The Development of the Rhabdom and the Appearance of the Electrical Response in the Insect Eye

EISUKE EGUCHI, KÉN-ICHI NAKA, and MASUTARO KUWABARA

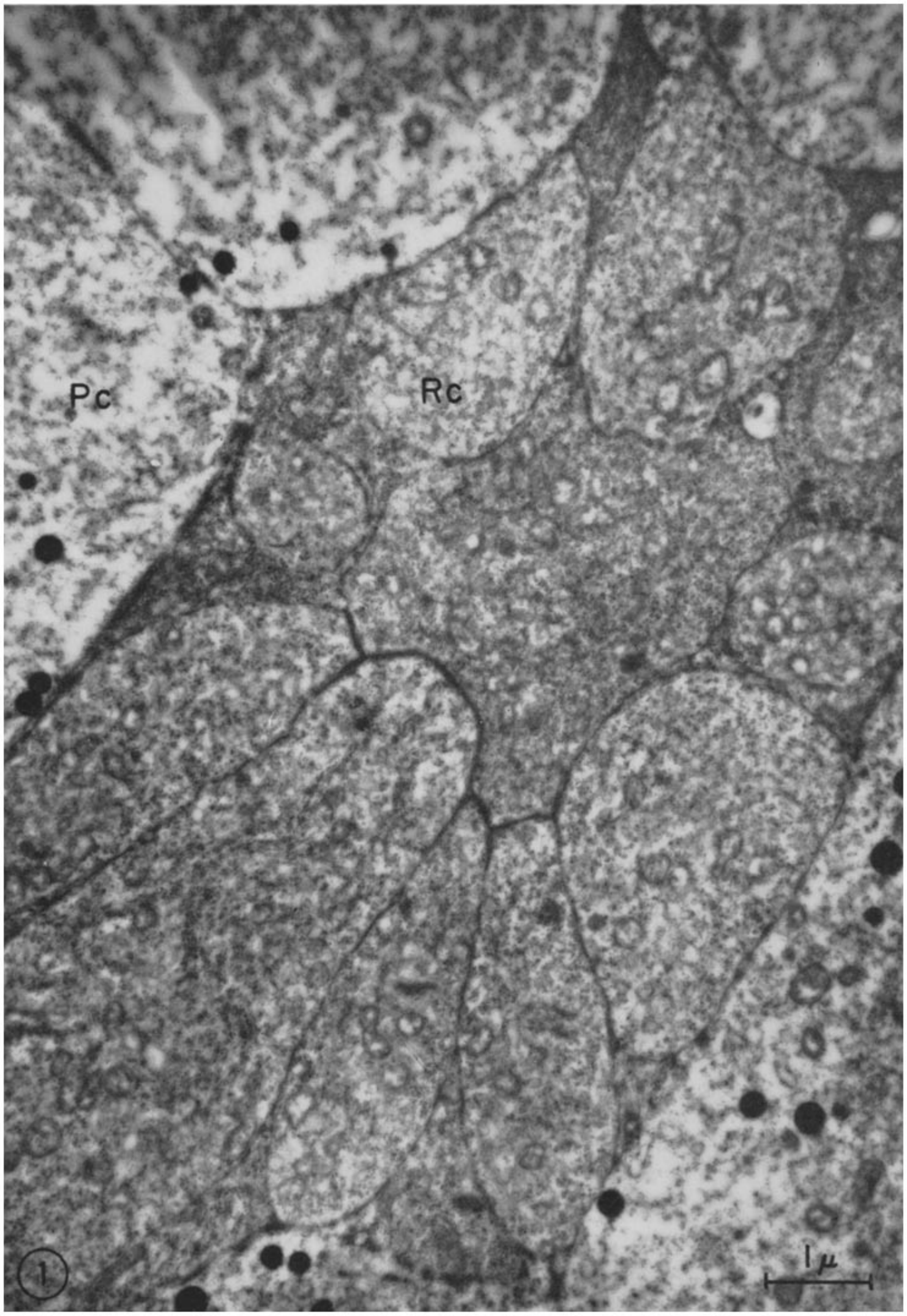
From the Department of Biology, Faculty of Science, Kyushu University, Fukuoka, Japan. Dr. Eguchi is from the Department of Anatomy, Faculty of Medicine, Kyushu University, Fukuoka, Japan. Dr. Naka's present address is the Department of Anatomy, School of Medicine, Medical Center, University of California, Los Angeles

ABSTRACT Electron microscopic studies on the development of the rhabdom in the compound eye of the silkworm moth and pupa (*Bombyx mori*) were carried out in parallel with the recording of the electrical response to photic stimulation. No electrical response to photic stimulation was recorded from the pupal compound eye which had no trace of differentiation of the rhabdom. With the differentiation of development of the rhabdom in the pupal compound eye, electrical responses could be recorded, and the amplitude of such electrical responses increased with the progress of development of the rhabdom. These observations suggest that the rhabdom is probably the site of the photochemical reaction which leads to the generation of the slow retinal action potentials.

INTRODUCTION

Recent studies on the structure of the photoreceptor in the compound eye have revealed that the rhabdom is composed of many tubular structures which are "microvilli" (Miller, 1957), and the rhabdom resembles a "honeycomb" (Goldsmith and Philpott, 1957; Fernández-Morán, 1958). Several authors have suggested that these tubular structures might be analogous to the fine dics or flattened sacs of the outer segments of the rods and cones in the eyes of vertebrates (Sjöstrand, 1959; Yamada, 1960). Although there is no conclusive evidence, it is generally thought that the rhabdom contains photosensitive pigments, and is related directly to the photochemical reaction which precedes the generation of the slow retinal action potential.

Naka (1961) and Naka and Eguchi (1962) have shown that the slow retinal action potential is the depolarization of the retinula cell membrane by photic stimulation and in the drone this depolarization triggers spike potentials in


Pc
Rc
1
1 μ

the axon portion of the cell. However, with an intracellular microelectrode in the retinula cell, it is impossible to decide whether or not the rhabdom is responsible for some of the reactions leading to the depolarization of the cell membrane. To ascertain the contribution of the rhabdom to the retinal action potential, this paper will describe the relation between the development of the rhabdom and the appearance of the electrical response in the compound eye of the silkworm pupa and moth.

MATERIAL AND METHODS

Experiments were performed on pupae and adults of the silkworm (*Bombyx mori*) obtained from the Laboratory of Sericulture, Faculty of Agriculture, Kyushu University. The pupae were kept at a constant temperature (25°C), so that the pupal stage was completed in 10 days.

For morphological observations, discs of the compound eye were fixed at 24 hour intervals from pupation to the adult stage. Eye discs were cut into several small pieces and immediately immersed into 2 per cent osmium tetroxide buffered with veronal acetate pH 7.4 to 7.6. The discs were fixed for 1 hour at 0 to 4°C, washed in distilled water, and dehydrated with an ethanol series. After embedding in *n*-butyl methacrylate, thin sections were cut with a Porter-Blum microtome and were observed with an Hitachi electron microscope (HU-11) and an Akashi tronscope (50-EB).

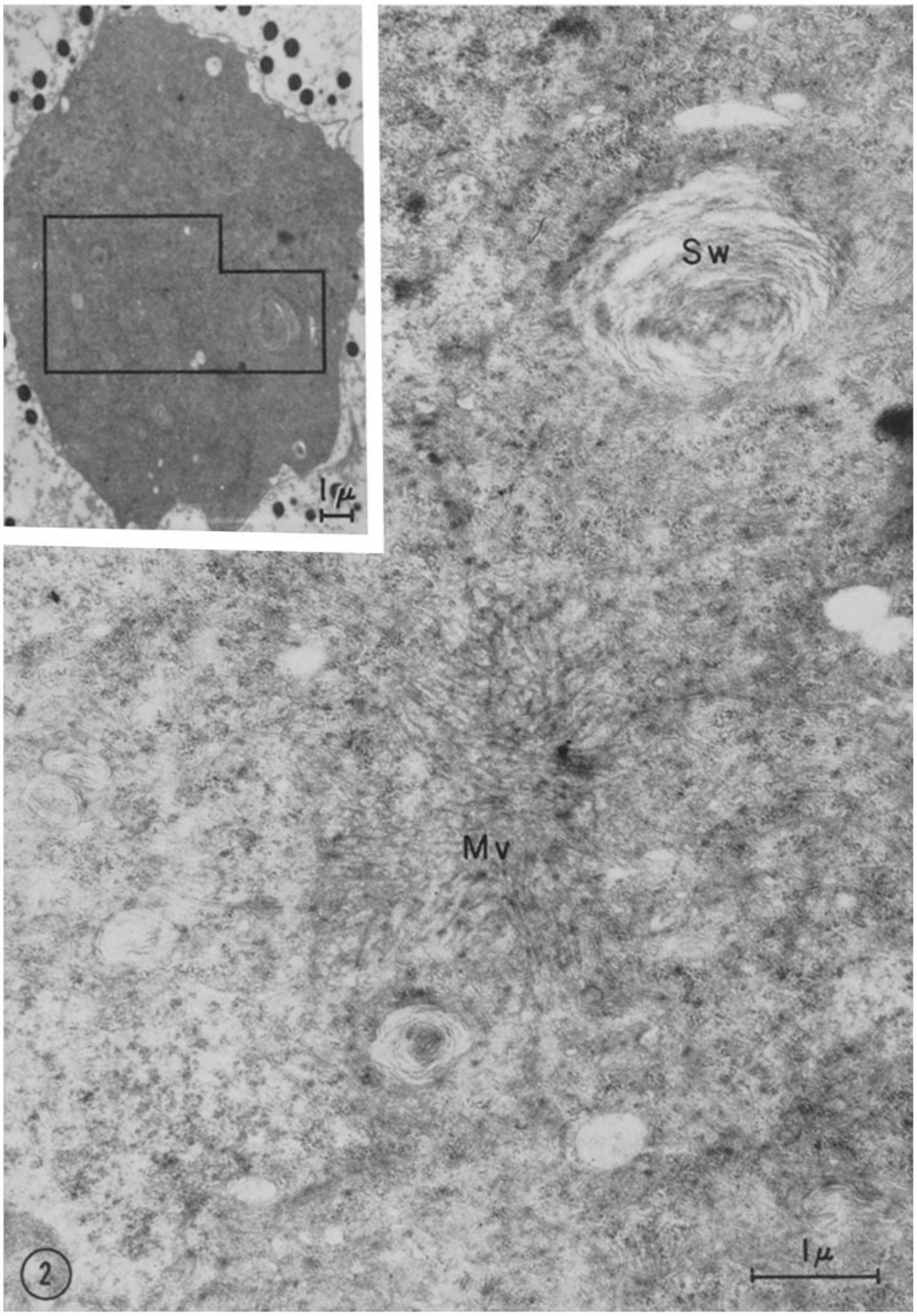
Methods for recording electrical responses from the insect compound eyes were almost the same as those described in previous papers except that all responses were obtained extracellularly (Naka, 1961; Naka and Eguchi, 1962). A microelectrode (3 M KCl-filled capillary electrode) was inserted into the receptor layer through a hole made on the corneal surface. Electrodes (1 to 2 μ tip diameter) were selected from ones having an ohmic resistance of around 5 megohms. An indifferent lead was taken from a Ringer pool in which the eye was fixed with wax. Potentials were picked up by a cathode follower input stage, and then fed into a two-beam oscilloscope (Nihon Koden VC-6). Illumination from a tungsten lamp with a ribbon filament was interrupted either by a camera shutter or by a turning sector wheel. Both duration and intensity of illumination were monitored by a photodiode placed near the eye and displaced on the lower beam of the oscilloscope.

Room temperature was about 20°C throughout the experiments.

RESULTS

For convenience of description, the pupal stage of the silkworm will be divided into two stages, namely, the first stage (1st to 5th day) in which no trace of rhabdom was found in the ommatidium, and the second stage or the rhabdom-

FIGURE 1. Oblique cross-section through the retinula cell layer of a single ommatidium of the compound eye of a 4 day old pupa of the silkworm. Ten retinula cells (*Rc*) surround a single cell lying at the center of the ommatidium. At this stage, the microvilli of the rhabdom are not yet observed. (*Pc*) pigment cell. $\times$ 15,000.



Sw
Mv
1μ
1μ
2

forming stage (6th to 10th day) in which the rhabdom was being formed in the ommatidium.

1. *The First Stage*

It was found to be extremely difficult to dissect and to fix the eye discs of 1 to 3 day old pupae due to the semifluid state of the tissue.

Fig. 1 shows a cross-section through the retinula cell layer of an ommatidium from a 4 day old pupa. In the figure, the ommatidium is seen to be composed of eleven cells; ten cells surround one lying at the center. In this ommatidium, there is no trace of microvilli and each cell has a smooth cell membrane. The arrangement of the cells suggests that the cell at the center of the ommatidium will develop into an eccentric retinula cell.

No electrical response to photic stimulation could be recorded from the eye disc at this stage.

2. *The Second Stage*

From the 5th day after pupation, the ommatidium began to show a trace of the rhabdom.

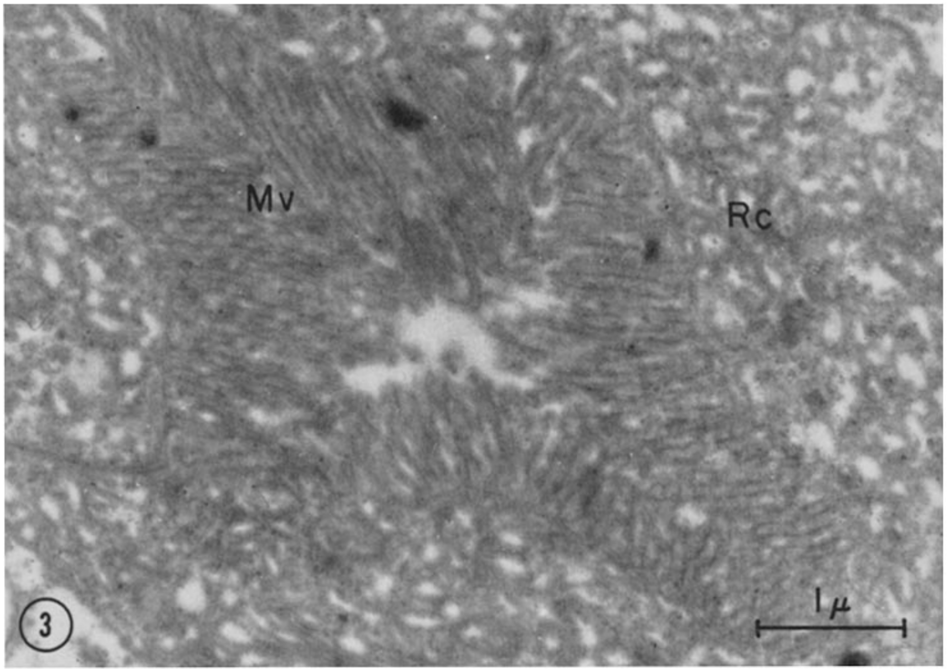
(A) FINE STRUCTURE

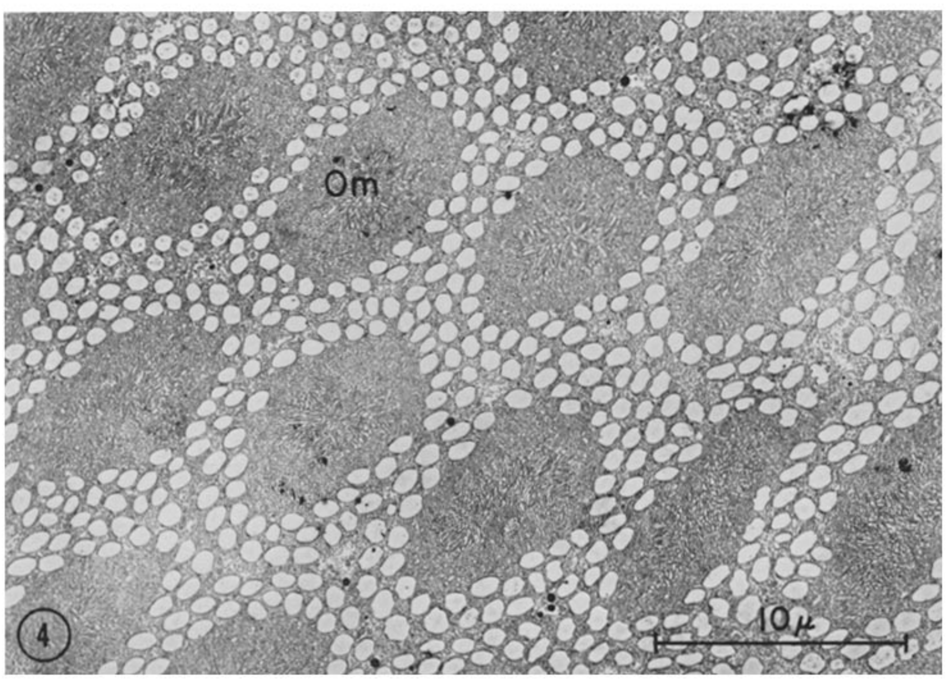
Fig. 2 shows an oblique cross-section of the central part of an ommatidium through the retinula cell layer of the compound eye of a 5 day old pupa. A small number of microvilli are seen in the central portion of this ommatidium, but the arrangement is not as orderly as that of the adult stage. This observation suggests that the rhabdom arises from the integration of the specialized parts of the retinula cell membrane. The retinula cell of this stage contains a considerable amount of rough surfaced endoplasmic reticulum which causes a rather dense staining of the cell as seen in the insert of Fig. 2. The swirling endoplasmic reticulum is also frequently observed in the retinula cell at this stage. There are no dark particles in the swirling endoplasmic reticulum, therefore these regions are composed of the so called smooth surfaced variety of endoplasmic reticulum.

The dense particles observed in Fig. 2 may represent the RNP particles. The membranes of the swirling endoplasmic reticulum are continuous with those of the rough surfaced endoplasmic reticulum at the margins of the

---

FIGURE 2. Oblique cross-section of the central part of an ommatidium through the retinula cell layer of the compound eye of a 5 day old pupa of the silkworm. At this stage, small number of microvilli (*Mv*) are seen at the central part of the figure, and there are numerous membranous structures, the endoplasmic reticulum. Characteristic swirling structures (*Sw*) of smooth surfaced endoplasmic reticulum are seen in the retinula cells. × 19,000. Inset: Low magnification photograph of a single ommatidium of this stage. Fig. 2 is the enlarged photograph of a marked area in this figure. × 4,200


Mv
Rc
1 μ
3


Om
10 μ
4

swirls. The endoplasmic reticulum in the cells may be possibly related to the synthesis of photosensitive substances.

Fig. 3 shows an oblique cross-section of the rhabdom of an ommatidium of the compound eye of 7 day old pupa. In this stage, the microvilli are quite well developed, and it is seen that these microvilli protrude to the center of the ommatidium.

Fig. 4 is a cross-section through the retinula cell layer of an ommatidium of the compound eye of a 7 day old pupa. This electron micrograph was obtained from the same compound eye that provided the electroretinogram of Fig. 6. Ommatidia are arranged symmetrically and surrounded by numerous cross-sectioned fine tracheoles. The central region of each ommatidium is occupied by many microvilli, but the region occupied by these microvilli is about half that of the moth ommatidium.

(B) ELECTRICAL RESPONSE

An electrical response to photic stimulation could be obtained from the pupa of the 5th day stage. However, it was very difficult to record electrical responses from the compound eye of this stage because of the semifluid state of the eye. The electrical responses recorded were unstable with an amplitude less than 0.5 mv.

Typical responses from the compound eye of this stage are shown in Fig. 5. Trace A shows a response to intermittent illumination of 0.1 sec. duration at 0.6 sec. intervals, and B is a response to illumination of 1.5 sec. duration. The response was maintained during illumination, though its amplitude decreased slightly. The response returned to the base line immediately after cessation of illumination.

Consistent and stable electrical responses to photic stimulation were obtained from the compound eye of the 7th day pupa. As stated above, the two records in Fig. 6 were obtained from the compound eye which provided the electron micrograph of Fig. 4. The electrical response from the eye of this stage was a slow negative potential of about 5 mv, which decreased in negativity during continuous illumination (Fig. 6 B); the repeated illuminations by short flashes also resulted in decreased amplitude (Fig. 6 A). The potential

---

FIGURE 3. Oblique cross-section of the central part of an ommatidium through the retinula cell layer of the compound eye of a 7 day old pupa. It is seen that many elongated microvilli (*Mv*) protrude from the retinula cell membranes. (*Rc*) retinula cell. × 20,000.

FIGURE 4. Oblique cross-section through the retinula cell layer of the compound eye of a 7 day old pupa of the silkworm. Each ommatidium (*Om*) is surrounded by many tracheoles. At this stage, the ommatidium is occupied with developing rhabdom showing reticular and tubular structures corresponding to the microvilli of the adult rhabdom. This electron micrograph was obtained from the same compound eye that provided the ERG of Fig. 6. × 3,300.

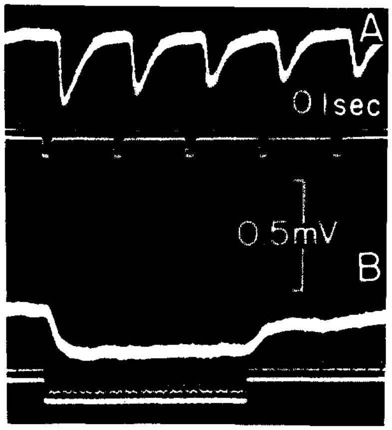


FIGURE 5. Electrical response to photic stimulation from the compound eye of a 5 day old pupa. A, the response to intermittent illumination, 0.1 sec. light, 0.5 sec. dark; B, the response to illumination for 1.5 sec.

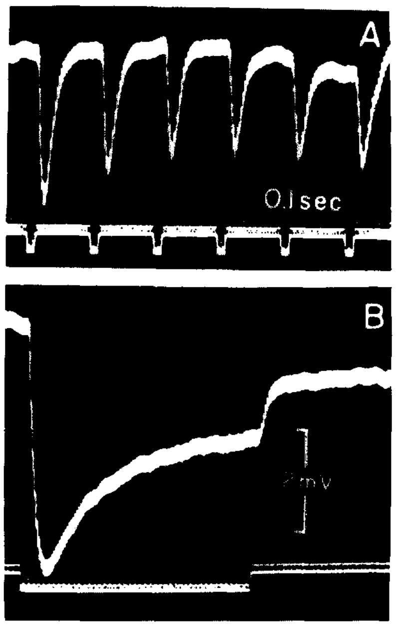


FIGURE 6. Electrical response to photic stimulation from the compound eye of a 7 day old pupa, which provided the electron micrograph for Fig. 4. After recording the electrical response, this eye was fixed by osmium tetroxide. A, the response to intermittent illumination, 0.1 sec. light, 0.5 sec. dark; B, the response to illumination for 2 sec.

returned almost to the base line after each period of illumination as in the case of the response from 5 day old pupa (Fig. 5).

3. *Adult*

(A) MORPHOLOGY

More detailed information on the fine structure of the compound eye of the silkworm moth is contained in the paper by Eguchi (1962). A brief summary of this paper follows: an ommatidium of the compound eye of the silkworm moth consists of eleven sensory cells, ten cells are usual retinula cells, but the last one resembles the eccentric cell observed only in the horseshoe crab compound eye. This particular cell is described as the eccentric retinula cell (Eguchi, 1962). Like a bipolar neuron, this cell sends a dendrite into the distal portion of the retinula cell layer and a neurite fiber into the proximal layer through the basement membrane. The perikaryon of this cell lies more proximal in the retinula cell layer than do those of retinula cells. The rhabdom of the moth eye is formed by microvilli of the retinula cells, the eccentric retinula cells, and its dendrite.

Fig. 7 shows an oblique section through the retinula cell layer of an ommatidium ot the compound eye of the silkworm moth. About half the area of this ommatidium is occupied by the eccentric retinula cell, its dendrite, and the microvilli, which protrude from both parts. In the dendrite of the eccentric cell, several mitochondria are seen. It is also apparent that in the adult eye, the microvilli are packed much more densely than in the pupa.

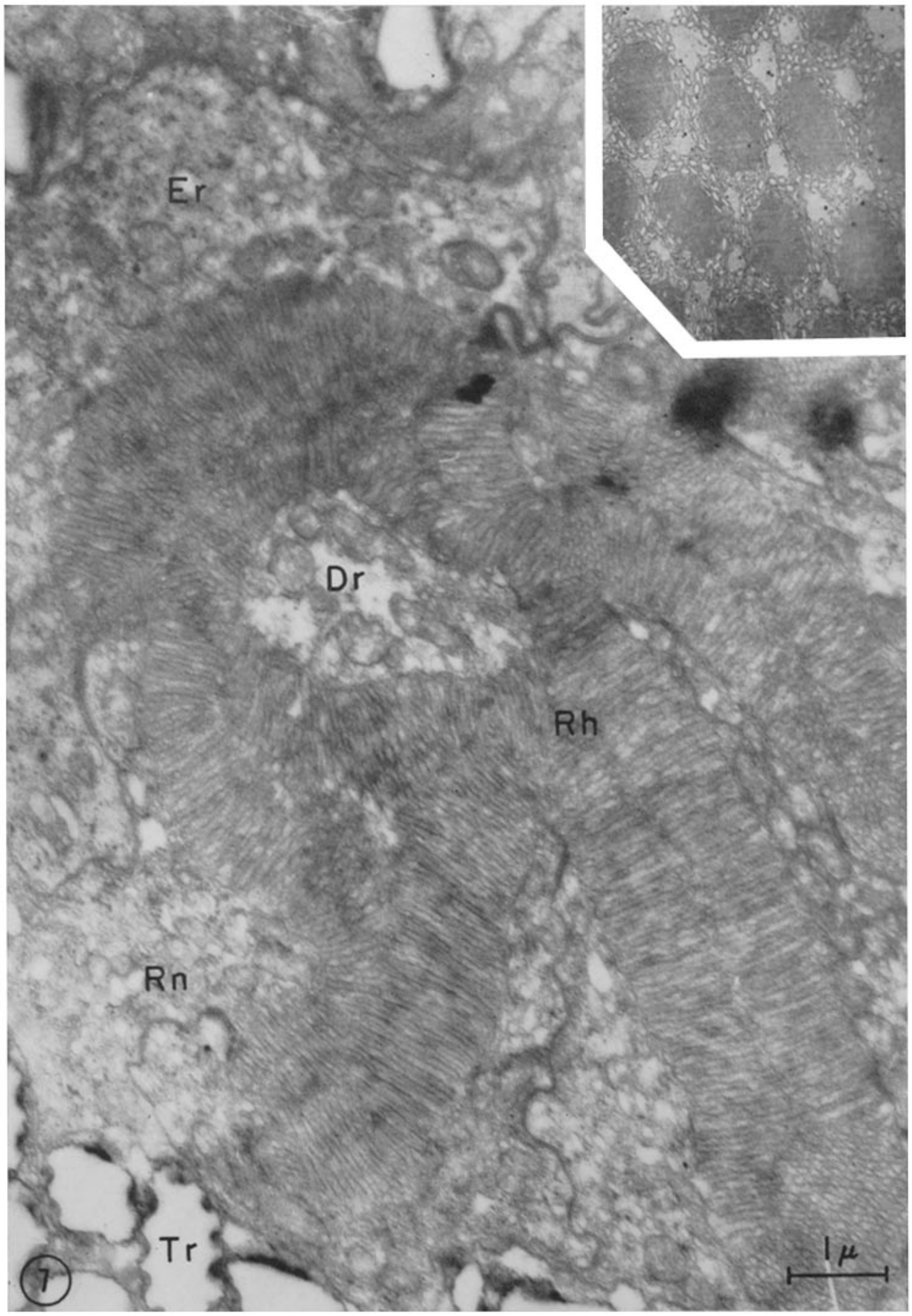
(B) ELECTRICAL RESPONSE

The electrical response from the compound eye of the adult silkworm was a slow negative potential. The amplitude of the response (usually about 10 mv) gradually increased during continued illumination (Fig. 8 C). After cessation of illumination, the slow potential did not return to the base line; a prolonged negativity continued. For example, in Fig. 8 C, the 9 mv slow potential showed an off-return of only 3 mv, and the prolonged negativity was about 6 mv in amplitude. This prolonged negativity was a characteristic feature of the electrical response from the adult silkworm. Fig. 8 B shows the electrical response to intermittent illumination of 0.1 sec. duration at 0.6 sec. intervals. In the case shown in Fig. 8 A, frequency of intermittent light is increased to 0.1 sec. on and 0.1 sec. off. With repeated illumination of high frequency, the amplitude of the response and that of the after negativity increased gradually as if the stimulation were continuous light.

DISCUSSION

The compound eye of the silkworm moth used in this experiment is of the superposition type. An ommatidium of the silkworm moth consists of eleven sensory cells, ten retinula cells, and one eccentric retinula cell. The eccentric retinula cell sends a dendrite along the central axis of the ommatidium into the distal portion (Fig. 7). The rhadbom is formed by microvilli of the retinula cells, of the eccentric retinula cell, and of its dendrite.

No reports on the existence of the particular type of cell like an eccentric retinula cell found in the silkworm moth has been reported from the other insect compound eyes. However, many workers (Watase, 1890; Miller, 1957, and others) have reported that an ommatidium of the compound eye of the


Er
Dr
Rh
Rn
Tr
7
1 μ

horseshoe crab has a bipolar neuron named the eccentric cell in addition to the normal retinula cells, and it is generally believed that only the eccentric cell responds electrically with spike potentials to photic stimulation (Hartline *et al.*, 1952; Tomita, 1956).

From the morphological point of view, the eccentric retinula cell in the silkworm moth resembles the eccentric cell of the horseshoe crab compound eye. While the eccentric cell of the horseshoe crab ommatidium does not contribute to the rhabdom formation (W. H. Miller, personal communication), the eccentric retinula cell contributes to the rhabdom formation by extending microvilli from its cell body and dendrite.

(*A*) *Morphogenesis of the Rhabdom during Pupal Stage*

In the early stage of development of the compound eye (1st to 4th day of pupal stage), an ommatidium is composed of eleven sensory cells containing no trace of the rhabdom. From the 5th day copious endoplasmic reticulum begins to appear in the retinula cells. At the same time, the microvilli extend from the retinula cells and the eccentric retinula cell, and occupy the central region of the ommatidium. These observations lead to the conclusion that formation of the rhabdom in the adult eye results from the increase in the number and length of the microvilli.

From the study of the morphogenesis of the pupal compound eye of *Drosophila*, Waddington (1960) suggested that the rhabdom might be constructed with vesicles which were made of nuclear membrane. On the morphogenesis of the outer segment of the retinal rod and cone of the vertebrate eye, Tokuyasu and Yamada (1959) and Moody and Robertson (1960) presented evidence that the lamellae of the outer segment were formed through in-tuckings of the surface membrane of the visual cell and were continuous with the extentions of the plasma membrane. Thus, there is an interesting similarity between the rhabdom and the outer segment of the rod and cone; that is, they are both formed by the differentiation of the visual cell membrane.

(*B*) *Relation between Rhabdom and Electrical Response*

In this study it was shown that even before emergence the compound eye is able to respond to photic stimulation with a slow negative potential, the

---

FIGURE 7. Oblique cross-section through the retinula cell layer of an ommatidium of the compound eye of a silkworm moth. The dendrite of the eccentric retinula cell is seen at the center of the ommatidium, and it is seen that the dendrite forms a rhabdomere. Several mitochondria are seen in the dendrite. (*Er*) eccentric retinula cell, (*Rn*) retinula cell, (*Dr*) dendrite of the eccentric retinula cell, (*Rh*) rhabdom., (*Tr*) trachea. × 14,000. Inset: Low magnification photograph of the cross-section through the retinula cell layer of the compound eye of silkworm moth. Each ommatidium is surrounded by pigment cells and many tracheae. × 1,300.

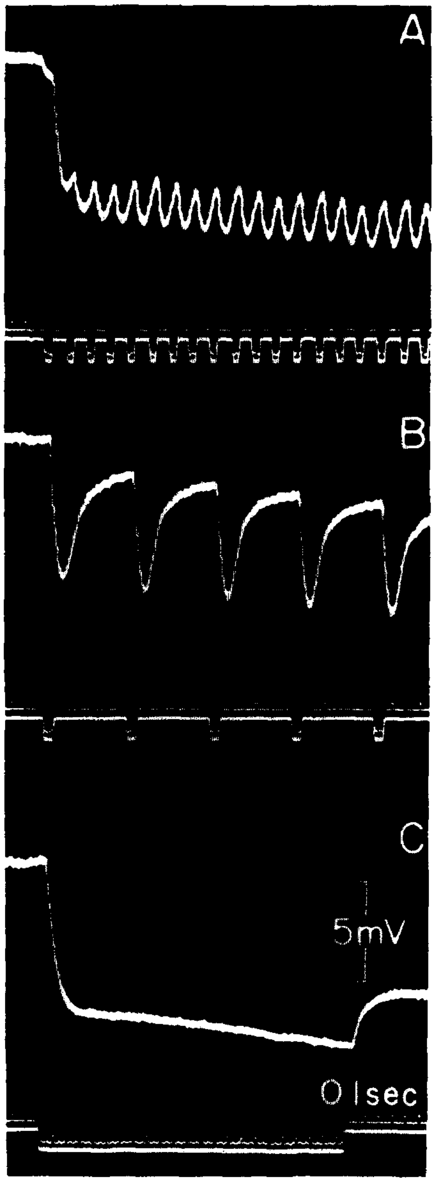


FIGURE 8. Electrical response to photic stimulation from the adult compound eye. A, the response to high frequency intermittent illumination, 0.1 sec. light, 0.1 sec. dark. B, the response to the intermittent illumination, 0.1 sec. light, 0.5 sec. dark. C, the response to 2.5 sec. illumination.

retinal action potential. The results obtained in several kinds of insects (Burkhardt and Autrum, 1960; Naka, 1961; Naka and Eguchi, 1962) show that this slow potential reflects the depolarization of the retinula cell membrane. As described in the results, the appearance of the electrical response during the pupal stage has a close relation with the differentiation of the microvilli in the retinula cells. It was also confirmed that the amplitude of the response increased with the increase in the number of microvilli of the retinula cells. These results are shown diagrammatically in Fig. 9, in which A illustrates the longitudinal section of an ommatidium at different stages of development and B illustrates the electrical responses recorded from the compound eye at corresponding stages.

This suggests that the generation of the slow potential or depolarization of the retinula cell membrane requires the presence of the microvilli. However, in the silkworm compound eye, the situation is complicated by the presence of the eccentric retinula cell (Eguchi, 1962). Though the functional significance of the eccentric retinula cell is presently unknown, the results obtained in the other insects which lack the eccentric retinula cell or from which no such cell has been reported so far, show that it is the retinula cell membrane that is depolarized by the photic stimulation (Naka, 1961; Naka and Eguchi, 1962). The results obtained from the *Procambarus* compound eye also support this

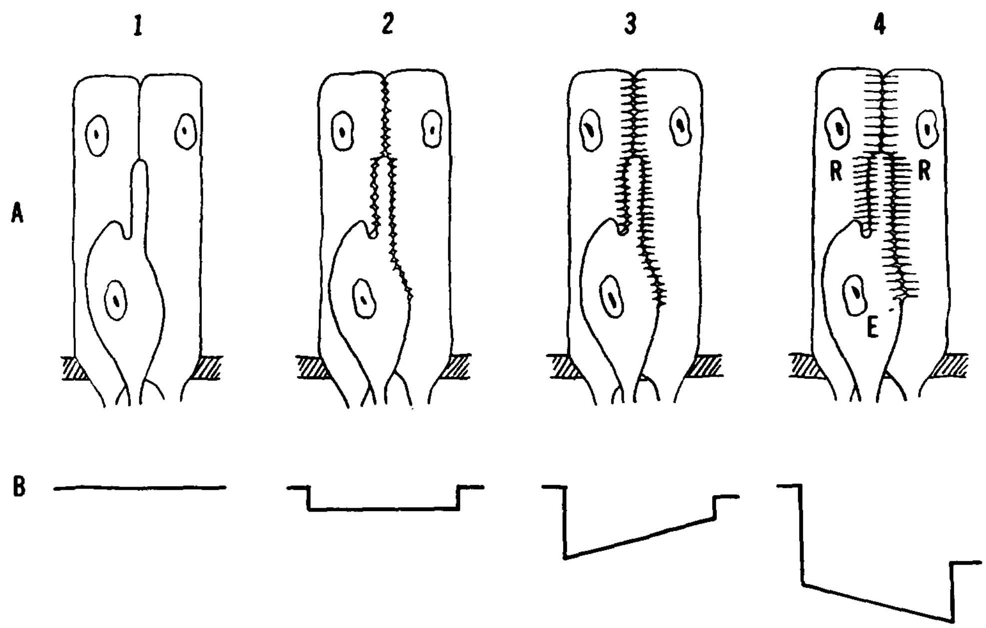


FIGURE 9. Schematic illustration of the relation between the development of the rhabdom and the electrical response. A, longitudinal section through the ommatidium. (*E*) eccentric retinula cell, (*R*) retinula cell. B, electrical response. 1, smooth surface of retinula cell membrane at the 4th day pupal stage, rhabdom is not formed at all. No electrical response can be recorded. 2, the first step of rhabdom formation, appearance of many small microvilli on the surface of the retinula cell membranes (5th day pupa), electrical response is a slow negative monophasic wave. 3, formation of microvilli (7th day pupa). Electrical response is a slow negative monophasic wave. 4, rhabdom at adult stage, tightly packed with many tubes. Electrical response is a slow negative monophasic wave. The amplitude of the electrical response and the prolonged after potential from the adult compound eye are larger than those from the pupal compound eye.

conclusion (Naka and Kuwabara, 1959). However, this observation does not exclude the possibility of slow or spike potential generation in the eccentric retinula cell. In *Limulus* it is the eccentric cell that produces spike potentials (Hartline, Wagner, and MacNichol, 1952).

Although there is no conclusive evidence, the results obtained in this experiment tempt us to postulate that the microvilli might be the site of the photochemical reaction which leads to the depolarization of the retinula cell membrane. In *Limulus*, Fuortes (1959) came to the conclusion that the retinula cell might liberate chemical substances which cause the membrane resistance of the eccentric cell to decrease. In the insect, the chemical substances contained in the microvilli might precipitate the decrease in the resistance of the retinula cell membrane by photic stimulation.

According to the classification of the insect ERG by Autrum (1950) the compound eye of the silkworm is of the slow type. An interesting difference between the responses from the compound eye of the adult and the pupa was observed, *i.e.* the response from the pupa compound eye decreased its amplitude during continued illumination, but that from the adult silkworm increased its amplitude. There were also some differences in the after potential. This after potential seems to be due to a long lasting component similar to the S component in *Dytiscus* (Bernhard, 1942). This component is absent in the response from the compound eye of the very early rhabdom-forming stage. Therefore it may be suggested that the mechanism responsible for this component develops at the late pupal stage.

The electron microscopic observations in this work were carried out in the Department of Anatomy, Faculty of Medicine, Kyushu University. We wish to thank Dr. Eichi Yamada, Professor of Anatomy, Kyushu University, for his suggestions during the course of this work and for his critical review of this paper. We are grateful to Associate Professor H. Tsukushi of the Department of Agriculture, Kyushu University, for his kindness in supplying the material.
This work was supported in part by a grant from The Rockefeller Foundation. It is Contribution No. 128 from the Department of Biology, Faculty of Science, Kyushu University, Japan.
Received for publication, March 13, 1962.

REFERENCES

AUTRUM, H., 1950, Die Belichtungspotentiale und das Sehen der Insekten, *Z. vergleich Physiol.*, **32**, 176.

BERNHARD, C. G., 1942, Isolation of retinal and optic ganglion response in the eye of *Dytiscus*, *J. Neurophysiol.*, **5**, 32.

BURKHARDT, D., and AUTRUM, H., 1960, Die Belichtungspotentiale einzelner Sehzellen von *Calliphora erythrocephala* Meig. *Z. Naturforsch.*, **15**, 612.

EGUCHI, E., 1962, The fine structure of the eccentric retinula cell of insect compound eye (*Bombyx mori*), *J. Ultrastructure Research*, in press.

FERNÁNDEZ-MORÁN, H., 1958, Fine structure of the light receptor in the compound eyes of insects, *Exp. Cell Research*, suppl. **5**, 586.

FUORTES, M. G. F., 1959, Initiation of impulses in visual cells of *Limulus, J. Physiol.*, **148**, 14.

GOLDSMITH, T. H., and Philpott, D. E., 1957, The microstructure of the compound eyes of insects. *J. Biophysic. and Biochem. Cytol.*, **3**, 429.

HARTLINE, H. K., WAGNER, H. G., and MACNICHOL, E. F., 1952, The peripheral origin of nervous activity in the visual system, *Cold Spring Harbor Symp. Quant. Biol.*, **17**, 125.

MILLER, W. H., 1957, Morphology of the compound eye of *Limulus, J. Biophysic. and Biochem. Cytol.*, **3**, 421.

MOODY, M. F., and ROBERTSON, J. D., 1960, The fine structure of some retinal photoreceptors, *J. Biophysic. and Biochem. Cytol.*, **7**, 87.

NAKA, K., 1961, Recording of retinula action potentials from single cells in the insect compound eye, *J. Gen. Physiol.*, **44**, 571.

NAKA, K., and EGUCHI, E., 1962, Spike potentials recorded from the insect photoreceptor, *J. Gen. Physiol.*, **45**, 663.

NAKA, K., and KUWABARA, M., 1959, Two components from the compound eye of the crayfish, *J. Exp. Biol.*, **36**, 51.

SJÖSTRAND, F. S., 1959, The ultrastructure of the retinal receptors of the vertebrate eye, *Ergebn. Biol.*, **21**, 128.

TOKUYASU, K., and YAMADA, E., 1959, The fine structure of the retina studied with the electron microscope. IV. Morphogenesis of outer segments of retinal rods, *J. Biophysic. and Biochem. Cytol.*, **6**, 225.

TOMITA, T., 1956, The nature of action potentials in the lateral eye of the horseshoe crab as revealed by simultaneous intra- and extracellular recording, *Japan J. Physiol.*, **6**, 327.

WADDINGTON, C. H., 1960, The morphology of developing systems at the ultramicroscopical level, New Approaches in Cell Biology, New York, Academic Press, Inc., 45.

WATASE, S., 1890, On the morphology of the compound eyes of arthropods, *Studies Biol. Lab. John Hopkins Univ.*, **4**, 287.

YAMADA, E., 1960, Observations on the fine structure of photoreceptive elements in the vertebrate eye, *J. Electronmicroscopy, Japan*, **9**, 1.