MORPHOLOGICAL OBSERVATION ON THE DEVELOPMENT OF THE COMPOUND EYE IN THE SILKWORM (*Bombyx mori* L.) (2) *

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INTRODUCTION

Previously the authors have reported on the growth process of the compound eye in the silkworm with special reference to its development from the prematured larva to the just moulted pupa (KOYAMA & TANAKA, 1954) pointing out the subjects shown briefly in the following topics.

1. The original optic layer of the compound eye has been formed by ten hours before pupation.

2. The formative origin of the imaginal compound eye is not correlated to the lateral ocelli but is originated from the division of hypodermis in the region of so-called optic disc, when each ocellus, being decomposed by histolysis, sinks into the inner part of the hypodermis.

3. By retarding of each ocellus into hypodermal layer the suture which separates the optic disc into anterior and posterior parts appears on the dorsoventral direction of the disc.

In the present paper the succeeding process of the growth and differentiation of the compound eye i. e. from the pupation to the emergence was mainly delt with to complete the research on the postembryonic development of the silkworm eye, on which only two papers (IKEDA, 1913 ; WOLSKY, 1949) have already been published, so far as the authors looked over.

Before proceeding further the authors wish to express their sincere gratitude to Professor Dr. N. YAGI, Shinshu University, for his cordial direction given in the course of the work and for reading the original manuscript.

Thanks are also due to Miss R. YAMAZAKI and Mr. K. KOBAYASHI, who kindly assisted the experimental operation.

MATERIALS AND METHODS

The materials used for the research were the hybrid between the

* Contribution No. 32 from the Laboratory of Biology and Entomology, Faculty of Textile and Sericulture, Shinshu University.

Japanese bivoltine strain and the Chinese one (Nichi No. 115×Shi No. 108) and the Chinese bivoltine strain (Senka), of the silkworm, majority of which were confined under the constant illumination (100 lux), temperature (25°C) and humidity (R. H. about 70%), minority of which were put in the constant darkness with the same conditions as abovedescribed. Ten pupae grown to each stage which is shown in the following respective topic were fixed with Carnoy's solution and the tissues of the compound eye were sectioned in 7～10μ thickness regardless the sexuality. Most of the sections were stained with Heidenhein's iron haematoxylin after being depigmented by Grenacher's solution and the others were stained by eosin without depigmentation to observe the formation and migration of the retinal pigments.

HISTOLOGICAL OBSERVATION ON THE DEVELOPMENT OF THE COMPOUND EYE

1. The first day pupa

The original disc of the compound eye in the pupa just moulted can superficially detected, being held between the mouth and the antenna as shown in Fig. 1. On the surface of the eye disc, the suture on which WOLSKY (1949) has firstly pointed out that the differentiation center lies under it, is

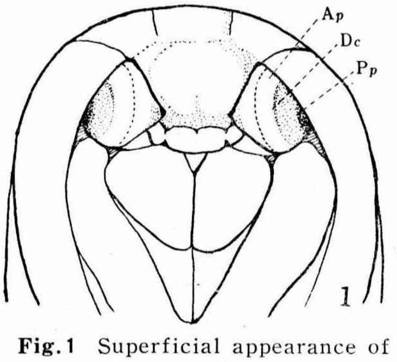

Fig. 1 Superficial appearance of the optic disc, ×6.

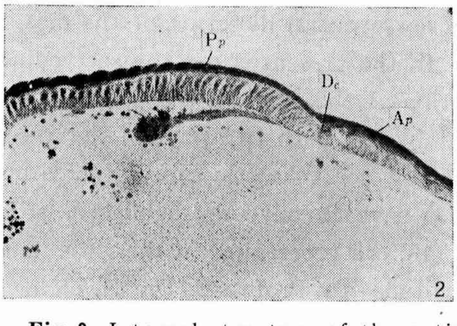

Fig. 2 Internal structure of the optic disc, ×100.

Ap : Anterior portion
Pp : Posterior portion
Dc : Differentiation center

seen in dorsoventral direction near the anterior margin of the eye anlage. The anterior and the posterior portion in the disc show a different velocity for the differentiation so as to be thought that the most potential part exists on the proximity to the furrow in the posterior portion, where its hypodermal layer becomes thicker (about 65μ in the center and 45μ in the margin) than that in the anterior one ($25\sim30\mu$). Each group of several original cells in the eye disc makes one bundle which seems to form an ommatidium in the future, being arranged in two rows (retinal layer), the external cells of which contain large slender nuclei (Fig. 2. Photo. 1, 2).

Under the retinal layer there appears the original nervous cells which are growing as the nerve cords here and there (Fig. 2). In this period any phagocytes can hardly be observed near the inner region of the eye disc. The nerve cords which enclose the remnant of ocellei are developing into the periopticon.

A pigmented part can be recognized at an adjacent tissue of the optic hypodermis.

The optic hypodermal cells at the posterior portion more and more divide as the pupa grows older. In the minority of the cells their protoplasma unite each other remaining only the nuclei which show elliptic shape in the middle part. The union of the cells happens remarkably in the upper part of the layer (Photo 3). When it reaches twelve hours after pupation the optic layer ($40\sim45\mu$) becomes a little thinner expanding rapidly its surface.

2. The second day pupa

The each cell of the optic hypodermis has almost fused not only at the upper layer but also at the under one which contains the nuclei and the chromatin granules abundantly (Photo 4).

The original nerve cells increase their number under the optic layer (Photo 4), but still scattered. Now the phagocytes have entirely disappeared. The ocular sclerite is a little produced at the boundary of the optic plate forming the ocular suture.

3. The third day pupa

The union of the cells has already finished (Photo 5, 25). The optic hypodermis becomes about 50μ the thickness which continues unchangeably until the sixth day after pupation. The small nuclei ($1.4\sim2.0\mu$) distribute extensively in the original retinal layer where the two layers of the cells which have been pointed out by Ikeda (1913) can scarcely be observed.

The older cells near the center of the posterior portion make several bundles, each top of which is somewhat rising to form the border-line for the adjacent cells and there a larger nucleus ($3.2\sim3.8\mu$) appears. This is a bud of the Semper's cell (Photo 6). The optic ganglion in this time is about 200μ width in cross section, being enveloped by a nervous membrane, where the tracheal truncks can be seen (Photo 31). The original nerve cells under the optic layer grow gradually towards the parallel direction to the layer and those in the inner region elongate towards the distal way of the eye.

4. **The fourth day pupa**

The united posterior retina divides longitudinally into the original ommatidial bundles from the posterior towards the anterior parts (Photo 7, 8). An anlage of ommatidium is 12～15μ in diameter being composed of about six cells (Photo 26, 27). The Semper's cell, beneath which the precursor of the crystalline cone, can often be recognized. The optical nerve cords, in which the fibers can be pointed out, develop immadiately distributing in the whole inner region of the compound eye and several main nervous cords start from the periopticon towards the distal part of the eye, yet the original nerve cells exist plentifully near round of the optic ganglion (Photo 30). In this period the chitin plate which is inserted into the ocular suture is formed.

5. **The fifth day pupa**

In this stage the number of ommatidia in the imaginal compound eye has entirely determined (Photo 9, 27) and the transition process of ommatidial differentiation can clearly be observed in the retinal layer (Fig. 4), where the anterior portion which was seen in the previous stage has reduced so narrow as it can hardly be detected, while the posterior one expands as the modification in ommatidia proceeds from its anterior towards its posterior.

The crystalline cone buds which are easily stainable are identified at the most grown ommatidia, being enclosed with the Semper's cells which consist of four in number. Under the Semper's cells nine to thirteen retinular cells exist forming an elongated ovoidal shape. One of the retinular cells which becomes the rhabdom in the future lies into their axis (Photo 9). The proximal part of the Semper's cells is covered with the original retinular pigment cells, without pigments, upon which the nuclei of iris cell appear. Connecting with the retinular pigment cells, the triangulate cells fill up the interstices among ommatidia.

The basal nerve cells beneath the basement membrane, it scarcely recognized, branch out and the nerve fibers connect with the proximal end of retinular cells penetrating the basement membrane.

There are another layer of the nerve cells under the basal nerve layer, between the two layers abovedescribed a membrane comes in sight (Photo 10). The branches of nerve cord have successively arisen by means of the cellular division of the nuclear cell ($8\sim15\mu$) produced at the surface of the nerve cord as shown in Fig. 3.

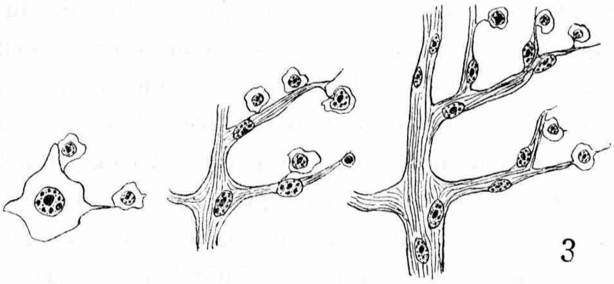

Fig. 3 Developmental process of the nerve cord

When the pupa aged twelve hours from the above stage, the axial cell in the retinular pigment cell goes downwards accompanying with the development of the ommatidium. The crystalline cones come out in nearly a half of the ommatidial layer (Fig. 4, Photo 10).

The inner nerve cords are more stretched, their nuclei being a little lengthened.

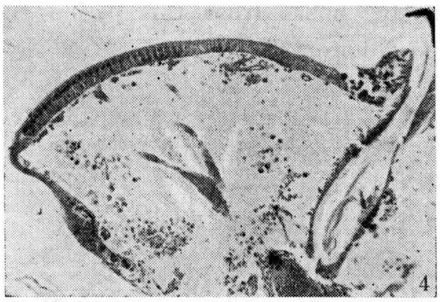

Fig. 4 Compound eye of the 5.5th day pupa, ×75.

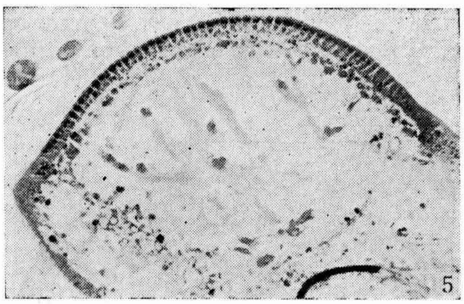

Fig. 5 Ditto of the 6th day pupa, ×80.

6. The sixth day pupa

The membranous corneal lens have been produced by the Semper's cells which are depressed (in about 7μ thickness) as the growth of the crystalline cone. The degree of differentiation in the ommatidia becomes almost parallel in the whole range of the eye (Fig. 5, Photo 28). At this time the differentiation seems to be temporarily retarded.

The basal nerve cells, each of which contains a large spherical nucleus, communicate one by one to connect with each ommatidium (Photo 11, 21).

After twelve hours have passed since the above time each cornal lens reaches to one μ thickness taking a hexagonal shape. The crystalline cone increases the volume forming the ellipsoid (size ; 12~15μ in horizontal, 7~8μ in vertical). The basal pigment cell becomes more slender and its distal part is ascertained to connect with the retinular pigment cell, the distal end of which is inserted into the interstice between the cones.

In longitudinal section the main nerve cords starting from the periopticon can be numbered as about six. The optic ganglion has not completed its growth, but the regions such as the periopticon, the external chiasma, the epiopticon, and the opticon can be discriminated as in the case of the imaginal compound eye (KOYAMA, 1954).

7. The seventh day pupa

Reaching to this time the differentiation more advances and each part in an ommatidium is easily recognizable (Photo 12). The thickness of the eye layer is estimated as about 50μ. The cornea and the crystalline cone increase their volume on the contrary to the Semper's cell being more or less flattened. In cross section the nuclei of the retinular cells arrange as like as a rosette at the medial position of the ommatidium (Photo 29). The eye layer is clearly distinguished as the two layers, i. e. the cone layer and the retinular one (Fig. 6). After twelve hours from this period the basal pigment cells begin to be pigmented (Photo 18).

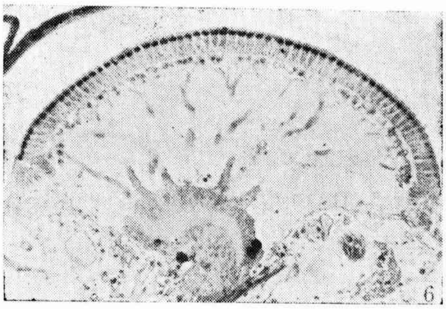

Fig. 6 Compound eye of the 7th day pupa, ×80.

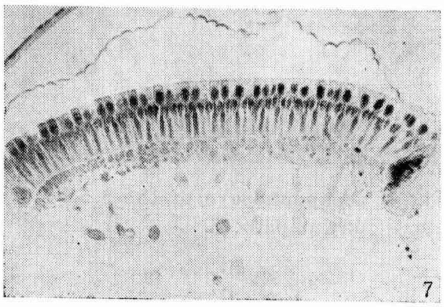

Fig. 7 Ditto of the 7.5th day pupa, ×75.

8. The eighth day pupa

Approaching this period the mass of granules begins to appear in the basal nerve cell (about 13μ) which takes an amoeboid shape, and in the inner nerve cords. Succeedingly the pigmentation occurs primarily in the basal pigment cell and the surface of the periopticon (Photo 17), secondarily in the retinular pigment cell.

Making this period a turning-point, the remarkable progress of development happens; the eye layer gets about twice thickness (about 100μ) to that of the seventh day pupa, the corneal lens (about 4μ) being more convex, the crystalline cone (about 25μ) elongating to elliptical form, the Semper's cell reduced, and the retinular cell and the rhabdom lengthened very much (Fig 7). In the just eighth day pupa (Photo 13) the eye layer reaches about 115μ in thickness and the cone 30$\sim$35μ in length. The iris cell located at the upper side of the crystalline cone develops so as to enclose the cone, at the end of which the nucleus of the iris cell underlies. Surrouding the rhabdom the tracheal bush is seen. The basal nerve nucleus decreases its size situating just under the ommatidium (Fig. 8, Photo 22, 33). Further the retinular pigment cells, as the development proceeds, become to be more pigmented (Photo 19), finally the iris cell is coloured with purplish pigments (Photo 20).

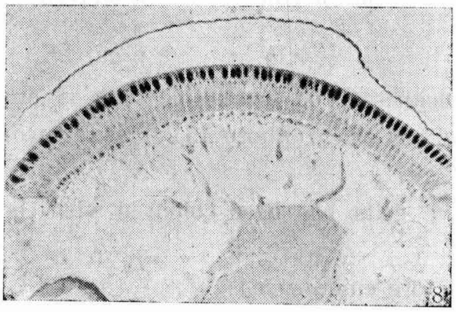

Fig. 8 Compound eye of the 8th day pupa, ×65.

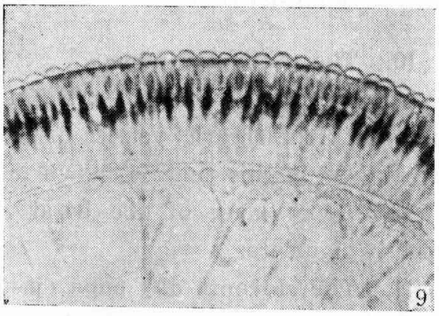

Fig. 9 Ditto of the 8.5th day pupa (unstained), ×80.

9. **The ninth day pupa**

With the elongation of each part, the whole length of an ommatidium is measured as about 180μ. The cone takes just like a spindle shape and the cornea consists apparently of two layers (Photo 14). The nucleus of the retinular pigment cell is remained under the proximity to the cone. The rhabdom, the nucleus of which sits near the basement membrane, is stretched about 50μ length. In this time the tracheoles have finished their distribution (Photo 24). The connection between the retinular pigment cell and the basal one is about to break (Fig. 9).

The nervous membrane under the basal nerve layer seems to disappear because of attaching with the basal nerve cell. The optic nervous system is considerably pigmented and has almost differentiated as in the imaginal eye (Photo 32), but by this time the upper layer of the basal nerve cells is pigmentless.

The whole eye is completely encircled with the chitin plate in the ocular suture (Fig 10).

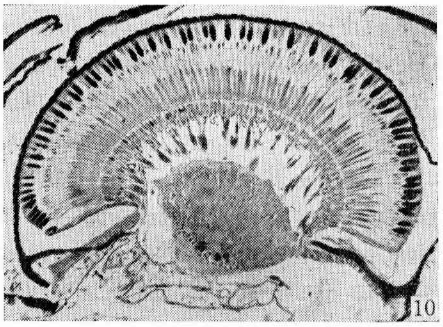

Fig. 10 Compound eye of the 9th day pupa, ×50.

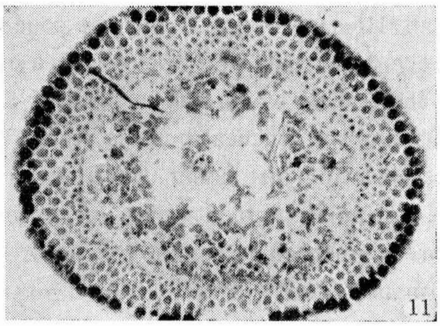

Fig. 11 Ditto of 10th day pupa showing in cross section, ×50.

10. The tenth day pupa

The eye plate shows 270∼280μ in thickness. The crystalline cone is enclosed with the sheath, yet its distal part is sharp (Photo 15). The form of a whole eye in this time is shown in Fig. 11.

The upper layer of the basal nerve cells has been coloured with the purplish pigments.

11. The eleventh day pupa (just before emergence)

The thickness of the eye plate is about 300μ. The difference of the structure between this pupal stage and the imaginal one is not evident except the cone shape which is more convex in the former than in the latter (Photo 16).

Soon or later the imago will break out.

From the results abovementioned the postembryonic development of the compound eye in the pupal stage can be divided into the four stages as described in the followings (see also Fig. 12, 13).

I. Divisional stage (1∼4 days after pupation)

In this stage the hypodermal cells, their protoplasma uniting each other, divide most actively and finally the original ommatidia are formed.

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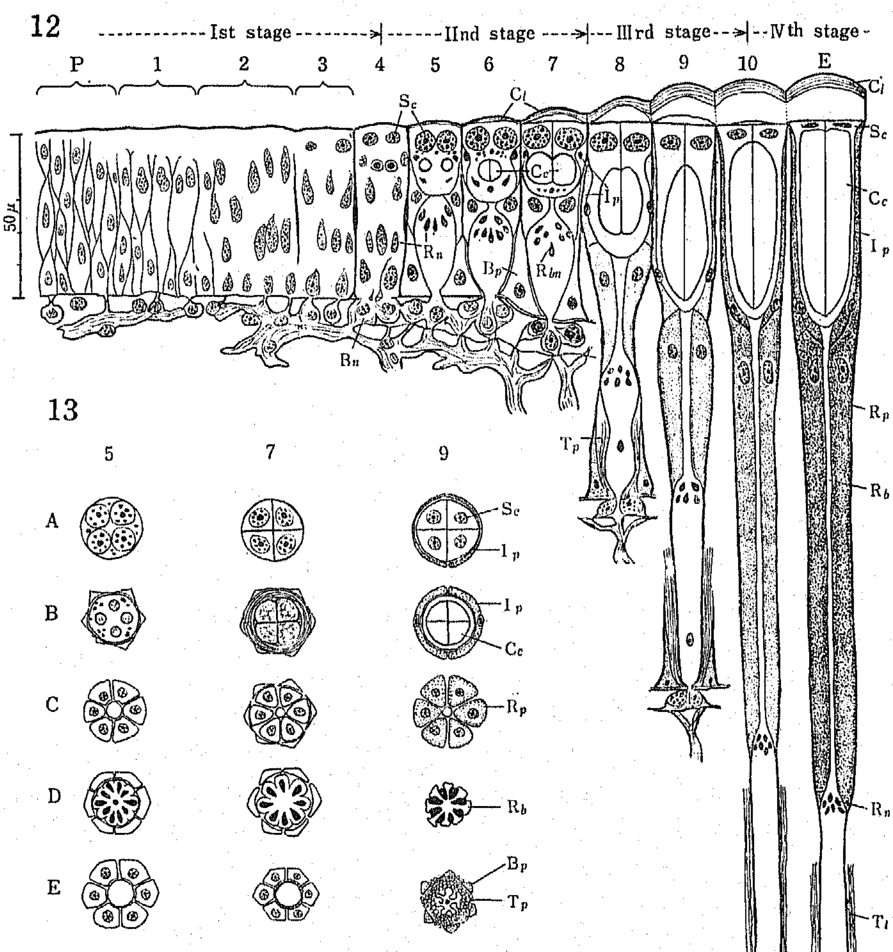

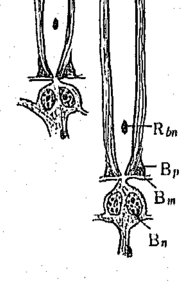

Fig. 12 Semidiagrammatic explanation of the development of the compound eye
P : 4 hours before pupation, 1~10 : 1st~10th day pupa (numbers concide with each pupal stage).
E : Just before emergence

Fig. 13 Cross section of the ommatidia in 5, 7, and 9 stages shown in Fig. 13.
A : Part of Semper's cell **B** : Crystalline part
C : Part of retinular pigment cell
D : Retinular part **E** : Basal part

Bm : Basement membrane	Rb : Rhabdom
Bn : Basal nucleus	Rbn : Rhabdom nucleus
Bp : Basal pigment cell	Rn : Retinular nucleus
Cc : Crystalline cone	Rp : Retinular pigment cell
Cl : Corneal lens	Sc : Semper's cell
Ip : Iris pigment cell	Tt : Tracheal tapetum

The division proceeds dorsoventrally from the anterior portion towards the posterior one.

II. Differentiation stage (5~7 days after pupation)

The multiplication of the cells is over and the ommatidial number in the eye has been determined. The differentiation in the ommatidial parts is remarkable, but the thickness of the layer of optic cells (about 50μ) does not increase. In this stage the following facts have been observed.

1. The Semper's cell produces the crystalline cone and corneal lens.
2. The retinular pigment cell develops originally from the same cell as the basal pigment one though each is separated in the future, while the iris pigment one is derived from the independent cell for the both cells.
3. The rhabdom is composed of the united retinular cells, each of which is named "Rhabdomere". The rhabdom nucleus near the basement membrane is nothing but one of the retinular nuclei.

III. Elongation stage (8~9 days after pupation)

The differentiation in the ommatidial parts has almost ceased and the ommatidium strikingly elongates accompanying with increase of the pigments. The pigments come to first appear in this time.

IV. Completive stage (10 days after pupation to emergence)

The structural change is scarcely recognized though the ommatidia are a little lengthened and the pigments become abundantly in the pigmentary tissues. The compound eye in this period is almost the same superficially as the imaginal eye but has not shown the photoreceptive function.

EFFECT OF THE LIGHT UPON THE GROWTH AND THE FUNCTION OF THE PUPAL EYE

The authors examined the effect of the light and the darkness upon the growth of the pupal eye along the methods described in the page 2 and the following results were obtained.

1. Significant differences were hardly recognized on the growth rate of the eye between the conditions of the light and the darkness.
2. The light brought no evidental effect on the formation of the pigments.
3. The retinular pigments did not move upwards even if the pupa was confined in the darkness, continuing the light adapted distribution.

The occurrence of pigment granules in the retinula advances from the internal part towards the external one and is ascertained to have few relation to presence or absence of the light. According to KATO's work (1952), the pupa of *Samia ricini* belonging to *Saturniidae*, when subjected in the darkness, did not produce the pigments in the compound eye since the melanin pigments are photosynthesized.

In the present research the purplish pigments, as much as in the eye of the illuminated pupa, appeared in the pupal eye under continuous darkness. So it must be said that the above statement has maintained a question to restudy with the reason that *Bombyx* and *Samia* are comparatively akin to each other in phylogenetical relationship.

Furthermore the fact that the eye in the oldest pupa showed no adaptation to the darkness exhibits it has not yet the photoreceptive function. In the Bombyx moth eye it has been reported the retinal pigments show the light adapted type in absence of the light when the moth eye is laid under physiological inadequate conditions (KOYAMA, 1954, '55).

Taking such phenomenon into the consideration on this study, it is assumed that the upward migration of the pigments (dark adapted type) could not happen because the pupal eye had been physiologically in an immatured condition though it seems to have finished its construction.

SUMMARY

The development and differentiation of the compound eye in the pupa of the silkworm were mainly described.

1. The compound eye is differentiated from the cellular division of the optic hypodermal disc situated at the seat of the lateral ocellei. At the cellular division the disc is divided dorsoventrally into the anterior portion and the posterior one by the furrow, under which the differentiation center lies.
2. The developmental process of the compound eye can be divided into the four stages ; those are Divisional stage during which the multiplication of the optic cells has finished, Differentiation stage in which the differentiation in every ommatidial part happens remarkably, Elongation stage in which the striking elongation can be seen in the ommatidia, and Completive stage during which the compound eye has almost completed.
3. The noticeable facts in regard to the differentiation of every ommatidial part are as follows:

   The corneal lens and the crystalline cone are produced by the Semper's cell.

The pigment cells of the retinula and the basement are arisen from the same origin, but that of the iris is originated from the unrelated cell to the both cells.

The rhabdom is formed by the retinular cells.

4. The illumination and the darkness to the pupal eye scarcely effects upon the growth rate of tissues, and the formation and the migration of the pigments.

5. It is assumed that the photoreceptive function has not yet been exhibited even in the almost completed eye.

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ABBREVIATIONS USED IN PLATES

Ap : Anterior portion
Bm : Basement membrane
Bnc : Basal nerve cell
Bp : Basal pigment
Bpc : Basal pigment cell
Br : Brain
Cc : Crystalline cone
Cl : Corneal lens
Dc : Differentiation center
Ek : External chiasma
Ep : Epiopticon
Ik : Internal chiasma
Ip : Iris pigment
Ipc : Iris pigment cell
Mn : Membranous nerve
Nc : Nerve cord
Noc : Original nerve cell
Np : Nervous pigment
Ob : Ommatidial bundle
Or : Remnant of ocellei
Ph : Phagocyte
Po : Periopticon
Pp : Posterior portion
Rc : Retinular cell
Rh : Rhabdom
Rhm : Rhabdomere
Rp : Retinular pigment
Rpc : Retinular pigment cell
Sc : Semper's cell
Tr : Trachea
Tt : Tracheal tapetum

EXPLANATION OF PHOTOGRAPHS

PLATE I AND II

Longitudinal sections of the optic hypodermis showing the differentiational process of the compound eye.

Photo 1. Just moulted pupa, showing the differentiation center, ×300.
Photo 2. The 1st day pupa, showing posterior portion, ×300.
Photo 3. The 1.5th day pupa, the upper layer united, ×300.
Photo 4. The 2nd day pupa, the optic layer almost united, ×300.
Photo 5. The 3rd day pupa, showing especially the differentiation center, ×500.
Photo 6. The 3.5th day pupa, the Semper's cell appears, ×440.
Photo 7. The 4th day pupa, the layer dividing longitudinally, ×440.
Photo 8. The 4.5th day pupa, the ommatidial bundles begin to be composed, ×440.
Photo 9. The 5th day pupa, ×290.
Photo 10. The 5.5th day pupa, the crystalline cone appears, ×290.
Photo 11. The 6th day pupa, ×390.
Photo 12. The 7th day pupa, ×450.
Photo 13. The 8th day pupa, ×480.
Photo 14. The 9th day pupa, ×220.
Photo 15. The 10th day pupa, ×300.
Photo 16. The 11th day pupa (unstained), ×55.

PLATE III

Photo 17. Periopticon in 7.5th day pupa, the pigments begin to appear (unstained), ×640.
Photo 18. Retinal layer in ditto, pigment masses being seen in the basal nerve cells, ×430.
Photo 19. Pigmentation in the 8th day pupa, ×420.
Photo 20. Ditto in the 8.5th day pupa, ×420.
Photo 21. Nervous system under the basement membrane in the 6th day pupa, ×450.
Photo 22. Ditto in the 8th day pupa, ×400.
Photo 23. Ditto in the cross section, ×600.
Photo 24. Cross section of the tracheal tapetum in the 9th day pupa, ×350.

PIATE IV

Photo 25~29. Cross sections of the optic layer showing differentiational process,
Photo 25. The 3rd day pupa, ×400.
Photo 26. The 4th day pupa, ×400.
Photo 27. The 5th day pupa, ×350.

Photo 28. The 6th day pupa, ×350.
Photo 29. The 7th day pupa, ×600.
Photo 30. Inner part of the eye in the 4th day pupa, ×300.
Photo 31. Cross section of the brain in the 3rd day pupa, ×200.
Photo 32. Optic ganglion of the 9th day pupa, ×80.

PLATE I

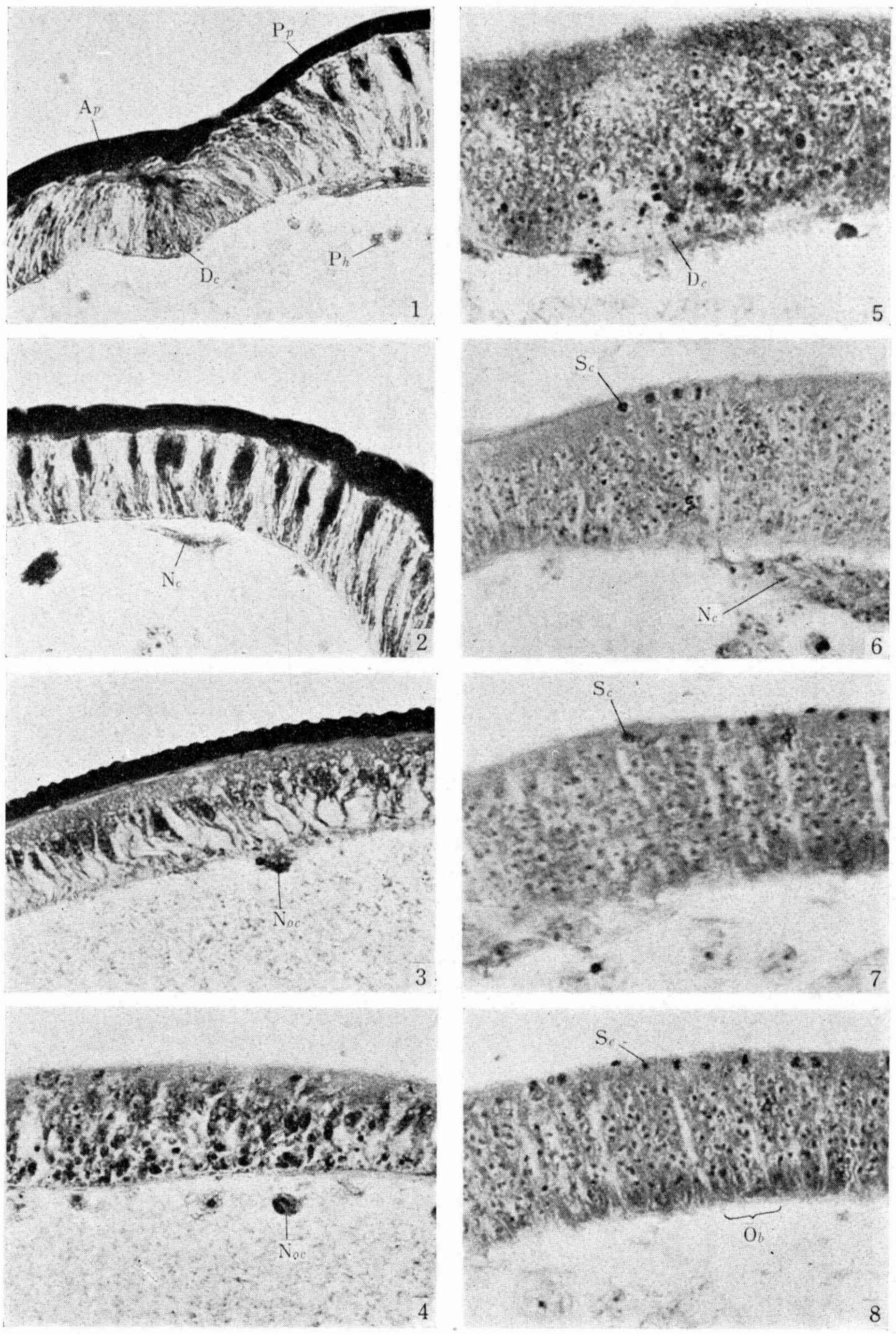
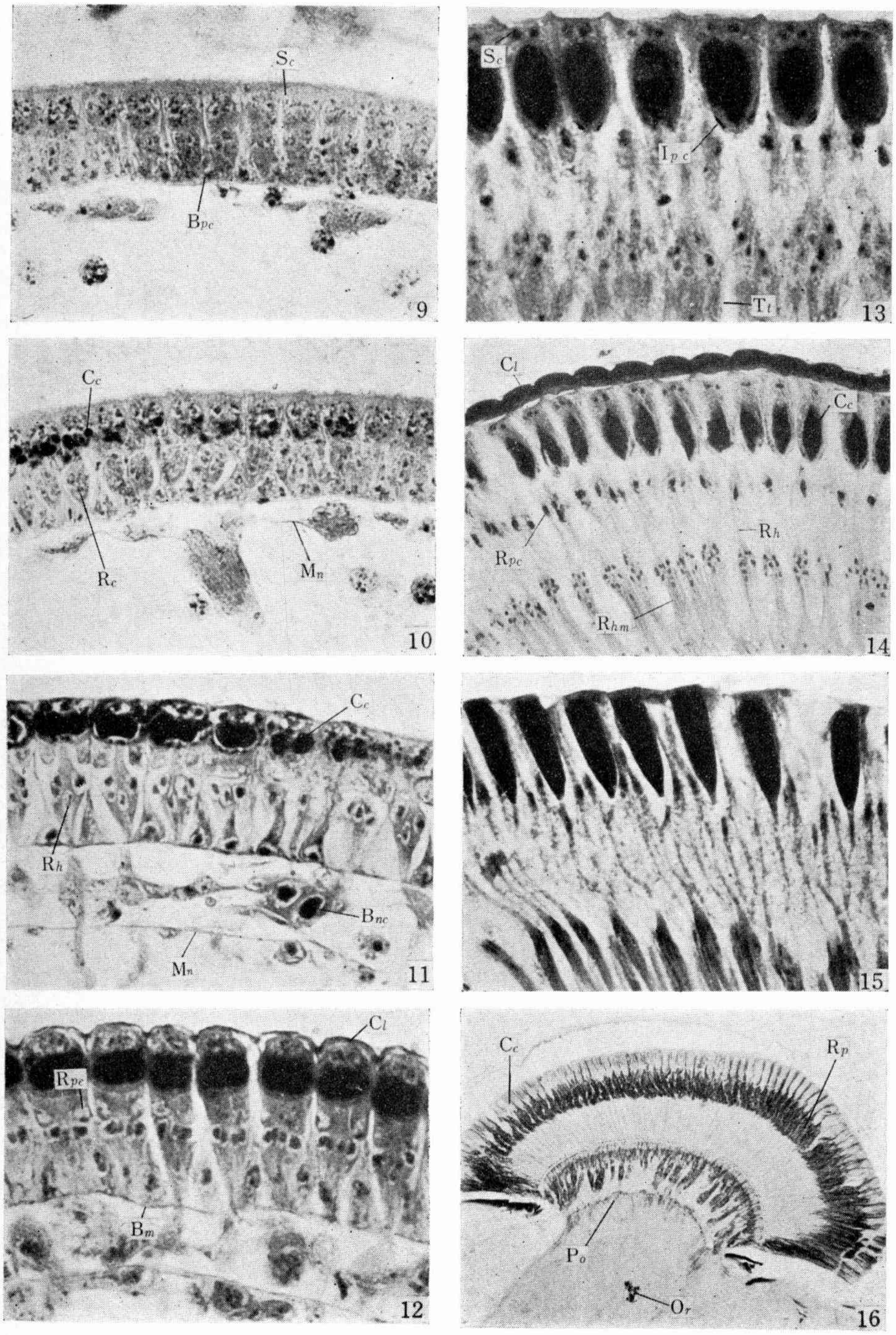


PLATE II



Sc
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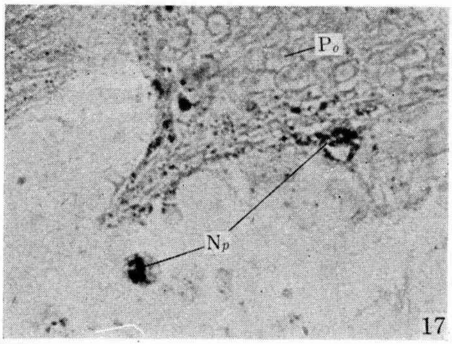
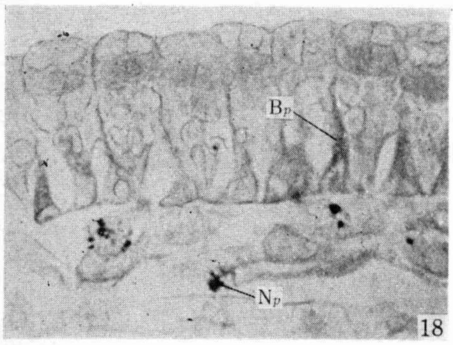
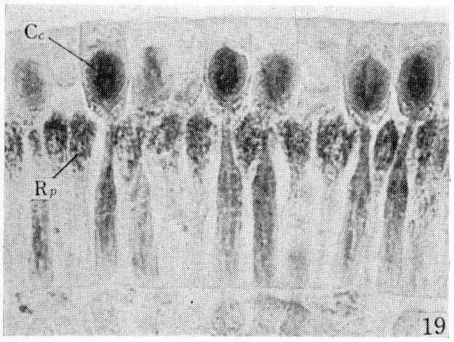
PLATE III



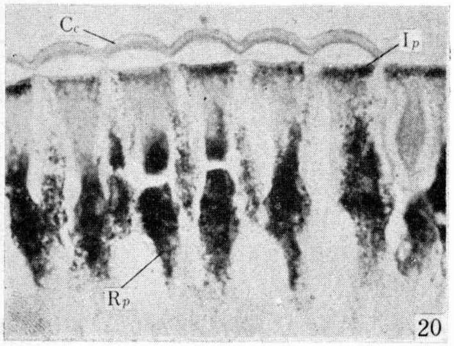
P$_{o}$
N$_{p}$
17



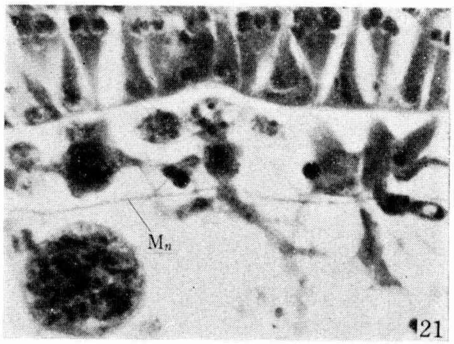
B$_{p}$
N$_{p}$
18



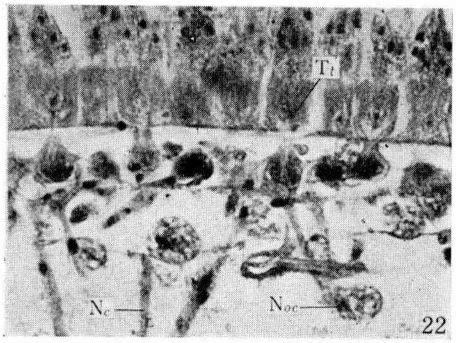
C$_{c}$
R$_{p}$
19



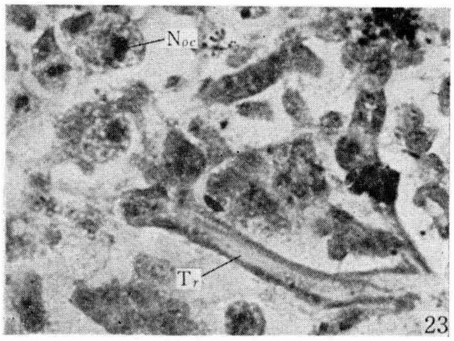
C$_{c}$
I$_{p}$
R$_{p}$
20



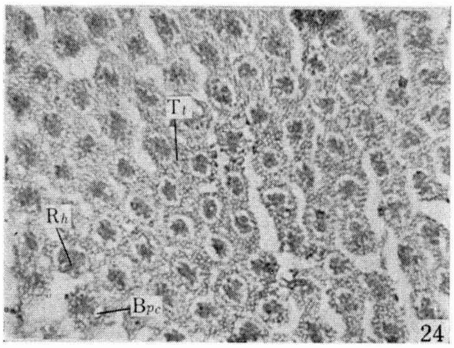
M$_{n}$
21



T$_{t}$
N$_{c}$
N$_{oc}$
22



N$_{oc}$
T$_{r}$
23



T$_{t}$
R$_{h}$
B$_{pc}$
24

PLATE IV