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Electron Microscpic Studies on the Planarian Eye
II. Fine structures of the regenerating eye.

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Introduction

It has been well known that the fresh water planarians are favorable meterial for the studies of regeneration, because of their strongly regenerative power. A wealth of investigations, therefore, have hitherto been reported as to the histological studies of the regenerating sequence in various organs of this worm. Especially the eye has been utilized as a convenient criterion of regenerating head, and regenerating process of this organ itself has also been investigated in various species of planarians (Carrière, 1881; Jähnichen, 1897; Schultz, 1902; Stevens, 1902; Lang, 1913; Steinmann, 1926, Bandier, 1936; Lender, 1952).

Most of the previous investigators' conclusions about the eye regeneration are in agreement well with one another in a view point that the pigment cells appear first in regeneration blastema and the visual cells appear and finally these visual cells are connected with the brain by the optic nerve. However, the histological descriptions about the regenerating sequence are far from satisfaction. This may be ascribed to the fact that precise observation of the planarian eye is hardly possible due to difficulty of the technical procedures for histological preparation. The author has pointed out in the previous papers (1965, 1966) that the structures of the planarian eye could not be exactly revealed by a routine way of light microscopy. In fact, the pigment layer of the eye can only be seen as a cup-shaped pigment pile after pigment formation, and indeed it is almost impossible to specify the primitive pigment cells without the pigment granules in the early stage of regeneration. Furthermore, the form of the visual cell can easily be modified during preparating procedures by paraffin method, and its stainabilities are not always specific. Only we can distinguish them from the primitive pigment cells prior to the pigment formation by means of the electron microscope. Moreover, since the electron microscopic observation has revealed in the previous study that the rhabdome of the visual cell is composed of numerous microvilli, it can be expected that rudimentary form of the visual cells may be easily defined by these microvili.

Under these considerations, electron microscopic study was undertaken for the analysis of mechanism of eye-formation in the planarian regeneration. Special attention was particularly focused to the following problem. (1) What are the morphogenetic sequences of the pigment cell and visual cell ? (2) How the mutual relation can be realized between the pigment cells and visual cells ? (3) What, if possible, are the cellular origins of the eye pigment cells and visual cells ?

Material and Methods

The material used was a sexual form of *Dugesia japonica* collected at the suburb of Kanazawa city. After the worms were starved for ten days, they were decapitated at the level of the neck posterior to the auricles and reared in decalcified tap water at temperatures of 20-22°C. The worms during one to fifteen days after decapitation were provided for histological and electron microscopic studies.

For histological examination, the worms in various regenerating stages were fixed in Helly's solution, dehydrated in graded ethanol from 20% to 100% after being washed in running water, desublimated with iodine, cleared with toluol, embedded in paraffin and then sectioned 5-7 μ in thickness. Staining methods used were Mallory's triple staining and Heidenhain iron haematoxylin-light green.

For electron microscopic examination, regenerating worms were previously fixed in cold 6% glutaraldehyde buffered with phosphate buffer for 2 hours and washed in the same buffer and then cut into small pieces containing a regenerating eye. These pieces were post-fixed in 1% osmium tetroxide for 1 hour. After dehydration in acetone series, they were embedded in Epon 812 (Luft, 1961). The sections were cut with glass knives on JUM-5A type ultramicrotome and were mounted on collodion-coated copper grids stabilized with carbon or naked grids. Some thin sections were stained only with saturated uranyl acetate in water and the others with uranyl acetate followed by lead citrate according to Reynolds (1963). The preparations were examined with JEM-6A electron microscope.

Observation

Light microscopic observation

The process of head regeneration of decapitated worms was first observed with binocular dissective microscope. On the first day after decapitation, regeneration blastema was not formed, but accumulation of the subepidermal pigments was seen at the cut end of the worm. On the second day, the blastema was first recognized as a pigment-free cone, but it lacked the eye in every case. On the third day, the blastema grew up to the hemicircular form and the eyes were visible as grey or brown spots in some but not in other cases. On the fourth day, the brown eye spots appeared in the posterior border of the blastema in all regenerating worms, but they were easily confused with some of subepidermal pigments. On the fifth to seventh day, the eye spots became more black and larger in size. On the fifteenth day, the blastema developed to a brown triangular form, and the clear space around the eye spot was recognized.

On the first to second day of regenration, the paraffin sections indicated that the dorsal subepidermal mesenchyme cells began to migrate ventrad (Fig. 1 a), while the cut ends of the nerve cords were extending dorsad to meet them. However, there was no sign of the eye formation in this stage.

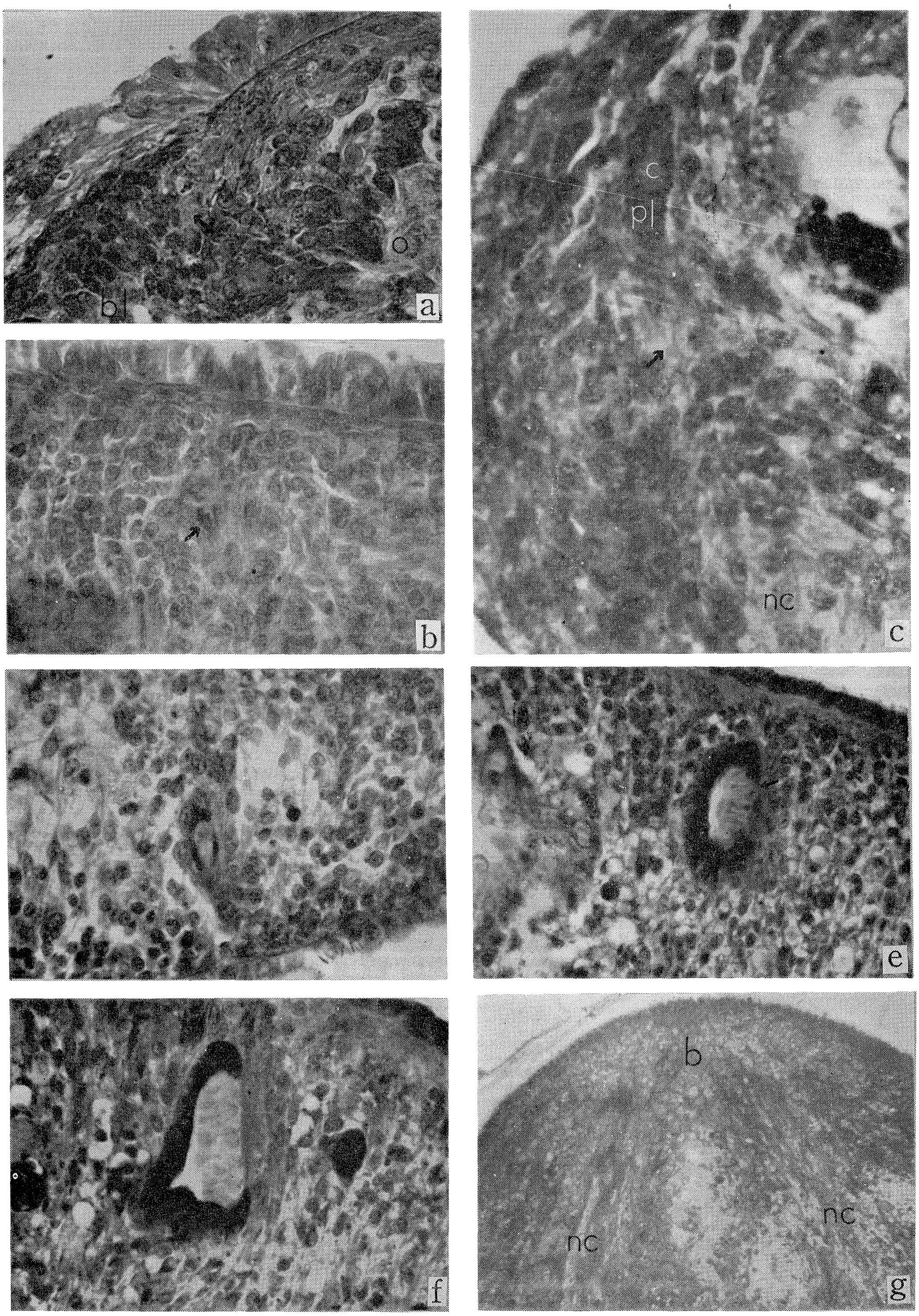
On the third day of regeneration, a few eye pigment cells were found sometimes scattering in mesenchyme (Fig. 1 b) and sometimes, as a small vesicle at a place where the aforesaid two kinds of cells had met (designated eye-vesicle hereafter) (Fig. 1c). The interior of the eye-vesicle was homogeneously stained light blue with the Mallory's triple stain. This seems to imply that in this stage no structures are yet developed in the eye-vesicle. The cut ends of the nerve cords extended several fine nerve fibers toward the dorsal side of the worm and connected them with the eye rudiment (Fig. 1c). On the other hand, the holizontal section of the blastema in the same stage revealed that the ends of the nerve cords of both sides fused together to give rise the brain (Fig. 1g).

On the fourth to fifth day of regeneration, it was found that the tip portions of the visual cells already existed in the eye-vesicle, so its interior showed heterogeneous staining (Fig. 1 d). On the sixth to seventh day, so-called "opening region" of the eye was clearly formed and consequently the eye-vesicle became cup-shaped with respect to its pigmentation. The fan-shaped rhabdomes of the visual cells were also distinguishable. Namely, the regenerating eye in this stage appeared to become the normal eye in its configulation but for their small sizes (Fig. 1 e). On the fifteenth day, the regenerating eye was not different at all from the normal eye (Fig. 1 f).

Electron microscopic observation

The third day of regeneration. The regenerating eye rudiment was observed with electron microscope, and found that several but not all eye pigment cells contained a small number of pigment granules (Fig. 2). These pigment cells were elongated and in contact with one another. But they were connected with one another by the septate desmosomes only at the portions near the rhabdomes described just below. Here it should be particularly noted that in this stage the rhabdome of the visual cell is already differentiated, and it is composed of many microvilli (Fig. 2). The rhabdomes were not, however, gathered together into a single mass as in the normal eye but were kept apart by one or two.

The nucleus of the pigment cell was in a long oval form with deep infoldings and several remarkable chromatin masses were seen in it (Fig. 3). Some of the chromatin



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strands were in contact with the nuclear envelope. This nucleus appeared as a whole considerable electron dense because of the small dense particles of about 100-200 Å. Moderate electron dense granules (about 200 mμ in diameter) were seen frequently in the deep nuclear infoldings.

It was noteworthy that the pigment cell nucleus contained a very large nucleolus (1-1.5 μ in diameter) of a ring form due to close aggregation of electron dense particles of about 150 Å in the periphery, while pale in the center due to lack of these particles. These ring-formed nucleoli of the eye pigment cells were recognizable only in the earlier stages than 10 days of regeneration.

The pigment granules specific in the pigment cell were yet rarely seen. Instead, vacuoles were often encountered (about 0.5 μ in diameter) in which highly electron dense subunit particles with pigment (about 50 mμ) were loosely accumulated. In addition, empty vacuoles of the same size with the pigment granules, the vacuoles including the small vesicles and pigment subunit particles (Figs. 4 and 5) were visible; these vacuoles may be referred to as "pigment-vacuoles", because they will be in future filled with the pigment subunit particles to give rise the perfect pigment granules.

Free ribosomal particles were very abundant and the rough-surfaced endoplasmic reticulum of thread form were also numerous. The elongated sausage-like mitcchondria were distributed throughout the cytoplasm but especially in plenty near the nucleus. Several Golgi apparatus which were composed of lamellae and vesicles were found in each pigment cell (Fig. 6).

As described previously, the rhabdomes of the visual cells were not differentiated to the normal structure of fan-shape but to crowding of many microvilli (Fig. 7). Each microvillus was 12-14 mμ in thickness and a distance between them was about 1.3 mμ. They were not arranged in parallel as in the normal.

Accumulation of large mitochondria characteristic in a conical body was not yet

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Fig. 1. Explanation of the light photomicrographs of the regenerating eye.

a. Presumptive eye region being shown by influx of the subepidermal tissues (indicated by arrow) into the region between the blastema (bl) and old tissue (o) on the second day of regeneration. ×400

b. Onset of the eye-formation on the third day of regeneration. The eye pigment cells (indicated by arrow) appear but any visual cells are not discernible. ×400

c. Showing connection between an eye-rudiment and the nerve cord on the third day of regeneration. The pigment layer (pl) is vesicular form in which the cavity (c) is homogeneously stained. Notice the vesicle being connected with a nerve cord (nc) by a fine nerve fibers (indicated by arrow). ×700

d. An eye on the fourth day of regeneration. The eye cavity is heterogeneously stained. ×315

e. An eye on the seventh day of regeneration. The cup-shaped pigment layer and thin pluged membrane (indicated by arrow) are observable. The eye-cavity contains considerable numbers of the tip portions of the visual cells. Fan-shaped rhabdomes and conical bodies are darkly stained. ×315

f. An eye on the fifteenth day of regeneration. Structure of the eye is almost the same with that of the normal eye. X 315

g. Holizontal section representing the formation of the brain on the second day of regeneration. The brain (b) is an area in which two nerve cords (nc) are fused together. ×100

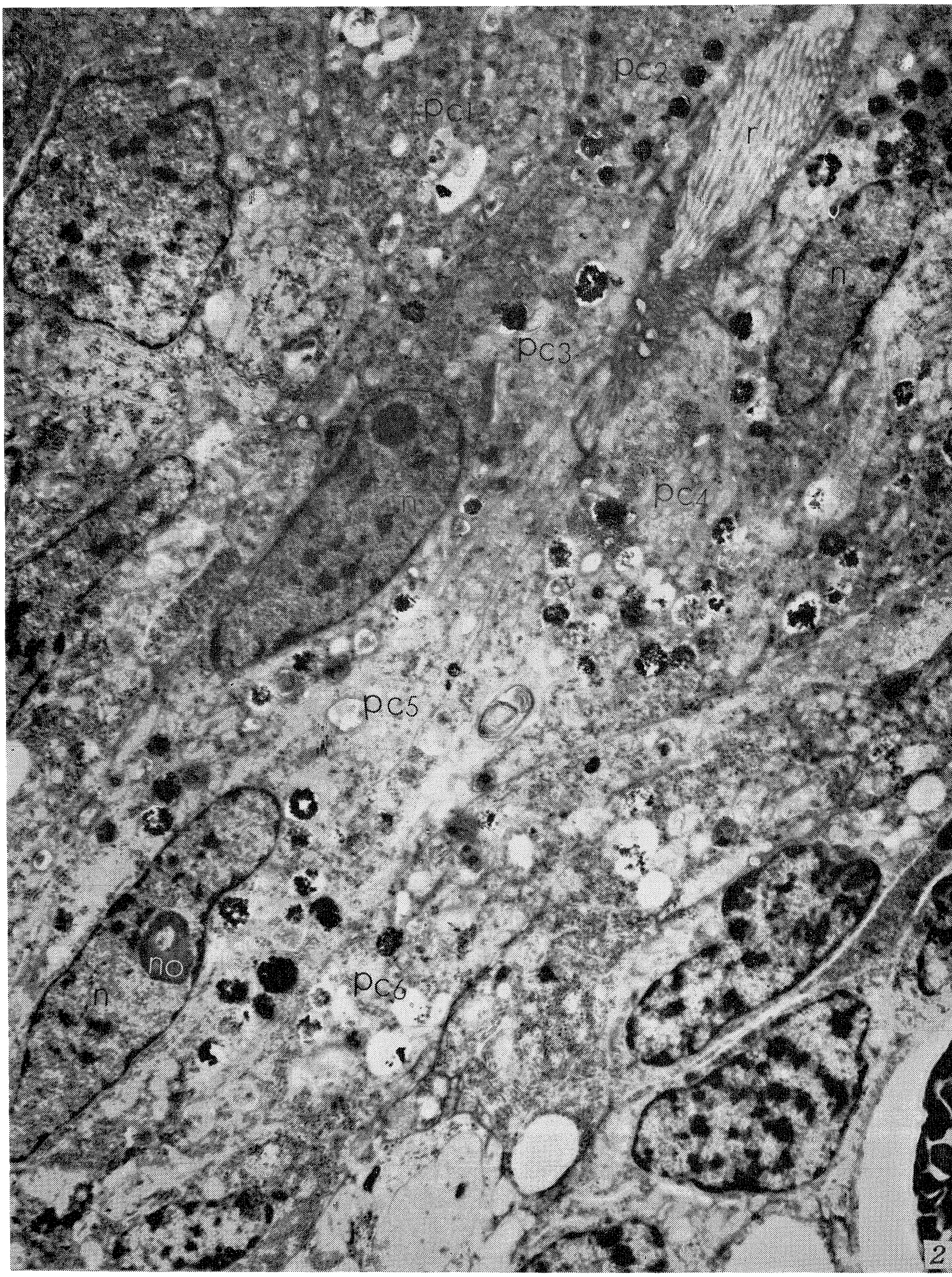
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Fig. 3. Infolding of the nucleus (n) of the eye pigment cell on the third day of regeneration. Moderate electron dense granules (gr) are seen in the infloding and its neighbourhood. ×42000

Fig. 2. Electron micrograph showing an eye-rudiment on the third day of regeneration. Pigment cells (pc 1–6) gather in close contact. Rhabdome of a visual cell is observed to lie singly between the pigment cells (pc 2,3 and 4). Pigment cells elongate themselves and their nuclei (n) are of long ellipsoid-form, one of which includes a ring-formed nucleolus (no). ×6200


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shown. It was, however, observed that some of the limiting membranes of microvilli of the rhabdome were intruded deeply into this conical body as in the normal cells. The conical body contained the vesicles of 100-200 mμ in diameter and fine filaments (Fig. 8).

The stalks of the visual cells were about 1.3 μ in thickness, in which numerous typical neurotubules (20-30 mμ thick) ran longitudinally, and free ribosomal particles and some large mitochondria were also clearly visible in it (Fig. 9). However, such small vesicles, vacuoles and especially multivesicular bodies as were seen in the stalk of a normal visual cell were not yet found. Furthermore, there were not developed any connecting apparatus between stalk and pigment cell.

The fourth to fifth day of regeneration. The cells of the eye-vesicle were roughly arranged in a single cell layer to form the hollow pigment globe. The septate desmosomes were found connecting these adjacent cells with each other (Fig. 14).

The nucleus of the pigment cells was irregular and lobulate in form with several deep infoldings (Fig. 11).

Free ribosomal particles were found in cluster (Fig. 12). The cisternae of rough-surfaced endoplasmic reticulum were strikingly enlarged here and there into vesicles. In their neighbouring well developed Golgi apparatus were frequently encountered.

Pigment vacuoles increased in number and some of them were laden with electron dense subunit particles to grow up into the pigment granules in true sense. The figures were occasionally observed that indicated a possible relationship between the development of the Golgi apparatus and the formation of pigment (Fig. 13).

The rhabdomes of the visual cells gathered together in the cavity of the pigmented globe described above (Fig. 10), while their proximal portions outside the globe were tied up together into a band.

The microvilli of the rhabdome were developed very well, and their arrangement was nearly normal (Fig. 15). A few large mitochondria with short cristae were observed in the conical body, but pronounced accumulation of large mitochondria specific to this region was only at the start (Fig. 16). The stalk contained a large number of neurotubules and some mitochondria just as in that of the established visual cell.

The sixth to seventh day of regeneration. The microvilli of the rhabdome were arranged in completely regular form (Fig. 17), while a number of mitochondria and numerous small vesicles of about 10-50 mμ were arranged longitudinally in the conical body (Fig. 18). The stalks contained many vacuoles, large multivesicular bodies and

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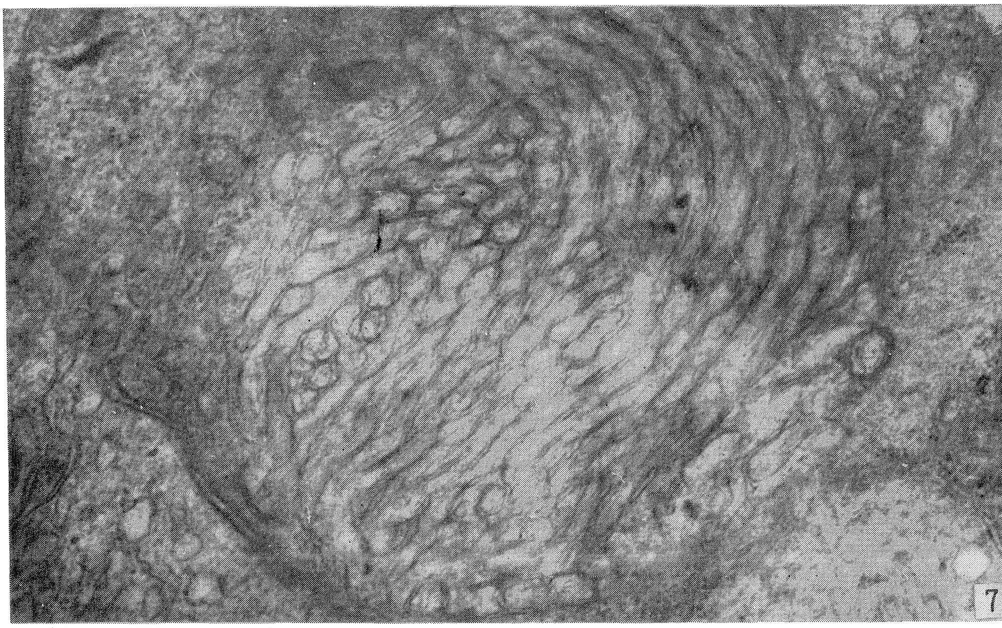
Fig. 4. Pigment vacuoles in the eye pigment cells on the third day of regeneration. Each vacuole is enveloped with a limiting membrane and contains small vesicles and pigment particles (p). ×28000

Fig. 5. Additional type of pigment vacuoles which contain vesicles and mediated electron dense substance. ×54000

Fig. 6. Eye pigment cell on the third day of regeneration. Numerous free ribosomal particles, Golgi apparatus (g) and rough-surfaced endoplasmic reticulum (er) are visible. n: nucleus, P: pigment granule, m: mitochondria. ×30000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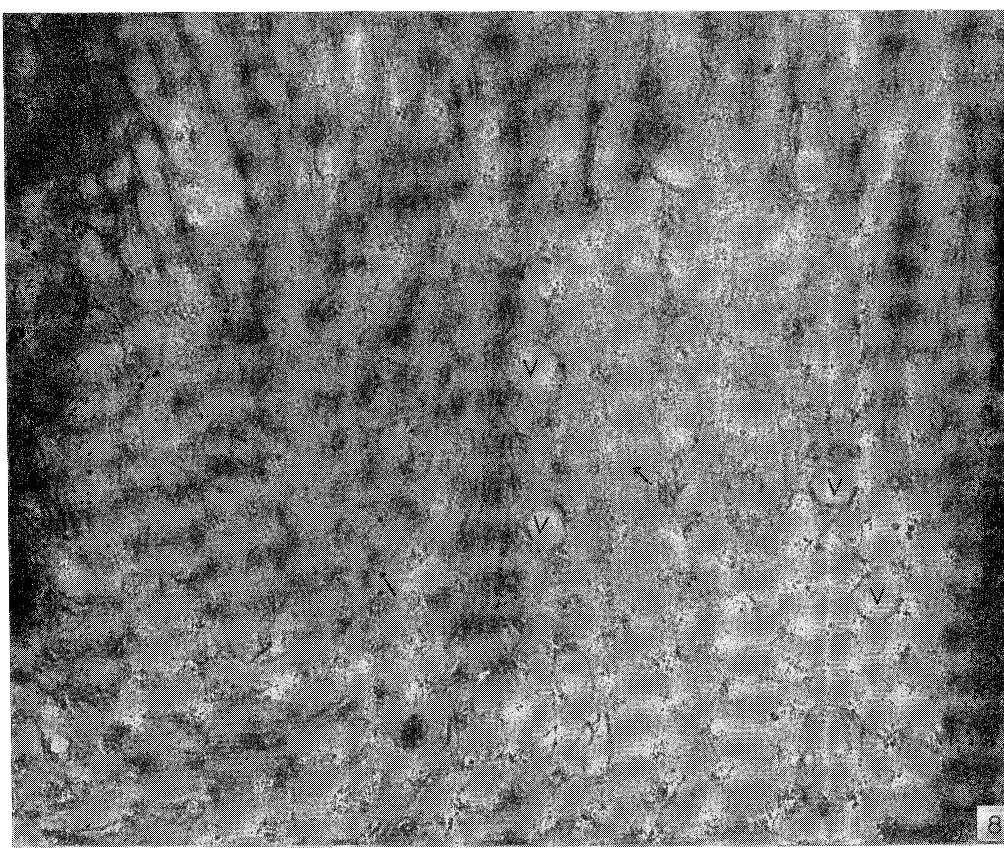
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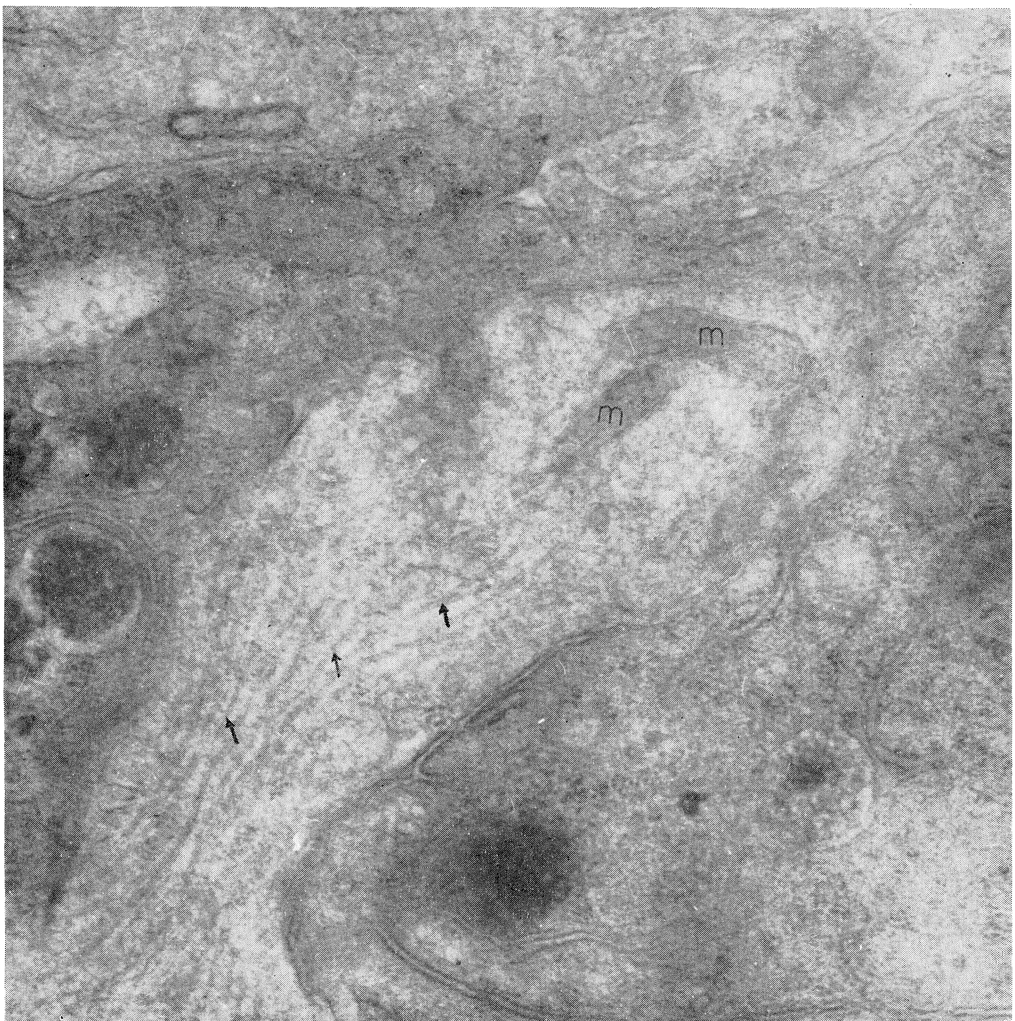

Fig. 9. Stalk of the visual cell on the third day of regeneration. The neurotubules (indicated by arrows) are developed as well as in the normal eye. m: mitochondria ×37200

Fig. 7. Rhabdome of the visual cell on the third day of regeneration. The microvilli are developed very well, but not arranged in parallel to each other, so transverse and longitudinal sections of microvilli are observed at random. ×38700

Fig. 8. Conical body of the visual cell on the third day of regeneration. The conical body contains vacuoles (v), fine filaments (indicated by arrows), but not mitochondria. It is seen that membranes of the microvilli of the rhabdome intrude deeply into the conical body (as interpreted in the text). ×42000


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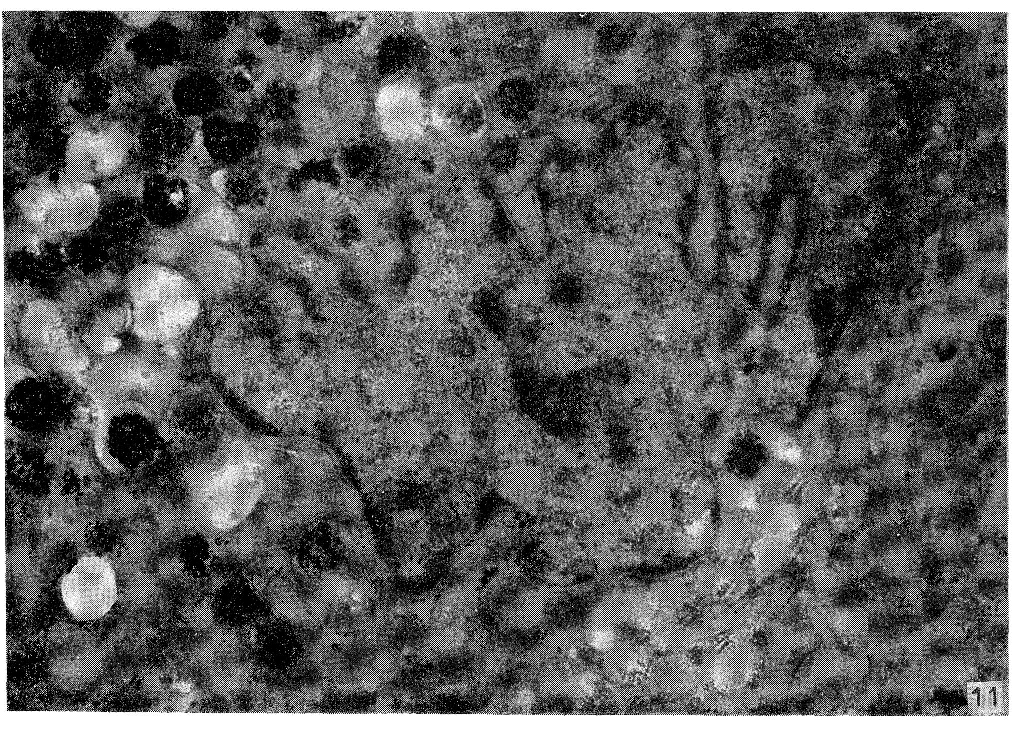
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numerous neurotubules (Fig. 19). The structure of the visual cell in this stage was almost the same with that of the normal visual cell observed previously (Kishida, 1967).

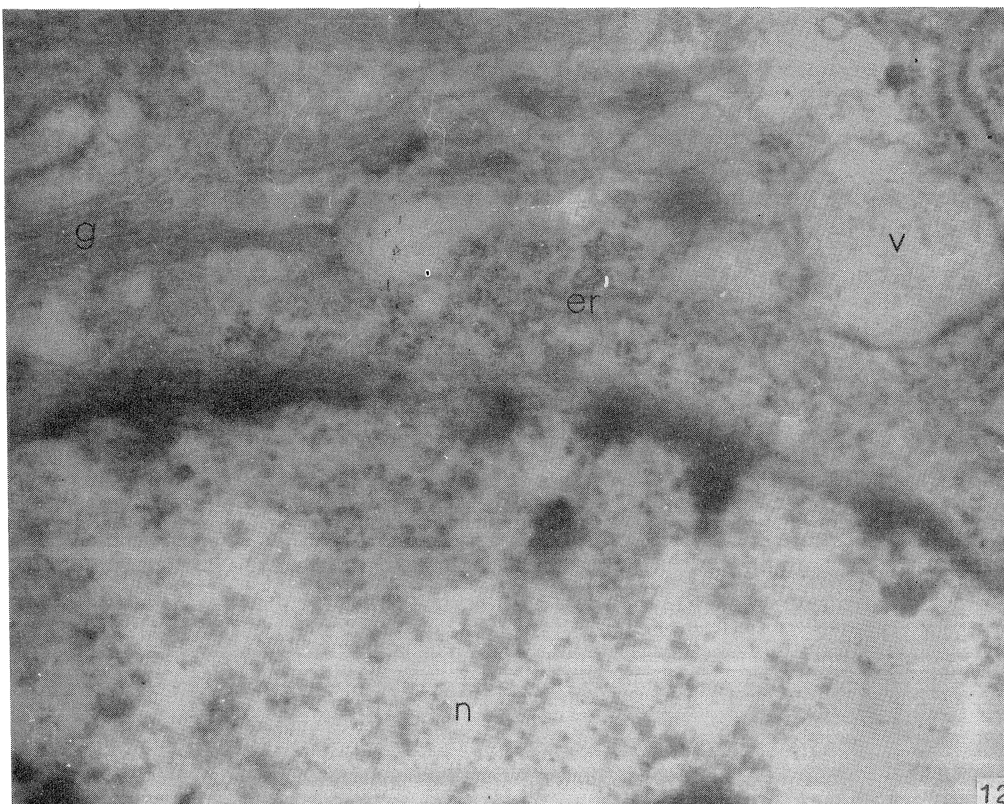
On the other hand, the pigment cells in this stage were rather in primitive state and their nuclei had deep infoldings as descibed previously, though the infoldings became less remarkable than those on the fifth day of regeneration (Fig. 20). In addition, a ring-formed nucleolus was still recognizable in the nucleus of the pigment cell. The pigment vacuoles with a few sub-pigment particles were fairly abundant and the rough-surfaced endoplasmic reticulum were well developed with enlarged cisternae (100-150 mμ in thickness) in a rosary fashion. They arranged themselves concentrically near the nucleus (Fig. 21). Several Golgi apparatus were visible in each cell (Fig. 20). A considerably large number of free ribosomal particles were scattering in the cytoplasmic matrix (Fig. 21). Comparing with the normal eye, the features such as described above were typical as the regenerating eye pigment cell. Some of the wall cells, pigment cells, of the eye-cavity became thinner and contained only a few pigment granules. From the position and the ultrastructures of cells thus modified, it is sure that these cells will form the pluged membrane of the eye. It was further ascertained that the growth of the pigmet layer was performed by addition of the presumptive pigment cells from outside between the pigment cells. As in Fig. 22, cytoplasmic protrusion of such inserted pigment cell had well-developed rough-surfaced endoplasmic reticulum, and scarce pigment granules.

The tenth to fifteenth day of regeneration. The pigment cells were normally arranged in a layer (Fig. 23) and the pluged membrane appeared as pieces in the histological sections because of existence of the stalks of the visual cells (Figs. 23 and 24). The incisions of the pigment cell nuclei disappeared and the nucleoli were not always observed. Most of the pigment vacuoles grew up to the pigment granules and were made of numerous sub-pigment particles of high and homogeneous electron density (Fig. 25). Neverthless, several Golgi apparatus were found in each cell (Fig. 26) and rough-surfaced endoplasmic reticulum were still recognizable, although small in number (Fig. 27).

Concerning the visual cells in this stage, arrangement of the microvilli of the rhabdome and other features were all identical with in the normal eye (Fig. 28).

Fig. 10. The regenerating eye on the fifth day. Pigment cell layer (Pl) covers a mass of the microvilli of the rhabdome (r) arranged almost in parallel. Stalks (st) of the visual cells are running out of the eye-cavity through the pigment layer. Notice desmosomes (indicated by arrows) between the stalks and the pigment cells. ×10800

Fig. 11. Nucleus (n) of the pigment cell on the fifth day of regeneration. The conspicuous infoldings are seen. ×15900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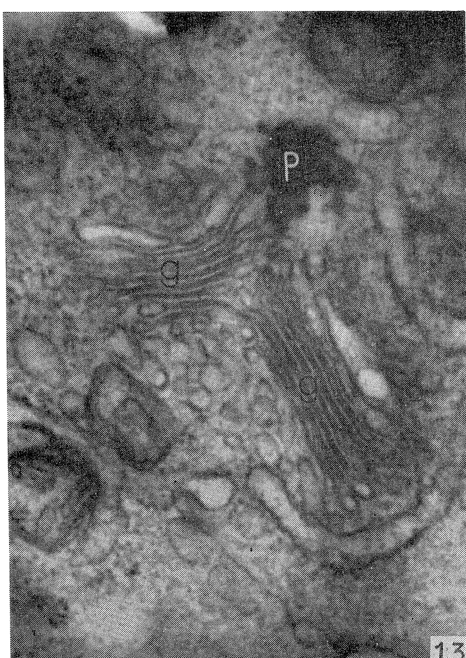
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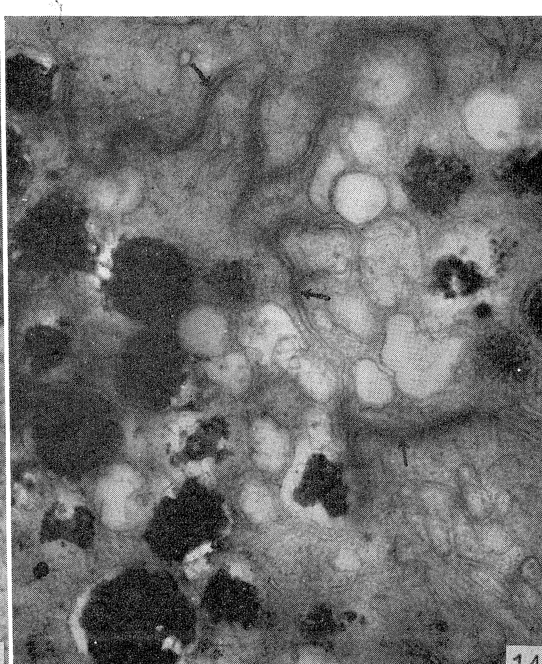
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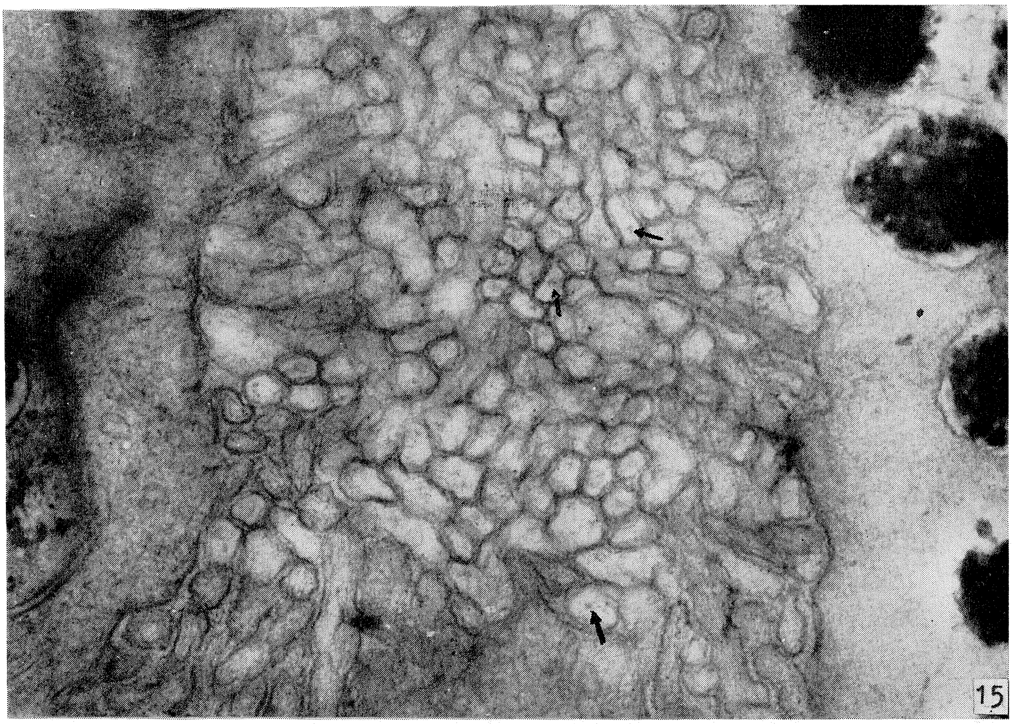


Fig. 15. Transverse section of the rhabdome of a visual cell on the fifth day of regeneration. The microvilli contain a moderate electron dense spot (indicated by arrows). ×42000

Fig. 12. Cytoplasm of the eye pigment cell on the fifth day of regeneration. Numerous ribosomal particles, rough-surfaced endoplasmic reticulum (er) and a Golgi apparatus (g) are visible. Cisternae of the endoplasmic reticulum enlarge partly and gives a impression to give rise a vesicle (v). n: nucleus. ×42000

Fig. 13. Golgi apparatus (g) showing a possible relationship between it and the formation of the pigment granules (P) on the fifth day of regeneration. ×42000

Fig. 14. Showing the complicated contact between adjacent pigment cells with the septate desmosomes (indicated by arrows) on the fifth day of regeneration. ×23700

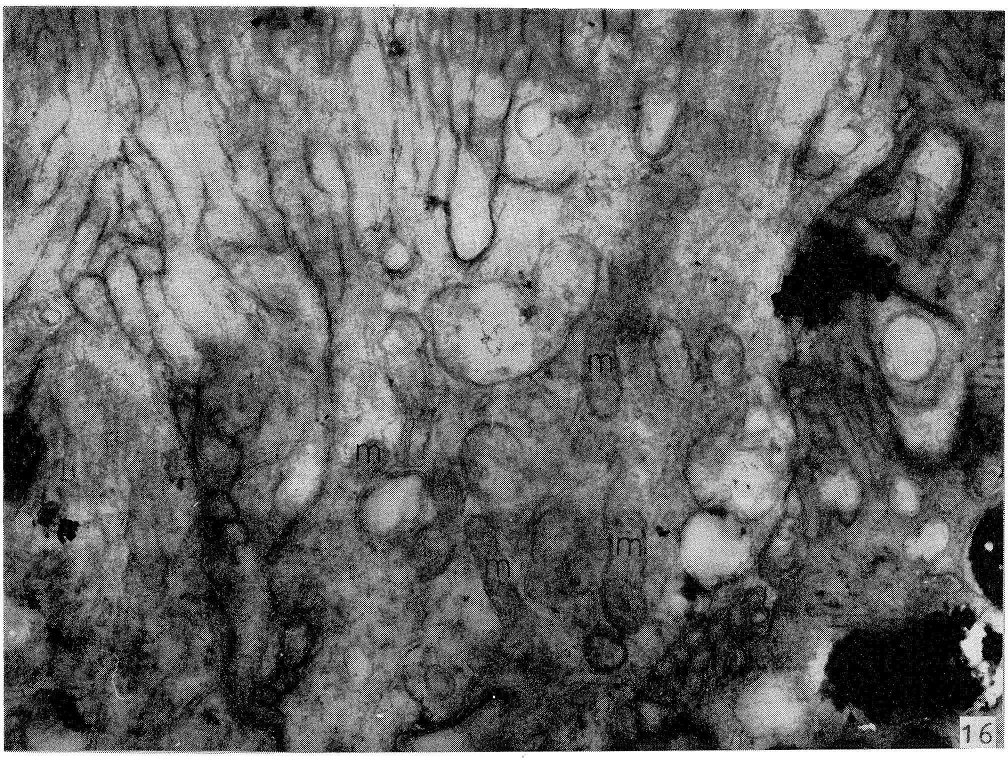
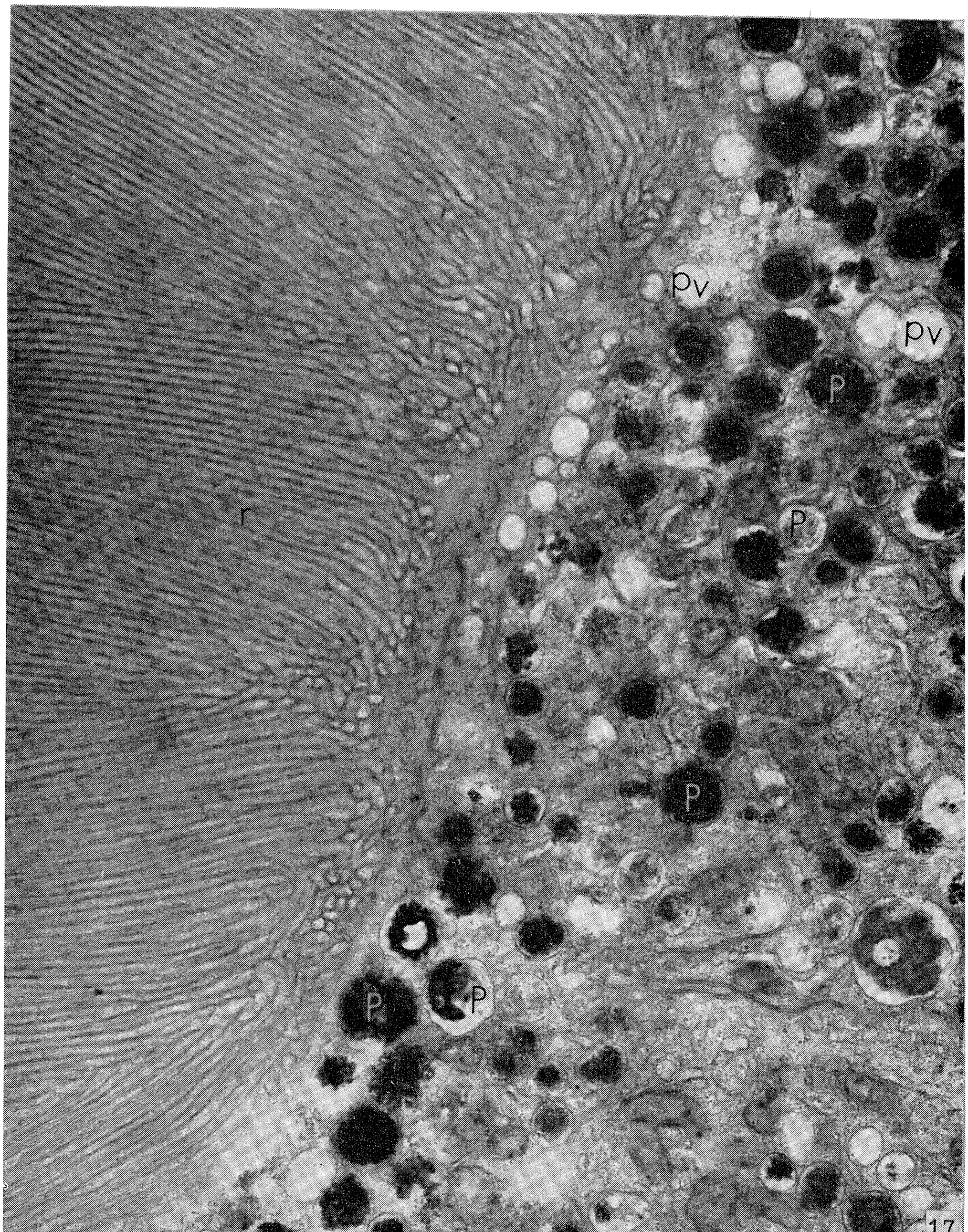


Fig. 16. Conical body of a visual cell on the fifth day of regeneration. The mitochondria (m) begin to increase in number. ×23700

Fig. 17. Showing inner surface of the pigment cell layer and tip ends of the visual cells on the seventh day of regeneration. The pigment cells contain less numerous pigment granules (P) than in the normal eye but each pigment granule is not yet enough filled with the pigment substance. Considerable numbers of the empty vacuoles (pv) which may become pigment vacuoles are visible. Microvilli of the rhabdome (r) are developed very well. ×15450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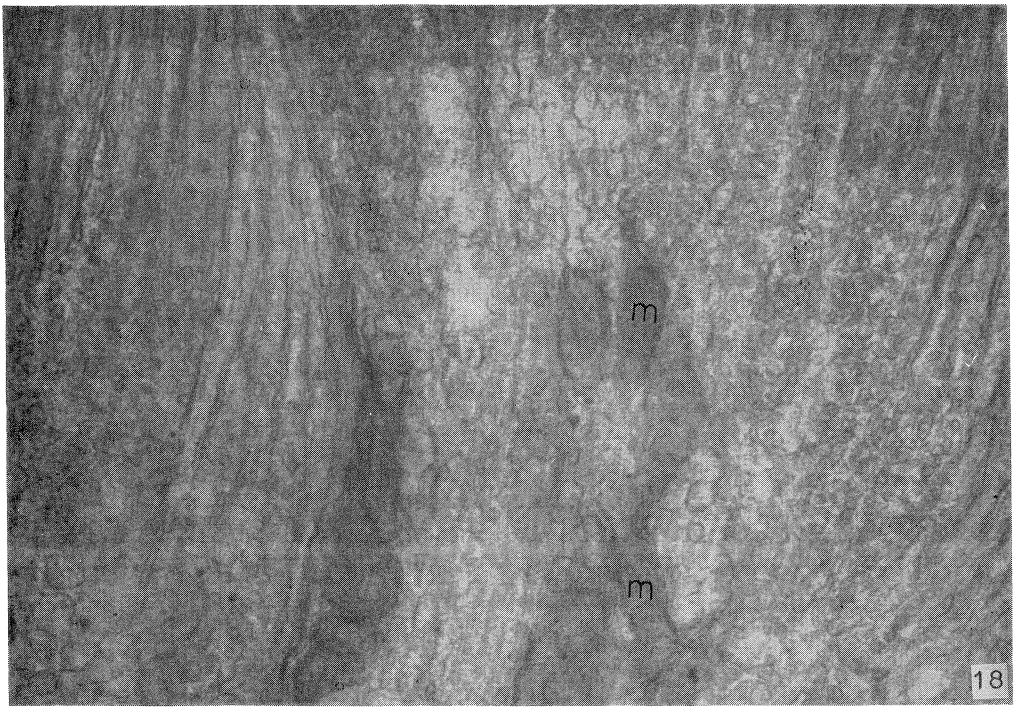
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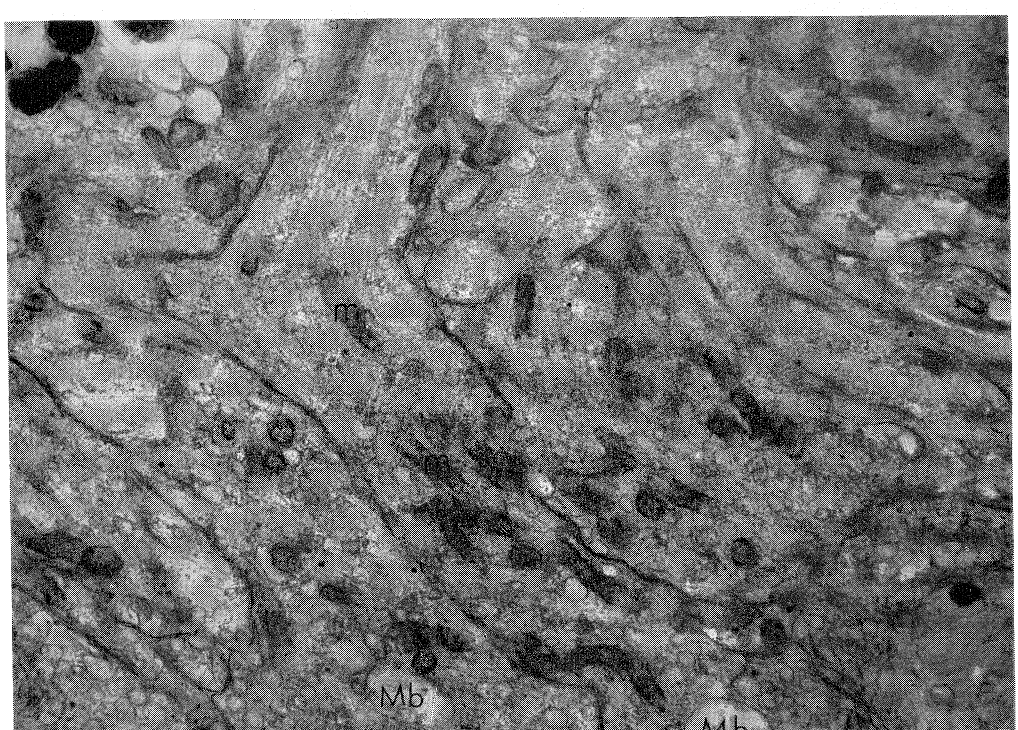
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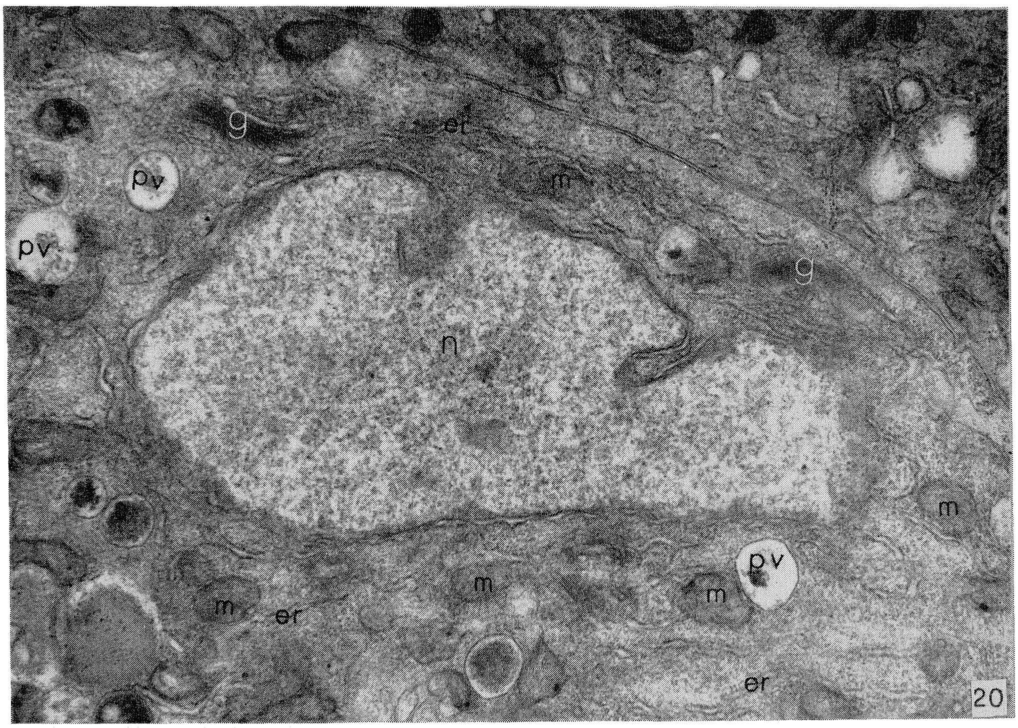


Fig. 20. Region near the nucleus of the pigment cell on the seventh day of regeneration. Well-develobed Golgi apparatus (g)are visible near the nucleus (n) with deep infoldings. Numerous free ribosomal particles, endoplasmic reticulum (er), mitochondria (m) and pigment vacuoles (pv) are observed. ×17100

Fig. 18. Conical body of the visual cell on the seventh day of regeneration. The mitochondria (m) and numerous small vesicles are seen in matrix. ×42000

Fig. 19. Stalks of the visual cells on the seventh day of regeneration. The stalks contain numerous neurotubules, well-developed mitochondria (m), many vesicles and some multivesicular bodies (Mb) as in the normal stalk. ×12300

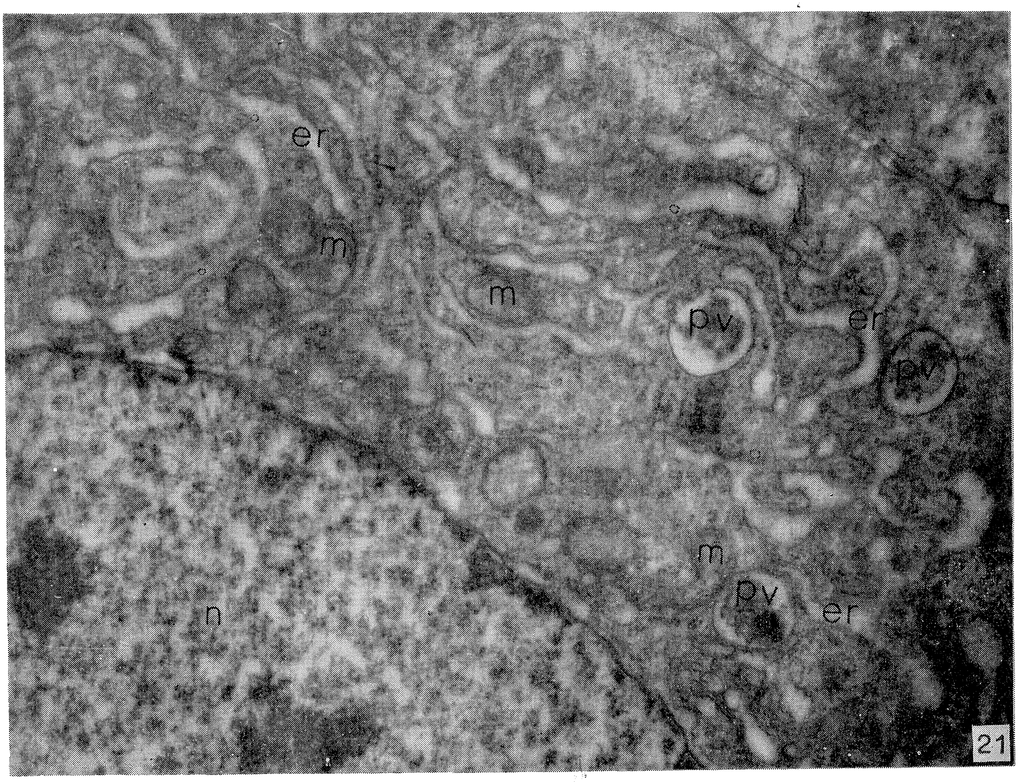
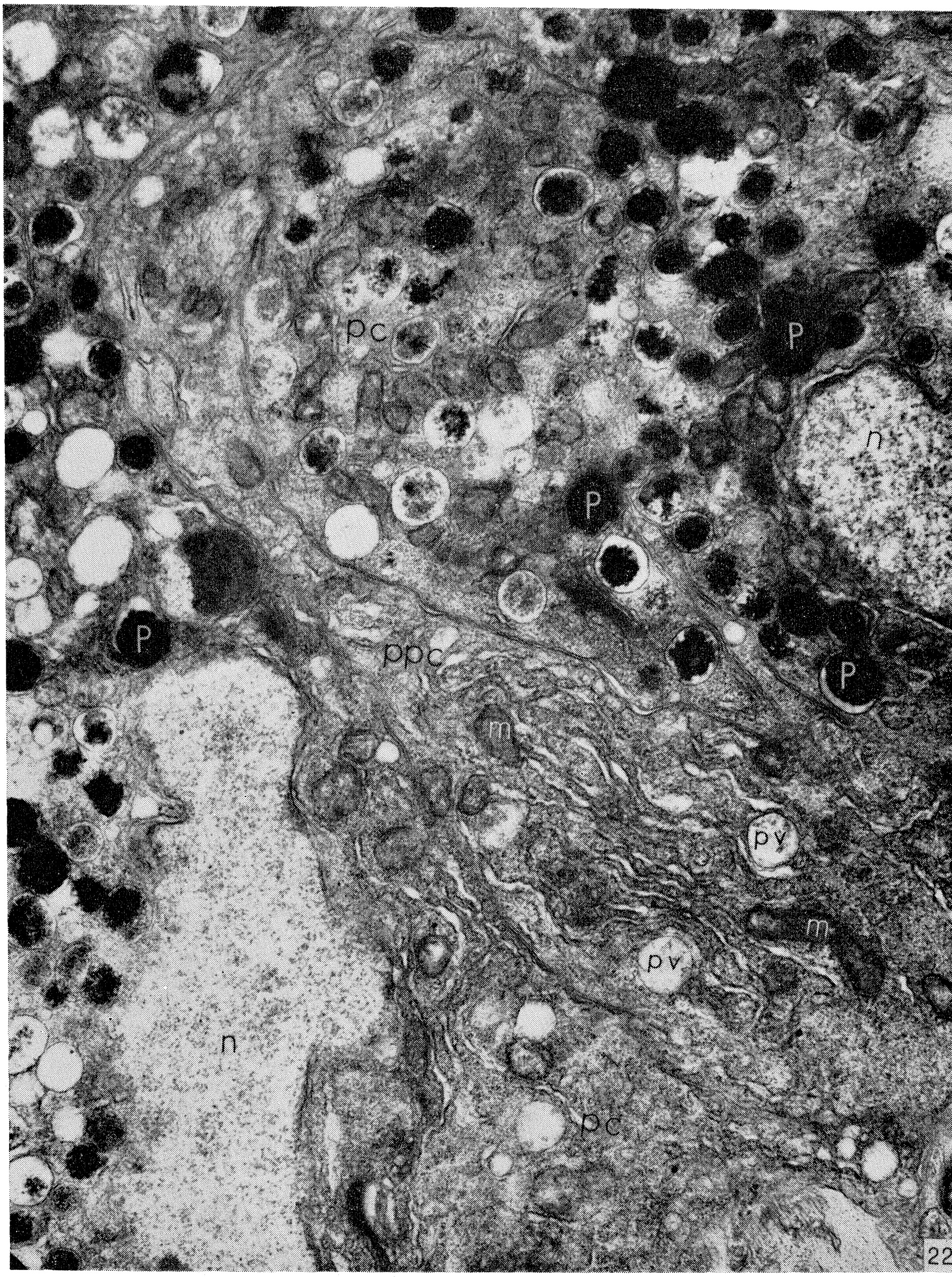


Fig. 21. Enlargement of the similar photograph showing the complicated profile of the cytoplasm near the nucleus (n) of the pigment cell on the seventh day of regeneration. Numerous free ribosomal particles scattered throughout cytoplasm and rough-surfaced endoplasmic reticulum (er) are evidently observed. The pigment vesicles (pv) are presented near the rough-surfaced endoplasmic reticulum and mitochondria (m). ×36000

Fig. 22. Primitive pigment cell wedging itself between two pigment cells to take share in the construction of the pigment cell layer of the eye on the sevneth day of regeneration. Elongated cytoplasm of the primitive pigment cell (ppc) does not yet contain the pigment granules but a few pigment vacuoles (pv). m : mitochondria, n : nucleus, pc : pigment cell, P : pigment granule. ×11400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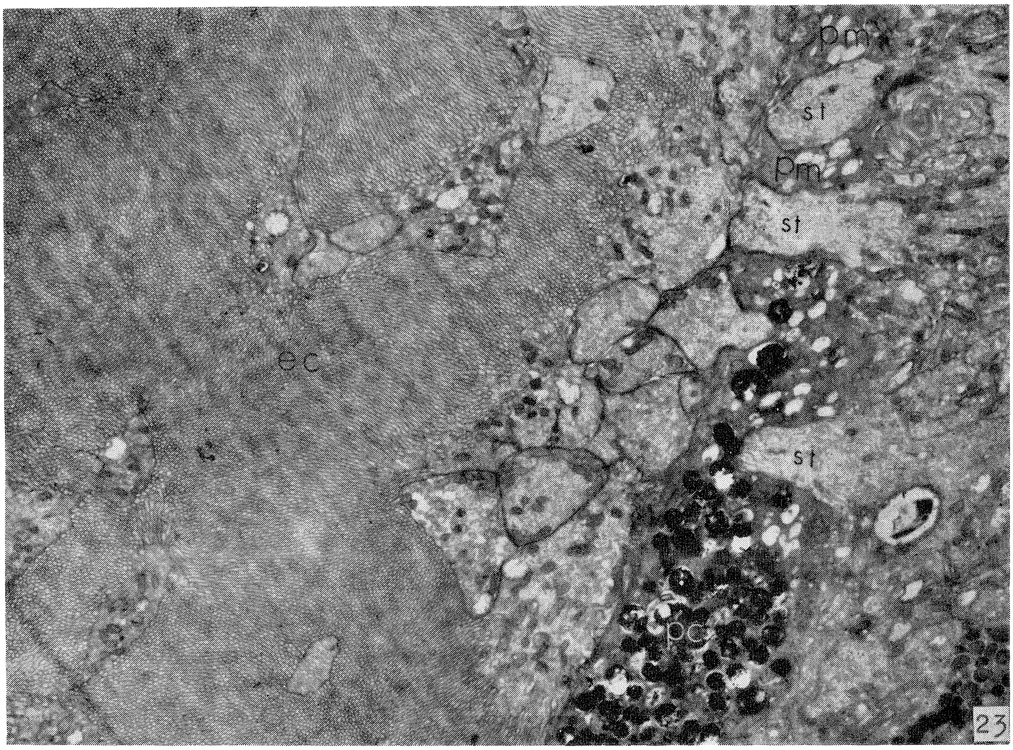
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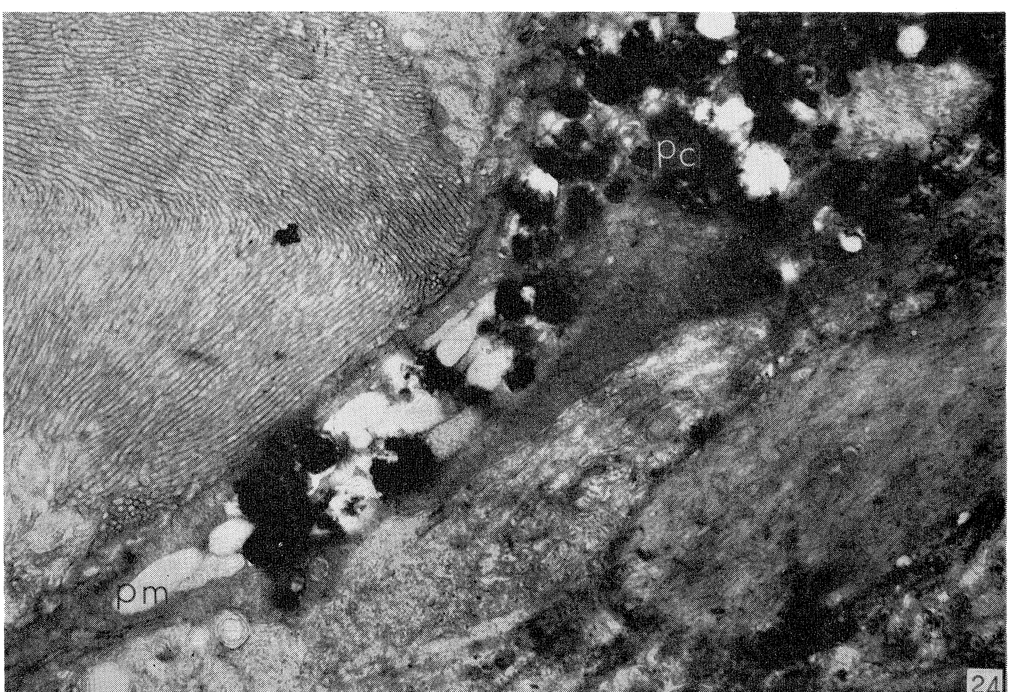
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Discussion

The planarian eye is composed of two components, i. e., the visual cells and the pigment cells. Special regardness, therefore, should be paid for the morphogenetic interrelation between the two components in the study of eye regeneration. Neverthless, most the previous investigators have devoted themselves to study separately the formation of each component, and therefore they have made almost no contribution to their mutual relationship, save for a few descriptions. Prielgauskiene (1933) stated with *Polycelis nigra* that the pigment cells were induced by the optic nerve, but his view was denied by Bandier (1936) and others. Lender (1952) claimed that the eye pigment cells were first induced by the neurosecretory substance coming from the brain without any morphological connection, for he could not histologically recognize the visual cells and optic nerves in the early stage of eye regeneration.

Indeed, it may be impossible to ascertain either presence or absence of the visual cells with light microscope in this stage of regeneration, for the form and stainabilities of the visual cell are rather shifty as previously pointed out (Kishida, 1967). Furthermore, the action of the neurosecretory substance from the brain alone may not be necessarily responsible for the eye regeneration. If the neurosecretory substance is possible agent for the eye regenration, it is desirable to elucidate first how the locality of the eye formation is settled. Needless to say that the present study and other recent electron microscopic studies (Morita and Best, 1965; Oosaki and Ishii, 1965) could not demonstrate the neurosecretory granules liberating from the brain.

In addition, it was found in the present study, that fairly well developed visual cells were in contact with the primitive pigment cells even in the early eye rudiment without a cavity in it. Moreover, it was observed that the visual cells had already well developed microvilli in the rhabdome and numerous neurotubules in the stalk even in the stage in which the pigment cells were only in the biginning of the pigment granule formation. In these situations, it may be reasonable to consider that first appearance of the visual cells is earlier than the pigment cells, and that differentiation of the pigment cells may be under the influence of the visual cells but not of the neurosecretory substance from the brain. This problem is related closely to the origin of the visual cell and the pigment cell as is discussed below.

There are few investigators who have paid their attention to the origin of the cells which participate in the formation of the eye in the planarian regeneration. Jähnichen (1897) described the mesodermal or endodermal contribution to the formation of the eye pigment cells, but no comment about the origin of the visual cells. Lender (1952) stated that the visual cells and pigment cells are derived from the

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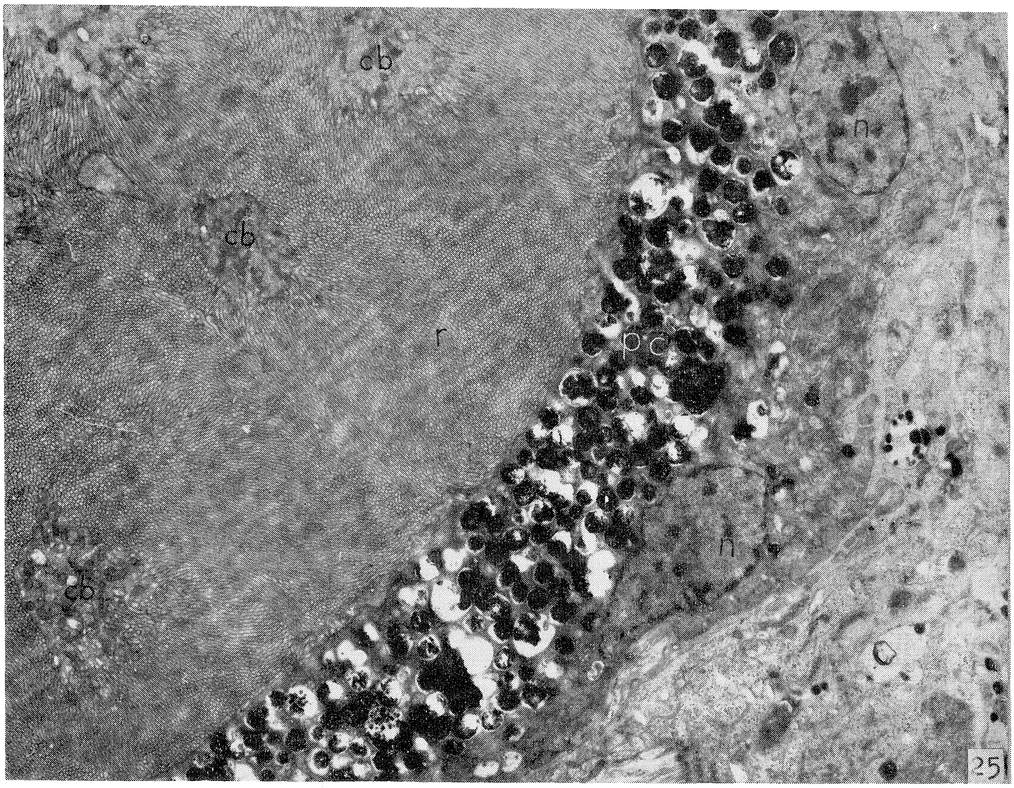
Fig. 23. Region near the pluged membrane of the eye on the fifteenth day of regenration. Stalks (st) go away from the eye-cavity (ec) through the pluged membrane to the outer side. pm: pluged membrane, pc: pigment cell. ×4500

Fig. 24. Extention of the pigment cell (pc) as the pluged membrane (pm) of the eye on the fifteenth day of regeneration. ×8400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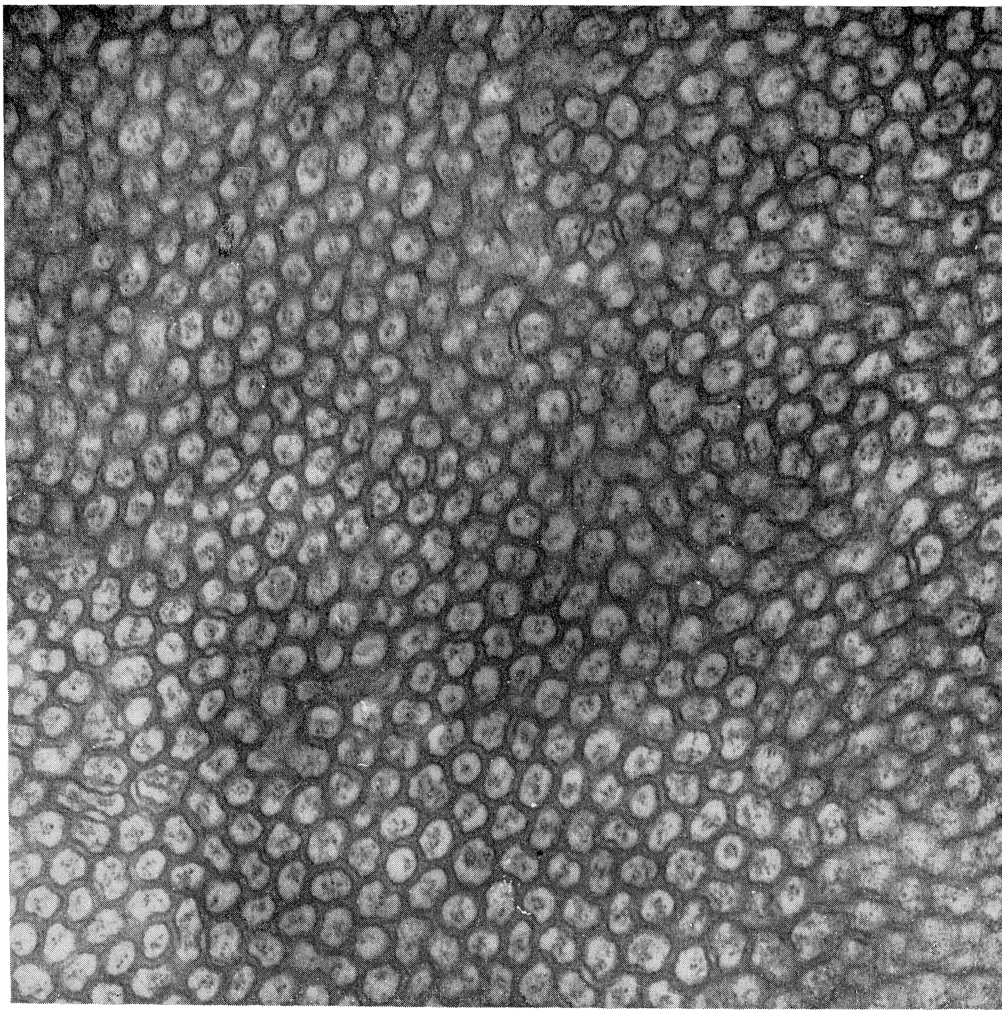
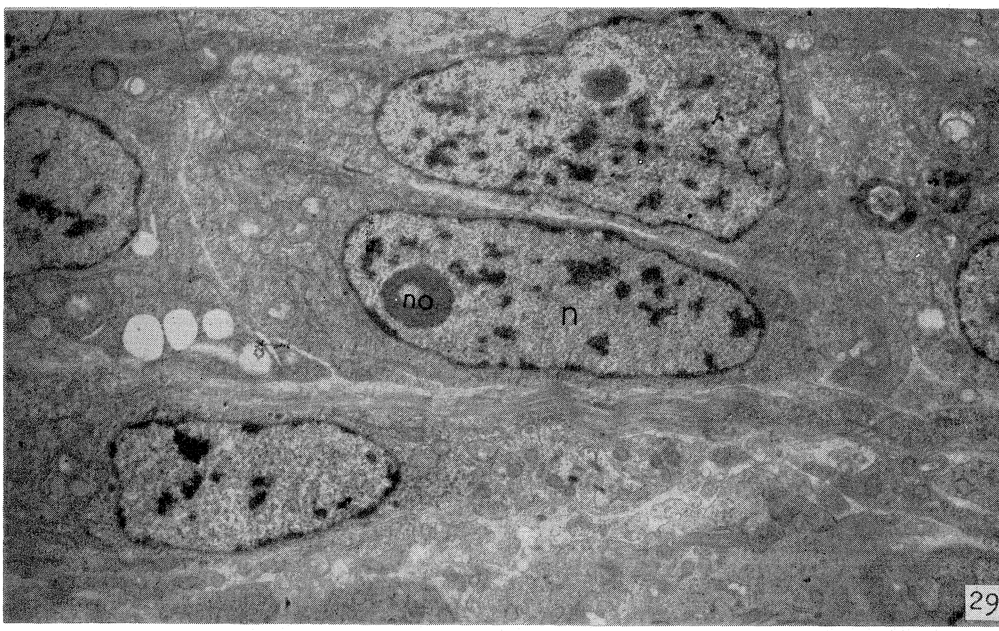


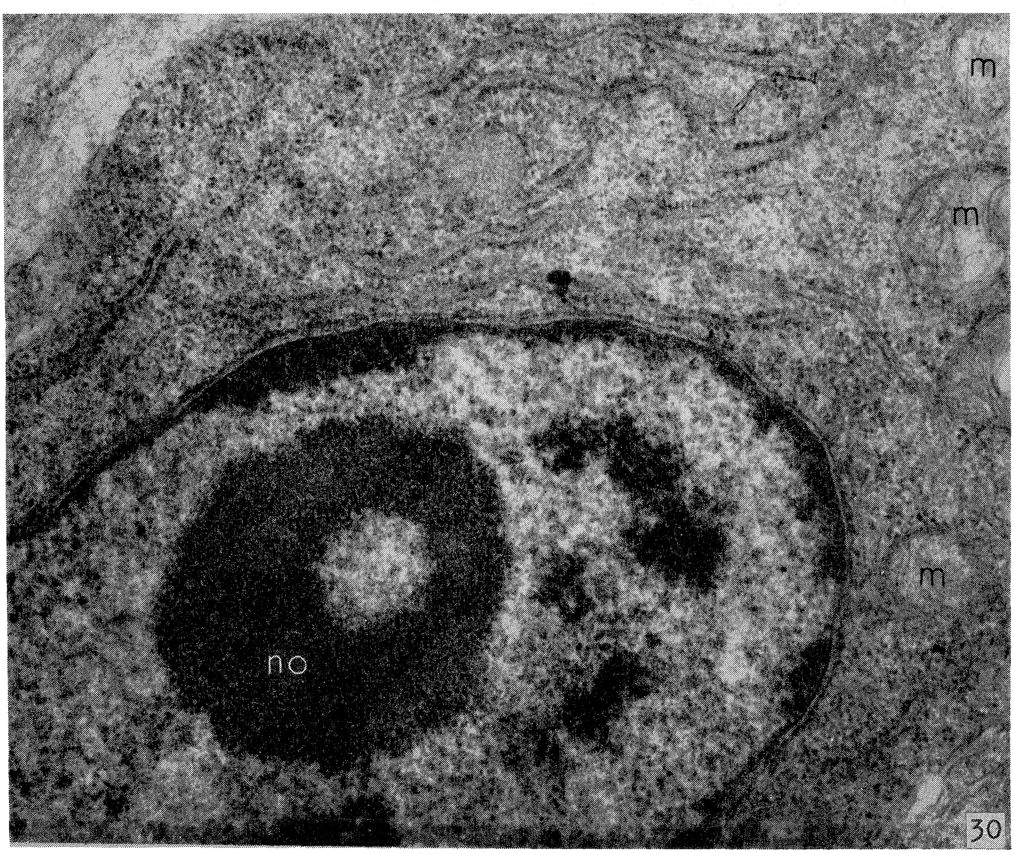
Fig. 28. Transverse section of the rhabdome on the fifteenth day of regeneration. Each microvilli contains spots of mediated electron density. ×54000

Fig. 25. A part of the eye on the fifteenth day of regeneration. Pigment cells (pc) is packed with a lot of pigment granules except for juxtaposition to the nucleus. Eye-cavity is closely packed with the rhabdomes of the visual cells. r: rhabdome, cb: conical body, n: nucleus. ×4500

Fig. 26. Golgi apparatus the eye pigment cell on the fifteenth day of regeneration. Two Golgi apparatus (g) cisternae of which are considerable electron dense are visible. ×40800

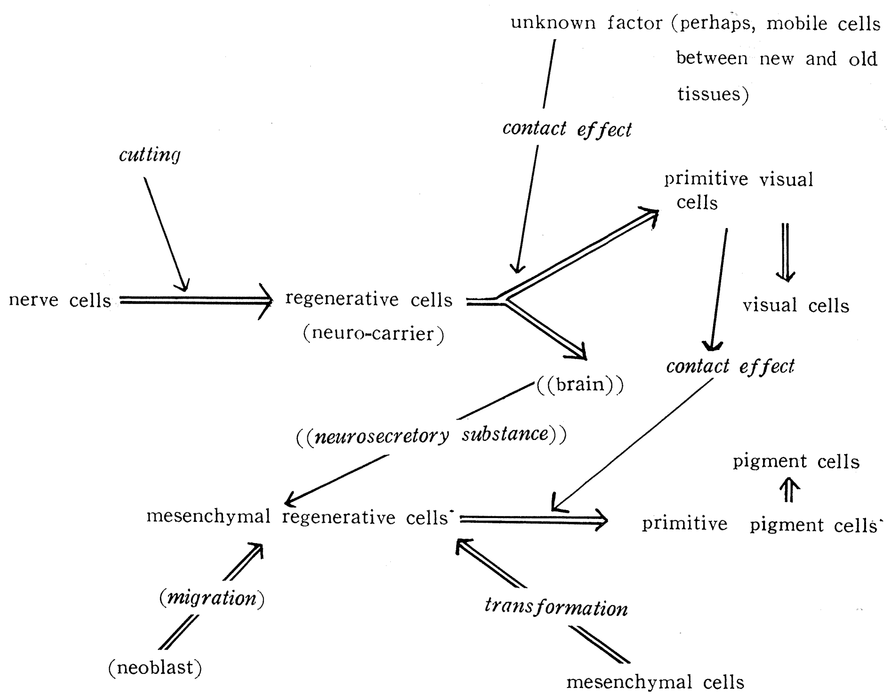
Fig. 27. Endoplasmic reticulum of the eye pigment cell on the fifteenth day of regeneration. Rough-surfaced endoplasmic reticulum are developed very well and sometimes arranged concentrically. Free ribosomal particles are seen. ×40800


no
n
29


m
m
m
no
30

totipotent neoblasts. Although it is very difficult to determine the origin of cells only from electron microscopic observation, we shall be able to give some suggestion. As described above, the neurotules in the stalk were already present as many as those of the normal eye in the very early stage of development of the visual cells. This may imply that the visual cells are originated from the nerve cells rather than from the totipotent neoblasts, because the nerve cells are provided with numerous neurotubules. When the planarians are decapitated, the wound is reduced by contraction of the muscles near the cutting surface, and tip end of the worm body turns dorsad. In accordance with it, the nerve cords turn dorsad too. Regenerative cells derived from the nerve cords are scattering loosely near the cut end and a part of them reaggregate in the anterior region of the blastema to make up the brain, as was reported by Kido (1958, 1961), while the other regenerative cells of nerve cord origin are pushed to the dorsal side and they give origin to the visual cells. In fact, the fine nerve fibers

Fig. 31. Scheme representing the mechanism of the eye-formation in planarian regeneration.



(( )) course is of Lender's view (1952).
( ) course is of Dubois' view (1949).

Fig. 29. Blastema cell on the second day of regeneration. Ring-formed nucleolus (no) is clearly presented in a nucleus (n). ×6400

Fig. 30. Enlarged photomicrograph showing the blastema cell on the second day of regeneration. Nucleolus (no) comprises a ring-formed aggregate of numerous electron dense particles. Numerous free ribosomal particles and rough-surfaced endoplasmic reticulum are visible. Mitochondria (m) are considerably large and of oval form. ×45000

were found to link the eye rudiment to the primitive brain in the blastema (Fig. 1c).

On the other hand, the pigment cells may be considered to the derivative of the blastema cells of the mesenchymal origin, by comparing the ultrastuctures of the primitive pigment cells in various stages of differentiation.

The ulrastructures characteristic to the primitive pigment cells will be summarized as follows; (1) the nucleus has several deep infoldings, (2) the nucleolus appears in a large ring form, (3) there are numerous free ribosomes in cytoplasm, (4) the rough-surfaced endoplasmic reticulum are developed very well, (5) the mitochondria are comparatively small in size and are not numerous, (6) Golgi apparatus in a single cell are several in number.

So far the electron microscopic studies of the regeneration blastema cell of the planarians are rare, but for Pedersen's observation of the neoblast (1959). Pedersen described in detail concerning the so-called neoblast in blastema, although it is questionable to ascribe all of the regenerative cells to the neoblasts alone. Comparing the ultrastructures of the primitive pigment cell with that of the neoblast described by Pedersen, both sorts of cells are different in some points. The former has well-developed endoplasmic reticulum and a ring-formed nucleolus, while the latter lacks both of these fine structures, only that these cells are like in the point of being provided with numerous free ribosomes. In the present examination, there were often found the blastema cells which have free ribosomes, rough-surfaced endoplasmic reticulum and ring-formed nucleolus in 1 to 2 days of regeneration (Figs. 29 and 30). Accordingly, it is reasonable to assume that such blastema cells give origin to the pigment cells. Since similar cells are found in the mesenchyme of the normal planarian tissue (Kishida, unpublished), such cells may be denoted tentatively as the mesenchymal regenerative cell, and its further natures are left to be inquired in future. From the view point so far described, the mechanism of the eye-formation in planarian regeneration is illustrated as in Fig. 31.

Fig. 32. Scheme showing the sequence of eye-formation in regeneration.

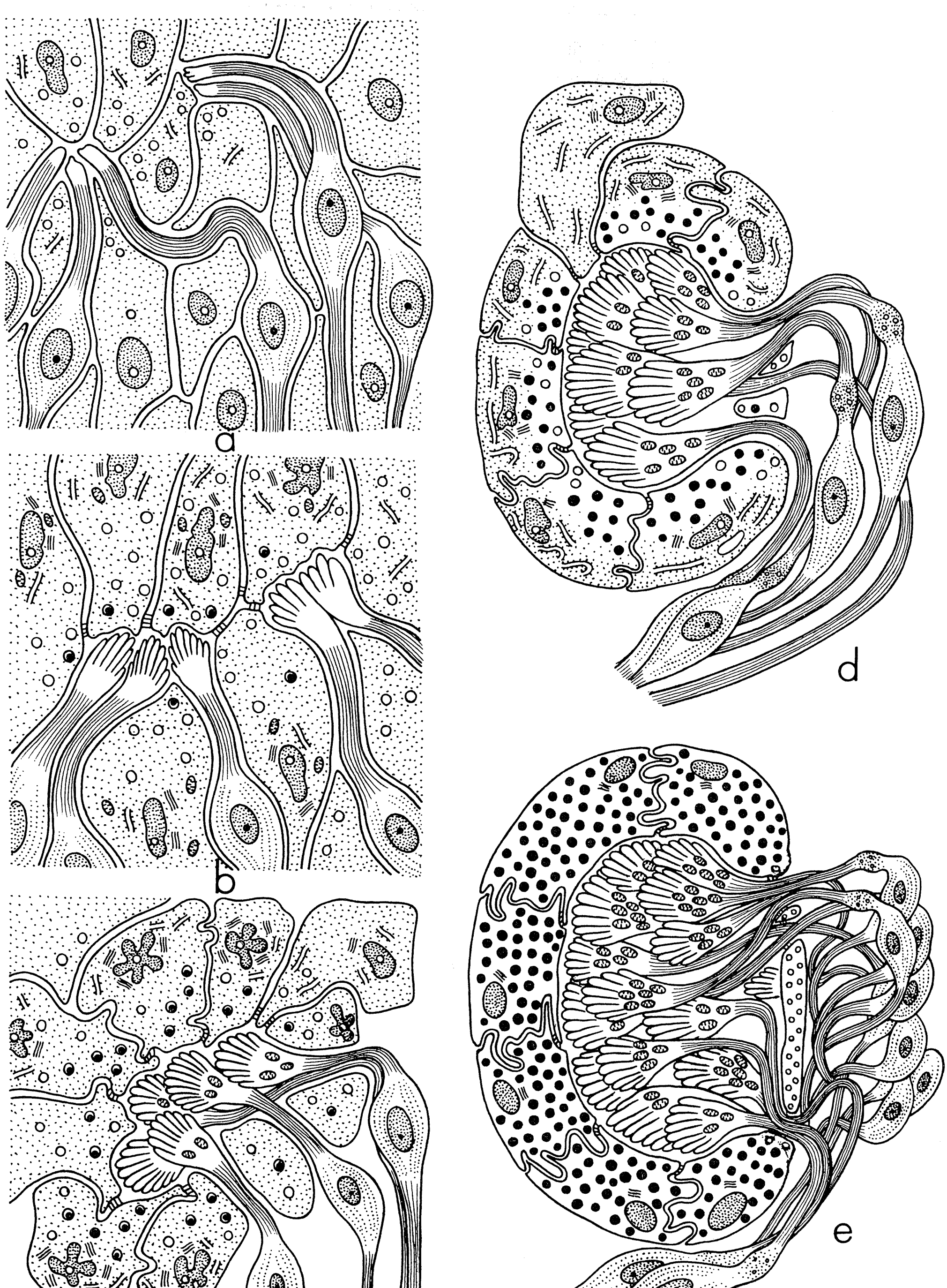
a. Initial stage of eye-formation on the second day of regeneration. Eye-rudiment consists of the bipolar primitive visual cells with many neurotubules and the ptimitive pigment cells with ring-formed nucleolus. The latter contains numerous free ribosomes, rough-surfaced endoplasmic reticulum, several Golgi apparatus and empty pigment vacuoles.

b. Eye-rudiment on the third day of regeneration. The visual cells develop the rhabdomes consisting of many microvilli and the primitive pigment cells contain the pigment vacuoles which begin to form the pigment subunit particles. The primitive pigment cells are connected with each other by the septate desmosomes.

c. Onset of the eye-vesicle formation on the fourth to fifth day of regeneration. Pigment cell has a nucleus with remarkable infoldings, numerous free ribosomes, rough-surfaced endoplasmic reticulum and several Golgi apparatus. While, visual cell develops itself quite similar to that of the normal eye, in which rhabdomes consist of numerous microvilli, conical body packed with mitochondria and stalks filled with neurotubules.

d. Eye on the seventh dey of regeneration. The pigment cell layer are of cup-form and the pluged membrane is being developed, but the pigment cells are in the primitive status in which free ribosomes, rough-surfaced endoplasmic reticulum, ring-formed nucleolus are present. Occasionally, it is seen that primitive pigment cells are intruding between the definite pigment cells.

e. Eye on the fifteenth day of regeneration, structure of it being completed.


a
b
c
d
e

In the early stage of eye regeneration, the primitive pigment cells originate from the mesenchymal regenerative cells in mingle with the primitive visual cells derived from the nerve cells, without special relationship between them. Then, the mingled cells rearrange themselves at their respective positions to establish the normal relationship, like a sorting-out in the dissociated sponge cells (Gartsoff, 1925) and amphibian embryonic cells (Townes and Holtfreter, 1955).

After establishment of fundamental form of the eye by the segregation of cells, formation of the pigment granules is actively performed in the primitive pigment cells. Namely, the pigment granules appear first as the empty vacuoles and then small vesicles which contain the pre-pigment substance are formed in them. These small vesicles give rise to the subunits of the pigment granules including the pigment converted from the pre-pigment substance. Thus, the vacuoles are gradually filled with numerous subunits of the pigment granules and finally become the pigment granules. In fact, pigment-forming cells are provided with remarkably numerous free ribosomes and rough-surfaced endoplasmic reticulum. A part of cisternae of such endoplasmic reticulum is frequently enlarged to make vacuoles. This may suggest the formation of the pre-pigment vacuole. It was also found that the cells in the active formation of the pigment granules develope Golgi apparatus very well.

After the seventh day of regenration, the regenerating eye grows gradually in size. This growth appears to be done by addition of the new cells from surroundings, because the cells of the eye do not show any mitotic figures. In fact, it was clearly observed that the primitive pigment cells were entering between the cells of the pigment layer.

As the eye growth proceeds, some of the pigment cells which are in contact with the stalks of the visual cells extend toward the mouth of eye-cup as thin folds. The folds are transformed in due course into the pluged membrane. The above relationship is shown in Fig. 32.

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Summary

The eye formation of the regenerating planarian, *Dugesia japonica*, was examined with electron microscope during first 15 days after decapitation, and some mechanism of the eye formation was discussed.

1. On the third day of regeneration, the mingled mass of primitive pigment cells and primitive visual cells can be observed. At that time, the visual cell has already differentiated the rhabdome and stalk. The rhabdome is composed of many microvilli and the stalk contains numerous neurotubules. On the other hand, the primitive pigment cells are just in the beginning of the pigment formation. Accordingly, differentiation of the visual cell is earlier than that of the eye pigment cell. The pigment cell is considered to be induced by the contact effect of the visual cells.

2. Basic form of the eye is established by segregation of visual cells and pigment cells in the mingled cell-mass of the eye rudiment.

3. Growth of regenerating eye is effected by addition of the pigment cells between the cells already differentiated into the pigment cell layer.

4. Primitive pigment cells contain numerous free ribosomes, rough-surfaced endoplasmic reticlum with vacuoles, several Golgi apparatus and elongated oval nucleus with deep infoldings and large ring-formed nucleolus. The rough-surfaced endoplasmic reticulum increase in number in the stage previous to the formation of the pigment granules. The pigment granule appears first as a simple vacuole and then small vesicles are formed in it. These vesicles give rise to the subunits of the pigment granule including the pigment. Thus, the vacuoles are gradually filled with these subunits and finally become the pigment granules. Numerous crowding of mitochondria which is particularly characteristic in the conical body of the visual cell occurs after the differentiation of the rhabdome and stalk.

5. Comparing the ultrastructures of the primitive pigment cells with those of the surrounding mesenchymal regenerative cells, it is assumed that eye pigment cells are derived from the mesenchymal regenerative cells. Likewise, the visual cells is considered to originate from the nerve cells.

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