Morphological Observation on the Development of the Compound Eye in the Silkworm(*Bombyx mori* L.) (1)*

By

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(1954. 9. 5)

A lot of papers have been reported on the development of the compound eye in insects, i. e. on musca (WEISMANN, 1846), on *Lepidoptera* (CARRIÉRE, 1886: JOHANSEN , 1893: KOFÉC, 1922: UMBACH, 1934), on *Hymenoptera* (PATTEN, 1887, '88: PHILLIPS, 1905), on *Drosophila* (KRAFKA, 1924: ENZMAN and HASKINS, 1938: HAUSMAN, 1949), on mosquitoes (SATO, 1951, '53) etc. In addition to these researches, the works concerning the silkworm's have been done, so far as we know, by IKEDA (1913) and WOLSKY (1949) maintaining many questionable problems.

The present paper deals with the observation on the growth process of the compound eye in the larval stage of the silkworm with special reference to its development from the prematured larva to the just moulted pupa.

Before going further the authors must express their hearty thanks to prof. Dr. N. YAGI for his cordial guidance and for correction of the manuscript. Thanks are also due to prof. Dr. H. SATO, who afforded facilities for the experiment.

MATERIALS AND METHODS

The materials used for the experiment were the Chinese bivoltine strain (Senka) of *Bombyx mori*, which were bred under the condition of constant temperature (25°C), humidity (RH55%), and light intensity (100 lux). Five individuals of each larval stage described in the following topics were sectioned in 9μ thickness regardless on the sexuality and observed the change of optical tissue along the development. The sections were stained with eosin and Heidenhein's iron hemathoxylin after fixation by Bouin's fluid.

RESULTS

1. Prematured larva

In this stage the tissue of optic disc which consists of the lateral ocelli and its boundary plate has a little difference from that of the immature larva (Fig. 1-1), on the structure of which several researches have been done by BLANC (1891), TOYAMA and ISHIWATA (1899), IKEDA (1913), and KOIKE (1952, '53). According to the authors' observation a certain number of phagocytes are always found beneath the hypodermis and near around the optic nerve cord, at that time the internal part of hypodermis contains the purplish pigment.

2. Matured larva

The number of phagocytes distributed under the hypodermis increases in this stage than

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in the preceding stage. Some of them are seen attacking the internal part of the hypodermis, which begins to divide at some parts, while the superficial structure of the ocelli does not change (Photo 1, Fig. 1-2). Rarely in this stage, the authors came across a case to observe roundish granular bodies in various tissues of the head (Photo 2, 3) though they were scarcely found in the muscles. The size of the granular bodies is estimated about 4. 0_{μ} in diameter and they are readily stained by eosin and Heidenhein's iron hematoxylin. Some quantities of the granules are observed scattering in the interspace of the tissue. It is not certain whether the granule is composed of pure substance or not, however it seems to be composed of lipoid-like substance according to its affinity to the stain. When metamorphosis turns to the succeeding stage it will be produced in autolizing tissues as a reserving phase of autolized substance.

3. Larva aged 1~5 hours since spinning has begun

In this stage the superficial form of the ocelli is elongated a little accompanying with shortening of the optic nerve cord, the boundary of the latter is filled entirely with the abundant phagocytes which increase enormously in whole parts of the tissues (Photo 4).

4. Larva aged 10 hours since spinning has begun

The cellular division of hypodermis surrounding the ocelli is advanced, while the pigment in the internal part of the hypodermis decreases by getting attack of the phagocytes, some of which grow as a granular ball (Photo 5, Fig. 1-3) named primarily as "Kornchenkugel" by WEISMANN, (1864) in *Diptera*.

5. Larva aged 20 hours since spinning has begun

The nerve cord is gradually thickened and its pigment decreases. A few ocelli covered with the thin layer of the hypodermal cells can be detected (Photo 6, 7, Fig. 1-4), though the entire decomposition of the ocelli is not seen yet. The histolysis goes on anteriorly along the optic disc.

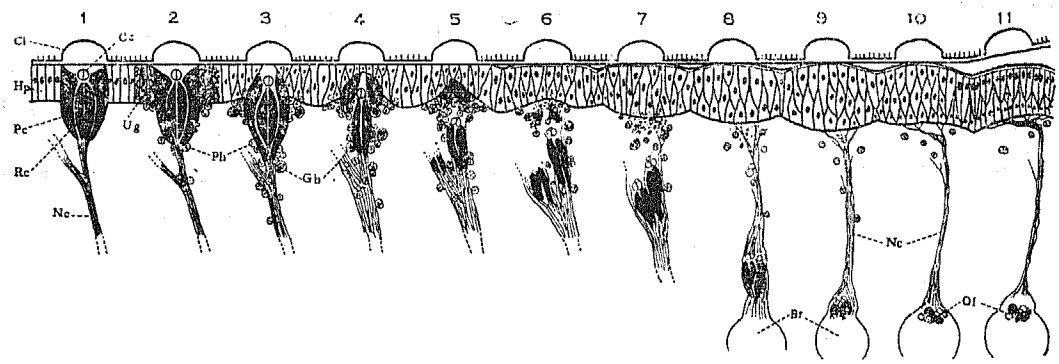


Fig. 1 Diagrammatic figure of the developmental process of the compound eye

1, Immature larva 2, Matured larva 3, Larva aged 10 hours since spinning has begun 4, Ditto 20 hours 5, Ditto 30 hours 6, Ditto 40 hours 7, Prepupa 8, 10 hours aged prepupa 9, 20 hours aged prepupa 10, 26 hours aged prepupa (4 hours before pupation) 11, Just moulted pupa Br: Brain Cc: Crystalline cone Cl: Corneal lens Gb: Granular ball Hp: Hypodermis Nc: Nerve cord Ol: Ocellus remnant Pc: Pigment cell Ph: Phagocyte Rc: Retinular cell Ug: Unknown granular body

6. Larva aged 30 hours since spinning has begun (20 hours before prepupation)

The upper layer of five ocelli excluding the nearest one from the antenna is covered with the hypodermal cells (Photo 8, Fig. 1-5) The pigment cell which envelops the retinula deforms a little. Succeeding to the above stage the ocelli will presumably be considered to have lost its photoreceptive function notwithstanding the cocooning behavior still exists.

7. Larva aged 40 hours since spinning has begun (10 hours before prepupation)

Each ocellus assembles beneath the center of the optic disc (Photo 9, 10, Fig. 1-6), where a suture of the hypodermis appears dorsoventrally, differing from IKEDA's description, in which the sinking trace was observed in each ocellar seat. In this stage the pigment cell separated from the retinular cell is remained under the hypodermis. The crystalline cone and the rhabdome, being enveloped by the retinular cell, fall into the main nerve cord as if it is drawn in. The number of the phagocytes decreases at the inner surface of the hypodermis. It is certain that the phagocyte destroys the tissues in the silkworm, though BERLESE (1900) and BREED (1903) denied it in the other insects. The thin layer is newly formed on the external surface of the hypodermis in this stage. Thus, the stage will be called ''the starting stage of the compound eye" viewing from the external aspect.

8. Prepupa (30 hours before pupation)

The anterior part of hypodermis of the optic disc is thickened, and the ocelli completely separate from the hypodermal layer (Photo 11, Fig. 1-7), where the pigment has scarcely been observed. The pigment granules spread from the destroyed pigment cell of the ocelli are attacked by the phagocytes, which grow as the granular balls enclosing the pigmemt. The nerve cord is thickened and the majority of tracheal branches disappear.

9. 10 hours aged prepupa (20 hours before pupation)

In this stage each ocellus is enclosed entirely by the nerve fibers assembling at a part near the brain as a mass of remnant of the ocelli (Photo 12, Fig. 1-8).

10. 20~22 hours aged prepupa (8~10 hours before pupation)

The remnant of ocelli has reached the surface of the brain (Photo 13, Fig. 1-9). The epidermal layer between the old cuticle and the hypodermal layers is much more thickened and the cuticular layer becomes very separately from the hypodermis. The pigment granules distributed under the hypodermis have not yet disappeared (Photo 14). Before or after of this stage the tissues under the posterior portion of the optic disc have shown perfect histolysis, but those under the anterior one have still been remained unchangeably.

11. 26 hours aged prepupa (4 hours before pupation)

All the pigments but the remnant of ocellus have disappeared (Photo 15, Fig. 1-10). The old cuticle is nearly to be separated from the newly developed retinal layer, in which several bundled cells are recognized. The main nerve cord which connects the brain with the ocelli, seems to be remained without decomposition.

12. Pupa (just pupated)

The original plate of the compound eye expands just after entering pupation and its surface is seen as if being divided into anterior and posterior portions by a groove dorsoventrally extended as WOLSKY have observed in 1949 (Photo 16, Fig. 1-11). The hypodermis under the posterior portion produces two layers of the retinal cells, whereas the part becomes thicker than the part of the anterior; the former part develops into the ommatidia succeedingly. The hypodermis in the anterior portion maintains almost the same construction as the preceding stage. Under the retinal layer there appears the original nerve cord, from which the nerve fibers can be observed being connected with the retinal cell through the basement membrane. In this stage no phagocytes are seen at the inner part of the eye but scanty numbers of them are scattered under the retinular layer. The nerve cords which enclose the ocellus remnant are assumed to be grown into the periopticon in future.

DISCUSSION

There are two opinions concerning the post embryonic development of the compound eye of insects; the one is supported by CARRIÉRE (1886) on *Vespa*, in which the compound eye develops by means of the differentiation of the ectodermal layer of the head, and the other is persisted by PATTEN (1888) on *Acilius*, in which the imaginal eye can not grow without relation of the larval ocelli. Taking the case of Bombyx eye into consideration, we have some facts to support CARRIÉRE'S opinion because the hypodermis between the lateral ocelli turns into the retinular layer of the imaginal eye as have already been shown in No. 6~8 topics and further more each ocellus assembles together in falling into the inner part of the optic disc so deeply as to reach the brain surface (KOYAMA, 1953). Thus the original disc of the compound eye has been completed at the time of 10 hours earlier to enter the prepupal stage,i.e. 40 hours since silk-spinning has begun. It may be interesting to note that the ocellus function has been lost even the cocooning conduct still continuing. Accordingly the effect of the light concerning the behavior of the silkworm (non-photodermatic) is limited in frontal half of the cocooning.

The developmental consideration of the imaginal eye of the silkworm in the pupal stage suggested by WOLSKY (1949) might be worth mentioning. According to his observation on the existence of a furrow, the eye disc is separated into anterior and posterior portions, each showing a different velocity for the differentiation as to be thought that the most potential part lies on the proximity to the furrow in the posterior portion, from where the differentiation proceeds to the anterior and the posterior portions. These two portions are ascertained by the present authors, however the progress of the differentiation has not been detected exactly.

SUMMARY

In this account the post embryonic development of the compound eye in the silkworm was observed histologically by using the premature, mature, prepupal, and pupal materials. The results obtained are summarized as follows:

1. The original retinular layer of the compound eye has been formed by 10 hours before pupation (40 hours since silk-spinning has begun), so the function of the lateral ocelli has been lost before the end of cocooning.

2. The formative origin of the imaginal retinular layer is not correlated to the lateral ocelli but is originated to the division of hypodermis in the region of so-called optic disc, when each ocellus, being decomposed by histolysis, falls into the inner part of the hypodermis assembling at the surface of the brain.

3. At the sinking of lateral ocelli into the inner part under the hypodermal layer, the pigment cells which envelops the retinular cell and the rhabdome are remained beneath the hypodermal layer separating from the above two sense cells and finally disappear by autolysis.

4. The nerve cords connecting the ocelli with the optic ganglion envelop the assembled ocelli (ocellus remnant) and they are assumed to be grown into the periopticon in future.

5. By retarding of each ocellus into the hypodermal layer a suture appears on the dorsoventral direction in the optic disc. This furrow separates the disc into anterior and posterior parts which were pointed out by WOLSKY (1949) in the pupal eye of the silkworm.

6. The occurring of histolysis of the optic disc caused by the attack of the phagocytes can be seen around the posterior towards the anterior portions of the eye.

7. In the tissue of the matured larval head, an unknown granule which is easily stained with eosin and Heidenhein's hemathoxylin was observed in a short period. It is considered to be a substance of lipochrome.

LITERATURE CITED

* BERLESE, A. ; Zool. Anz., 23 (1900)

BLANC, L.; Trav. Lab. Étud. Soie, 1889-1890: 1-180 (1891)

* BREED, R. B. ; Bull. Mus. Comp. Zool., 40 (1903)

* CARRIÉRE, J. ; Zool. Anz., 9 (1886)

ENZMAN, E. V. & C. P. HASKINS ; Jour. Morph., 63:53-73 (1938)

HAUSMAN, S. A. ; Trans. Am. Micros. Soc., 68:154-162) 1949)

IKEDA, A.; Anatomy and physiology of silkworm (1913)

JOHANSEN, J. ; Zool. Jahrb. Anat., 6:445-480) 1893)

KOIKE, A. ; Chubu Sanshigakkai Seri., 5:27-28 (1952)

———— ; Jour. Seric. Sci. Jap., 22:123 (1953)

KOPÉC, S.; Jour. Expt. Zool., 36:459-466 (1922)

KOYAMA, N. ; Res. Rep. Facul. Text. & Seric., Shinshu Univ., 3: 44-47 (1953)

KRAFKA, J. ; Biol. Bull., 47:143—149 (1924)

PATTEN, W. ; Jour. Morph., 1:193—226 (1887)

———— ; ibid., 2:97—190 (1888)

PHILLIPS, E. F. ; Proc. Acad. Nat. Sci. Philadelphia, 57:123—157 (1905)

SATO, S. ; Sci. Rep. Tōhoku Univ., Biol., 19:23—28 (1915)

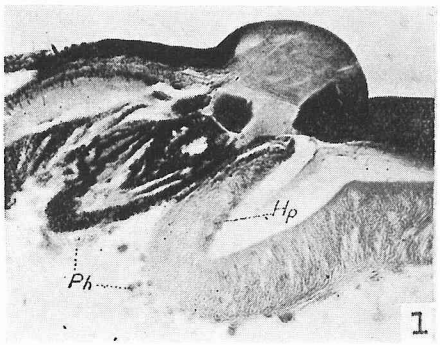
———— ibid., 20:33—44; 45—35 (1953)

TOYAMA, K. & S. ISHIWATA ; Experimental anatomy of silkworm (1899)

UMBACH, W. ; Zeit. Morph. ökol. Tiere., 28:561 -594 (1934)

WEISMANN, A. ; Zeits. wiss. Zool., 14:187—36 (1864)

PLATE I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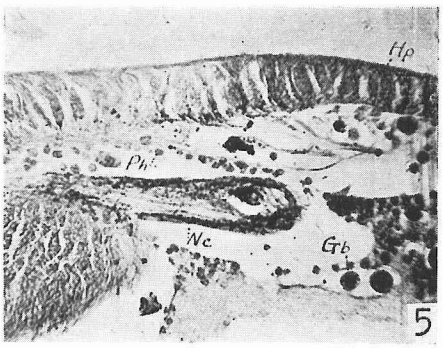
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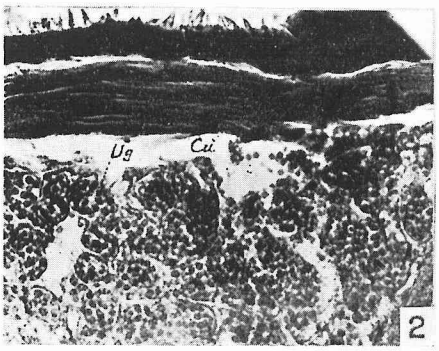
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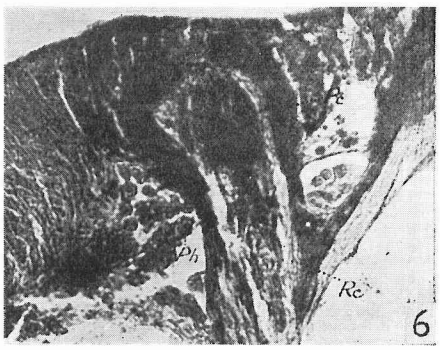
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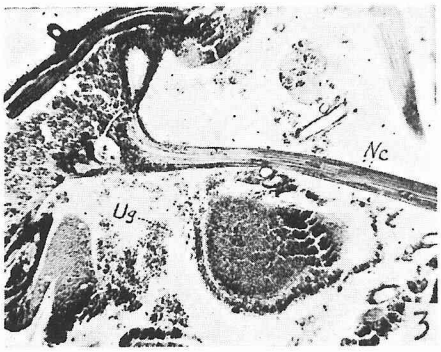
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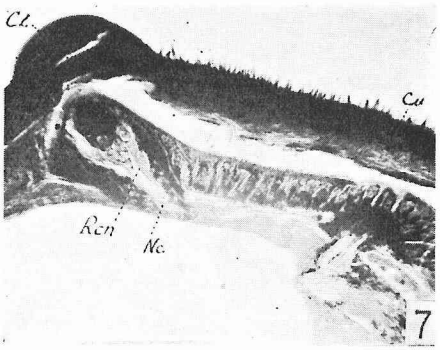
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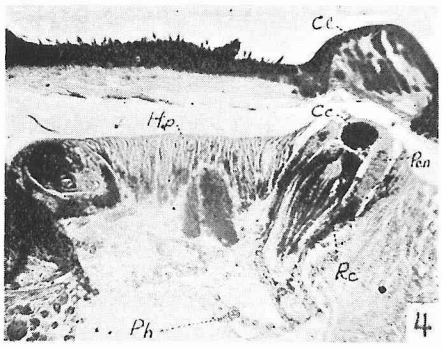
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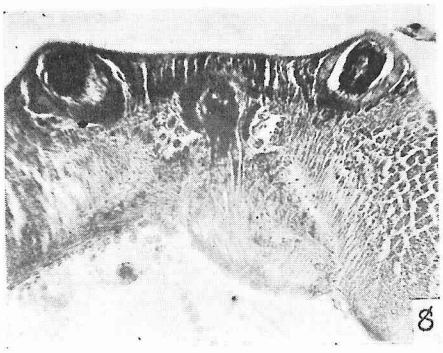
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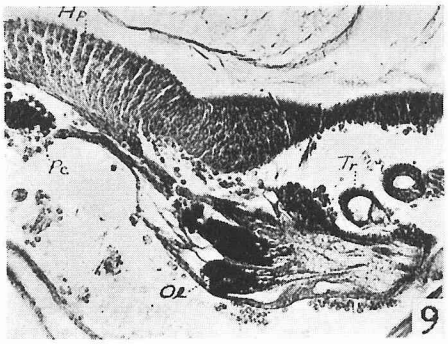
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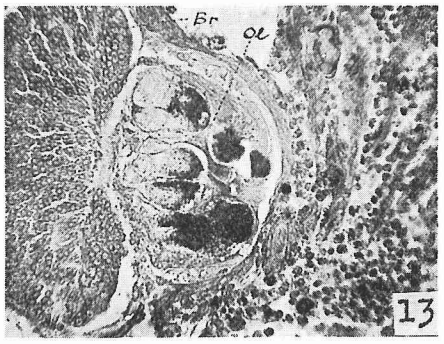
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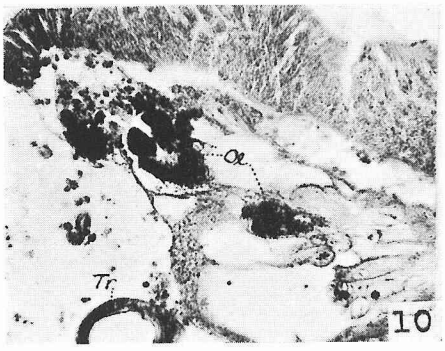
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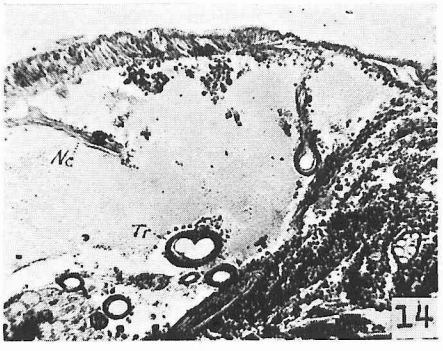
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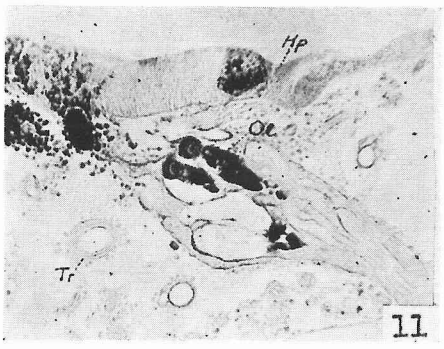
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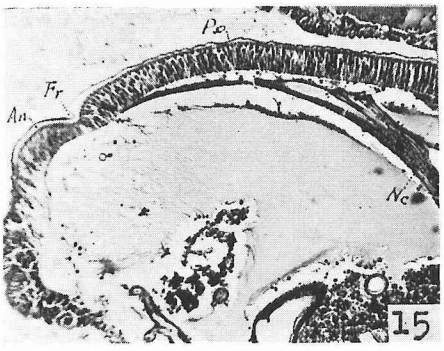
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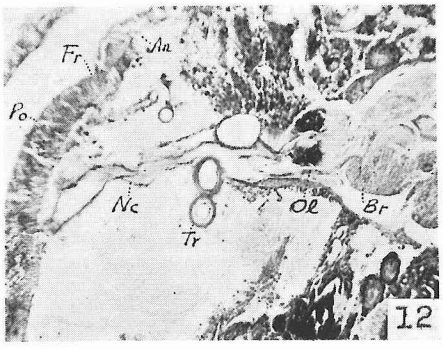
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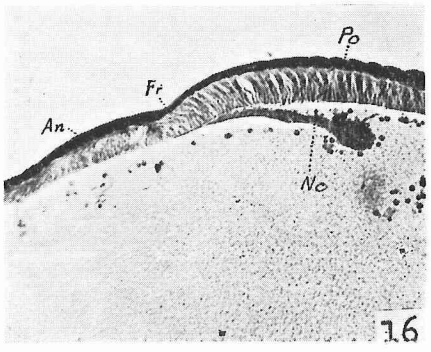
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WOLSKY, A. ; Expt. Cell. Res. Suppl., 1:549—554(1949)
* showing indirect reference

Explanation of the photos

All the photos show the developmental process of the compound eye in each larval stage undermentioned.

Photo 1. Matured larva
〃 2. Ditto (showing unknown granular bodies in the tissue)
〃 3. Ditto(showing the main nerve cord)
〃 4. Larva aged 5 hours since spinning has begun (phagocyte increases)
〃 5. Larva aged 10 hours since spinning has begun (granular ball appears)
〃 6. Larva aged 20 hours since spinning has begun (ocellus deforms)
〃 7. Ditto (ocellus is sinking)
〃 8. Larva aged 30 hours since spinnig has begun (in cross section)
〃 9. Larva of 10 hours before prepupation
〃 10. Ditto (in cross section)
〃 11. Prepupa (spinning has just finished)
〃 12. 10 hours aged prepupa
〃 13. 20 hours aged prepupa (showing the remnant of ocelli in cross section)
〃 14. Ditto (in longitudinal section)
〃 15. 26 hours aged prepupa (4 hours before pupation)
〃 16. Pupa (just pupated)

Abbreviations

An; Anterior portion of eye
Br; Brain
Cc; Crystalline cone
Cl; Corneal lens
Cu; Cuticle
Fr; Furrow
Gb; Granular ball
Hp; Hypodermis
Nc; Optic nerve cord
Ol; Ocellus remnant
Pc; Pigment cell
Pcn; Pigment cell nucleus
Ph; Phagocyte
Po; Posterior portion of eye
Rc; Retinular cell
Rcn; Retinular cell nucleus
Tr; Trachea
Ug; Unknown granular body