Diminution and Enlargement of the Mosquito Rhabdom in Light and Darkness

RICHARD H. WHITE and ELIZABETH LORD

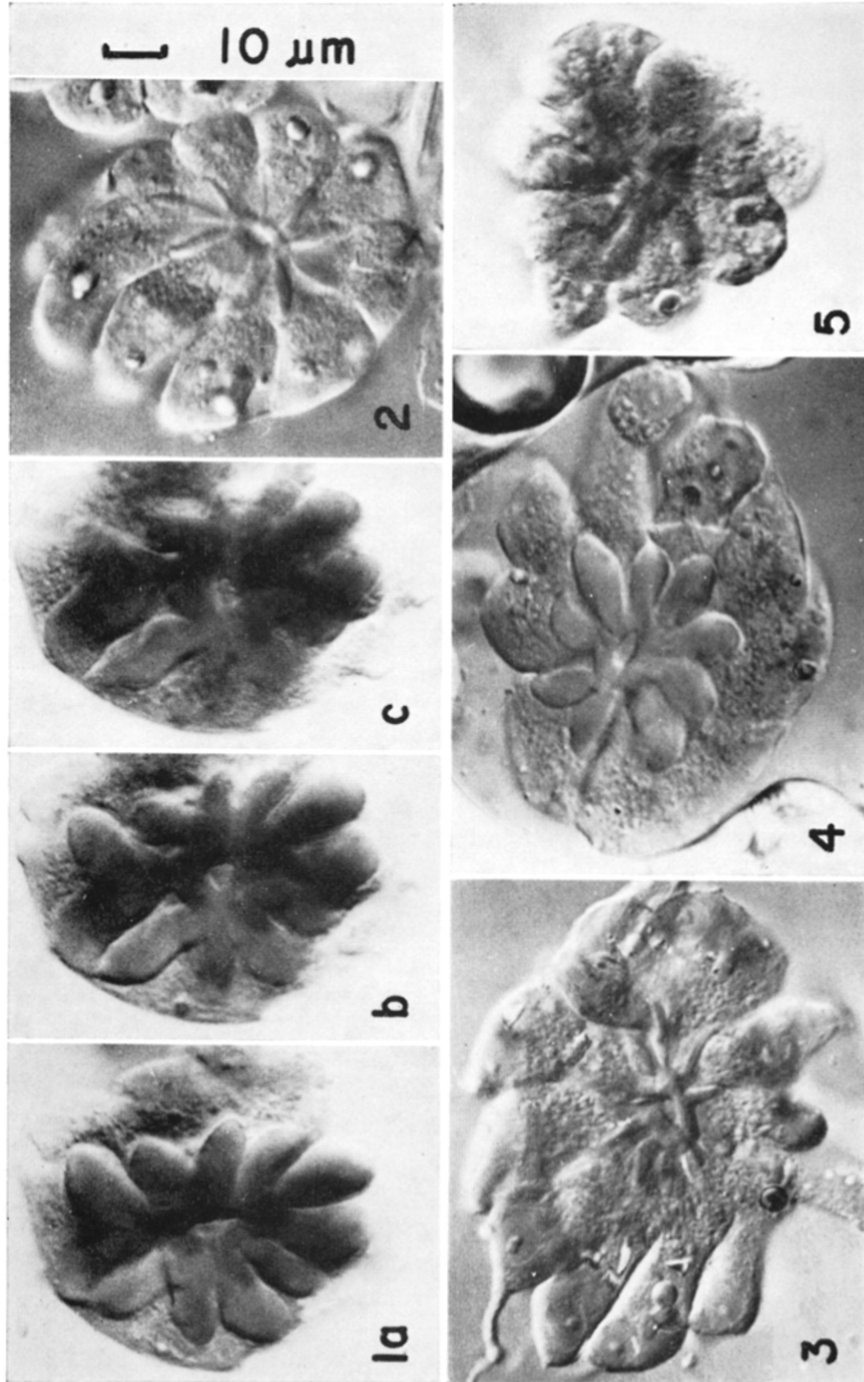
From the Biology Department, University of Massachusetts, Boston, Massachusetts 02125

ABSTRACT The rhabdoms of the larval ocelli of the mosquito *Aedes aegypti* undergo morphological light and dark adaptation over periods of hours. The rhabdom enlarges during dark adaptation and grows smaller during light adaptation. Diminution is exponential, enlargement linear, and rates of change are proportional to log light intensity. Rhabdoms maintained at a constant intensity level off at a constant volume inversely proportional to log intensity. We argue that changes in rhabdom volume after changes in light intensity reflect an influence of light on the turnover of photoreceptor membrane, and that the volumes at which rhabdoms level off represent equilibria between opposed processes of membrane loss and renewal.

INTRODUCTION

Inquiry into the turnover of photoreceptor membrane has centered on the renewal of outer segments in the vertebrate retina (Young, 1967, 1968, 1969, 1971 *a*, *b*; Young and Bok, 1969; Young and Droz, 1968). Only a few studies have followed the course of protein synthesis in arthropod photoreceptors (Burnel et al., 1970; Pepe and Baumann, 1972; Perrelet, 1972; Brammer et al., 1974). Not surprisingly, these experiments, as well as inference from electron microscopy, suggest that invertebrate photoreceptor membrane must also undergo degradation and renewal. However, there is this interesting difference: outer segment renewal in vetebrates is scarcely affected by the conditions of illumination (Young, 1967), whereas the autoradiography of Burnel et al. (1970), and various ultrastructural observations (Röhlich and Törö, 1965; Tuurala, et al., 1966; Eguchi and Waterman, 1967; Eguchi et al., 1973; White, 1967, 1968) suggest that the turnover of photoreceptor membrane in arthropods is influenced by light.

The larval ocellus of the mosquito is one of the photoreceptors in which light-dependent modulations of ultrastructure seem most easily interpreted in terms of membrane turnover. The ocellar rhabdom becomes smaller in the light as vesicles of photoreceptor membrane bud off and accumulate in lysosome-like bodies within the receptor cells. It has been argued that this


10 µm
2
c
b
1a
5
4
3

process might be the degradative limb of a cycle of membrane loss and renewal (White, 1967, 1968).

Following up this idea, we have now measured the rhabdoms of mosquito ocelli under various conditions of illumination. Our measurements establish the relationship between light intensity and rhabdom size, as well as the rates of change going from one intensity to another. Our data suggest that the amount of photoreceptor membrane in an ocellus that has been for some time at a particular level of illumination is a steady-state balance between processes of membrane assembly and degradation. This study prepares the way for the direct analysis of membrane turnover.

MATERIALS AND METHODS

Eggs of the yellow fever mosquito *Aedes aegypti* were kindly provided by Professor George B. Craig, Jr., Biology Department, Notre Dame University. White eye mutants were used, since the internal structure of their photoreceptor cells is visible owing to the absence of accessory pigment (Fig. 1). Larvae were grown at 32°C on the standard diet of dry dog food (Hartz Dog Yummies, Hartz Mountain Products, New York). In order to parallel previous experiments (White, 1967, 1968; Brown and White, 1972), larvae were hatched and maintained in darkness before experimentation. In setting up experiments, animals were exposed briefly to dim red light, which does not measurably bleach mosquito rhodopsin (Brown and White, 1972).

The larval ocelli of the mosquito are typical arthropod photoreceptors. An ocellus is a rosette of receptor cells each bearing an array of microvilli termed the *rhabdomere.* There is now abundant evidence that the visual pigment is a component of the rhabdomere membrane, that is, that the rhabdomeric microvilli correspond to the membraneous disks of the vertebrate outer segment. The joined rhabdomeres of the cells making up an ocellus are collectively its *rhabdom* (Figs. 1–4). There is a cluster of four ocelli on each side of the larval head (Brown and White, 1972). We found it easiest in these experiments to measure the rhabdoms of the two dorsal-most ocelli. However, the rhabdoms of the central and ventral ocelli respond to light in the same way (Fig. 6).

FIGURES 1–5. Photomicrographs from working negatives used in measuring rhabdom volume. All show dorsal ocelli from fourth instar mosquito larvae. The stellate rhabdom stands out at the center of each ocellus in the apparent relief provided by Nomarski optics. The granules in the surrounding cytoplasm are mitochondria and multivesicular bodies. The larger dark or refractile spheres are oil droplets. The dozen or so receptor cells making up an ocellus can be distinguished particularly well in Fig. 2. The ocellus of Fig. 3 has been unusually compressed and the nuclei at the proximal ends of the cells, and an axon, are seen in the upper left corner. Fig. 1 *a*, *b*, *c*. Successive 4-μm optical sections (from a series of six) of a fully *dark-adapted* ocellus. Figure 2. Ocellus *light adapted* for 24 h (I = 1 mW/cm^2). Figure 3. Ocellus *light adapted* for 72 h at the same intensity. Figure 4. Ocellus *dark adapted* for 4 h following 24 h of light adaptation as in Fig. 2. Figure 5. Ocellus exposed to very bright light (I = 100 mW/cm^2) for 8 h. The rhabdom, coarsely striated and hard to distinguish from the cytoplasm, appears to be disintegrating.

The size of the rhabdom of an ocellus depends upon the age of the larva as well as the conditions of illumination. The receptor cells and their rhabdomeres enlarge as the larva grows. In order to minimize this source of variability, our measurements were restricted to fourth (last) instar larvae, when, according to control measurements, growth of the rhabdom has ceased.

For measuring rhabdoms, ocelli were fixed in 4% glutaraldehyde in 0.12 M phosphate buffer (pH 7.2), lightly stained with osmium tetroxide, and stored at 4°C in buffer. Dissected ocelli were mounted in buffer on microscope slides beneath no. 1 coverslips held in place with Vaseline, and were viewed in a Zeiss WL microscope (Carl Zeiss, Inc., New York) equipped with Nomarski differential interference optics (Allen et al., 1969). The rhabdom stands out against the granular cytoplasm of the receptor cells, outlined by the illusion of low relief (Figs. 1–4) that is characteristic of Nomarski optics. As the depth of focus is very shallow, the distinctly outlined rhabdom is presented in a sequence of optical sections as one focuses through an ocellus (Fig. 1 *a*, *b*, *c*). The volume of the rhabdom was measured from photographs of such serial, optical sections.

The equipment was calibrated and the method tested as follows. The accuracy of the micrometer scale on the fine focus control of the microscope was assessed by comparing the vertical diameter of an isodiametric micropipette, measured by moving focus from one side to the other, with its horizontal diameter measured by an ocular micrometer. We found that the fine focus micrometer accurately measured the displacement of the objective as it was focused up and down, and so could be used to set the "thickness" of optical sections. The optics for measuring rhabdoms consisted of a Zeiss planapochromat $\times$ 100/1.3 oil immersion objective coupled with the appropriate differential interference condenser, the condenser diaphragm being fully open. The depth of field of this system was estimated to be 2–3 μm from focusing across motes of dirt adherent on the walls of a micropipette, and this agrees with theory (Allen et al., 1969). Hence we decided to measure rhabdom volume from optical sections taken at 4-μm intervals. We further tested our system in practice by comparing measurements taken optically with measurements from conventional serial sections. Contralateral ocelli from the same animal were compared. At most, measurements differed by about 15%. As the working distance of the objective we used for optical sectioning is short, compression or shifting of an ocellus as the objective moves downward is a possible source of error. We found it essential that ocelli be caught firmly between slide and coverslip, and that the thinnest coverslips be used.

To summarize our procedure for measuring rhabdoms: fixed ocelli were photographed at 4-μm intervals of focus. The outline of the rhabdom in each optical section was traced from images of the negatives projected in a photographic enlarger. These outlines were measured with a planimeter and the total volume of the rhabdom calculated from the appropriate magnification factors.

A "daylight" fluorescent lamp (ITT FIST 12/cw, ITT Electro-Optical Products Div., Roanoke, Va.) was used for illuminating larvae in most experiments. A halogen lamp (Westinghouse "Sungun," Westinghouse Electric Corp., Pittsburgh, Pa.) was also used in some experiments for higher intensities. The fluorescent lamp was positioned so as to avoid the heat-emitting ends. Light from the halogen lamp was filtered

through 17 cm of running water and 4 cm of 0.2 M $CuSO_4$ to remove most of the heat. In both setups, the chambers containing experimental animals were immersed in water baths maintaining the temperature of the animals at 32°C. The chambers were either the metal cannisters that package 35-mm film cassettes, or glass slide staining dishes wrapped in aluminum foil. Light intensity was measured at the level of the water surface in the experimental chambers with an Epply thermopile or a calibrated photodiode (PIN-10, UV enhanced, United Detector Technology Inc., Santa Monica, Calif.). The intensity of the unfiltered fluorescent lamp was 1 mW/cm^2. Illumination was adjusted in various experiments to logarithmic multiples of this intensity by means of Wratten neutral density filters.

RESULTS

Samples of photomicrographs used for measuring rhabdoms are presented in the plate, Figs. 1–4 illustrating morphological light and dark adaptation. The fully dark-adapted rhabdom in larvae grown to the last instar in darkness takes up about a third of the volume of an ocellus (Fig. 1). If such rhabdoms are then continuously illuminated at constant intensity they become smaller (Fig. 2), finally leveling off after several hours to a constant size (Fig. 3). When larvae with small light-adapted rhabdoms are put back in darkness, their rhabdoms enlarge again (Fig. 4).

Fig. 6 follows the diminution of rhabdoms under the bright light of our unfiltered fluorescent lamp. Rhabdom volume decreased exponentially by about 60% during 24 h of illumination. Similar experiments continued longer (e.g., the curve labeled 1 mW/cm^2 in Fig. 8) showed that rhabdom volume then levels off. There was some variability in the initial response of the rhabdom to light. In some experiments, such as that of Fig. 6, the rhabdoms fell immediately into exponential diminution from the dark-adapted state. Usually however, as in Fig. 8, the volume fell more rapidly during the first few hours of illumination, so that the dark-adapted volume lay well above the extrapolated exponential curve.

Figs. 7 and 9 show the growth of the rhabdom when larvae that have been illuminated for 24 h are returned to darkness. Rhabdom enlargement is linear and more rapid than its diminution in light, leveling off at full volume after about 4 h of darkness.

We found that the rate of rhabdom diminution depends upon light intensity. Fig. 8 combines the results of two experiments, one using the halogen lamp to provide the higher intensities (broken lines), and the other the fluorescent lamp for the lower range of intensities (solid lines). Although the lamps differed in spectral quality, the rate of rhabdom diminution under either was the same at the same intensity (curves labeled 1 mW/cm^2). Over the intensity range from 10 to 10^{-4} mW/cm^2, rhabdoms lost volume exponentially, with rates depending on brightness. The rate constants of rhab-

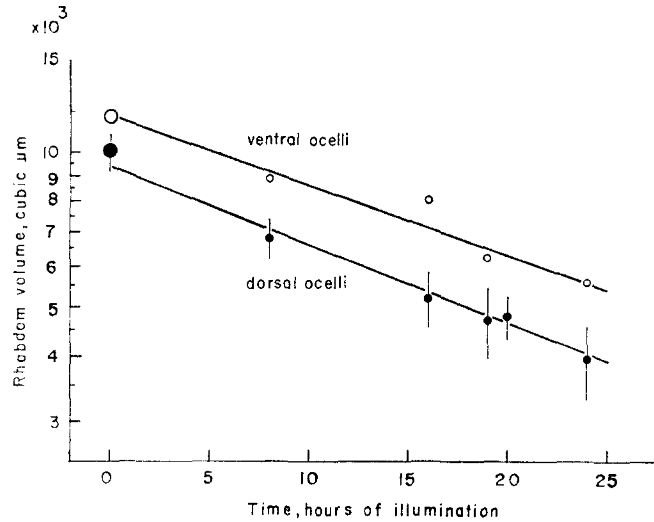


FIGURE 6. Diminution of rhabdoms in larvae reared in darkness, and then exposed to continuous illumination (I = 1 mW/cm^2). The open circles represent the rhabdoms of ventral ocelli, the closed circles dorsal ocelli. The larger circles represent fully dark-adapted rhabdoms. The standard error values shown for the measurements of dorsal ocelli are typical of our data. Under the conditions of this experiment, rhabdom volume decreased exponentially for about 24 h. Other experiments (cf. Fig. 8), show that the volume remains constant thereafter.

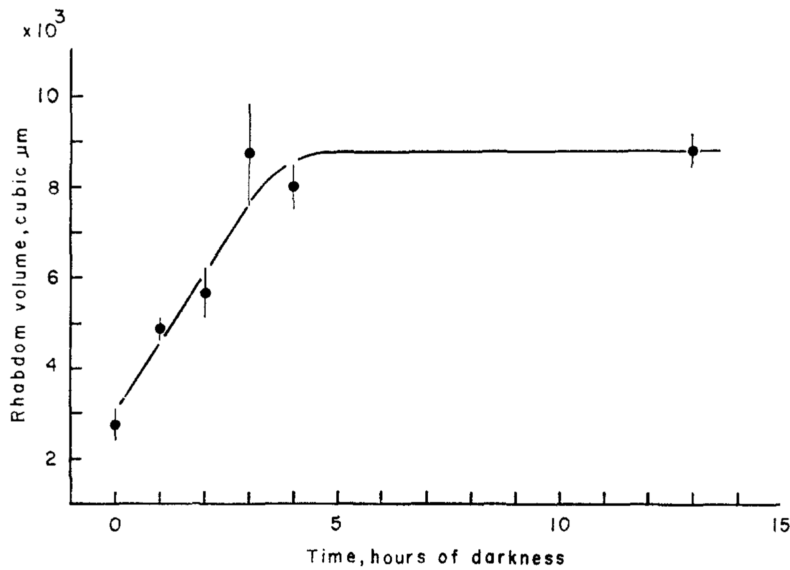


FIGURE 7. Enlargement of rhabdoms of dorsall oceli in darkness. Larvae were grown in darkness, exposed to light for 24 h as in Fig. 1, and then returned to darkness.

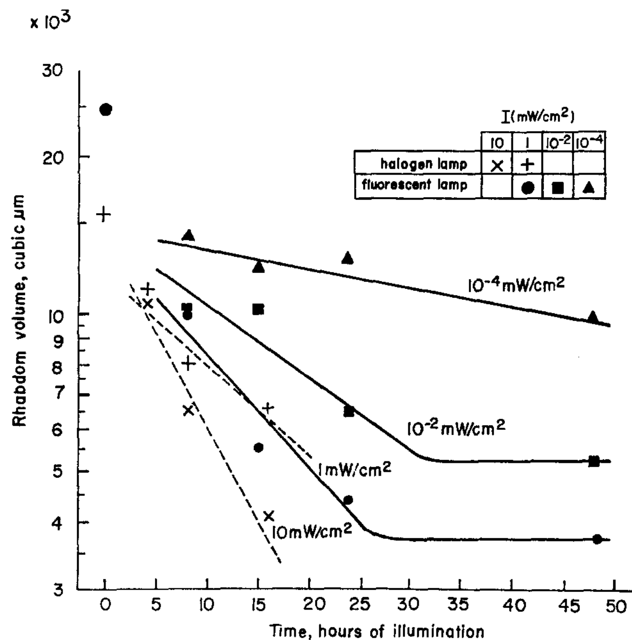


FIGURE 8. Relation of rate of rhabdom diminution to light intensity. The solid lines represent data from one experiment using a white fluorescent lamp, the broken lines represent another experiment using a heat filtered halogen lamp. Intensity was adjusted in relation to the unfiltered fluorescent lamp (I = 1 mW/cm²) with neutral density filters. Curves were fitted by the method of least squares to the data points representing the period of diminution, but omitting the dark-adapted volumes and those after leveling off.

dom diminution averaged from several such experiments were approximately proportional to log light intensity.

Light 10 times brighter (100 mW/cm², the full output of the halogen lamp) appeared to damage the rhabdoms. They first appeared swollen, coarsely striated and indistinct (Fig. 5), and by 16 h of illumination had disappeared. The larvae themselves seemed healthy. We have not yet examined the fine structure of these ocelli, but at the level of light microscopy this apparent disintegration of the rhabdom at very high intensity is distinctly different from the changes in rhabdom size that occur at lower intensities.

Fig. 8 also shows that the volume at which the rhabdom eventually levels off depends upon the intensity of the adapting light. As the preceeding experiments were begun and completed in last instar larvae they could not be continued longer than 2 days, since the larvae are then approaching pupation. This provides barely enough time at low light levels for a rhabdom to diminish to the volume at which it remains constant. Therefore, we used a somewhat different procedure to determine more exactly the relationship between in-

tensity and the constant volume of full morphological light adaptation. Larvae were hatched in darkness, transferred to differentially illuminated chambers in the second instar, and allowed to develop to the early fourth instar. They were thus exposed to the selected levels of brightness for at least 72 h. Only the range of intensities provided by the fluorescent lamp could be used for these prolonged experiments owing to the short lives of our halogen bulbs. The results of such an experiment are shown as the upper curve in Fig. 10. Log rhabdom volume after full light adaptation is inversely proportional to log light intensity.

The rate of rhabdom enlargement also varies with intensity when larvae go from brighter to dimmer light. Fig. 9 shows an experiment in which larvae were illuminated at 1 mW/cm² for 24 h, and then transferred to darkness or to dimmer chambers illuminated at 10^{-2} and 10^{-4} mW/cm², respectively. Rhabdom volume increased most rapidly in darkness and more slowly at higher light levels. The rate of enlargement was proportional to the logarithm of the intensity change.

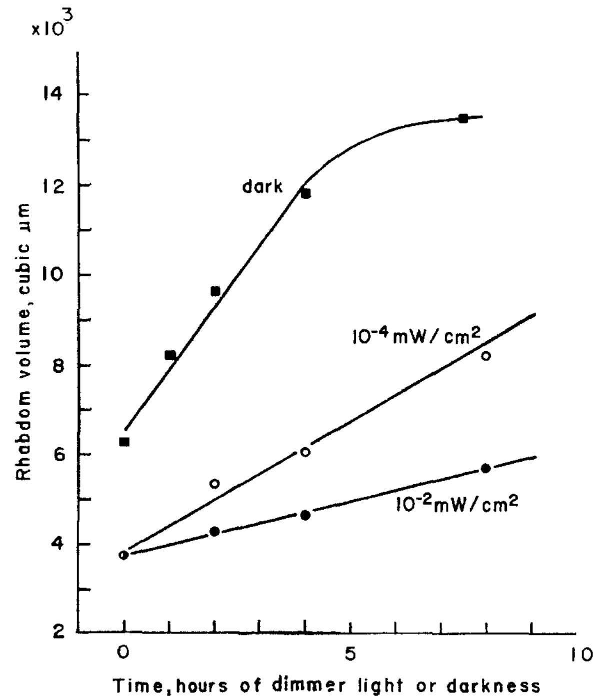


FIGURE 9. Enlargement of rhabdoms transferred from brighter to dimmer light. Larvae were exposed to bright light (I = 1 mW/cm²) for 48 h. The light was then attenuated as indicated by the labels on the curves. The growth of rhabdoms in darkness from another experiment (in which the rhabdoms were larger to begin with) done at the same time is shown for comparison.

The following procedure established the relationship between intensity and the constant volume at which enlarging rhabdoms level off. Larvae were light adapted for 24 h at 1 mW/cm², then divided into four groups, one maintained at the same intensity (1 mW/cm²), the others transferred, respectively, to 10^{-2} and 10^{-4} mW/cm², and to darkness for an additional 24 h. The results are shown by the lower curve in Fig. 10. Rhabdoms illuminated at the original intensity remained the same size since they had already reached constant volume. Those transferred to dimmer light ceased enlarging at volumes

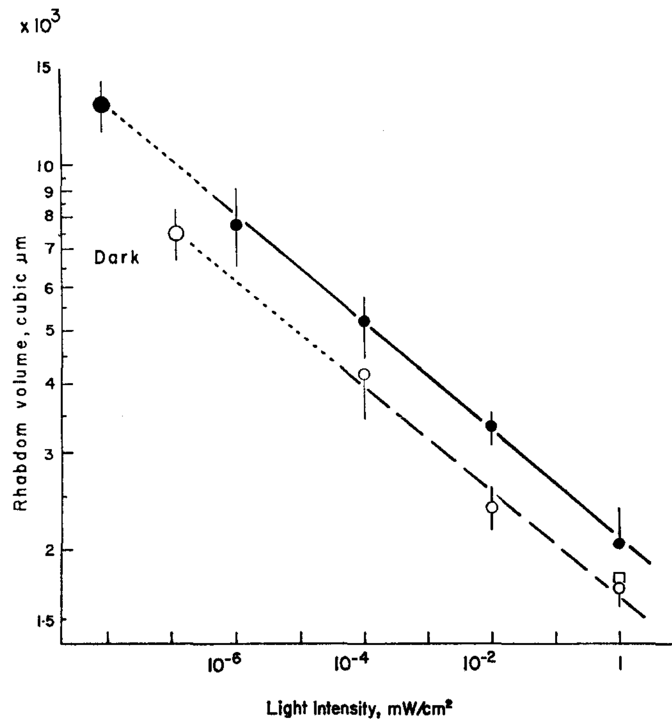


FIGURE 10. Relation of steady-state rhabdom volumes to light intensity. The *upper curve* shows the results of an experiment in which initially dark-adapted larvae were exposed to different light levels for 72 h. In the experiment shown by the *lower curve*, larvae were illuminated at 1 mW/cm² for 48 h, and then some were maintained at the same intensity while others were transferred to dimmer light or darkness for an additional 24 h. The open square represents rhabdom volume at the beginning of the experiment, i.e., after 48 h of bright light, showing that rhabdom volume did not change in those larvae left at the original intensity for the additional 24 h (corresponding open circle). Both curves have been extrapolated to the volumes of dark-adapted rhabdoms (larger symbols to the left) measured for each experiment: in the upper curve, rhabdoms never exposed to light; in the lower curve light-adapted rhabdoms returned to darkness for 24 h. The extrapolations indicate the approximate threshold of the response. Some standard error bars have been left off where they would overlap.

inversely proportional to the levels of illumination into which they were transferred.

The differences between the comparable points on two curves in fig. 10 lie well within the range of variation of larval populations. Hence the volume at which a rhabdom levels off depends only on the intensity of the adapting light. The same constant volume is attained at a given intensity whether the rhabdom has enlarged during morphological dark adaptation or diminished during light adaptation.

The curves in Fig. 10 have been extrapolated (broken lines) to the rhabdom volumes of the fully dark-adapted controls in each experiment. For the upper curve that is the volume before the beginning of the light adaptation experiment; for the lower curve it is the volume after 24 h of darkness. The extrapolation allows us to estimate that the threshold of morphologidal light adaptation lies between 10^{-8} and 10^{-6} mW/cm^2.

In order to reassure ourselves that changes in rhabdom volume were not fixation artifacts, we examined light- and dark-adapted unfixed mosquito heads dissected in Schneider's medium. Fixed and unfixed rhabdoms appear identical. We also made a few measurements of wild type larvae with normal screening pigment. Although we could not make very accurate measurements, it was clear that the rhabdoms of the wild type mosquitoes respond to light and darkness in the same way as do those of the white eye mutants.

DISCUSSION

We were prompted to measure the dependancy between rhabdom size and illumination by an earlier study on the ultrastructural effects of light in the mosquito photoreceptor (White, 1967, 1968; White and Sundeen, 1967). Those observations describe the cellular events that underlie changes in rhabdom volume, and suggest likely mechanisms.

In the earlier ultrastructural experiments, as for most of the present measurements, larvae were grown in darkness to the fourth instar before initial illumination. In such dark-reared animals, the diameter of rhabdom microvilli are unusually large, about 1,000 Å. During the first hours of illumination they shrink to their normal size, about 500 Å in diameter. It is likely that this relatively rapid change in microvillous geometry accounts for the initial rapid drop in rhabdom volume measured in most ocelli illuminated for the first time (cf. Fig. 8). The abnormal enlargement of microvillous diameter in prolonged darkness is variable (unpublished observations), and so too is the initial decrease in volume; it was occasionally not seen at all (cf. Fig. 6). We did not make closely timed measurements during the course of this first phase of rhabdom diminution, and it will not be considered further in this paper. We have generally ignored initial dark-adapted volumes in fitting light-adaptation curves (cf. Fig. 8) to the second prolonged phase of rhabdom diminution.

After about 4 h of illumination, there is no further decrease in microvillous diameter. During the subsequent second phase of rhabdom diminution, which is the concern of this paper, the microvilli become shorter and fewer. We have argued before (White, 1968) that the microvilli shorten because they lose membrane to the cytoplasm by endocytosis. With initial illumination, coated vesicles form at the inner ends of the microvilli, and multivesicular bodies appear near the rhabdom and accumulate. These bodies are abundant in light adaptation, but are nearly absent from fully dark-adapted cells. We have presented evidence (White, 1968) that the multivesicular bodies incorporate fragments of membrane pinched off the rhabdom. According to our reconstruction of events, the coated vesicles budding off the inner ends of the illuminated microvilli coalesce into the multivesicular bodies within which the photoreceptor membrane appears to be progressively degraded. After the first few hours of illumination, during the second phase of rhabdom diminution, loss of volume can be most simply accounted for by the transfer of membrane from the rhabdom to multivesicular bodies.

We have proposed (White, 1967, 1968) that this process corresponds to the degradation of photoreceptor membrane in vertebrates. Autoradiography has shown that the outer segments of vertebrate rods are continually renewed. Protein synthesized by the inner segment is assembled into disks of photoreceptor membrane at the base of the outer segment (Young, 1967, 1968, 1969, 1971 b; Young and Droz, 1968). As new disks are added behind them, the older disks migrate to the tip of the outer segment, where they are intermittently phagocytized by the cells of the overlying pigment epithelium, (Young, 1971 a; Young and Bok, 1969; Spitznas and Hogan, 1970; Ishikawa and Yamada, 1970). Disk turnover may be slightly speeded by light (Young, 1967). We have suggested that the loss of rhabdom membrane to multivesicular bodies in the mosquito may be equivalent to the phagocytosis of rod outer segment disks by pigment epithelium in vertebrates except that in the mosquito, photoreceptor membrane is engulfed by the receptor cell itself, and the process is strongly stimulated by light.

Electron microscopy shows that endocytosis still continues in fully light-adapted rhabdoms stabilized at constant volume. Therefore it seems likely that a process of membrane renewal balances degradation under continuous illumination. Although we have no direct evidence showing membrane assembly under constant illumination, renewal is betrayed morphologically by the immediate rapid growth of the rhabdom when a light-adapted ocellus is returned to darkness. Moreover, when ocelli are transferred from brighter to dimmer light, rhabdom volume increases at a rate proportional to the change of intensity, leveling off at the constant proportional volume characteristic of the new lower intensity. This behavior suggests that the constant volume at which a continuously illuminated rhabdom settles is a steady-state equi-

librium that may be approached from either direction, depending upon opposed continuous processes of membrane assembly and loss.

When a rhabdom grows larger, electron microscopy shows that its microvilli lengthen, that new microvilli are probably added along its periphery, and that the formation of multivesicular bodies declines. A subsequent paper will deal with the cytology of morphological dark adaptation. None of our observations show the source of new rhabdom membrane, whether, for instance, it is synthesized *de novo* or reassembled from preexisting units. However, appropriate labeling experiments should answer this question, and test the hypothesis advanced above.

A few studies of protein synthesis in arthropod eyes have been published which bear upon our interpretation of our data. Perrelet (1972) has followed the incorporation of labeled protein into honeybee photoreceptors by autoradiography. After an injection of radioactive leucine, label first appears in the cytoplasm and then in the rhabdom, where it reaches maximal intensity after 48 h and then declines. The bees were maintained under normal alternation of day and night, and effects of illumination were not studied. However, other measurements of incorporation of radioactive leucine into the honeybee retina are consistent with the notion that light increases the rate of degradation of rhabdom membrane (Pepe and Baumann, 1972). The proportion of label bound in protein was greater in darkness than in light. Experiments with inhibition of protein synthesis by puromycin lead the authors to suggest that the main effect of light is to increase the rate of protein breakdown rather than to influence protein synthesis. Brammer et al. (1974) found by autoradiography that rhabdoms of adult mosquitoes incorporate more tritiated leucine in light than in darkness. On the other hand, Burnel et al., (1970) found that incorporation of amino acids into *Limulus* rhabdoms is greater in darkness than in light. However, it is particularly high in those exposed to light before being put in darkness. The latter result is consistent with our observation that the mosquito rhabdom grows most rapidly under similar conditions. These studies on rhabdoms present a fragmentary and inconsistent picture, especially when contrasted with what we know of membrane turnover in vertebrate photoreceptors. If our conclusions are correct and can be generalized, the picture will be clarified only if illumination is included as a variable in incorporation experiments.

Assuming that the growth of the newly darkened mosquito rhabdom reflects the assembly of rhabdom membrane, it is interesting to compare its rate with that of the assembly of disk membrane in the rod outer segment. The amount of photoreceptor membrane in a rhabdom is most appropriately expressed as area of membrane, which can be calculated from measurements of rhabdom volume. With the exception, as described above, of those larvae kept for long periods in darkness, the rhabdom is made up of more or less

uniform, approximately cylindrical microvilli about 500 Å in diameter (White, 1967). Since the area of membrane in a microvillus is its circumference times length, the total area of membrane in a rhabdom is expressed by

$$A = 2\pi rln, \tag{1}$$

r representing the average microvillous radius, l the average microvillous length, and n the number of microvilli in the rhabdom. Neglecting the space between microvilli, the volume of the rhabdom is represented by

$$V = \pi r^2 ln. \tag{2}$$

Rearranging and substituting, the area of photoreceptor membrane in the rhabdom is then

$$A = (2\pi rl)\,(V/\pi r^2 l) = 2\,V/r = 2\,V/2.50 \times 10^{-2}\ \mu\text{m}. \tag{3}$$

As Eq. 3 does not take into account intervillous space or irregularities of microvillous geometry, we estimated the likely error empirically by measuring the total circumference of microvilli in selected areas of several electron micrographs and calculated the area of membrane per unit rhabdom volume. These sample measurements indicate that Eq. 3 probably overestimates the area of membrane, but by no more than a factor of 1.5 or 2.

Applying Eq. 3 to the data of Fig. 7, the rate of increase of photoreceptor membrane in dorsal ocelli in darkness is $1.2 \times 10^5\ \mu m^2/h$ at 32°C. Since a dorsal ocellus consists of about a dozen cells, the rate per cell is about $10^4\ \mu m^2/h$. This should be taken as a minimum rate of assembly of rhabdom membrane, since we do not know if membrane loss continues in the just-darkened ocellus.

For comparison we calculated the rate of assembly of disks into the outer segments of frog rods from the data of Young (1967, 1968). Frog rod cells are about the same size as mosquito photoreceptors. The frog rod assembles about 36 disks per day at 22.5°C. Taking the radius of an outer segment as 2.5 μm, the area of membrane in a disk, which is made up of two layers of membrane, is $2\,\pi(2.5)^2 = 40\ \mu m^2$, and membrane is assembled at the rate of about 60 $\mu m^2/h$. As the rate doubles with an increase of 10°C it would be about $1.2 \times 10^2\ \mu m^2/h$ at 32°. This is of course an uncertain comparison, since we cannot express membrane assembly in directly comparable units of biosynthesis. However, it suggests that rhabdom formation in darkness after a period of illumination may be as much as 100 times faster than the continuous assembly of frog rod outer segment.

Rhabdom size in the compound eye of adult *Aedes aegypti* also varies inversely with illumination (Brammer et al., 1974). Do rhabdoms in general

respond to the level of illumination in this way? The phenomenon has been well documented in the ocelli of sowbugs (*Isopoda*) (Tuurala and Lehtinen, 1965, 1968; Tuurala et al., 1966), and the compound eye of *Daphnia* (Crustacea) (Debasieux, 1944; Röhlich and Törö, 1965; Röhlich, 1968) with both light and electron microscopy. As in the mosquito, diminution of these rhabdoms occurs through shortening of the microvilli with vesiculation. Such changes in rhabdom size have been reported, from light microscopy alone, in other arthropods (e.g., Debasieux, 1944) and in cephalopod mollusks (Young, 1962). However, at the resolution of light microscopy the picture may be confused by pigment migration, and it is difficult to distinguish between size changes and photomechanical movements or distortions of the rhabdom (Behrens, 1974). Thus the particular response of the rhabdom to light that we are concerned with occurs in diverse arthropods, but despite the vast literature on rhabdomeric photoreceptors, we cannot say if it is common.

It certainly seems that striking size changes do not occur in many of the well-studied arthropod rhabdoms, since they have not been reported. For example, Eguchi and Waterman (1967), in a careful ultrastructural study of the crab *Libinia*, found that the dimensions of the rhabdom remained constant during light and dark adaptation. However, the number of multivesicular and lamellar bodies increased in the light. According to our interpretation given above, the formation of these lysosome-like bodies (recently used by Eguchi et al., 1973, to identify spectral cell types in the crayfish eye) would be the more basic cytological expression of the underlying response to light. Rhabdom volume would change only if the rates of assembly and degradation differed during a period of adaptation.

Among the arthropods (and cephalopods), if the rhabdom changes size it becomes smaller in the light. By contrast, the rhabdoms of planaria atrophy in darkness, enlarging again in the light (Röhlich and Tar, 1968). The authors suggest that this might result if light regulated the turnover of photoreceptor membrane.

Diminution of the arthropod rhabdom during morphological light adaptation has been spoken of as if it were a pathological effect of light or an artifact of unphysiological illumination (e.g. Röhlich, 1968) much as the changes in vertebrate retinas exposed to continuous illumination are viewed as degenerative (e.g., Shear et al., 1973). Very bright light (100 mW/cm^2) does seem to destroy the rhabdom (Fig. 5) but rhabdom diminution at lower levels of illumination is distinctly different. Whether or not the phenomenon described in this paper turns out to be in some sense a pathological side effect of light it seems likely that the size of the rhabdom changes under "normal" conditions. Mosquitoes in their usual environment would be exposed to light fluctuations sufficient to affect the rhabdom, and the ocelli of wild type larvae respond as do those of white eye mutants. The threshold of rhabdom diminution

is relatively low: below 10^{-6} mW/cm^2 (Fig. 10), or about at the threshold of human cone vision (Wald et al., 1963, and confirmed by our own observation of our attenuated light source). Moreover, steady-state rhabdom volume, and rates of diminution and enlargement are functions of log light intensity, paralleling the characteristic physiological nonlinearity of photoreceptor response. We believe that the morphological response of the rhabdom to light that we have dealt with here cannot be relegated to pathology. The open question is the physiological significance of the regulation of rhabdom size or rate of turnover.

We are grateful for the assistance of Dian Gifford, and the helpful comments of Ruth Bennett and Paul K. Brown.
This research was supported by U.S.P.H.S. Research Grant 1 R01 EY01012-01.

Received for publication 12 July 1974.

REFERENCES

ALLEN, R. D., G. B. DAVID, and G. NOMARSKI. 1969. The Zeiss-Nomarski differential interference equipment for transmitted-light microscopy. *Z. Wiss. Mikrosk. Mikrosk. Tech.* **69**:193.

BEHRENS, M. E. 1974. Photomechanical changes in *Limulus* lateral eye. *J. Comp. Physiol.* **89**:45.

BRAMMER, J. D., R. A. ANDERSON, and P. J. STEIN. 1974. Membrane turnover in mosquito rhabdoms. *Assoc. Res. Vision Ophthalmology, Spring Meeting Abstracts.* 33.

BROWN, P. K., and R. H. WHITE. 1972. Rhodopsin of the larval mosquito. *J. Gen. Physiol.* **59**:401.

BURNEL, M., H. R. MAHLER, and W. J. MOORE. 1970. Protein synthesis in visual cells of *Limulus*. *J. Neurochem.* **17**:1493.

DEBAISIEUX, P. 1944. Les yeux de Crustaces. *Cellule.* **50**:9.

EGUCHI, E., and T. H. WATERMAN. 1967. Changes in retinal fine structure induced in the crab *Libinia* by light and dark adaptation. *Z. Zellforsch.* **79**:209.

EGUCHI, E., T. H. WATERMAN, and A. AKIYAMA. 1973. Localization of the violet and yellow receptor cells in the crayfish retinula. *J. Gen. Physiol.* **62**:355.

ISHIKAWA, T., and E. YAMADA. 1970. The degradation of the photoreceptor outer segment within the pigment epithelial cell of the rat retina. *J. Electron Microsc.* **19**:85.

PEPE, I. M., and F. BAUMANN. 1972. Incorporation of ^{3}H-labelled leucine into the protein fraction in the retina of the honeybee drone. *J. Neurochem.* **19**:507.

PERRELET, A. 1972. Protein synthesis in the visual cells of the honeybee drone as studied with electron microscope radioautography. *J. Cell Biol.* **55**:595.

RÖHLICH, P. 1968. Fine structural changes induced in photoreceptors by light and prolonged darkness. *In* Symposium on Neurobiology of Invertebrates. J. Salanki, editor. Plenum Press New York. 95.

RÖHLICH, P., and E. TAR. 1968. The effect of prolonged light-deprivation on the fine structure of planarian photoreceptors. *Z. Zellforsch.* **90**:507.

RÖHLICH, P., and I. TÖRÖ. 1965. Fine structure of the compound eye of *Daphnia* in normal, dark- and strongly light-adapted state. *In* The Structure of the Eye. J. W. Rohen. editor. F. K. Schattauer-Verlag, Stuttgart. 175.

SHEAR, C. R., W. K. O'STEEN, and K. V. ANDERSON. 1973. Effects of short-term low intensity light on the albino rat retina. An electron microscope study. *Am. J. Anat.* **138**:127.

SPITZNAS, M., and M. J. HOGAN. 1970. Outer segments of photoreceptors and the retinal pigment epithelium. *Arch. Ophthalmol.* **84**:810.

TUURALA, O., and A. LEHTINEN. 1965. Über die photomechanischen Erscheinungen in den Augen zweier Asselarten, *Oniscus asellus* L. und *Porcellio scaber* Latr. *Ann. Acad. Sci. Fenn. Ser. A IV Biol.* **77**:1.

TUURALA, O., and A. LEHTINEN. 1968. Über die Wandlungen in der Feinstruktur der Lichtsinneszellen bei der Hell-und Dunkeladaptation im Auge einer Asselart *Oniscus Asellus* L. *Ann. Acad. Sci. Fenn. Ser. A IV Biol.* **123**:1.

TUURALA, O., A. LEHTINEN, and M. NYHOLM. 1966. Zu den photomechanischen Erscheinungen im Auge einer Asserlart *Oniscus asellus* L. *Ann. Acad. Sci. Fenn. Ser. A IV Biol.* **99**:1.

WALD, G., P. K. BROWN, and I. R. GIBBONS. 1963. The problem of visual excitation. *J. Opt. Soc. Am.* **53**:20.

WHITE, R. H. 1967. The effect of light and light deprivation upon the ultrastructure of the larval mosquito eye. II. The rhabdom. *J. Exp Zool.* **166**:405.

WHITE, R. H. 1968. The effect of light and light deprivation upon the ultrastructure of the larval mosquito eye. III. Multivesicular bodies and protein uptake. *J. Exp. Zool.* **169**:261.

WHITE, R. H., and C. D. SUNDEEN. 1967. The effect of light and light deprivation upon the ultrastructure of the larval mosquito eye. I. Polyribosomes and endoplasmic reticulum. *J. Exp. Zool.* **164**:461.

YOUNG, J. Z. 1962. Light and dark adaptation in the eyes of some cephalopods. *Proc. Zool. Soc. Lond.* **140**:255.

YOUNG, R. W. 1967. The renewal of photoreceptor cell outer segments. *J. Cell Biol.* **33**:61.

YOUNG, R. W. 1968. Passage of newly formed protein through the connecting cilium of retinal rods in the frog. *J. Ultrastruct. Res.* **23**:462.

YOUNG, R. W. 1969. A difference between rods and cones in the renewal of outer segment protein. *Invest. Ophthalmol.* **8**:222.

YOUNG, R. W. 1971 *a*. Shedding of discs from rod outer segments in the rhesus monkey. *J. Ultrastruct. Res.* **34**:190.

YOUNG, R. W. 1971 *b*. The renewal of rod and cone outer segments in the rhesus monkey. *J. Cell Biol.* **49**:303.

YOUNG, R. W., and D. BOK. 1969. Participation of the retinal pigment epithelium in the rod outer segment renewal process. *J. Cell Biol.* **42**:392.

YOUNG, R. W., and B. DROZ. 1968. The renewal of protein in retinal rods and cones. *J. Cell Biol.* **39**:169.