Formation and Histochemical Analysis of the Crystalline Cone of Compound Eye in Pieris rapae L. (Lepidoptera)

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배추흰나비 複眼의 圓錐體發生과 그 組織化學的 硏究

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(Received on Sept. 5, 1964)

摘 要

1. 배추벌레를 써서 成蟲眼의 圓錐體의 起原과 그 形成에 관한 組織學的 硏究를 하는 同時에 圓錐體의 組織化學的 分析을 하였다.
2. 早期蛹의 視板 緣邊에 왕성하게 細胞分裂을 일으키는 곳이 두군데 있다. 하나는 視板의 內緣에 있어서 圓錐體細胞를 만들어내고 또하나는 外緣에 있어서 網膜細胞들을 形成한다.
3. 圓錐體는 脊椎動物의 角膜과 마찬가지로 mucopolysaccharide-protein complex로 되어 있다.
4. 圓錐體細胞속 polysaccharides는 처음에는 可溶狀態로 圓錐體 속에 分泌되나 後에 그 重合과 蛋白質의 結合으로 不溶狀態로 된다.

INTRODUCTION

A number of descriptive studies on the development of the compound eye in Endopterygotes were given by many authors(Bodenstein, 1954; Pflugfelder, 1958; Wigglesworth, 1953; Imms, 1957; Kim, 1963) and it is now generally accepted that Lepidoptera has the eucone eyes, each ommatidium of which contains a hard refractive crystalline cone formed as an intracellular product of the cone cells. However, the origin of the cone cells and the chemical component of the cone itself are not given detailed account.

Recently Yasuzumi and Deguchi (1958) clarified the submicroscopic structure of rhabdomeres of the compound eyes in *Drosophila* and an experimental analysis of the development of the compound eyes in the mosquito, *Aedes*, was carried out by White (1961, 1963) to confirm the existence of a differentiation centre in developing compound eye, just similar one which was described by the present author in the leg rudiment from the larval leg in *Pieris* (Kim, 1959).

This paper reports an investigation in which the origin and normal formation of the crystalline cone is accounted for and histochemical analysis of it done.

MATERIALS AND METHODS

The materials, *Pieris rapae*, came from the breeding room at the Department of Biology, Korea University, were fixed at the prepupal and pupal stages (just after ecdysis, at 5 hour intervals till 60 hours, thereafter 10 hour intervals till 120 hours after pupation respectively and just before emergence). The Carnoy's fluid and formalin buffered were used as fixatives. Sections were cut in paraffin at 3 to 5 microns.

Eosin and Heidenhain's haematoxylin were adopted for usual staining and periodic acid Schiff (PAS) reaction (McManus and Cason, 1950; Hotchikiss, 1948), protein reaction (Gurr, 1956), methyl-green-pyronin staining for RNA (Kurnick, 1952) and Mallory's triple stain (Pantin, 1948) for histochemical works.

Heads of the 5th instar larvae were irradiated by X-ray (total doses 1362 r) for the purpose of inhibiting the differentiation into the elements of compound eye in adult. X-rays were delivered by North American Philips Co.

generator at the Department of Physics, Korea University, which operated at 15 m.amp. and 35 kv., using target Cu without filter. The motionless animal narcotized with ether was sticked mesothorax and end of abdomen to a short wire with wax and the head of the animal was fixed at the front of collimeter in 1cm. distance.

RESULTS

1. Development of the crystalline cone cells.

Optic placode appears by thickening epidermis between larval lateral ocelli at the prepupal stage. Epidermal cells around the larval lateral ocelli in *Pieris* become elongated to differentiate into elements of the ommatidia, nuclei of which are arranged at different levels. The nuclei of crystalline cone cells are located at the outmost of the placode, those of the pigment cells on the basement membrane and those of retinular cells between them. The crystalline cone cells appeared at the outer portion undergo cell-division to form an ommatidium.

The optic placode in the pupae to 5 hour stage after ecdysis has curved jewel shaped, the margin of which has a groove. The cells formed at the outer epidermal portion where mitosis occurs are added to the placode over the groove to be differentiated into the elements of eye. According to the growth of the placode these zones where mitosis occurs move outwards and the crystalline cone cells taken place under the cuticle at the inside of the groove are added to the elements that come from the outer epidermal portion over the groove (plate 1, figs. A and B). In other words, there are two portions where mitosis takes place intensely: One is at the out-edge of the groove to form retinular cells, while the other at the inside to form crystalline cone cell. The detailed account of it will be published in another paper.

As the retinular cells thicken and aggregate together to become spindle-shaped from 35 hour stage, the crystalline cone cells and iris cells are located around the distal end of the spindle, so ommatidium as a whole takes the appearance of a spindle (plate 1, fig. C). At this stage the cytoplasm of retinular cells contains more basophilic substances than that of the crystalline cone cells.

At 40 to 50 hour stage when the length of the epithelial cells are becoming shortened, the crystalline body is developing at the behind of the nuclei of cone cells and the cytoplasm around the crystalline body shows RNA reaction positive. At the stage of 60 hours when the pigment cells are vacuolated, crystalline cone increases in size and the nuclei of the cone cells located in front of it draw near the cuticle.

At about 80 hour stage the elements of compound eye except crystalline cone cells elongate again, and the cones of an ommatidium take a heart form to be cudgel-shaped thereafter (plate 2, figs. E,F,H and I).

2. Formation and chemical component of crystalline cones.

Histolysis taking place at about 25 hour stage of pupa, the outside of compound eye zone contains PAS positive granules scattered. At 35 hour stage when the crystalline cone cells are located at the end of the bundle of retinular cells, the cytoplasm in the behind of the nuclei contains RNA and PAS positive substances accumulated as the result of the metabolism (plate 1, fig. C). Thereafter the accumulation of PAS-positive substance is going on in the cytoplasm to be a clot, RNA being abundant in the cytoplasm around the cone at 50 hour stage. The cone looks like a vacuole containing a large globule PAS-positive (plate 1, figs. D, E and F). On the other hand the haemocytes and the basement membrane in this stage also show strong PAS positive.

However, in about 90 hours old pupal compound eyes the crystalline cones contain PAS-positive granules scattered at the margin of them as the results of elimination during the dehydration. There are aggregations of granules of PAS-positive in the crystalline cone cells and the cones of the compound eye, particularly at the inner margins, which have been irradiated with X-ray at the late 5th instar larva (plate 2, figs. C and D.). At the pupa before emergence the cones show not only PAS reaction homogenously positive but also protein reaction positive without elimination during the dehydration (plate 2, figs. G and K). The nuclei of cone cells occupying a great deal of the cytoplasm remained in the cell stain reddy by Mallory's triple stain as well as endocuticle layer.

DISCUSSION

It was generally known that a zone of larval epidermis undergoes cell division and invagination, from which the

compound eye develops entirely and that certain groups of cells move basalwards and eventually form the retinular cells while other conspicuous cells simultaneously become associated with distal parts of the developing retinulae and form the crystalline cone cells.

In *Pieris* the marginal zone of the optic placode has a groove which may be the invagination of the larval ocelli and the both edges of which undergo mitosis. It is very interesting to know that the definite region under the cuticle of the out-edge of the invagination undergoes cell divisions to produce retinular cells in insects, while multiplication of cells in vertebrates usually takes place on the basement membrane, and the cells at the inside edge of the invagination do to form crystalline cone cells.

In the pupae from 40 to 50 hour stages the granules accumulated in the crystalline cone cells react PAS-positive, so the substance may be polysaccharides. As the accumulation of the substances increases in volume at 60 hour stage, the cone contains a globule of polysaccharides in solution secreted by the cone cells (plate 1, fig. F). That the substances in the cone, thereafter, remain only at the margin of the cone may mean that the soluble polysaccharide might be eliminated during the procedure of dehydration.

In the view point of that the cytoplasm around cone being newly formed in the cone cells has RNA rich, RNA may be concerned in secretion of the polysaccharides into cone. In fact, Yasuzumi reported on the conversion of nucleoprotein into polysaccharide at the spermiogenesis in pond snail, *Cipangopaludina* (by a special lecture in Korea in 1964).

The pupa coming into emergence has cones showing strongly positive reaction of polysaccharides without elimination by dehydration and besides showing protein reaction positive, too. Therefore, presumably the crystalline cones consist of mucopolysaccharide-protein complex. In facts, polysaccharide, especially mucopolysaccharide, is known that it composes of the matrix of cornea, particularly the human cornea is known to contain sulphated mucopolysaccharide-protein complex.

So polysaccharide in cone cells is formed at soluble state at first to be secreted into cones but later the cones develop into adult's one, polymerization of polysacharides and combination with protein taking place in insoluble condition.

The part of epidermis which extends beneath the cornea is known as the corneagen layer. In *Pieris*, however, the conrneagen cells are wanting and the cornea is secreted by the outer ends of the crystalline cone cells, for the nuclei and cytoplasm in front of cones stain red by Mallory's triple stain as well as endocuticle layer.

SUMMARY

1. Histological study on the origin and formation and histochemical analysis of the crystalline cones in *Pieris* were done.

2. There are two portions where mitosis takes place intensely at the margin of the optic placode in early pupa. One is at the out-edge of the invagination to form retinular cells, while the other at the inside to form crystalline cone cells.

3. It was analyzed that the cones consist of mucopolysaccharide-protein complex as well as the cornea in vertebrates.

4. It was described that polysaccharides in cone cells are formed at soluble state at first to be secreted into cones but later developed into insoluble condition as the results of polymerization of polysaccharides and combination with proteins.

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EXPLANATION OF PLATES

(All scales mean 1μ in length)

Plate 1

Fig. A. Longitudinal section at the marginal portion of the optic placode of the prepupal stage just before ecdysis.
a: Mitosis to form the retinular cells. b: Mitosis to form the crystalline cone cells. Op: Optic placode. G: Groove (Invagination).

Fig. B. Cross section of the optic placode showing the outer edge of it where mitosis (a) takes place to form the retinular cells.

Fig. C. Longitudinal section at the stage of 35 hours, stained by periodic acid-Schiff.
N: Nucleus of the crystalline cone cells.
C: The crystalline cone (PAS-positive).

Fig. D. The same as fig. C at the 50 hour stage.

Figs. E. and **G.** Stage of 60 hours.
Fig. E: Stained by periodic acid-Schiff.
Fig. G: Stained by usual method.
R: Retinular cells. N: Nucleus of the cone cell. C: Cone (PAS-positive).

Figs. F and **H.** Stage of 70 hours.
Fig. F: Stained by periodic acid-Schiff.
Fig. H: Stained by usual method.

Plate 2

Fig. A. Section of the compound eye at the stage of 75 hours, stained by usual method.
C: Crystalline cone.

Fig. B. Section at the stage of about 90 hours, stained by usual method.

Figs. C and **D.** Section of the compound eye at about 80 hour stage in the pupa irradiated by X-ray.
Fig. C: Stained by uaual method.
Fig. D: Stained by periodic acid-Schiff.

Fig. E and **H.** The stage of about 100 hours, stained by usual method.
N: Nucleus of the cone. C: Crystalline cone.

Figs. F and **I.** The stage of about 120 hours, stained by usual method.

Figs. G and **K.** The stage just before emergence, stained by periodic acid-Schiff. The cone (c) PAS strongly positive.

PLATE 1

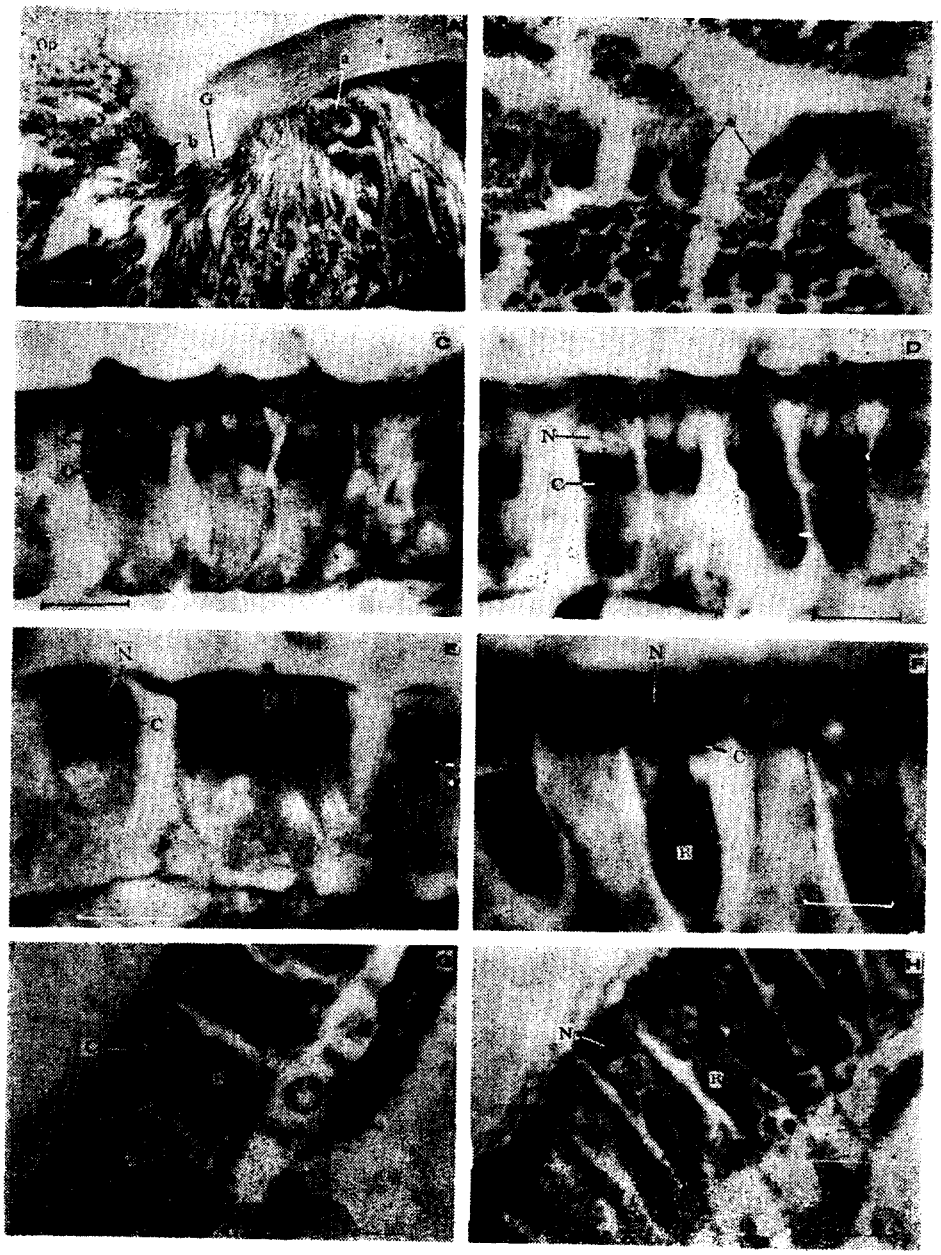

PLATE 2