Fig. 4. Viewer mounted on laser photocoagulator slit lamp.

fluorescein slide on and off so that the location of various features of a fluorescein angiogram can be better related to retinal landmarks of the color slide and hence to the patient’s retina. The ability to read fluorescein angiograms with simultaneous reference to the color photographic landmarks has also been useful in interpretation of retinal lesions causing “blocked fluorescence” of underlying fluorescein details.

From the Applied Physics Laboratory and The Wilmer Ophthalmological Institute of The Johns Hopkins University and Hospital, Baltimore. This work was supported by Grant No. EY-01008 from the National Eye Institute, National Institutes of Health, and a career award* from The Seeing Eye, Inc., Morristown, N. J. Submitted for publication Nov. 29, 1973. Reprint requests: Dr. R. W. Flower, The Johns Hopkins University, Applied Physics Laboratory, 8621 Georgia Ave., Silver Spring, Md. 20910.

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Pigment granule movement in Limulus photoreceptors. William H. Miller and David F. Cawthon.

Intense illumination causes centripetal movement of pigment granules into the region of the rhabdom within retinular cells so that the rhabdomeres are “coated” with light-absorbing pigment. Moderate illumination alone causes other changes, but little pigment migration. Colchicine moves the pigment to the light position where it stays after hours in the dark. Retinular cells have previously undescribed 240 Å diameter microtubules which are oriented radially. Because of the action of colchicine, we postulate that these microtubules control pigment position. There is no acute change in the electron microscopic appearance of the colchicine-treated microtubules in this preparation. Colchicine does not interfere with the electrophysiologic recording of nerve fiber activity.

Photomechanical responses in the retinas of vertebrates and invertebrates have been widely studied, but the underlying mechanisms are unknown. Shielding pigment granules of about 1 μm diameter migrate centripetally within the photoreceptor (retinular) cells of the compound eye of Limulus in response to bright illumination. We describe here: (1) radially oriented microtubules within the retinular cells as an underlying morphologic basis for this photomechanical response; and (2) a colchicine-produced pigment migration which does not interfere with electrophysiologic recording of the optic nerve response to illumination.

Methods. Limulus of about 6 inch carapace are maintained in artificial sea water at pH 7.5 on a 9-hour light, 15-hour dark cycle. Animals are removed from the tank near the end of the dark cycle. The eyes are excited and are injected with various solutions through a 27-gauge needle inserted through the cornea. One eye is injected with varying concentrations of colchicine dissolved in 1.0 to 5.0 per cent dimethyl sulfoxide (DMSO) made up in Limulus Ringer’s. The other eye is injected with the same solution but with the colchicine replaced by an equimolar concentration of dextrose (found to be without effect on pigment position). In some experiments the eyes are held in darkness during the injections. In other experiments the eyes are illuminated on an x-ray view box (450 μW per square centimeter) for varying periods of time, but usually 30 minutes, and are either immediately fixed, or are then held in darkness for periods of 30 to 90 minutes after which they are fixed or used in electrophysiologic preparations. The solutions to be injected are oxygenated to saturation and injected at a rate of 0.5 ml per 2.25 minutes in illumination and darkness. Fixative composed of 10 per cent glutaraldehyde in 0.1
Fig. 1. Schematic drawings of Limulus ommatidia. A, longitudinal section; 1, cornea; 2, retinular (photoreceptor) cell; 3, eccentric cell; 4, rhabdomere. B and C are drawings of cross-sections at level "a" of longitudinal section A. B shows pigment position in dark-adapted state; C shows the light-adapted state. Ommatidial diameter is about 70 μm.

M phosphate buffer (pH 7.2) is injected through the cornea and the eyes are then kept immersed in fixative for 45 minutes followed by a one-hour wash in 0.5 M dextrose in the 0.1 M phosphate buffer and a one-hour fixation in collidine-buffered 1.33 per cent OsO₄ and embedded in Spur’s medium. The eyes are sectioned for phase-contrast microscopy at 5 μm and for electron microscopy by conventional methods. Physiologic single-fiber preparations are also prepared by conventional methods.

Results. Pigment migration by illumination. The photoreceptor (retinular) cells of the Limulus compound eye are arranged in the sections of a grapefruit: the photoreceptor-organelle rhabdomeres are microvilli of retinular cell plasma membranes. The rhabdomeres appear as refractile rays in light-microscope cross-sections of ommatidia. Pigment granules tend to move centripetally within retinular cells in response to illumination. Fig. 1, A is a schematic drawing of an ommatidium in longitudinal section. The plane of the section is normal to the corneal surface. Figs. 1, B and 1, C are schematic cross-sections taken at depth "a" in Fig. 1, A. Fig. 1, B indicates pigment position in dark adaptation, Fig. 1, C in light adaptation. Illumination causes increased pigmentation in the rhabdomal region. Fig. 2, A is a cross-section from an excised eye held in darkness for 60 minutes. The ommatidium of Fig. 2, B is from the other eye of the animal which was excised and illuminated with bright light (10,000 lux per square centimeter) for 60 minutes. Illumination causes centripetal movement of pigment granules.

Two other photomechanical effects recently reported are confirmed. The rhabdomal rays are thinner in the light-adapted preparations and the eccentric cell dendrite is usually smaller in diameter as a result of light adaptation, though it is not in the ommatidium of Fig. 2, B. There is considerable variation, of unknown cause, in the retinular cell pigment position of dark-adapted eyes. Infrequently, the pigment is fully in the dark position in both the dark-adapted and illuminated eyes. Whenever there is a difference in pigment position it is as described above.

Retinular cell microtubules. Examination of the retinular cells in the rhabdomal region shows that they contain a high density of radially oriented 240 A microtubules which are illustrated at survey and higher magnifications of cross-sections of ommatidia in Figs. 3, A and 3, B, and of a longitudinal section of an ommatidium in Fig. 3, C.

Effect of colchicine. High concentrations of colchicine (5 x 10⁻³ M, or 10⁻² M) cause the pigment granules in the retinular cells to move to the light position. Fig. 2, D shows an example of an eye fixed after 70 minutes in darkness plus treatment with 10⁻² M colchicine and 2.5 per cent DMSO in comparison with the control eye in Fig. 2, C. In other experiments, we found that brief moderate illumination (450 jnm² per square centimeter) alone causes very little change in pigment position but facilitates the colchicine effect. The pigment migration occurs at every level of the retinular cell and the pigment stays in the light position after 90 minutes (and possibly longer) of darkness, following 30 minutes of moderate illumination. We reconstructed a series consisting of complete sets of 20 and 34 serial 5 μm sections, the colchicine-treated ommatidium being the shorter one. These sets show that the change in pigment position alone is reproducible; there is no definite difference in rhabdomere thickness or dendrite diameter that is attributable to colchicine, although there is occasional evidence of rhabdomal thickening, due to increased microvillar diameter, especially in experiments with very high concentrations of colchicine, i.e., 5 x 10⁻² M.

Radially oriented microtubules are easily detected in the retinular cells of colchicine material. Their numbers do not appear reduced in this early stage, a finding in keeping with results of other work with colchicine on axonal flow. The colchicine-treated eyes are alive and in functioning condition as judged from optic nerve fiber electrophysiologic experiments. Possible
Fig. 2. Ommatidia in cross-section approximately 50 μm from the cornea. The wheel-like spokes and hub are the rhabdom. Note particularly the pigment density between the rays (spokes). A, dark-adapted for 60 minutes; B, 60 minutes of intense illumination; C, 70 minutes of dark adaptation with 2.5 per cent DMSO. D, same as C but with 10⁻⁵ M colchicine and 2.5 per cent DMSO. Magnification marker, 20 μm.

physiologic effects of colchicine-induced pigment migration on sensitivity in light and dark adaptation are being investigated in this preparation.

Discussion. Because colchicine specifically binds the tubulin protein of the microtubules’ subunit,⁸ we conclude that the radially oriented microtubules of the retinular cells mediate the light- and dark-induced pigment migration. In other tissues, other complex factors are known to regulate pigment position. For example, β-adrenergic agents and adenosine 3',5' cyclic monophosphate (cyclic AMP) mediate the light-induced pigment dispersion in frog melanophores, and cytochalasin B, among other agents, prevents dispersion of pigment.⁹ The cytochalasin effect is exerted by disruption of microfilaments. Preliminary experiments in Limulus have failed to show a role for microfilaments in retinular cell pigment migration, and the effects of β-adrenergic agents and cyclic AMP are now being tested.

Colchicine alone causes the pigment to move to the light position. However, the use of brief illumination of moderate intensity and the addition of 1 to 5 per cent DMSO increase the reliability of the movement. The brief exposure to light helps trigger the movement, though our experiments have shown that 70 minutes in the dark with 10⁻² M colchicine and 2.5 or 1.0 per cent DMSO were sufficient, without any prior exposure to light. The DMSO presumably facilitates penetration of colchicine into the cells. The same concentration of DMSO was always added to the control eyes. Further, in separate control experiments DMSO was injected into one eye of the animal with no effect on pigment position relative to the control eye. We conclude that DMSO does not affect
Fig. 3. Retinular cell cytoplasm between rhabdomeres. Arrows point to microtubules. A and B are cross-sections of the ommatidium; C is a longitudinal section. A, survey picture. B, rhabdomere. Magnification marker, 1 μm. B, higher magnification. Magnification marker, 1,000 A. C, high magnification of cross-sectioned microtubules. Magnification marker, 1,000 A.
pigment position at these concentrations; however, it is interesting to note that one might expect DMSO acting alone to have an effect opposite to colchicine.11

The optical function of pigment migration and other photomechanical effects is presumably to control the amount of light in the photoreceptor organelle, i.e., the rhabdomere. These organelles are optical light guides.12 The amount of energy that is propagated in the organelle is a function of the refractive index difference between the organelle and surrounding material, and of the organelle's physical dimensions and the wavelength of light. As the radiant energy propagates within the organelle, a small fraction is outside the organelle. Thus, bringing pigment granules near the organelle has two effects. It tends to increase the refractive index of the surround, which frustrates total internal reflection and increases the intensity in the surround. Second, the pigment granules absorb a fraction of the light bled from the organelle. Additionally, the pigment will absorb light in the retinular cell that propagates outside the rhabdom.

In summary, light-induced pigment migration within the retinular cells of the compound eye of Limulus decreases the amount of light in the rhabdom. There are radially oriented microtubules within the retinular cells. Colchicine brings this pigment to the light position. Colchicine's action results from its binding to the tubulin protein subunits of microtubules. We conclude that the light-induced pigment migration in Limulus photoreceptor cells is mediated by radially oriented microtubules in these cells.


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