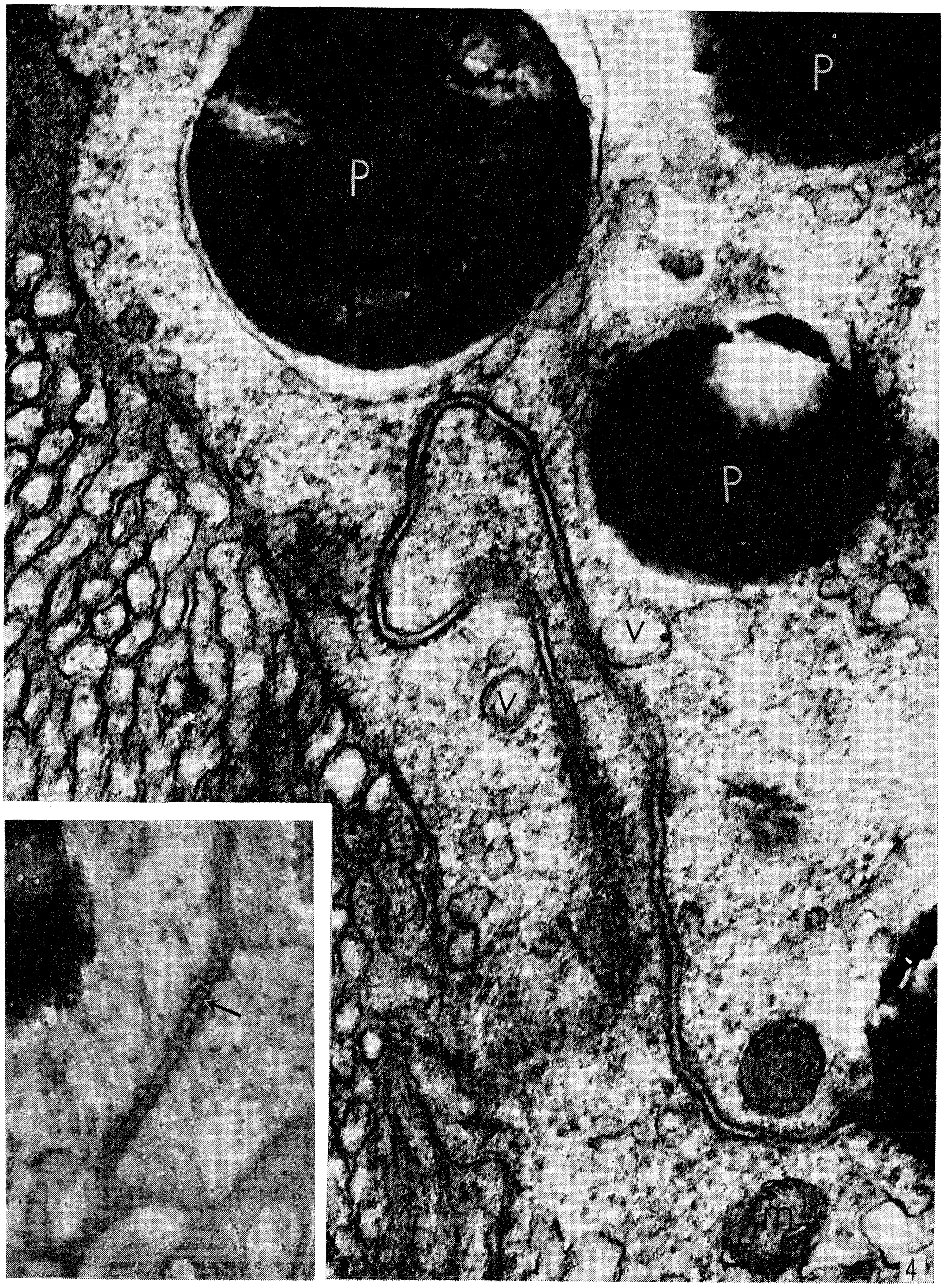
eye-cavity. They are ellipsoid in shape (10-13 μ in major axis and 2-5 μ in minor axis). The nuclei are limited by a double layered nuclear envelope with shallow pits in each of which sometimes a pigment granule is inlayed. The nucleus contains several chromatin masses and some of them attach themselves to the inner membrane of the nuclear envelope. The mitochondria with comparatively well developed cristae are distributed sparsely throughout the cytoplasm, but rather numerously around the nucleus. The mitochondria are generally globular (about 0.5 μ in diameter) or ovoid (about 0.8 μ in major axis and about 0.5 μ in minor axis) in section (Fig. 6). However, the elongated mitochondria in a sausage-form are occasionally found (Fig. 7). The globular and ovoid forms may be due to oblique sections of the sausage form. The endoplasmic reticulm are mostly vesicular in various sizes (70-170 mμ in diameter) but sometimes thready (Fig. 8). Golgi apparatus are often seen near the nucleus (Fig. 9).

The globular pigment granules in uniform size (about 1 μ in diameter) are homogeneously electron dense and enclosed with a limiting membrane (Fig. 10). The destructed figure due to preparation procedures indicates that pigment granule consists of numerous subunit particles of 50-70 mμ in diameter (Fig. 11). In addition, the pigment cells contain two sorts of granules which are larger (about 2 μ in diameter) than the pigment granules, and enclosed each with a limiting membrane. The first granule (designated as α-granule) is laden with numerous and mediated electron dense particles of 15-20 mμ in diameter (Figs. 12 and 13). The second granule (designated as β-granule) includes several kinds of globules of 400-1200 mμ in diameter. Some of them are made of electron dense and pale layers arranged concentrically (Figs. 14 and 15). Such a feature of the globule is occasionally found also in α-granule (Fig. 16). The α-granules are always seen, but the β-granules scarcely. Sometimes, oval bodies of various sizes which are complicatedly partitioned by membranes are also encountered in the pigment cells (Fig. 17). These bodies take various appearances, i. e., some of them are filled with small particles (Fig. 18), some of others contain small particles and vesicles (Fig. 19) and still others include heavy electron dense mass identical to the black pigment (Fig. 20).

It was ascertained that the pluged membrane of the eye is nothing but a part of cytoplasm of the pigment cells, and it contains mitochondria, small vesicles and large vacuoles (Fig. 23). However, the fine structures in the pluged membrane are not always so definite, for some cells are densely packed with many vacuoles of 100-800 mμ in diameter (Fig. 21), and others with numerous small vesicles of 50-100 mμ in diameter (Fig. 22). Furthermore, α- and β-granules are occasionally included in the membrane.

Fig. 4. Interdigitation of a pigment cell-boundary in the area faced to the eye-cavity. Several short connecting apparatus are visible. P: pigment granule, v: vesicular endoplasmic reticulum, m: mitochondrion. ×42000 Inset. Septate desmosome between the pigment cells (indicated by arrow). ×50000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The present observation indicates that the visual cells pass through the peripheral part, not through the central part, of the pluged membrane, and that there occur microtubular fibers in the cytoplasm of the pigment cell near the stalk (Fig. 24). It is also found that the stalks of the visual cells are ensheathed with the pigment cells (Fig. 25).

(b) *Visual cell.*

(1) Rhabdome.

Rhabdome is furnished with numerous tubular microvilli (70-120 mμ in width) running longitudinally. The microvilli in a single rhabdome are different in length, that is, they are longer in the median and shorter in the periphery of the rhabdome. The tip ends of the microvilli are enlarged to 200-300 mμ in width (Fig. 26). The transverse section of the rhabdome shows an electron dense spot in each microvillus (Fig. 27), but the longitudinal section reveals that it is not a spot but a thread. This dense thread becomes lucent at the enlarged tip end of the microvillus. Each microvillus is demarcated with the membrane of about 80 Å in thickness and the distance between the microvilli is about 100 Å in average. Röhlich und Török (1961) stated with *Dendrocoelum lacteum* that each microvillus is independently placed and does not branch off. However, the present observation indicates that the fusion of microvilli is of frequent occurrence (Fig. 27). In this point, therefore, the microvilli of *Dugesia japonica* may be different from those of *Dendrocoelum lacteum*.

In the boundary area between the rhabdome and conical body there are several vacuoles of various sizes (400-800 mμ in diameter) surrounded with limiting membranes (Fig. 28), and moderately electron dense material is attached to the inner wall of the vacuole. The limiting membrane of the vacuole is continuous to the membrane of microvillus. Some of the membrane of the microvilli invaginates deeply in the conical body.

(2) Conical body.

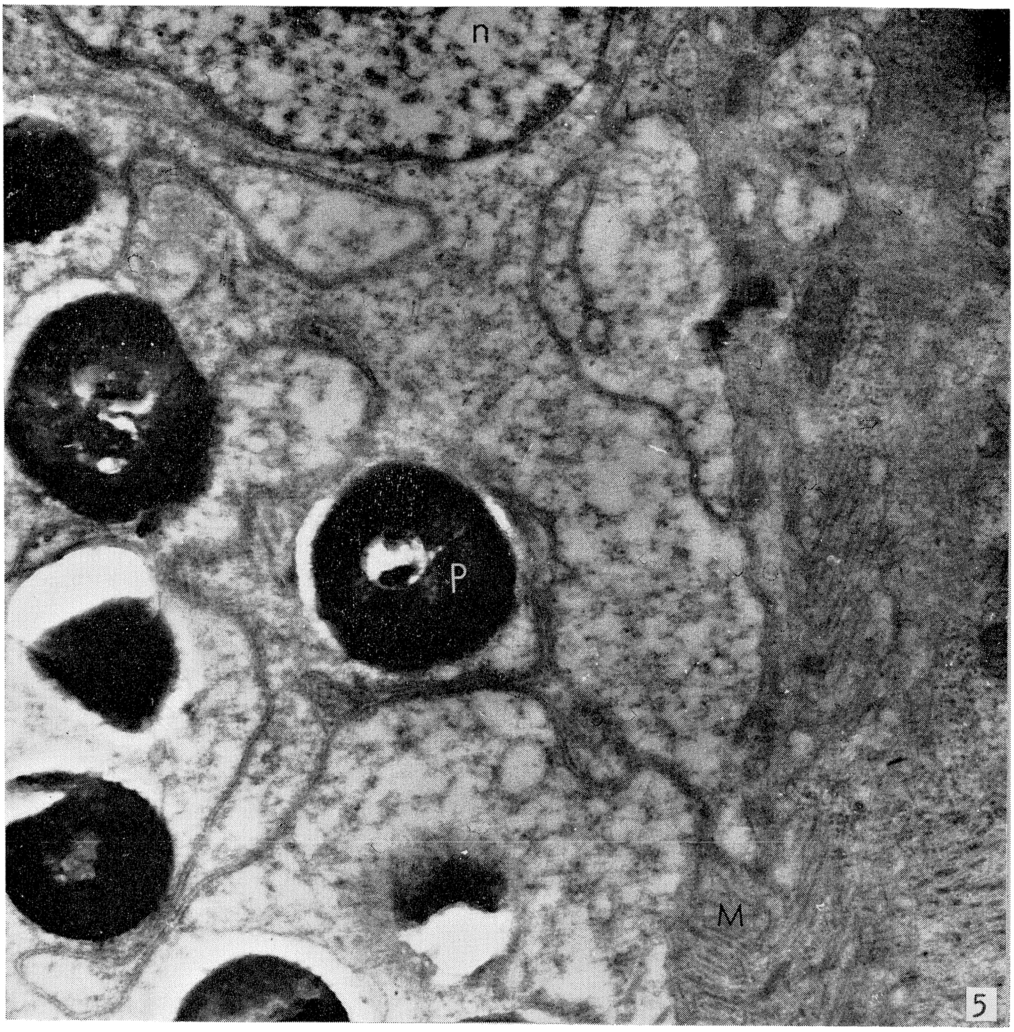
The conical body is a continuation of the rhabdome, but it is characterized by an assemblage of mitochondria (Fig. 28) which are generally in large size (2-3 μ in length and about 0,3 μ in width). They are apt to gather along the longitudinal axis of the conical body. In addition, the conical body contains a few fine particles (150 Å in diameter), fine tubular fibers (200-250 Å in width) and vacuoles of various sizes. Some of the vacuoles are arranged along the longitudinal axis and sometimes fused each other. The fine tubular fibers extend near to the rhabdome, but it can not be ascertained whether or not the other ends of these fibers are continuous to the neurotubules in the stalk.

(3) Stalk.

Fig. 5. Pigment cell-boundary showing conspicuous interdigitation in the area near the basal part of the pigment layer. M: muscle, n: nucleus, P: pigment granule. ×30000

Fig. 6. Mitochondria of the oval type in the pigment cell. ×54000

Fig. 7. Elongated mitochondrion of the sausage type. ×54000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The proximal portion of the conical body is continued with a long stalk. The stalks are filled with numerous long neurotubules, some vacuoles of 100-300 mμ in diameter (Fig. 29), considerable numbers of ribosomal particles (Fig. 30) and a few but well-developed mitochondria distributed in the peripheral part. The widths of neurotubules may be different in various animals (Schmidt and Geren, 1950, Fernández-Morán, 1958, Bertolini, 1964, Metuzals, 1963, Andersson-Cedergren and Karlsson, 1966), and those in *Dugesia japonica* ranges from 200 to 250 Å.

The stalk is connected with the adjacent pigment cell by septate desmoeomes located at the portion where the visual cells go out from the eye-cavity (Fig. 31). This portion in the stalk contains several multivesicular bodies of about 800 mμ in diameter, in which are found many vesicles of about 60 mμ in diameter.

It is frequently found that the stalks branch into two as soon as they go out of the eye-cavity (Fig. 32). The number of neurotubules in the stalks is diminished as they approach to the perikaryon. Several Golgi apparatus are visible in a portion of the stalk which is outside the eye-cavity (Fig. 33).

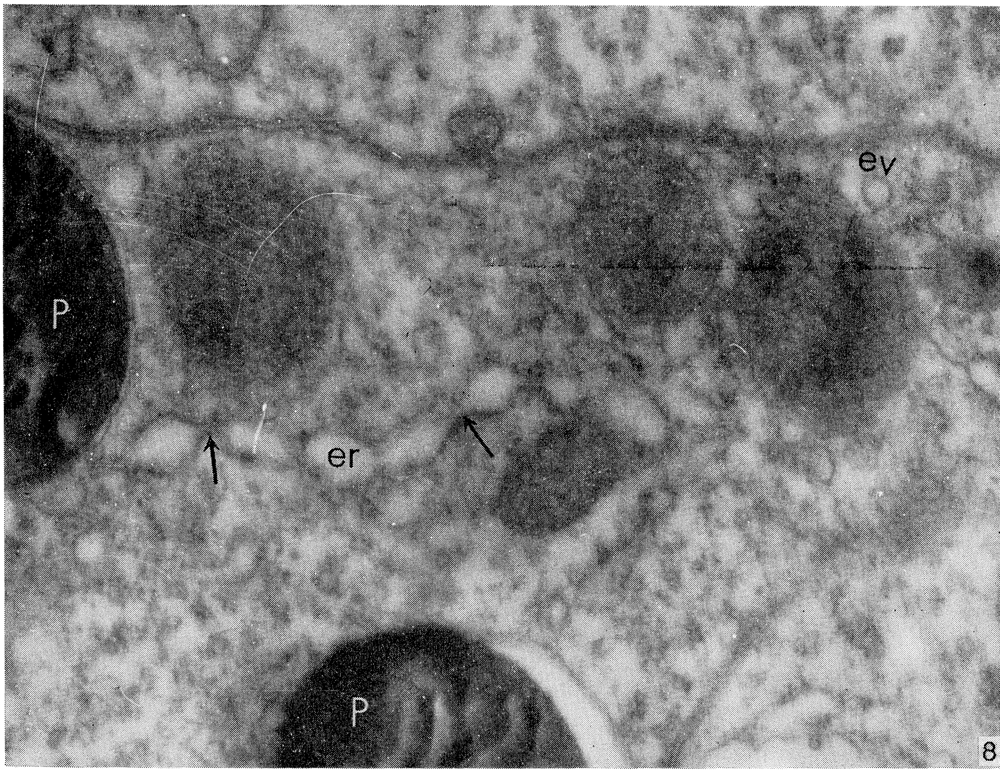
In a slightly enlarged proximal portion of the stalk which is continued with perikaryon, vacuoles, vesicles, mitochondria and abundant ribosomal particles are observed (Fig. 34). Some of ribosomal particles are scattered in cluster or singly, while others are attached to the membrane of the vesicles. The neurotubules can not be found in this portion.

(4) Perikaryon.

The perikaryon is a part of visual cell which contains nucleus. The nucleus contains the electron dense particles, a few fibrils which has moderate density and remarkable nucleolus made of fine particles of about 200 Å which are less electron dense than the chromation mass (Fig. 36). The outer undulated membrane of the nuclear envelope appears to be locally fused with the inner membrane. The ribosomal particles are scattered enormously in cytoplasm except that those are associated with the outer membrane of the nuclear envelope. The endoplasmic reticulum appears mostly in vesicular form and are arranged around the nucleus (Fig. 35), but some are in thread form with locally enlarged cisternae (100-350 mμ in width) and contrictions (about 60 mμ in width) at intervals. The cisternae are rather electron less dense but its inner side is attached here and there with electron dense material. A few small mitochondria are found in the perikaryon (Fig. 37). Marked accumulations of heavy electron dense particles of 90-100 mμ in diameter are sometimes encountered in the perikaryon. They are so closely associated with the elongated mitochondria that they pretend to be engulfed in mitochondria. Endoplasmic reticulum are sometimes arran-

Fig. 8. Endoplasmic reticulum in a pigment cell, being mostly vesicular (ev) but occasionally in thread form (er) and having enlarged cisternae with several constrictions (indicated by arrows). Moderate electron dense materials attach the innerside of the memdrane. P: pigment granule. ×41100

Fig. 9. Well-developed Golgi apparatus in the pigment cell, consisting of several lamellae and numerous vesicles. ×63000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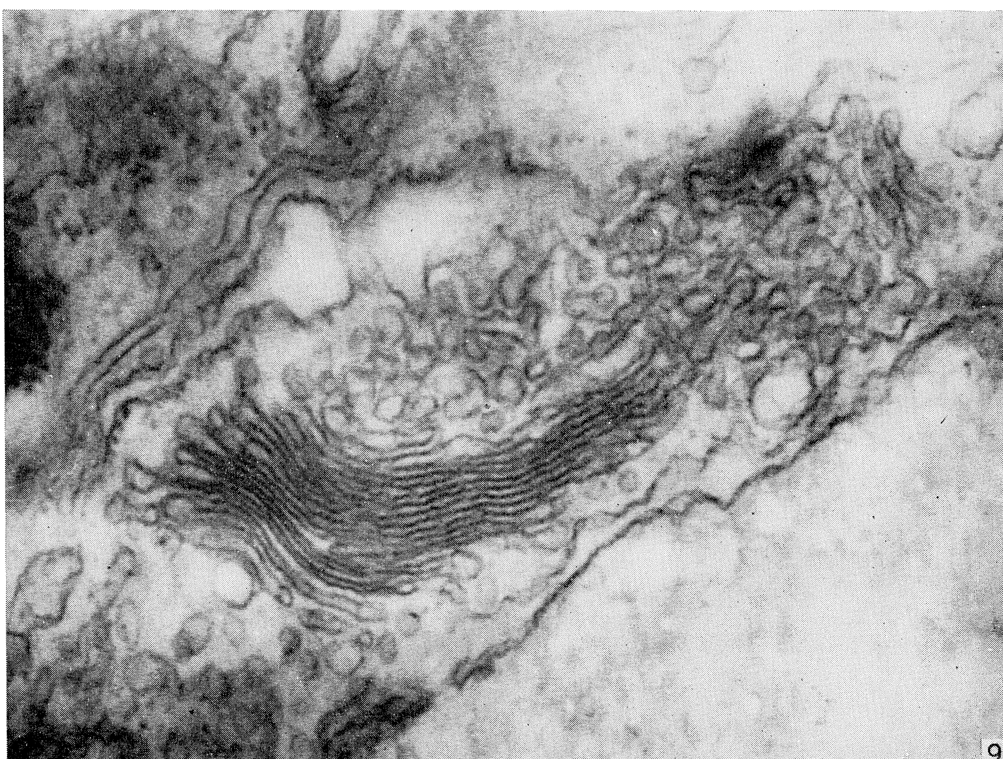
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ged concentrically around these accumulations. Enlargement of the figure of the accumulation indicates that the particles are divided into subunit particles of about 200 Å in diameter.

(5) Axon.

The axon of the visual cell includes mitochondria, vesicles and neurotubules. The transverse sections of the axon shows that its peripheral part is low electron dense but moderate in its central part. This difference of the density may be artifact (Fig. 39). The cytoplasmic components, i. e., neurotubules, mitochondria and vesicles, are contained exclusively in the central part. The neurotubules are fewer in number than in the stalk. The fine structure of the axon are similar to that of the nerve fiber in the neuropil of the brain but the former has not synaptic vesicles and neurosecretory granules which are included in the latter.

It was determined by an electron microscopy that the fibrous tissue outside the pluged membrane is made mostly of these axons and partly of the stalks of the visual cells (Fig. 39). Occasionally a large myelin figure is seen in this region.

Discussion

Since Hesse (1897) and Jähnichen (1897), it has been a long-pending question whether the planarian eye is of open cup-shaped or closed spherical, pluged with a membrane in front of the eye. Lender (1952) stated that the pluged membrane is probably difficult to be detected as it is extremely thin in some species. In the present examination, this membrane is not necessarily observed in paraffin sections, while in epon-embedded sections, it is always recognized. This fact suggests that the pluged membrane may be damaged by means cf fixation, dehydration or embedding in the procedure of paraffin sectioning. In fact, the enlarged figure of the pluged membrane sometimes indicates many large vacuoles in it. Therefore, it may be reasonable to consider that such pluged membrane may easily be disintegrated due to crushing of the vacuoles. In short, the planarian eye should not be refered to as "eye-cup" described by many authors, but as "spherical eye" with the pluged membrane in the front.

Röhlich und Török (1961) with *Dendrocoelum lacteum* and *Dugesia lugubris* concluded that the pluged membrane is made of cytoplasm of the pigment cells and that the stalks of the visual cells pierce through this membrane. The present study is

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Fig. 10. Pigment granule enveloped with a distinct limiting membrane. ×54000

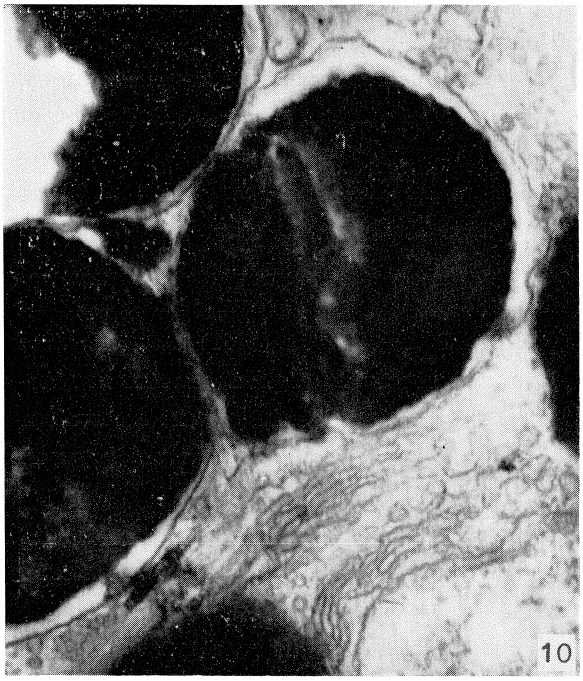
Fig. 11. Enlarged photograph showing a destructed part of a pigment granule. Note the pigment granule being composed of small subunit particles. ×66000

Fig. 12. Comparison of the α-granule and pigment granule. The former is larger than the latter (P) and enveloped with a limiting membrane also. ×20000

Fig. 13. Enlargement of a part of α-granule filled with fine particles. ×89700

Fig. 14. β-granule containing several small globules. ×20000

Fig. 15. Enlarged photograph of a globule in β-granule. Electron dense and pale layers are set concentrically. ×70000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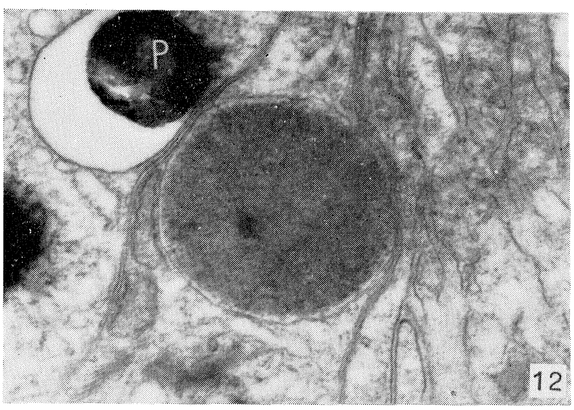

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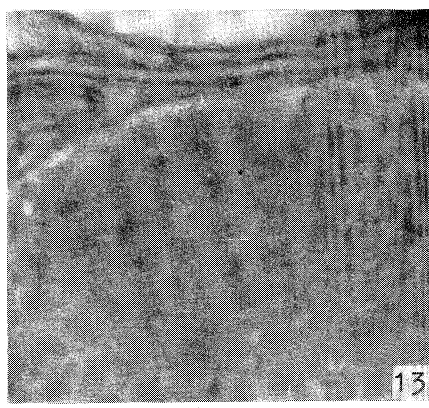
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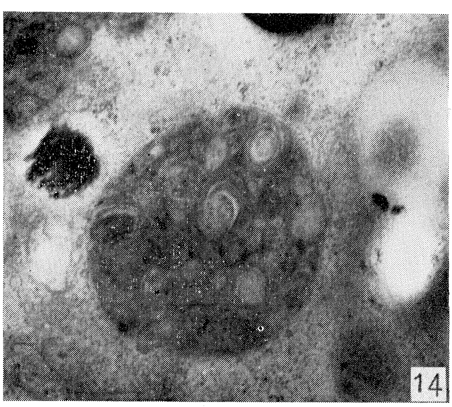
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in accordance with their conclusion in the point that the cytoplasm of the pigment cell spreads over the "opening region" of the eye-cup. Penetration of the stalks through the pluged membrane is, however, limited near the pigment layer and never in the central region of the pluged membrane. From these findings, the structure of the planarian eye will be illustrated as in Fig. 40.

The pigment granules in the animal eyes can be divided into two kinds of types, that is rod form as in frogs and bird (Tanaka, 1959, 1962) and globular form as in chaetognath (Eakin and Westfall, 1964) and *Helix pomatia* (Schwalbach, Lickfeld und Hahn, 1963). According to this classification, the pigment granules of the eye in *Dugesia japonica* are of the globular type. Rölich und Török (1961) assumed with the polarisation microscope that the protein matrix of the pigment particle in *Dendrocoelum lacteum* eye is a lamellar structure. However, in the present observation this point is not confirmed even with the electron microscpe, only that the pigment granule is subdivided into small particles.

Besides the pigment granules, two sorts of granules designated as α-granule and β-granule, are seen. These granules have not been described in *Dendrocoelum lacteum* and *Dugesia lugubris* (Röhlich und Török, 1961) and *Dugesia tigrina* (Röhlich, 1964). In the present material, however, light microscopic observation revealed the strongly stained α-granules in the epon-embedded and toluidine blue-stained sections of the pigment cells, though not in the paraffin sections. It is impossible to say their characters and functions. Probably, however, these granules are similar to the lipofuscin granules of sympathetic nerve cell (Cravioto, 1962). Since number of these granules appears to change depending on the conditions of the eye, they have probably something to do with physiological functions in the pigment cells. In addition, the bodies which are partitioned with membranes are also occasional occurrence in the pigment cells. It is likely that the bodies may be concerned with pigment granules, α-granule or β-granule because they appear associated with these granules.

Between the pigment cells there are often septate desmosomes. These desmosomes are also detectable in the epidermis of *Dugesia lugubris* (Török and Röhlich, 1959), of *Dugesia japonica* (Kishida, unpublished) and in the pharyngeal epithelium of *Bdellocephala brunnea* (Ishii, 1963), of *Dugesia japonica* (Kido, Kishida and Asakura, 1965). All the desmosomes hitherto known in the fresh water planarians are found between the same sort of cells, except for McRae's report (1964). She described the desmosomes of non-septate type between the visual cell and the pigment cell. The present observation added the septate desmosomes between the visual cell and the pigment cell.

Fig. 16. Modified type of α-granule containing a globule as observed in β-granule. ×42000

Fig. 17. Showing a granule partitioned complicatedly with membranes. ×42000

Fig. 18. Showing a granule filled partly with small electron dense particles and partitioned partly with mambranes. ×20000

Fig. 19. Showing a granule containing numerous vesicles and small particles. ×20000

Fig. 20. Showing a granule containing vesicles and pigment substance. ×43300


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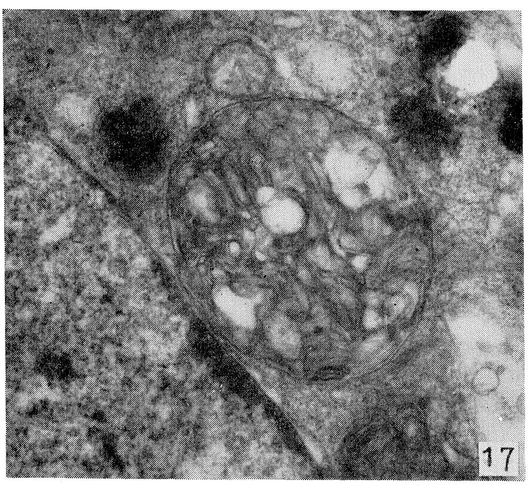
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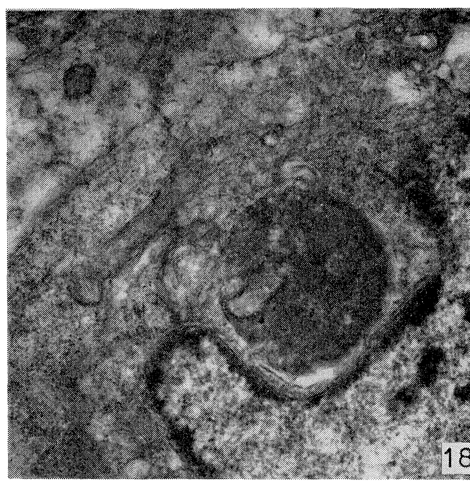
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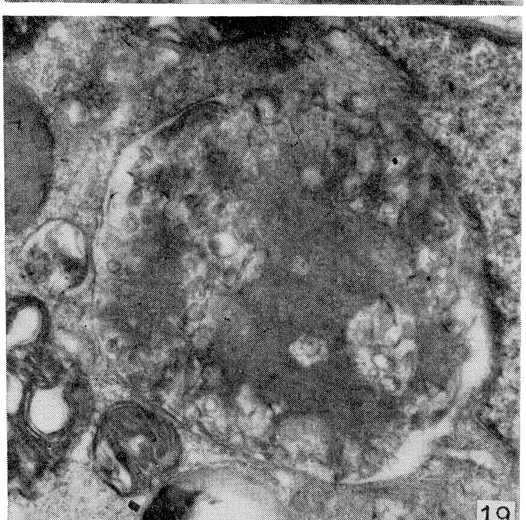
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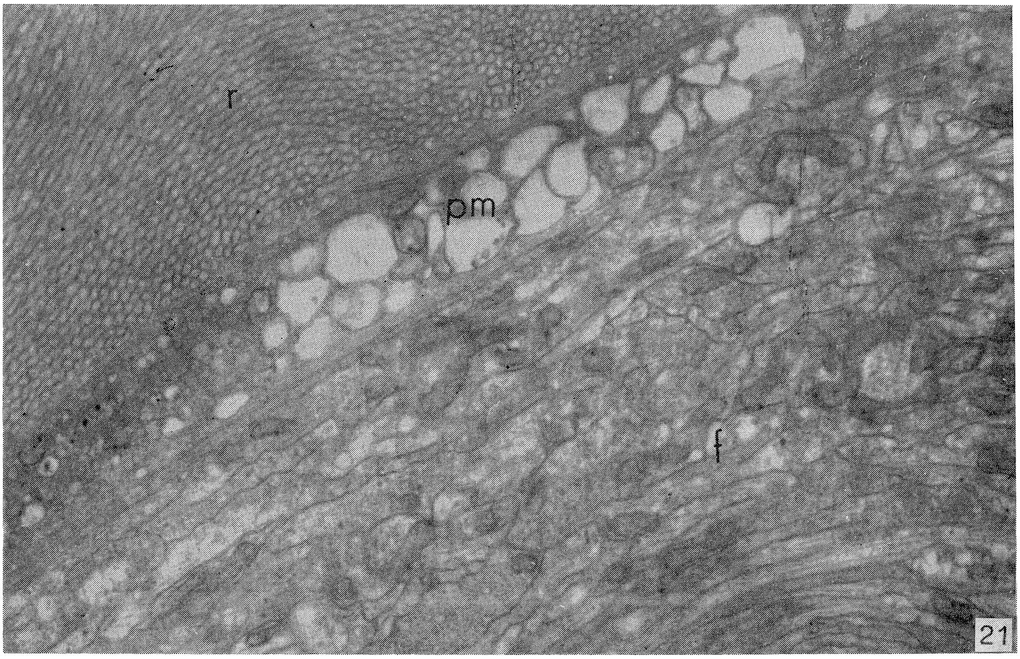
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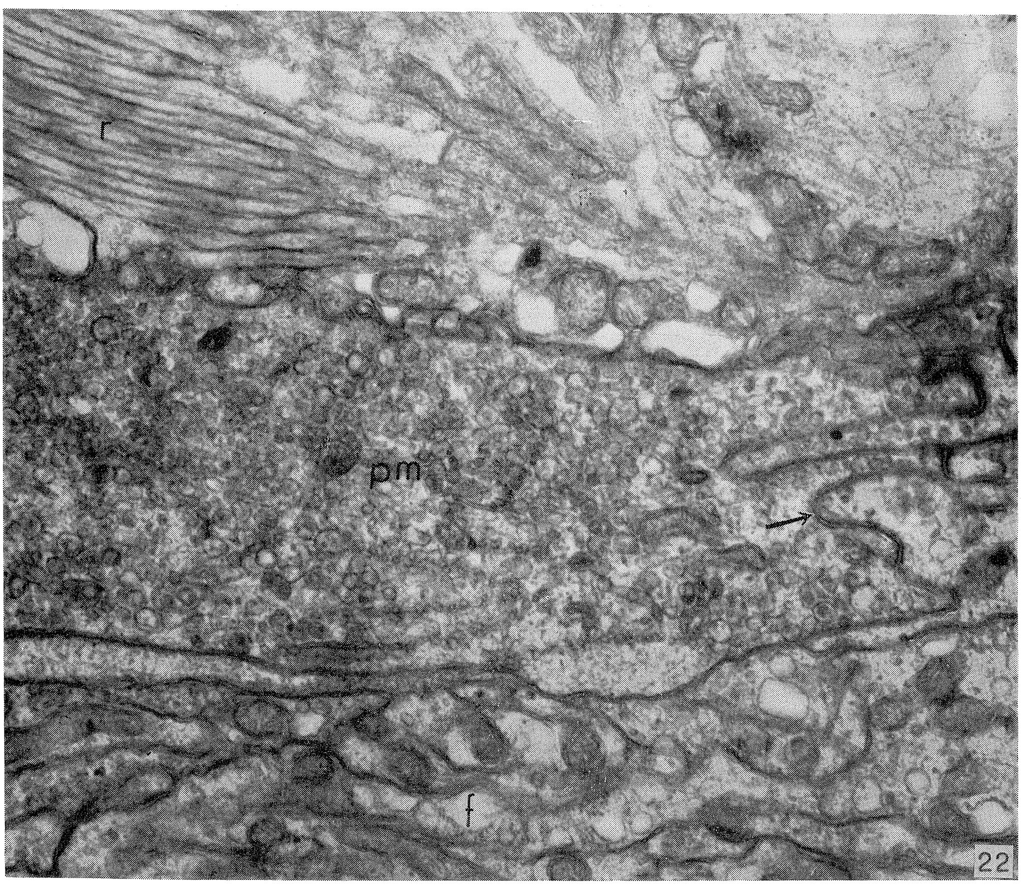
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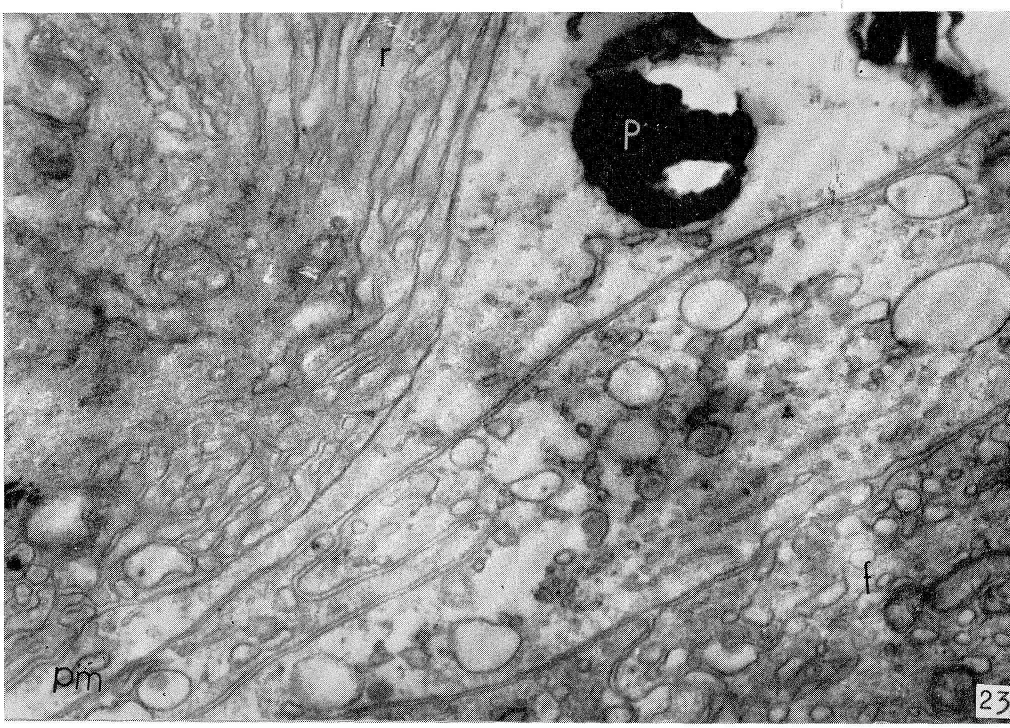
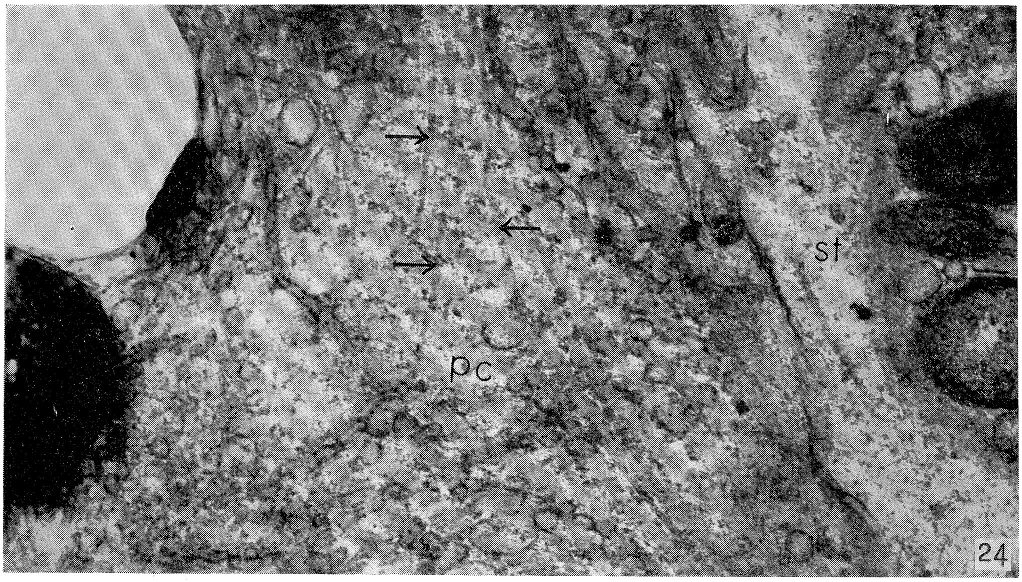


Fig. 23. Relationship of the pigment cell and the pluged membrane. Pigment cell extends itself as the pluged membrane (pm) toward so-called "opening region" of the eye (under left). P: pigment granule, r: rhabdome, f: fibrous tissue. ×15640

Fig. 21. Pluged membrane containing large vacuoles and its neighbors. The pluged membrane (pm) separates the rhabdomes (r) in the eye-cavity from the fibrous tissue (f) of the visual cells outside the eye-cavity. ×12300

Fig. 22. Pluged membrane (pm) containing numerous small vesicles and showing a interdigitating cell-boundary (indicated by arrow). r: rhabdome, f: fibrous tissue. ×22200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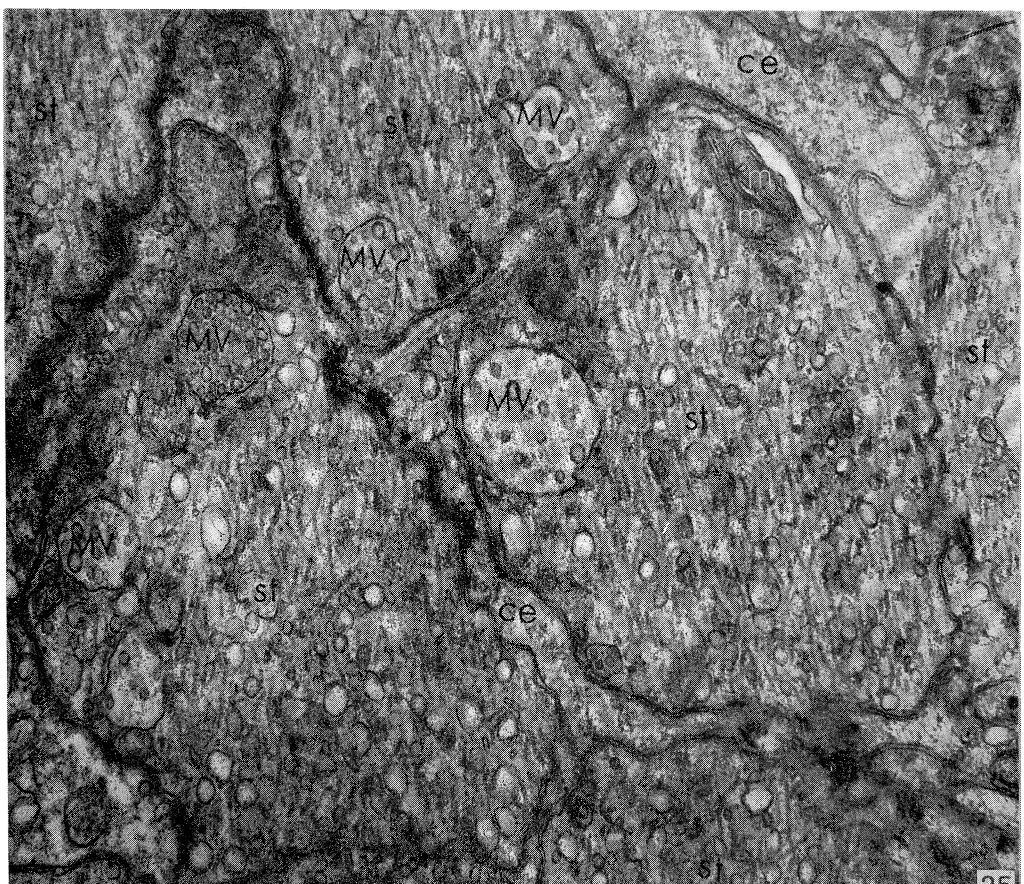
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It has been suggested that the smooth-surfaced endoplasmic reticulum is concerned with the carbohydrate metabolism (Porter and Bruni, 1960) or lipid metabolism (Muta, 1958, Palay, 1958, Christensen and Fawcett, 1960). Porter and Yamada (1960) considered that smooth-surfaced endoplasmic reticulum may be related to photoreceptic activity of the pigment cells in the amphibian eye. The endoplasmic reticulum in the pigment cells of the planarian eye is also of smooth-type but appears scarcely. Therefore, it is doubtful if they are concerned with the photoreceptic activity.

Hesse (1897) and other investigators pointed out that the rhabdome is a structure with the striated border. Moreover, Ude (1908) stated that the longitudinal fibrils are found clearly in the rhabdome fixed with sublimate but not with osmium. He considered, therefore, that the fibrils might be disintegrated in the osmium-fixation. In the present study, fibrils in the rhabdome are also seen with Helly's fixation but not with osmium-fixation in the case of light microscpic examination. However, electron microscopy reveald that the rhabdome consist of numerous microvilli of about 1000 Å in diameter which run in parallel to the longitudinal axis of the rhabdome. Each space between the microvilli is 100 Å, and it is below the resolution power of the light microscope. Accordingly it may be safe to say that the fibrils in the rhabdome observed with light microscopy are assemblage of the microvilli or their modified structure due to sublimate-fixation.

The present examination found that the microvilli are tubular and their inner cavity contains a substance probably related to photoreceptic function.

A boundary membrane between the rhabdome and conical body is not observed, though Press (1959) described it in *Dugesia tigrina*. The fact that some of basal portions of microvilli in the conical body are frequently associated with mitochondria seems to imply a significance for photoreception.

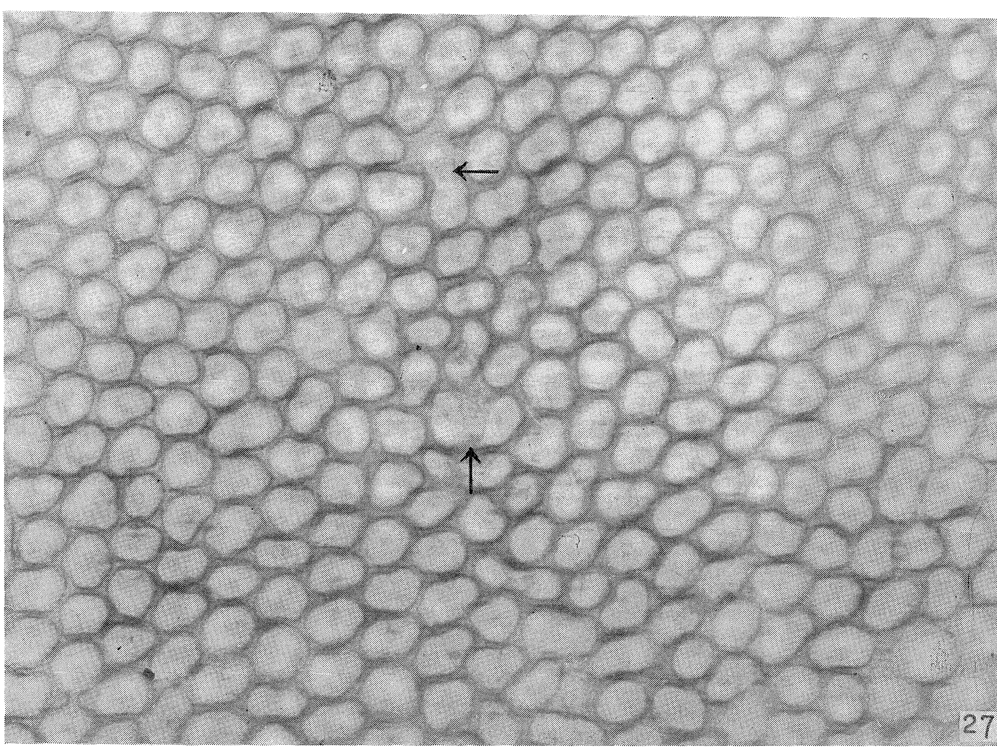
The fine structure of the conical body is agreed with the observations in the other species of the planarians by previous investigators, i. e., a lot of mitochondria accumulates in parallel lines. Thus, a strong stainability for certain basic dyes appears to be ascribed to such abundant accumulation of mitochondria in the conical body.

Taliaferro (1920) said that a visual rod of vertebrate eye and a visual cell of the planarian eye are identical. According to his consideration, rhabdome, conical body and stalk in planarian visual cell correspond respectively with outer segment, inner segment and myoid in vertebrate visual rod. It is well known with electron microscopy that in the vertebrate visual cell numerous large mitochondria are also accumulated in the inner segment (Sjöstrand, 1953), as in the conical body in planarian visual cell. On the other hand, Eakin and Westfall (1965) divided the animal eyes

Fig. 24. Pigment cell contacting with the stalk (st) of the visual cell. Pigment cell (pc) contains considerably long cytoplasmic microtubules (indicated by arrows) and small vesicles. ×32000

Fig. 25. Oblique sections through the stalks of the visual cells. Stalks (st) containing numerous neurotubules, vesicles, mitochondria (m) and multivesicular bodies (Mv) are ensheathed with the pigment cell cytoplasm (ce). ×21000

26



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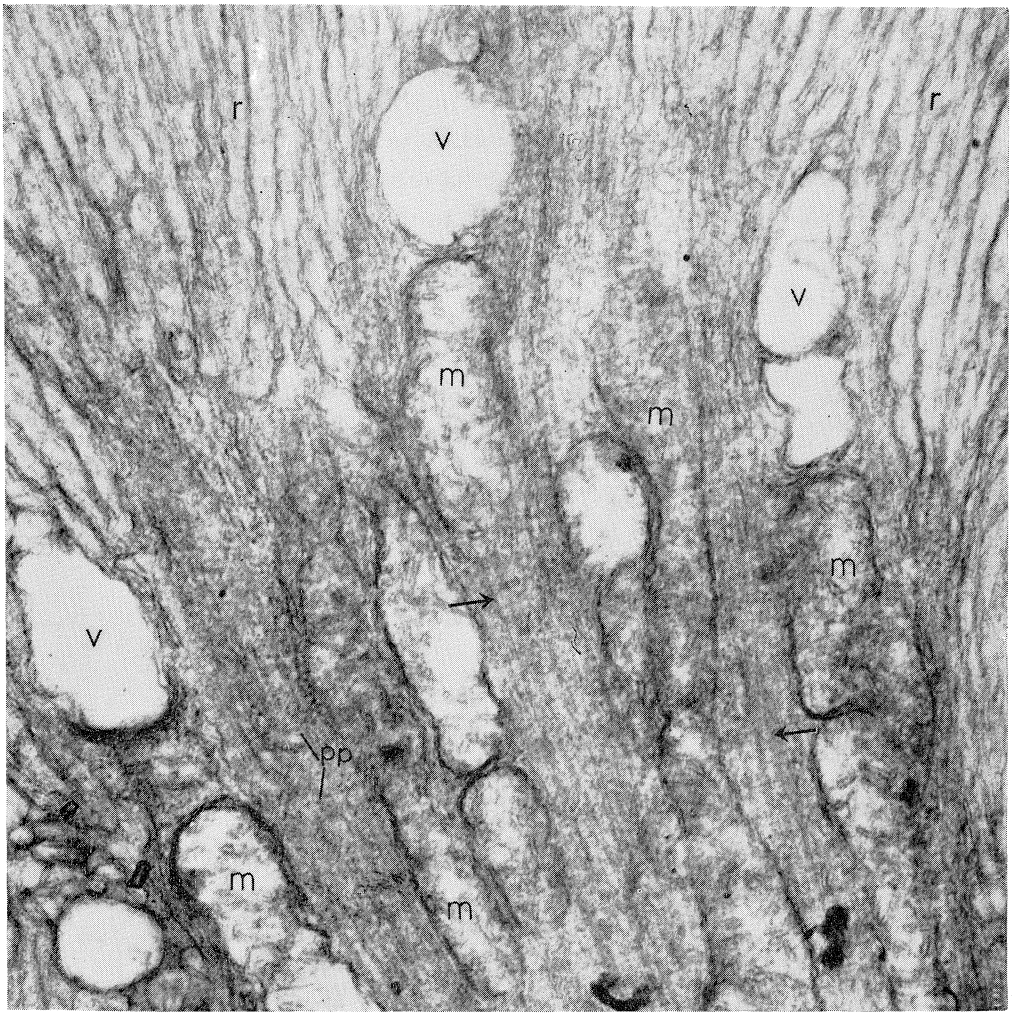


Fig. 28. Remarkable feature in the conical body of the visual cell. Large elongated mitochondria (m) are arranged longitudinally, and vesicles (v) are located in the area near the rhabdome (r). Microtubules (indicated by arrows) run longitudinally and small particles (pp) scatter. ×35700

Fig. 26. Longitudinal section through tip end of the rhabdome of the visual cell. The rhabdome (r) consists of numerous slender microvilli which run longitudinally. The tip ends of microvilli are enlarged and attach the pigment cell. ×63000

Fig. 27. Transverse section of the rhabdome consisting of numerous microvilli. Each microvillus includes a moderate electron dense spot. Some of contiguous microvilli fuse one another (indicated by arrows). ×60000

into two types phylogenically; (1) ciliary type in which the light-sensitive organelles arise from a cilium-like process and (2) rhabdomeric type in which the photoreceptive apparatus is not a modified cilium. The vertebrate visual rod belongs to the former type from the fact that the connecting part of it shows the typical structure of the cilium, while the planarian visual cell pertains to the latter type and has not any vestiges of the cilium. It is, therefore, doubtful to make any comparison between the vertebrate and the planarian eyes with respect to their origin. Form the morphological similarlity alone, it can be thought that they may correspond with each other concerning the important function such as resynthesis of photo-pigment.

Recently microtubules are found in cytoplasm not only of the nerve cell but of other tissue cells also. Slautterback (1963) was the first who described "cytoplasmic microtubules" of 220 Å thick in Hydra. Thereafter, it was found that the microtubules are presented in regular form in various tissue cells of the rat (Sandborn, Koen, McNabb and Moore, 1964, Behnke, 1964). Universal occurrence of the microtubule suggests its possible functions (a) supporting, (b) contractile or (c) as channel to transport fluid and fine particles.

As to the stalk, its proximal portion is likely related to protein synthesis, because it contains a wealth of ribosomal particles, several Golgi apparatus, many vacuoles, and enoplasmic reticulum appearing in rows. If so, this portion may perform exclusively synthesis of substance concerning photoreceptic function.

The structures of the perikaryon and axon are almost similar to those in *Dugesia tigrina* after McRae (1963). But they differ from each other in the following point, namely, there is a remarkable gathering of electron dense particles in the present species. It is considered to be glycogen referring to their sizes and density. Eakin and Westfall (1964) described a large number of single glycogen granules and they ascribed sole source of feuel to them in the rotifer eye. The electron dense particles in perikaryon of the planarian eye may bear similar function to the glycogen granules in the rotifer eye.

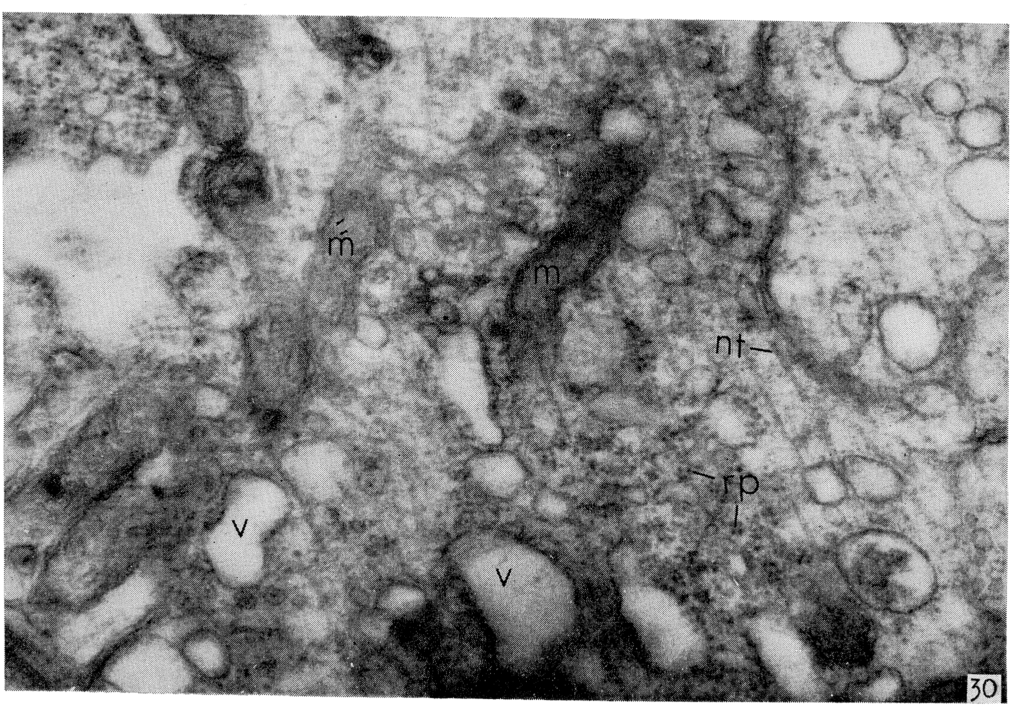
Acknowledgement

The writer wishes to express his sincere thanks to Prof. Dr. T. Kido of the University of Kanazawa for his invaluable guidance and encouragement. He is deeply indebted to Prof. Dr. M. Ichikawa of the University of Kyoto for his instructive criticism and kindness of reading the manuscript.

Fig. 29. Stalks of the visual cells in the eye-cavity, containing numerous neurotules, vesicles, vacuoles (v) and elongated mitochondria (m). ×34000

Fig. 30. Enlargement of a part of the previous figure. Notice the distribution of free ribosomal particles (rp). m: mitochondria, nt: neurotubules, v: vacuoles. ×40800


v
m
m
29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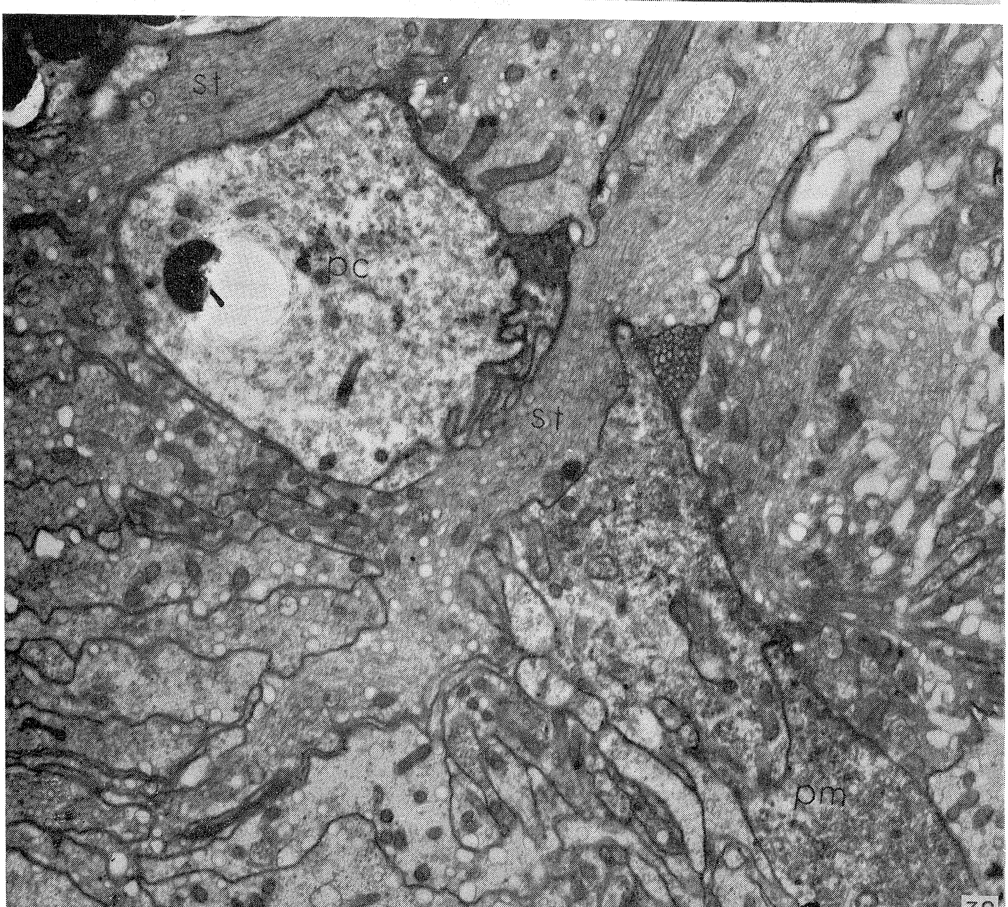
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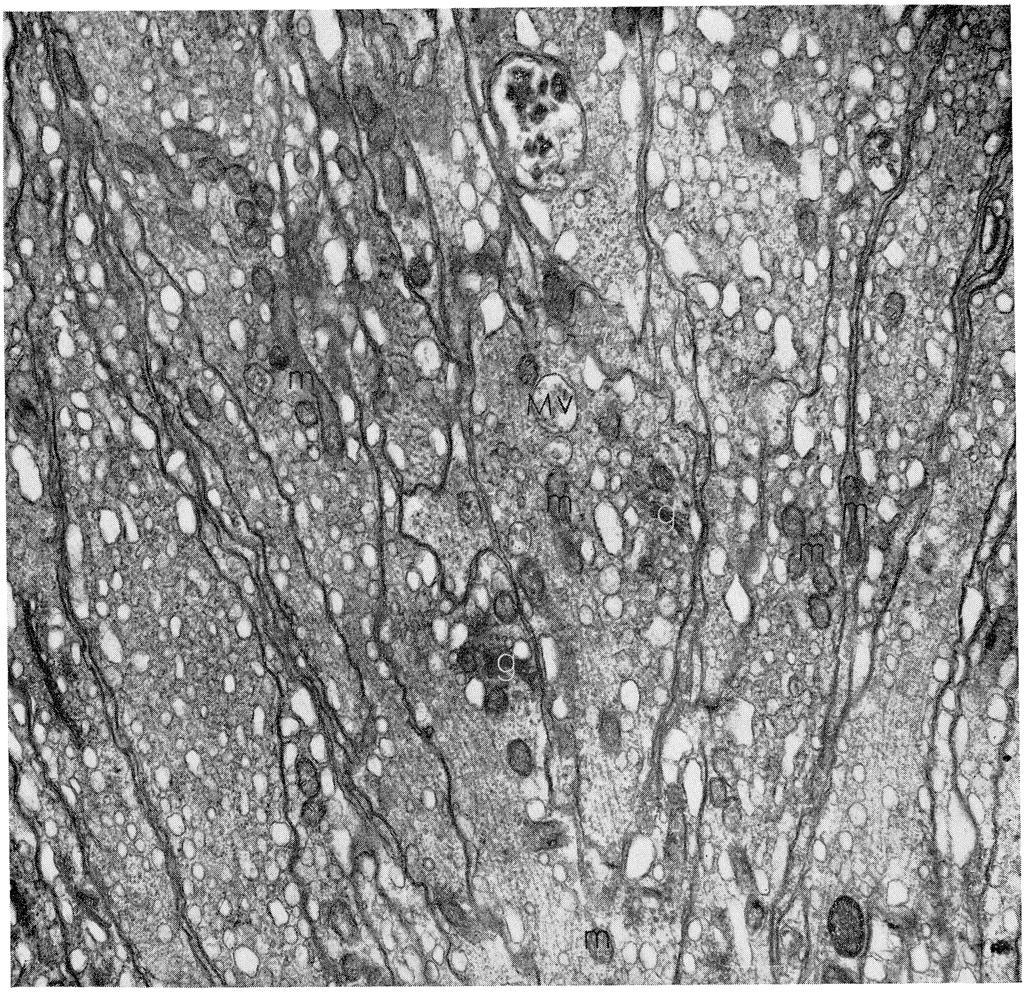

Fig. 33. Stalks outside the eye, running in parallel with each other and containing neurotubules, vacuoles, vesicles, mitochondria (m) and multivesicular bodies (Mv). Notice several, small Golgi apparatus (g) in each stalk. ×12900

Fig. 31. Stalk (st) being in contact with the pigment cells (pc) with septate desmosome (indicated by arrow). ×28200

Fig. 32. Marginal part of the pluged membrane (pm). Stalks (st) of the visual cells penetrate through the area between the pluged membrane and the pigment cell (pc). ×7800

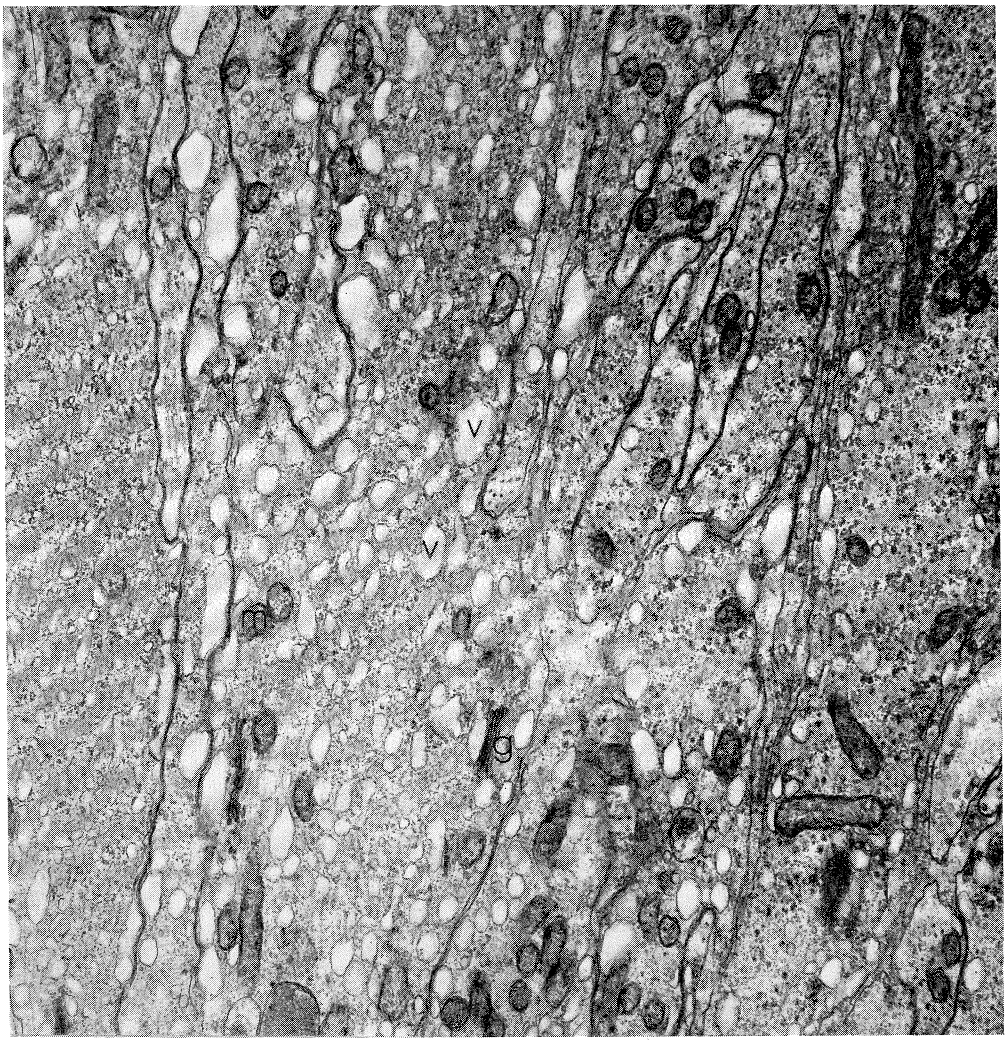

Fig. 34. Proximal portions of the stalks near the perikaryon showing slightly enlargement and containing numerous vacuoles (v), mitochondria (m) and Golgi apparatus (g). ×12900

Fig. 35. Perikaryon of the visual cell containing nucleus (n) with a nucleolus (ne) and chromatin masses. Endoplasmic reticulum are concentrically arranged arround the nucleus. ×8700

Fig. 36 Enlarged photograph showing the nucleus of the visual cell and the nucleolus made of numerous small electron dense particles. Some chromatin masses attach the inner membrane of the nuclear envelope (indicated by arrow). Outer membrane undulates and makes contact with the inner membrane at intervals. ×38400


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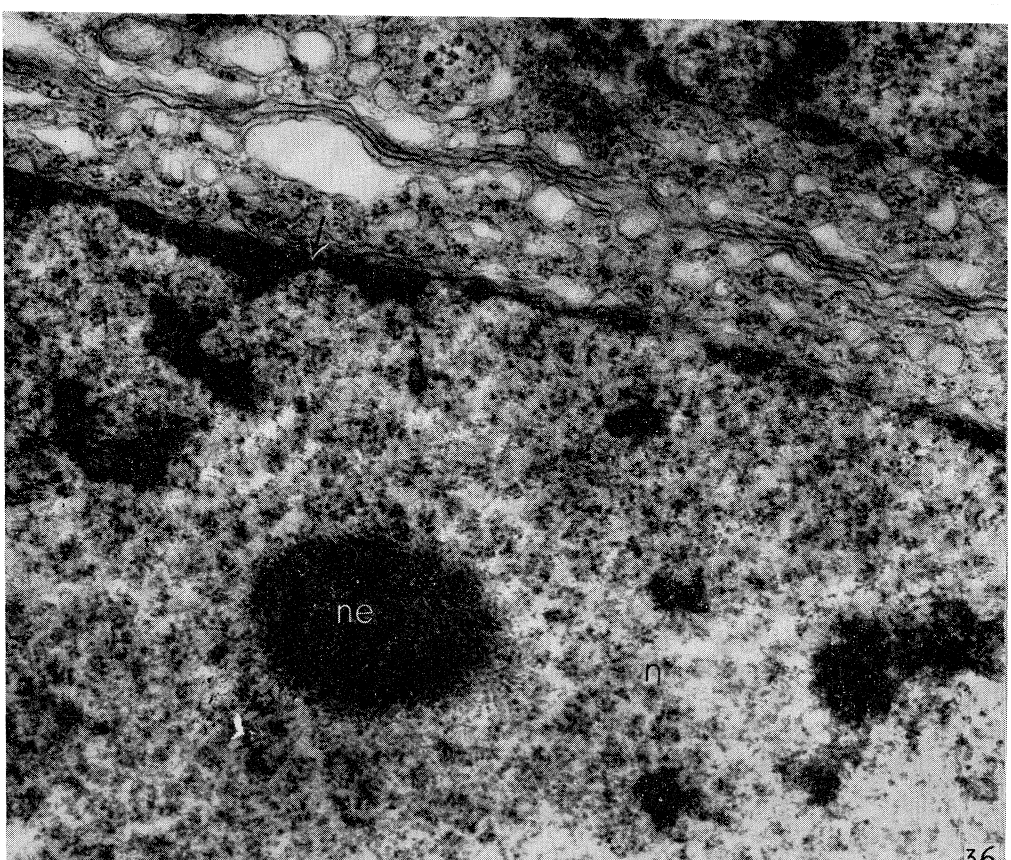
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n
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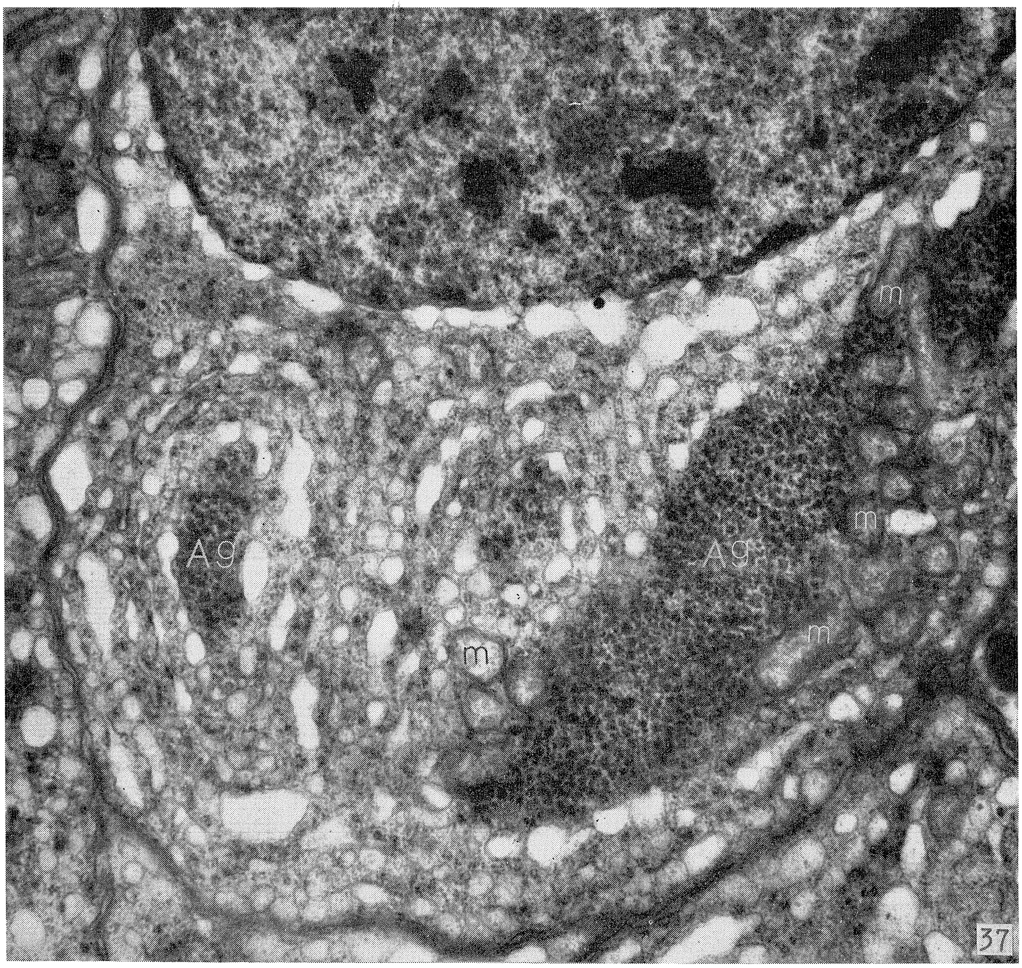
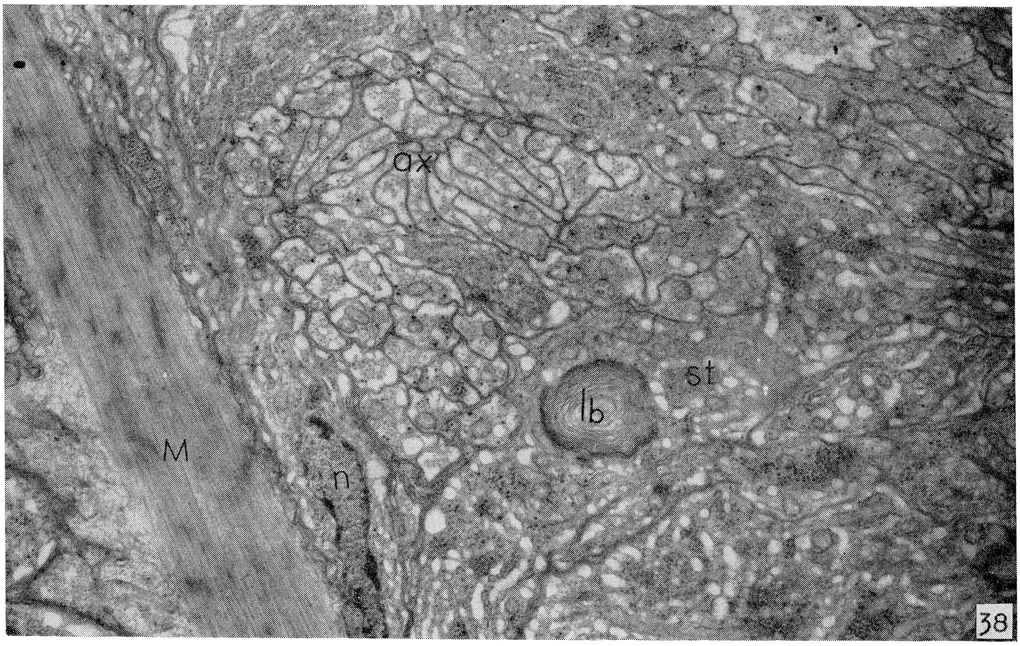


Fig. 37. A part of the perikaryon of the visual cell near the axon, containing aggregates (Ag) of electron dense particles. Endoplasmic reticulum are arranged in a helical fashion around the aggregates, and mitochondria (m) are associated with the aggregates. ×16500

Fig 38. Fibrous tissue in front of the eye, being an assembly of nervous fibers, some of which are stalks (st) and others are axon (ax) of the visual cells. Stalk possesses large myelinated body (Ib). Nuclei (n) are observable near the transverse muscle (M) in the outside of this tissue. ×6000

Fig. 39. Cross section of axons of the visual cells, containing the large vacuoles, mitochondria and neurotubules (nt). ×27000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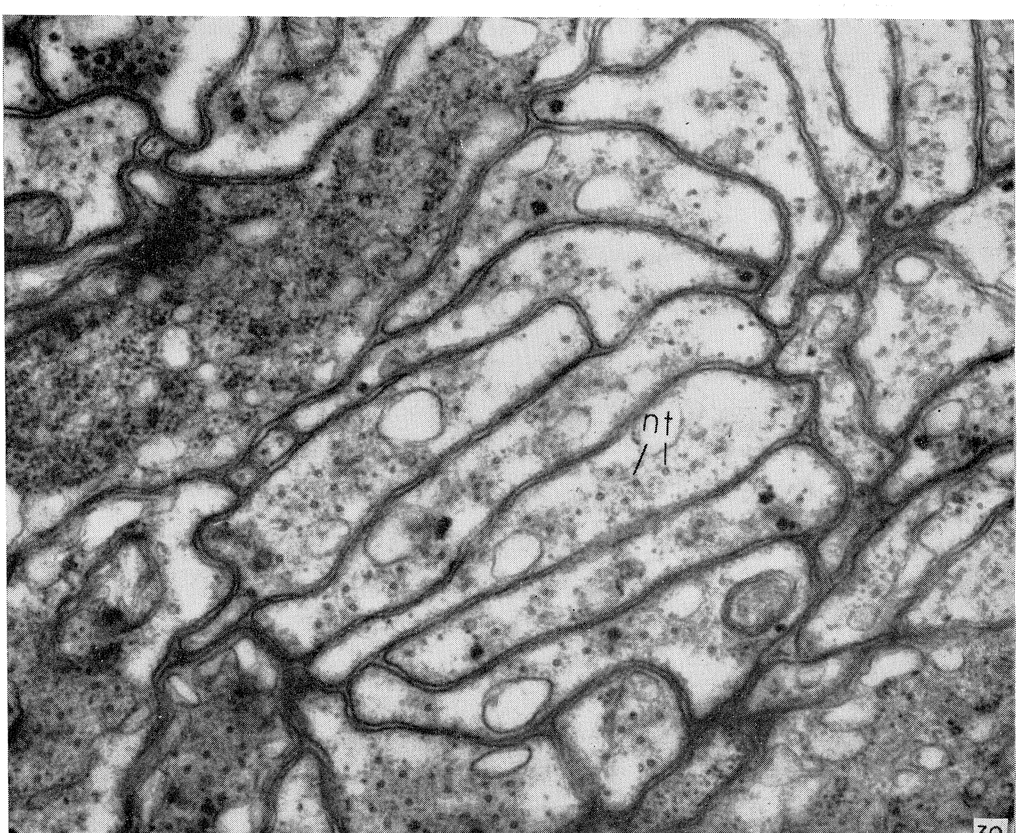
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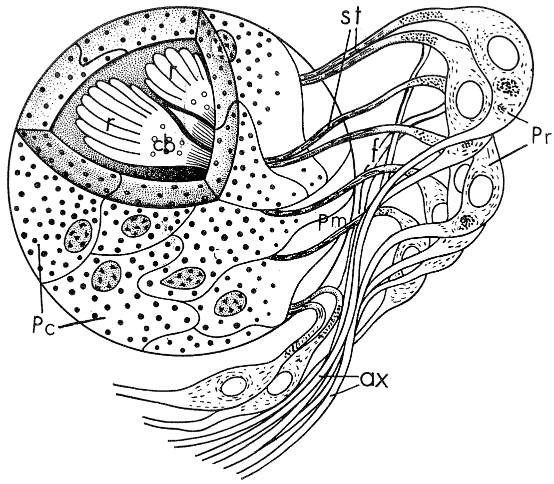

Fig. 40. Scheme showing the structure of the planarian eye. Pigment cells extend themselves to make the pluged membrane. Eye is spherical in total form. Tip portions of the rhabdomes located in the eye-cavity are exposed by removing a part of the pigment layer. Stalks of the visual cells run out through the pluged membrane. Fibrous tissue consists of the stalks and the axons of the visual cells.

ax: axon of the visual cell, cb: conical body of the visual cell, f: fibrous tissue in front of the eye, Pc: pigment cell, Pm: pluged membrane, pr: perikaryon of the visual cell, r: rhabdome of the visual cell, st: stalk of the visual cell.

Summary

1. The structure of the eye in planarian, *Dugesia japonica*, was studied mainly by electron microscope. The eye consists of two parts, i. e., one is the pigment cell layer and the other is the visual cell-mass. The visual cell is divided into five parts; rhabdome, conical body, stalk, perikaryon and axon.

2. The boundary between the eye pigment cells is very intricate, and the cells are strongly connected with each other by septate desmosomes. The endoplasmic reticulum in the pigment cell sometimes has the partially enlarged cisternae.

The eye pigment cells are packed with numerous pigment granules (about 1 μ in diameter) with high electron density. The granules are each enclosed by a limiting membrane, and can be divided into particular subunits of 50 mμ. In addition, two kinds of granules (designated as α-granule and β-granule) are found in the pigment cell. Besides, the large bodies which are divided into small chambers by the membranes are also seen. The pigment cells spread themselves over the so-called "opening region" of the eye and eventually form the pluged membrane. The pluged membrane contains occasionally a lot of vacuoles and small vesicles.

3. It is found that numerous tubular microvilli from 70-120 mμ in diameter are closely packed in rhabdome. Some of the microvilli can fuse partly one another, and others can branch into two. There occurs a thready material running longitudinally

in each microvillus. The proximal ends of the microvilli are sometimes intruded deeply into the conical body.

4. Numerous mitochondria, several vacuoles and vesicles with various sizes are seen in the conical body. It is noticed that the conical body contains a few neurotubules.

5. The stalk contains numerous neurotubules, multivesicular bodies and small vesicles. It pierces through the wall of the eye-cavity towards outside. Desmosomes are occasionally found between the stalk and the adjacent pigment cell. The proximal portion of the stalk enlarges itself and harvours numerous free ribosomes.

6. The stalks do not pass through a middle part of the pluged membrane, only through the peripheral part of the membrane.

7. The nucleus, generally ellipsoidal, is clear in the perikaryon, and the nucleolus in it is distinct. Most of the endoplasmic reticulum in thread form are of rough type, but the cisternae are partially enlarged and constricted. They are arranged concentrically around the nucleus. It is conspicuous feature that in the perikaryon near the axon, the mitochondria are found associated with mass of electron dene particles.

8. The profile of axon shows several mitochondria, some vesicles and neurotubules, like in the nerve fiber in the neuropil of the brain.

9. It is ascertained that the fibrous tissue in front of the eye-cavity is nothing but a mass of the stalks and axons of the visual cells.

10. The configuration of the planarian eye is depicted as the text illustration (Fig. 40).

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