ATPASE ACTIVITY IN THE CILIARY ROOTLET OF HUMAN RETINAL RODS

TOSHIHIKO MATSUSAKA. From the Ophthalmologic Clinic, the Center for Adult Diseases, Higashinari-ku, Osaka, Japan

Local specialization of the plasma membrane generally is becoming recognizable in retinal photoreceptors. For example, the outer segment has been noted as a site for a photochemical reaction with respect to visual function (Wald, 1961). And the synaptic membrane of the terminal end has been known to perform the transmission of an impulse (De Robertis and Franchi, 1956; Sjöstrand,

1958). Yamada (1960) and Matsusaka (1963 and 1966) emphasized that the plasma membrane of the inner segment may subserve metabolism in the photoreceptor. They also refer to a close correlation between the photoreceptor and the Müller cell and thereby suggest functional and biochemical interactions between the two. Since invaginations and protrusions in the plasma membrane of the inner segment are present, they may be involved in the absorption or release of metabolites rather than in the conduction of an excitatory state from the outer segment to the synapse (De Robertis, 1956). This may suggest an intracellular conduction on this level. Sjöstrand (1953) and Cohen (1960) have speculated that the connecting cilium and its rootlet might be the site of the conduction of an impulse. However, little evidence has been available to confirm such speculation.

This note reports an electron microscope study of a normal human retina following incubation in Wachstein-Meisel's ATPase medium (1957). The study has revealed deposition of reaction products at the ciliary rootlet of retinal rods.

MATERIALS AND METHODS

The eyes were obtained from a 79 yr old patient with cardiac disorders, 5 hr after his death. They were fixed in neutral 10% formalin at 4°C overnight. After treatment at 4°C for 1 day in 0.067 M phosphate buffer solution containing 5% sucrose, the retina from the posterior eye segment was washed for 1 day in Veronal-acetate buffer solution containing 5% sucrose at 4°C. The retina then was cut with a razor into small pieces 2 × 4 mm². The specimens were incubated in Wachstein-Meisel's medium with adenosine triphosphate as a substrate at room temperature for 25 min. Control sections were incubated without adenosine triphosphate. After incubation, the retina was washed briefly in Veronal-acetate buffer solution and postfixed in 1% osmium tetroxide, followed by embedding in epoxy-Epon resin. Ultrathin sections were cut on an LKB Ultrotome with glass knives. They were observed under a JEM-6C type electron microscope with or without staining with lead hydroxide.

RESULTS AND DISCUSSION

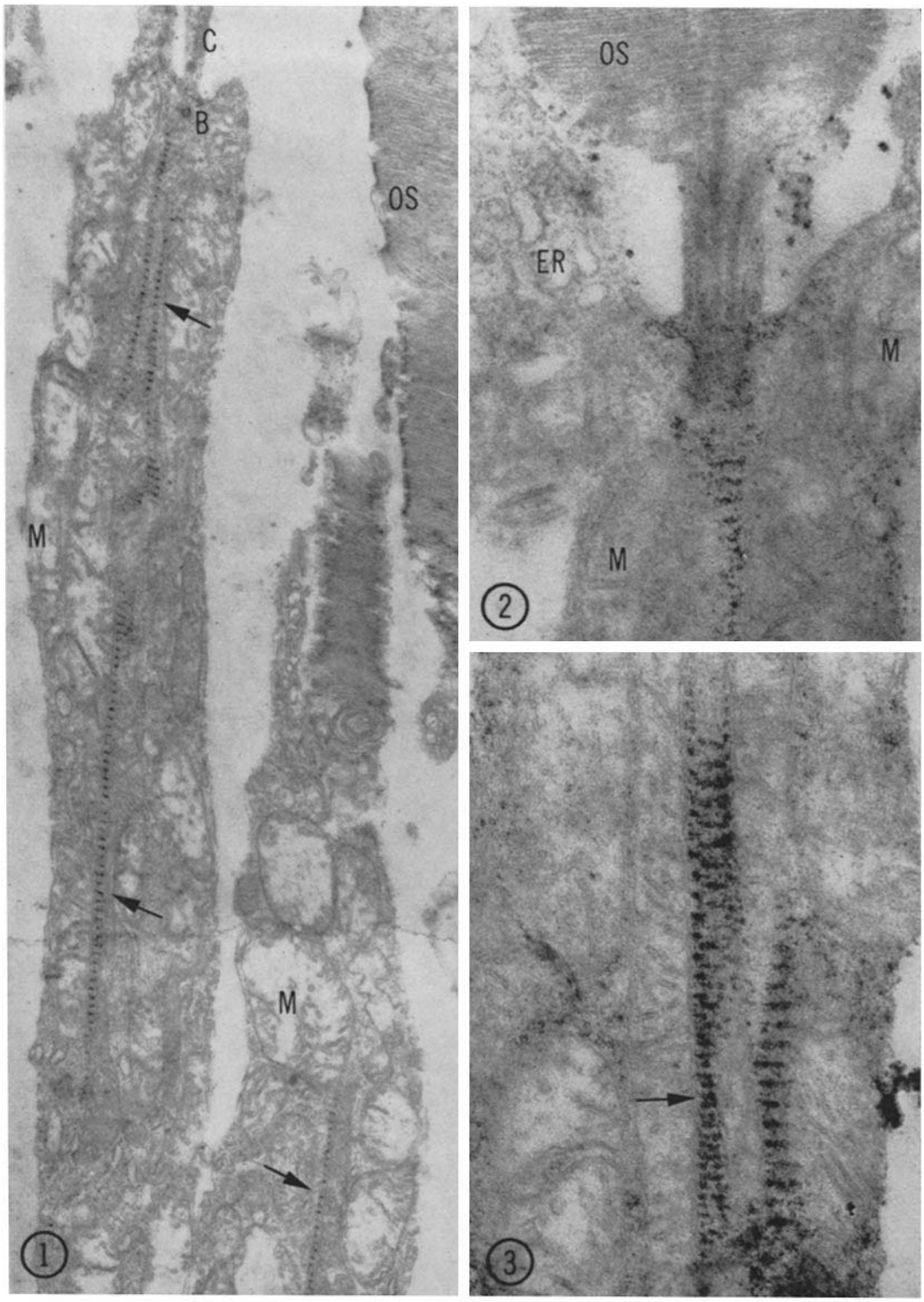
The distribution of the reaction products in the human retina is similar to that in the frog (Scarpelli and Craig, 1963) and rabbit retinae (Mazima, 1966), but the intensity of staining is lower than that in experimental animals. The poor preservation of the fine structure, as well as the low level of activity, seems to result from the long delay before fixation of the eye. In the rod inner segment, the location of reaction products corresponds to the position of the cross-striated bundles of the ciliary rootlet (Fig. 1). Since these deposits are very intensely electron-opaque, sections incubated for enzyme activity are convenient for demonstrating the structure and the course of the bundles as presented by Novikoff et al. (1966). Minute deposits of reaction products are seen where the cilium and basal body connect. An aggregation of these products extends for a short distance from the structure of the basal body along the cell surface. It seems to coincide with "Begleitgebilde" (Lerche, 1963).

Banded deposits at the ciliary rootlet start from

FIGURE 1 Longitudinal section through the outer and inner segments of human retinal rods. Reaction products are seen (*a*) at the basal body (*B*) and the ciliary rootlets (arrows) where they appear in the cross-striations, and (*b*) at a margin of the outer segment (OS). The ciliary rootlet runs down into the inner segment among profiles of mitochondria (*M*) which are swollen like vacuoles. *C*, connecting cilium. Lead hydroxide stain. × 16,000.

FIGURE 2 Distal end of a rod inner segment at high magnification. Minute deposits of reaction products are seen at the region of the basal body and connecting cilium, and at the filamentous structure of the ciliary rootlet. But gross deposits appear to be related to disrupted plasma membranes of the outer and inner segments. *M*, mitochondria; *ER*, endoplasmic reticulum; *OS*, outer segment. Unstained. × 47,000.

FIGURE 3 Longitudinal section through a proximal region of a rod inner segment. Banded deposits of reaction products are seen at bundles of the ciliary rootlet filaments. Note the change of the repeating pattern where two tracts of filament bundles conjugate (arrow). Unstained. × 41,000.


C
B
OS
M
M
1
OS
ER
M
M
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3

a site about 40 mμ away from the lower end of the basal body (Fig. 2). At this originating portion of the rootlet, its periodicity is measured as 630–650 A and the width of the deposit is 170 A as measured along the course of the filaments of the rootlet. The periodicity and the pattern of the deposit of reaction products gradually vary as the distance from the originating site increases, i.e., the periodicity becomes 750–780 A at the intermediate region of the course of the rootlet. In a 750 A period at this region, the width of the deposit is 270 A (Fig. 3). But after two tracts of filament bundles are conjugated, a 760 A period comprises a 250 A clear zone and a 510 A zone of the deposit which is subdivided by a 70 A clear zone into two 220 A zones of the deposit (Fig. 3, cited by an arrow). Furthermore, the periodicity is measured as 670–780 A at the proximal end, in which a dark zone of the deposit, showing a cross-striation by more dense lines, occupies a region 560 A wide (Fig. 7). Each filament is round in cross-section and is 95–120 A in diameter in both the clear and dark zones (Fig. 4).

When it passes through the inner segment, the bundle of the ciliary rootlet moves from the center to a lateral position, and the deposit of reaction products is confluent with the deposit at the cell surface (Figs. 5 and 6). In a section more advanced to show better the course of the ciliary rootlet, it can be seen that the bundle approaches the plasma membrane at the level of the outer, limiting membrane (Fig. 7). Moreover, a very intense accumulation of reaction products is observed at the cell surface in the proximal end of the rod inner segment (Fig. 7), and a small deposit of the products also appears at the cell surface (Figs. 2, 3 and 5–7). Both deposits need further study to determine whether they result from the diffusion of products which occurs at the plasma membrane of the proximal end of the inner segment. Whether the initial site of the reaction products is the plasma membrane of the inner segment, a membrane of microvilli of the Müller cell, or both membranes remains unknown.

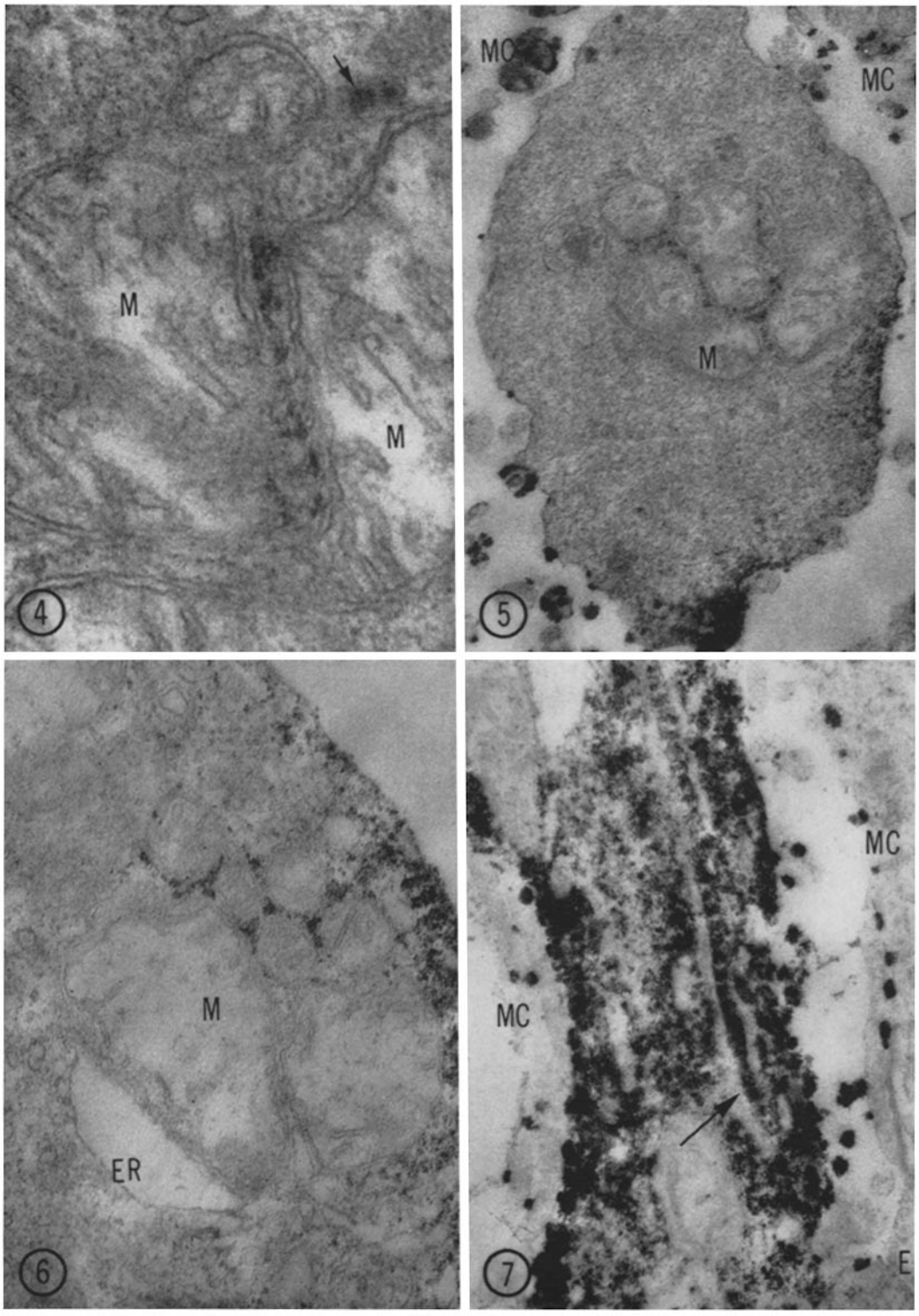
Regarding the existence and the filamentous structure of the ciliary rootlet, several descriptions have been presented in previous reports on the vertebrate retinae (Sjöstrand, 1953; Tokuyasu and Yamada, 1959; Cohen, 1960 and 1961; Missotten, 1960; Orzalesi and Bairati, 1964). The present

FIGURE 4 Cross-section of a bundle of ciliary rootlet filaments at the level of the distal region of a rod inner segment at higher magnification. Reaction products are seen at some filaments located in the center and the right upper portion of the figure among profiles of mitochondria (*M*). Between such grouped filaments with reaction products are seen less dense filaments in a clear zone of a cross-striation of the same diameter as that of a dark zone (arrow). Lead hydroxide stain. × 82,000.

FIGURE 5 Cross-section of a rod inner segment at the level of its distal region. Reaction products are seen (*a*) at a bundle of the ciliary rootlet as minute deposits, (*b*) at numerous microvilli of the Müller cell (*MC*) as gross deposits, and (*c*) at some regions of the rod cell surface as both minute and gross forms. The ciliary rootlet appears to be located in the center of the rod. *M*, mitochondria. Lead hydroxide stain. × 33,000.

FIGURE 6 Cross-section of a rod inner segment at the level of its proximal region. The ciliary rootlet, the electron density of which is enhanced by minute deposits of reaction products, appears eccentric at this level, and its lateral portion is confluent with the reaction products at the cell surface. *ER*, endoplasmic reticulum; *M*, mitochondria. Lead hydroxide stain. × 36,000.

FIGURE 7 Longitudinal section of the proximal end of a rod inner segment through a plane just beneath the plasma membrane. Heavy reaction products are seen through the cytoplasm of a rod situated between certain microvilli of the Müller cells (*MC*). Particularly, the proximal end of the ciliary rootlet appears suitable to show its course with deposits of the reaction product (arrow), and it approaches the plasma membrane of the rod at the outer limiting membrane, as judged by the existence of the distal end of the cell body of the Müller cell (*E*). Unstained. × 36,000.


M
M
4
MC
MC
M
5
M
ER
6
MC
MC
E
7

study shows, in accordance with these descriptions, that (*a*) the ciliary rootlet closely correlates with the structure of the cilium connecting the outer and inner segments, (*b*) penetrates the inner segment, (*c*) exhibits close contact with the mitochondria and the endoplasmic reticulum in the inner segment, and (*d*) shows a cross-striation with about a 700 A periodicity. An additional finding of the present study is that the end of the ciliary rootlet may touch the plasma membrane of the inner segment at the level of the outer, limiting membrane. The reaction products resulting from incubation in Wachstein-Meisel's medium with adenosine triphosphate as a substrate are observed at the ciliary rootlet in correspondence with its cross-striation. It may be assumed that the deposition of reaction products directly indicates the localization of a certain enzyme, since the various possibilities of deposition of diffusion artifacts (Novikoff et al. 1966) may be excluded. The enzyme, the activity of which is demonstrated, might be ATPase in the specific sense, but the alternative explanation remains that the deposit of lead phosphate may not be a direct product of ATPase reaction but may be caused by some further reactions originating from the addition of adenosine triphosphate. Further clarification and characterization of the reaction is now under way.

If the reaction products observed at the ciliary rootlet result from ATPase activity, their localization is significant because it lends support to the view that the rootlet filaments subserve a function that depends on the ready availability of energy. Whether this function is a mechanical one involving contraction, or the transmission of an impulse, is not clear at present. However, in view of the fact that mammalian receptors do not appear to undergo adaptive movements, it is reasonable to speculate that the rootlet may subserve a conductive function.

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