Dichroism of Photosensitive Pigment in Rhabdoms of the Crayfish *Orconectes*

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ABSTRACT Microspectrophotometric measurements of isolated crayfish rhabdoms illuminated transversely show that their photosensitive absorption exhibits a dichroic ratio of 2 *in situ*. The major absorption axis matches the axial direction of the closely parallel microvilli comprising the receptor organelle. Since these microvilli are regularly oriented transversely in about 24 layers, with the axes of the microvilli at 90° in alternate layers, transverse illumination of a properly oriented rhabdom displays alternate dichroic and isotropic bands. Because all the microvilli from any one cell share the same orientation, the layers of microvilli constitute two sets of orthogonal polarization analyzers when illuminated along the normal visual axis. Furthermore, since the dichroic ratio is 2 and transverse absorption in isotropic bands is the same as that in the minor absorbing axis of dichroic bands, the simplest explanation of the analyzer action is that the absorbing dipoles of the chromophores, as in rod and cone outer segments, lie parallel to the membrane surface but are otherwise randomly oriented. The rhabdom's functional dichroism thus arises from its specific fine structural geometry.

Much recent work suggests that the detection of polarized light by arthropods and cephalopods is based on the dichroism of their visual pigments (for reviews see Moody, 1964; Waterman, 1968). The fine structure of the rhabdom in decapod crustaceans makes these receptor organelles a particularly favorable object for examining this hypothesis directly by microspectrophotometry.

The rhabdom of decapod crustaceans, like those of all eyes with a high ability to analyze plane-polarized light, typically comprises a regular array of microvilli normal to the ommatidial axis and originating in a characteristic pattern from the neurosensory cells in each retinula (Fig. 1). But unlike all other animal groups, in decapods and some other crustaceans the rhabdom consists of an axial series of interleaved layers (Fig. 1 B; Fig. 2). Each layer is made up of closely parallel microvilli which are orthogonal to one another in alternate layers (Fig. 1 C; Fig. 2).

Where they have been studied, the orientation of retinular cells and the

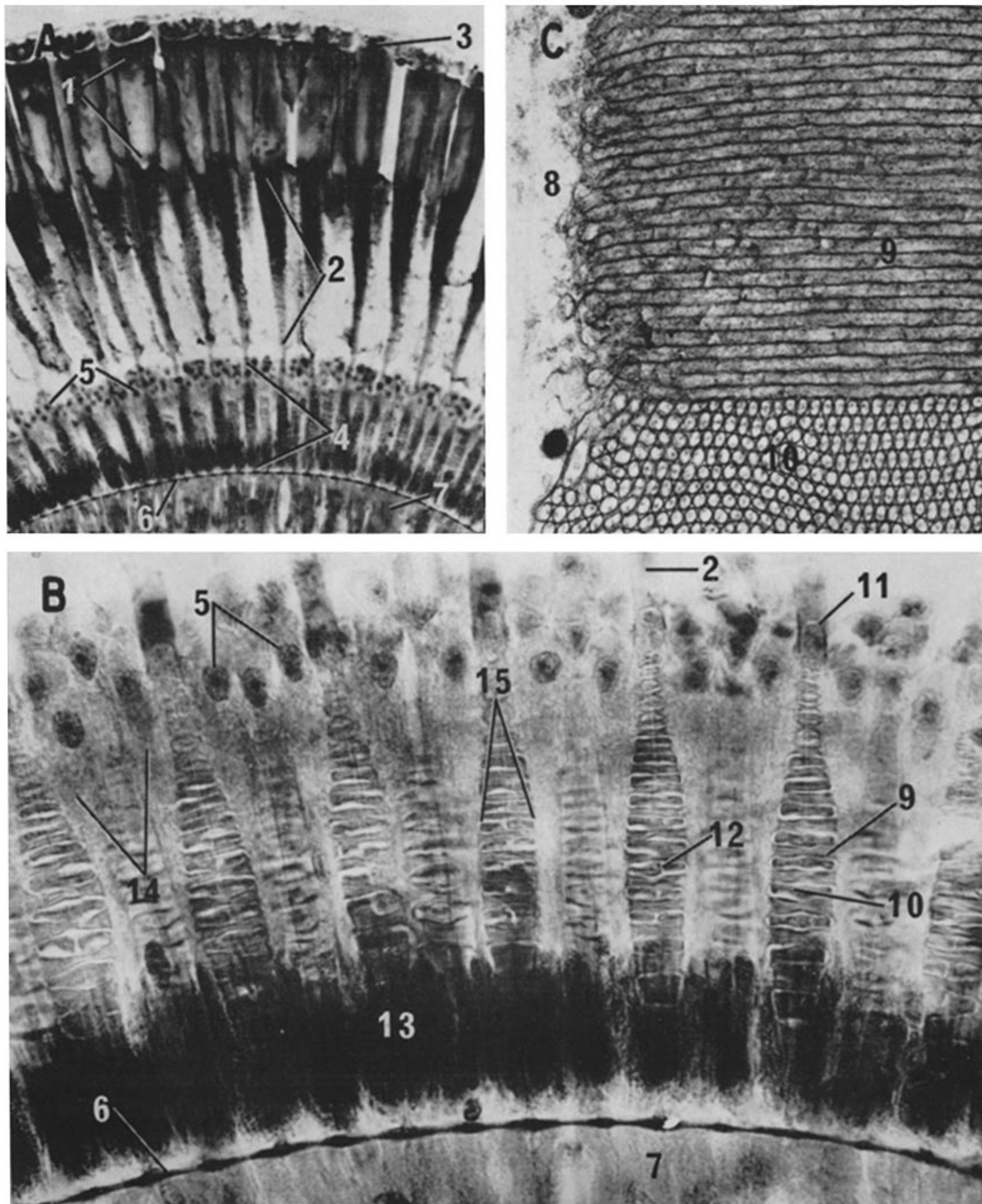

FIGURE 1. Basic structure of the crayfish retina. A, radial section through part of a compound eye showing the location of the rhabdoms in the ommatidia. Light normally enters from above through the subspherical, faceted corneal surface. (In this section the

consequent directions of the two sets of microvilli correspond (at least in the central retina) with the vertical axis and the horizontal plane of the animal's body (Parker, 1895; Rutherford and Horridge, 1965; Waterman and Horch,

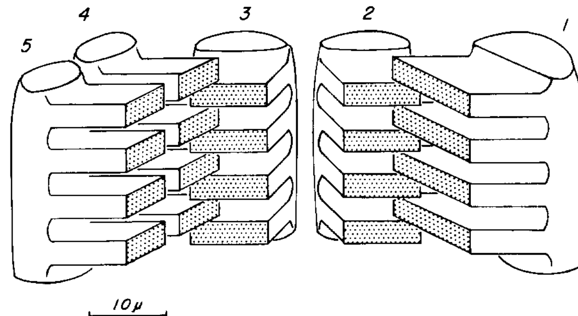

FIGURE 2. Stereodiagram of the middle third of a crayfish rhabdom. Two (6 and 7) of the seven retinular cells have been omitted and the remainder opened out to demonstrate the structure of the rhabdomeres and the way they fit together to form the multilayered rhabdom. When normally assembled the whole organelle is squarish in cross-section. Cell 1 contributes one-half of the layers of which it forms a part, but all the other six cells, just one-quarter each to their respective layers. The microvilli comprising the rhabdom are symbolized by the stippling, which represents their closed distal ends in each rhabdomeric plate. In the normal rhabdom the long axes of the microvilli are orthogonal in alternate layers (compare Fig. 1 C).

1966; Eguchi and Waterman, 1967; Horridge, 1967; Kunze, 1968). In various species the layers of the rhabdom range in number from less than 10 to

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cornea itself has been removed.) 20 μm section, depigmented, Mallory's triple stain. × 90 (prepared by Mabelita Campbell). B, detail of a similar section showing the rhabdom's alternate layered structure and evidence of its constituent rhabdomeres. × 400. C, electron micrograph of a rhabdom showing parts of two contiguous layers of orthogonal microvilli and a small region of the retinular cell of which layer 9 is part. Note that the scalloped outline of the rhabdom, often prominent in freshly isolated organelles (Fig. 3) and sometimes in fixed ones as well (Eguchi, 1965), appears but weakly here. Glutaraldehyde–osmic acid fixation, uranyl acetate, lead citrate stain. × 24,400 (prepared by Eisuke Eguchi). *1*, Crystalline cone; *2*, crystalline cone stalk; *3*, crystalline cone cells; *4*, rhabdom (length); *5*, retinular cell nuclei; *6*, basement lamina; *7*, primary optic fibers; *8*, cytoplasm of retinular cell from which microvilli labeled 9 (in C) originate; *9*, layer of rhabdomic microvilli with their long axes parallel to the plane of the photograph (corresponds to an indentation of a fresh rhabdom, Fig. 3); *10*, layer of microvilli with their long axes parallel to the line of sight (corresponds to a bulge in a fresh rhabdom, Fig. 3); *11*, distal cap of a rhabdom; *12*, break showing where microvilli of two rhabdomeres come together; *13*, vestige of proximal retinal (screening) pigment (mostly removed); *14*, retinular cell cytoplasm; *15*, rhabdom (width). The light microscopy is from eyes of *Cambarus bartonii* (Fabricius) which is used here because of the exceptional detail shown by the preparation in Fig. 1 B. Available evidence strongly indicates that the structure and function of the retinas of epigean crayfish in general are closely alike.

more than 400 (Chun, 1896; Eguchi and Waterman, 1966). All layers with horizontal microvilli consist of rhabdomeres of three specific retinular cells among the seven regular cells in each ommatidium, whereas the vertical microvilli are parts of the other four.

Rhabdoms of crayfish are especially suitable for the study of dichroism because they are large and the layers of microvilli are unusually thick. Taking advantage of this condition we have used a high sensitivity double beam microspectrophotometer and lateral illumination to determine the absorption spectra and dichroic ratios of pigment in single layers of isolated rhabdoms.

Our present results confirm the conclusions hypothesized previously on the basis of selective adaptation and single unit recordings: (*a*) that the two sets of microvilli (horizontal and vertical) constitute a pair of dichroic polarization analyzers with their major axes set at 90° to one another (Shaw, 1966; Waterman, 1966 *b*; Waterman and Horch, 1966; Horridge, 1967), and (*b*) that the major absorbing axis of these dichroic layers is parallel to the long axes of the microvilli (Eguchi and Waterman, 1968). Finally the data demonstrate quantitatively the dependence of the dichroism on photosensitive pigment present in each layer of the rhabdom.

THE ISOLATED RHABDOM

A. *Preparation*

Adult *Orconectes virilis* (Hagen) and occasionally *O. immunis* (Hagen) were dark-adapted for a minimum of 4 hr, and usually overnight, before rhabdoms were isolated. The first stages of the preparation were made under the dim light of ruby lamps. Then the field illuminator of the microscope, fitted with an interference filter (peak transmission 712 nm; half-band width 22 nm) was used to locate appropriate rhabdoms. Control preparations made entirely under infrared illumination showed that these red lights did not significantly affect the photosensitive pigment.

Single eyestalks were removed with scissors and squashed with a glass rod in the bottom of a test tube containing 2 ml of cold physiological saline (pH 7.8, van Harreveld, 1936). After standing 30 sec, the sediment that had collected in the bottom of the tube was drawn off with a Pasteur pipette and transferred to another tube containing 2 ml of either fresh saline or 5% glutaraldehyde in 0.04 M phosphate buffer, pH 7.0. A drop of this resuspended retinal sediment, containing rhabdoms and other fragments of cells, was placed on a glass cover slip on which a 1 cm ring of silicone grease had been made. A second, slightly smaller cover slip was carefully laid on top and pressed down just tightly enough to form a bubble-free seal. The resulting sandwich could then be picked up with forceps by the edge of the bottom cover slip without compressing the retinal fragments. Throughout most of our experiments the temperature of the specimen was held at 11 °C by a pair of Peltier cells.

Isolated rhabdoms are composite membranous organelles which in preparation have been torn free of the normal connections with the cytoplasm of seven surrounding retinular cells (Fig. 2). It is not surprising, therefore, that they are fragile and

deteriorate rapidly at room temperature, losing their optically clear appearance in a few minutes, sometimes twisting out of shape, and often blebbing prominently. This gross deterioration, which usually is marked within 15–20 min, is accompanied by sharp decreases in dichroism. Lowering the temperature (as described above) slows the rate of such structural changes.

In addition to these effects which occur in the dark, illumination was found to induce photomechanical alterations. Fixation in glutaraldehyde, described above, strongly stabilizes the rhabdoms without apparently altering their optical properties (see below for evidence); it also hastens photobleaching (unpublished data).

B. *Observations*

Freshly isolated by our procedure, an *Orconectes* rhabdom is a fusiform structure as much as 25–30 μm in width at its thickest point and 130 μm or more in length (Fig. 3). It appears conspicuously banded because of the presence of 24–25 transverse layers perpendicular to the long axis, each about 5 μm thick. Careful comparison of the fresh preparations with light and electron microscope sections (Figs. 1 and 3) demonstrates that the banding corresponds to the alternating layers of orthogonal microvilli. (This relationship was anticipated by the observation of Parker (1895), who thought that the layers were made of fibers but who described their orthogonal pattern and relations to the retinular cells with astonishing accuracy.)

When oriented appropriately the fresh rhabdom has a conspicuously scalloped outline (Fig. 3 B, C). Further correlation of such images with electron micrographs (Eguchi, 1965) shows that the bulges in the scalloping correspond to layers in which the microvilli lie perpendicular to the plane of the photograph, and the indentations, to microvilli parallel to this plane. Such scalloping provided a prime criterion in selecting favorably oriented rhabdoms for photometric measurement.

Before proceeding further, it is important that the following geometrical relationships be understood. A transversely incident microbeam focused on a *bulge* enters the rhabdom *parallel* to the microvilli, and because of their cylindrical symmetry, all planes of polarization might reasonably be expected to be equivalent. By contrast, rays propagating transversely through an *indented* layer are *perpendicular* to the microvilli. When the plane of polarization is at the same time perpendicular to the axis of the rhabdom (Fig. 3 C), the *e*-vector is *parallel* to the axes of the *microvilli*; when the *e*-vector is paralllel to the axis of the rhabdom (Fig. 3 B), it is *perpendicular* to the *microvilli*.

Note further that if the cylindrical symmetry of the microvilli is reflected in the distribution of pigment, in those layers (corresponding with indentations) in which the laterally incident beam is perpendicular to the microvilli, absorption will be the same (per unit path length in a single layer) as it is along the major axis of the rhabdom, the normal path of light in the living

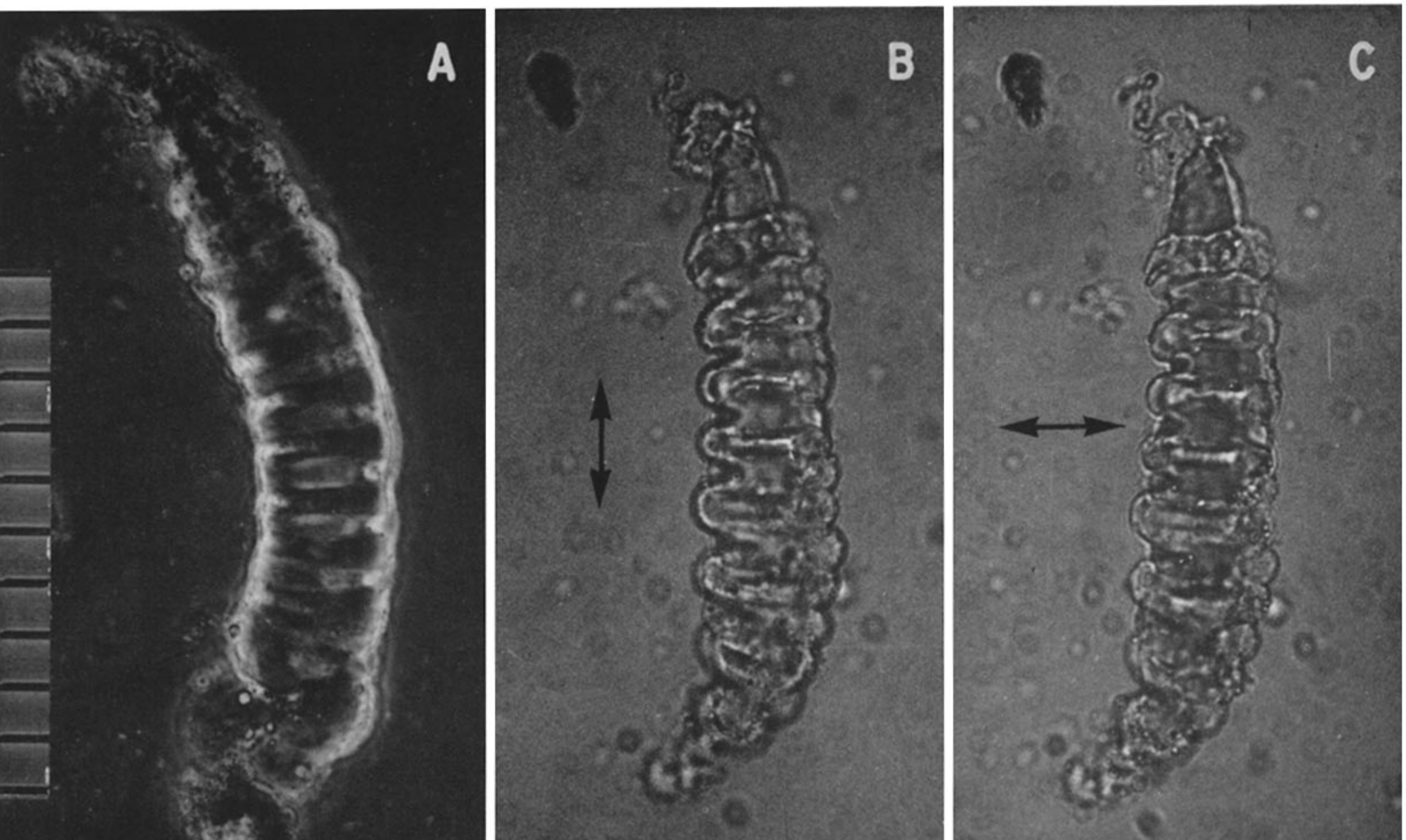

FIGURE 3. Fresh, isolated *Orconectes* rhabdoms in Ringer. A, appearance with phase-contrast optics. Scale marks represent 10 μm intervals. B and C, appearance in transmitted green light linearly polarized as shown by the double arrows. Note that the bands located at the level of indentations of the scalloped outline are dichroic. This is a qualitative demonstration that those bands with microvilli parallel to the plane of the photograph have their major absorption axis parallel to the long axes of the microvilli.

eye. This is an unusual advanatage because in photoreceptor organelles of vertebrates and most arthropods a transversely incident beam cannot be used to measure how axially incident light is normally absorbed.

Viewed by transmitted, linearly polarized light, the indented layers of the crayfish rhabdom absorb more light polarized parallel to the long axes of the microvilli (Fig. 3 C) than they do light polarized perpendicular to the microvilli (Fig. 3 B). This dichroism is described quantitatively below.

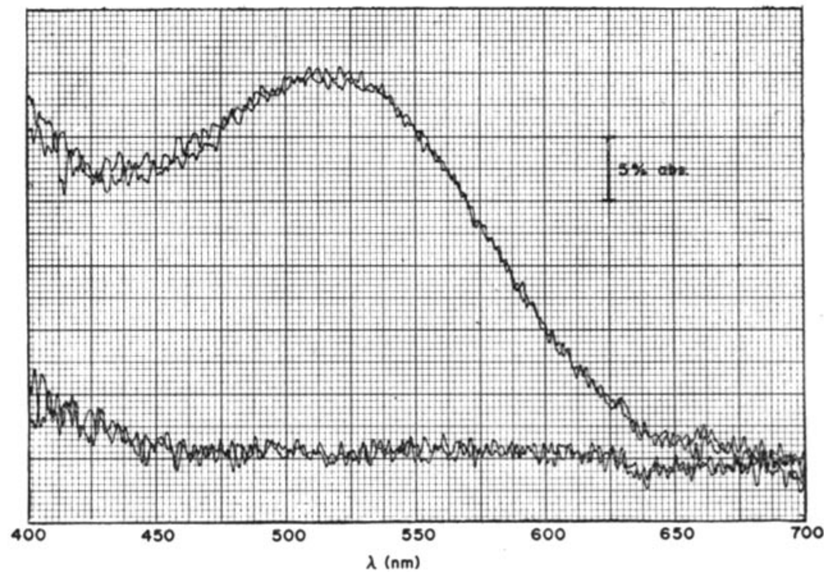

FIGURE 4. Microspectrophotometric recording which shows that no significant bleaching is caused by the measuring beam. The pair of forward and backward scans are virtually identical. The measuring beam was transverse to the normal optic axis of the rhabdom, the spot was 4 × 8 μm at the rhabdom, the scanning rate was 20 nm sec^{-1}, the spectral band width was 3.8 nm, the amplifier time constant 9 msec, and the temperature 11°C. The rhabdom was unfixed and suspended in saline. The base line was drawn (forward and backward scans) with the test beam traversing a clear region near the rhabdom. Scale mark represents 5% absorption (0.05 absorptance; 0.022 absorbance).

MICROSPECTROPHOTOMETRY

A. *Methods*

The microspectrophotometer used is a dual beam recording instrument whose operation is detailed elsewhere (Liebman and Entine, 1964; Goldsmith, Dizon, and Fernández, 1968). Its test beam, rectangular in shape and measuring 4 × 8 μm at the preparation, was linearly polarized with a dichroic sheet polarizer and directed to fall completely within one band of microvilli. The reference beam passed through an adjacent clear region of the cover slip.

The instrument was calibrated so that it graphed on an X-Y plotter transmittance (transmitted energy/incident energy) as a function of wavelength (isolated with a

grating monochromator). Control measurements demonstrated that under the conditions of our experiments, significant amounts of the substances under study were not destroyed by the test beam (Fig. 4).

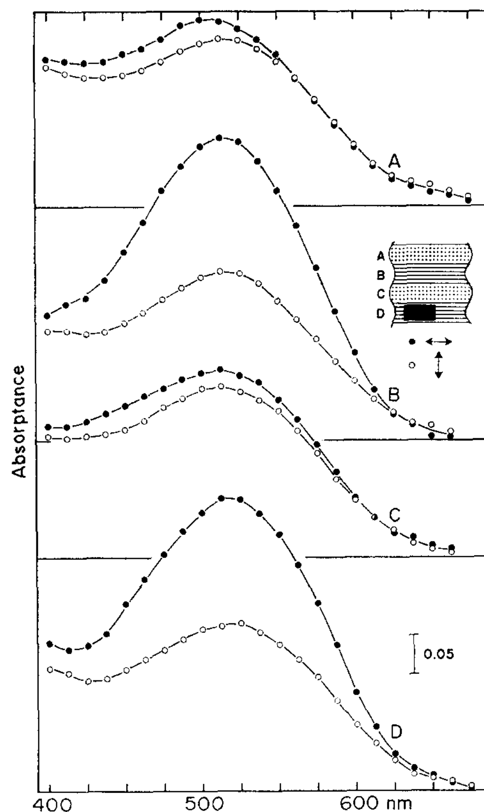

FIGURE 5. Absorptance spectra of four adjoining bands of microvilli in a single fresh rhabdom oriented so that the microvilli are either perpendicular to or parallel to the transverse direction of illumination (see insert diagram which also shows the approximate relative size of the measuring beam). The curves were obtained by subtracting the base lines from the absorption tracings of records made under conditions specified in Fig. 4. The illumination was linearly polarized in a direction either parallel to or perpendicular to the bands, as shown by the double arrows. See the text for further description.

B. *Results*

Favorably oriented rhabdoms with their microvilli parallel and perpendicular to the test beam were located under far red light and the rectangular test spot (set to a far red wavelength) was placed within a single layer of microvilli. Using light polarized in various directions but usually either parallel or perpendicular to the long axis of the rhabdom, it was then possible to measure the absorption of this or any other band which was free of adhering pigment and other debris.

The absorption spectra of four consecutive bands of a well-oriented rhabdom are shown in Fig. 5. As indicated by the diagram on the figure, bands B and D contained microvilli that were illuminated from the side, whereas bands A and C consisted of microvilli that were irradiated end on. The two orientations of the *e*-vector are indicated by the double-headed arrows. Those

layers in which the microvilli were illuminated from the side show positive dichroism, with maximal absorption when the light is polarized parallel to the axes of the microvilli (Fig. 5 B and D). Light polarized perpendicular to this direction gave minimum absorption and intermediate positions of the polarizer gave intermediate values of absorption. This result is qualitatively similar to that shown photographically in Fig. 3 B and C. For those layers in which the microvilli were illuminated axially, absorption is nearly independent of

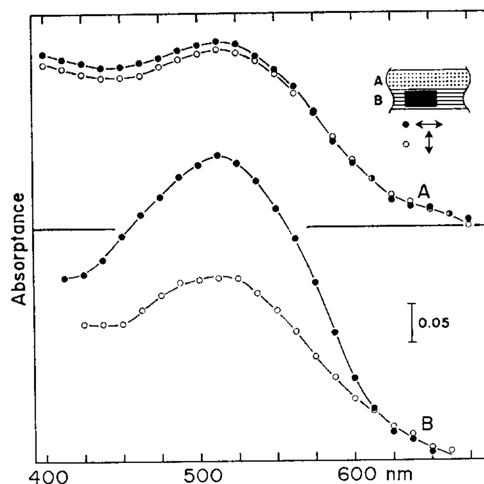

FIGURE 6. Absorptance spectra of two adjoining bands of microvilli measured and graphed as in Fig. 5 but with the rhabdom immersed in 5% glutaraldehyde. Note that neither dichroism nor spectral absorptance appears to be altered by this fixation.

the plane of polarization and is about the same in magnitude as that recorded in bands B and D with the *e*-vector oriented perpendicular to the axes of the microvilli.

The small difference between the curves in Fig. 5 A is thought to be caused by variations in light scattering rather than absorption. Similar differences in other well-oriented rhabdoms do not appear in the difference spectra of bleachable pigment yet persist in the transmission spectra of bleached rhabdoms.

It is evident by inspection that the dichroic ratio of absorption in bands B and D is approximately 2; however, because these spectra include wavelength-dependent scattering and possibly absorption by substances other than photopigment, a quantitative analysis requires that dichroism be related to changes in absorption associated with bleaching. In attempting this experiment, however, we initially ran into serious difficulties.

Irradiation of fresh crayfish rhabdoms produces a metarhodopsin which absorbs strongly in the blue-green region of the spectrum and persists for many minutes. Moreover, the rather rapid deterioration of the freshly isolated rhabdom (described above) precedes the decay of the metarhodopsin and makes precise spectral analysis of further bleaching very difficult.

Since squid rhodopsin is not bleached by glutaraldehyde fixation and the receptors maintain their capacity to generate fast photovoltages on illumination (Hagins and McGaughy, 1967), it seemed reasonable that glutaraldehyde might prevent structural changes in crayfish rhabdoms without altering the absorption and orientation of their photopigments. This technique proved to be successful, with the added benefit that in the presence of glutaraldehyde total bleaching can be accomplished in less than 2 min.

TABLE I
DICHROIC RATIOS OF RHABDOMS

Preparation	Absorptance*	Dichroic ratio‡
1	0.125	2.27
2	0.238	1.90
3	0.320	1.99
4	0.280	1.93
5	0.265	1.84
6	0.152	1.88
7	0.233	2.14
8	0.178	1.85
9	0.192	1.78
10	0.220	2.00
11	0.218	2.11
12	0.248	1.91
	$\bar{x}$= 0.222±0.016 SE	$\bar{x}$= 1.97±0.04 SE

* Measured at the λ_{max} of the difference spectrum with the *e*-vector parallel to the long axes of the microvilli.
‡ Measured at the λ_{max} of the difference spectrum with the *e*-vector parallel and perpendicular to the long axes of the microvilli.

Absorption measurements made on adjacent bands of glutaraldehyde-fixed rhabdoms (Fig. 6) yielded spectra similar to those obtained in analogous experiments on fresh, unfixed rhabdoms (Fig. 5). Clearly the amount of absorption, the position of the λ_{max}, the degree of dichroism, and its pattern of distribution in alternate bands are all unchanged by treatment with 5% glutaraldehyde.

In the presence of 5% glutaraldehyde, *Orconectes* rhabdoms are quickly bleached by light. The absorptance of totally bleachable material at the λ_{max} (525 nm) yields an average dichroic ratio of 1.97 (Table I) for parallel *e*-vector/perpendicular *e*-vector. The 12 rhabdoms in this table were selected because they were judged on both optical and spectrophotometric grounds to be well-oriented.

If our test beam were broad or divergent enough that some of its rays passed through contiguous bands in the rhabdom, this might reduce the apparent value of the actual dichroic ratio in a single band. If this had been

so, using a narrower test beam to reduce the amount of such possible overlap would increase the dichroic ratio. However, control tests using a 1–2 μm × 10 μm test beam having substantial clearance from the edges of the band being measured also yielded dichroic ratios of 2.

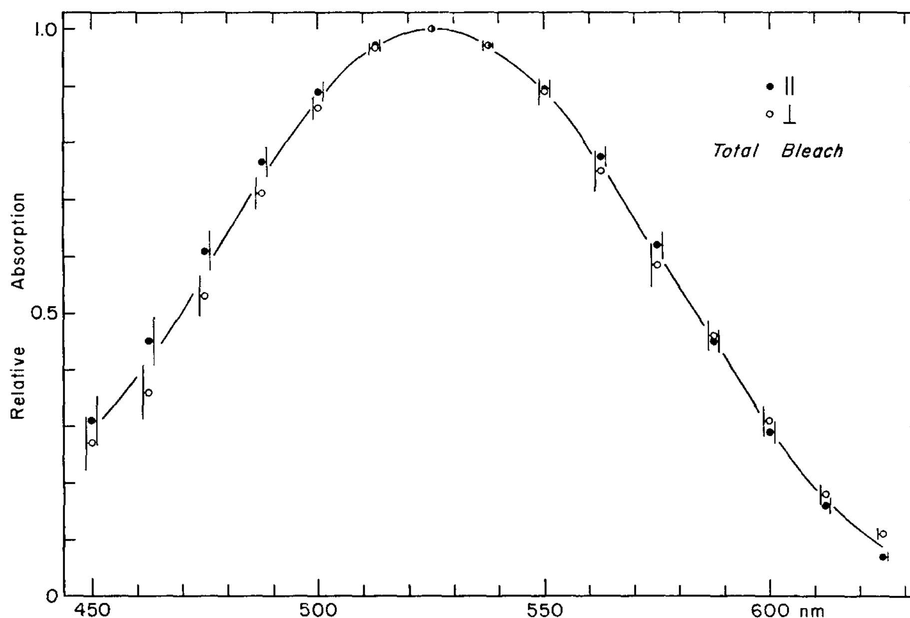

FIGURE 7. Evidence that if two photopigments are present they must share a common dichroic ratio of 2. The graph compares the difference spectra for the total bleach measured by transverse illumination when the *e*-vector is parallel to the long axes of the microvilli (filled circles) and when it is perpendicular to them (open circles). The data, which are the means of 10 experiments, have been normalized to eliminate the difference in absolute absorption (i.e. the dichroism). The shapes of the curves are closely similar throughout (the standard errors of the means are shown by the short vertical lines displaced to the sides of the data points).

Since there is evidence that this dichroism for total bleach is a composite of the properties of two pigments (Wald, 1967; Goldsmith, Fernández, and Waterman, unpublished data), it becomes critically important for the problem of polarized light analysis to ascertain the dichroic ratio of each pigment. If the ratios are the same, the difference spectrum for the total bleach as measured with the perpendicular *e*-vector should be identical to the difference spectrum as measured with the parallel *e*-vector, when scaled up by a constant equal to the dichroic ratio. This should be a sensitive test of whether the dichroic ratio is the same for the two pigments because it involves measuring values that are large in relation to the instrumental noise level.

Normalized difference spectra for the total bleach, measured with the two planes of polarization, are plotted in Fig. 7. Each set of points is an average of

10 experiments (these plus 2 others are also used in Table I). Examination of the standard errors indicates that the two sets are not significantly different at any place in the spectrum. That is, the absorption (difference) spectrum of light-sensitive pigments which one measures with light polarized parallel to the long axes of the microvilli is identical in shape to the absorption spectrum of light-sensitive material measured with light polarized perpendicular to the axes of the microvilli. Consequently, if two pigments are present, they must share a common dichroic ratio of about 2.

DISCUSSION

A. *Basis for the Rhabdom's Dichroism*

Any model that effectively explains the molecular and fine structural basis of the patterns of absorption and dichroism observed in the crayfish rhabdom must account for a number of observations. (*a*) Linear dichroism is prominent and is positive (prolate)[1] relative to the long axis (z, Fig. 8) of the rhabdom's microvilli. (*b*) The dichroic absorption ratio is 2 for light incident perpendicular to the z axis but only 1 parallel to z. (*c*) The absorptance of polarized light by a given path length is the same parallel to the z axis (independent of the plane of polarization) as it is for polarized light incident perpendicular to z when the e-vector is normal to z. (*d*) The spectrum of the dichroism corresponds to the photosensitive absorption of the dark-adapted rhabdom. (*e*) The dichroism is lost when the photosensitive pigments of the rhabdom are bleached.

A particular model which accounts very well for our observations on *Orconectes* can be derived as follows. It requires several assumptions, all reasonable. First *Orconectes*' photopigments are assumed to have a molecular dichroism which, like that of vertebrate rhodopsins, is uniaxial and fairly high. Second, the absorption axes of the chromophores are assumed to lie in the tangent planes of the membranes of the microvilli, just as the rhodopsin chromophores of vertebrate rods lie (with perhaps small deviation) in the planes of the discoidal membranes of the outer segments. Finally, the chromophores are assumed to be either fixed and randomly oriented or free to rotate about their point of attachment within the tangential planes of the cylinder.[2]

[1] For general reference to polarized light and related phenomena, including a substantial bibliography, see Shurcliff (1962).

[2] The present data and the model do not discriminate between random fixed orientation and free rotation. In the rod outer segments of vertebrates, however, failure to find selective bleaching of favorably oriented molecules by flashes of axially incident polarized light has been interpreted as evidence for free rotation (Hagins and Jennings, 1959). But more recent measurements on frog rod outer segments either fixed in glutaraldehyde or held at $-10°C$ also failed to show dichroism by the same criterion, which the authors interpret as some evidence against free rotation being the normal state (Pak and Helmrich, 1968).

A fairly simple analysis suggests that given these assumptions, a dichroic ratio of 2 would in fact be expected for linearly polarized light incident perpendicular to the cylinder's axis and a dichroic ratio of 1 for incidence along the axis.

To visualize this readily we need to consider the minimum necessary number of representative chromophores. For a random array lying in a plane, two

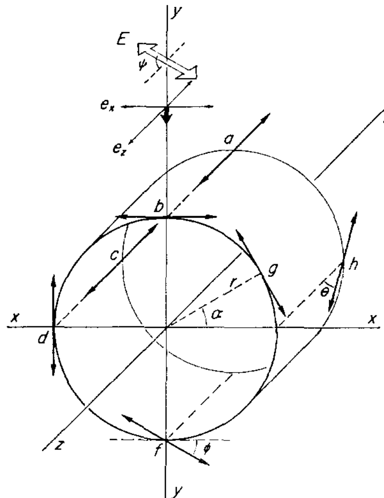

FIGURE 8. Diagram to illustrate the geometry of the illumination of a cylindrical microvillus with its long axis (z) perpendicular to the normal direction of irradiation *in situ* (y). The incoming light is polarized with its *e*-vector (E) making an angle ψ to the z axis. The absorption vectors for light parallel respectively to the x and z axes are indicated by e_x and e_z. Possible orientations of chromophores relative to the coordinate system are shown by seven double arrows, *a*, *b*, *c*, *d*, *f*, *g*, *h*, based on the assumption that each chromophore is part of or attached to the cylindrical membrane of the microvillus. All chromophores except *f* are shown as perpendicular to radius vectors *r* of the cylinder. The points of contact define the angle α relative to the x axis (e.g. $\alpha = 0°$ for *d*, 90° for *b*). Chromophore *f* is shown deviating from such a tangential orientation by the angle ϕ (which is therefore 0° for all the other chromophores drawn). The angle θ defines the axis of the chromophore relative to the z axis. For *a* and *c*, $\theta = 0°$; for *b*, *d*, *f*, and *g* it is 90°; for *h* it is 30°.

orthogonal dipoles are enough, but for a random array lying tangential to a cylinder, four are needed to represent the critical alternatives (Fig. 8). Two of these (*a* and *c*) lie parallel to the cylinder's axis (z) and two (*b* and *d*) perpendicular to it. For polarized light incident along the y axis with the *e*-vector parallel to z, half of these four chromophores (*a* and *c*) can absorb photons, whereas *b* and *d* cannot (assuming the dichroic ratio of the chromophores is positive and high). With the *e*-vector perpendicular to z, only *b* can absorb; *a*, *c*, and *d* cannot. Hence only one-quarter of the chromophores will absorb photons, and the dichroic ratio is 2. Q.E.D.

In support of this intuitive approach a formal proof that our set of assumptions leads to a dichroic ratio of 2 for rays perpendicular to the microvilli has been developed. But it will not be described here in detail since analogous demonstrations have previously been made (Moody and Parriss, 1961; Liebman, 1962). Instead some excerpts from this proof can be used to illustrate the limitations of the underlying model.

The ratio of absorption for *e*-vectors parallel and perpendicular to the long axis of the cylindrical membrane depends on the orientation of the individual chromophores, which we have assumed above to be uniaxial, and on their distribution function as a population. Two angles define the orientation of a dipole on the surface of a cylinder (Fig. 8): one, the angle (θ), between the dipole and z; the other, the angle (ϕ) between the dipole axis and the plane tangent to the cylinder at the point of contact. A variety of distribution functions are possible involving either fixed or freely rotating absorbing units.[2]

We have assumed above that θ is uniformly distributed. In this case the general expression for the dichroic ratio reduces to

$$R_A = 2 \frac{\cos^2\phi}{1 + \sin^2\phi} \tag{1}$$

which shows that its value can vary from 0 to 2 as ϕ varies from 90° (radial) to 0° (tangential as assumed in our model). For our observed $R_A = 1.97 \pm 0.04$ (Table I), the corresponding deviation from tangential would be 5° with the possibility, estimated from the 1% confidence interval in a *t*-table, that ϕ' might be as large as 11°. This value is similar to a previous estimate of coplanarity of rhodopsin chromophores with the receptor membranes of frog rods (Liebman, 1962).

Note that in addition to representing an angular deviation from tangential orientation of the chromophore, ϕ can also represent either departures of the surface of the microvillus from being strictly parallel to the z axis or some significant absorption by the chromophore along the radius vector of the cylinder. The latter is quite possible, as the chromophore is presumably the hindered 11-*cis* isomer of retinal, and therefore may not be describable by a single absorption vector on the surface of the membrane. Our detailed knowledge of the electronic structure of the absorbing system is, however, not adequate to evaluate the importance of these various alternative contributions. Yet the present measurements suggest that they cannot have much effect.

As alternatives to our assumption of a random distribution function for θ, there are two fixed parallel orientations of the chromophores (θ') which could yield $R_A = 2$. The relevant general expression is

$$R_A = 2 \frac{\cos^2\theta'}{\tan^2\phi + \sin^2\theta'} \tag{2}$$

If all absorbing dipoles were parallel and $\theta' = 0°$, R_A should represent the inherent molecular dichroism of the visual pigment. However, since both vertebrate and cephalopod receptors yield dichroic ratios substantially greater than 2 (namely 4–6), this alternative seems unlikely.

On the other hand, if ϕ were at or near zero, a dichroic ratio of 2 would occur according to Equation (2) when $\theta' = 45°$. In that case the chromophores would have a helical alignment like stripes on a barber pole. Although this alternative cannot now be eliminated, it seems to us less likely than a random distribution of θ (see footnote 2).

In the other two instances in which dichroic ratios are available for rhabdoms the situation is different. In *Calliphora* the observed ratio of 1.33 (Langer and Thorell, 1966) corresponds to a fixed $\phi' = 27°$, but various other alternatives (mentioned above) as well as twisting of the rhabdomeres may well be involved in such weak dichroism. For squid and octopus retinas Hagins and Liebman (personal communication cited by Moody, 1964; see also Hagins and Liebman, 1963) have found dichroic ratios of 6. (Although these are camera eyes, they nevertheless contain rhabdoms comprising two orthogonal sets of microvilli.) Such ratios must indicate considerable preferential orientation of chromophores parallel to the z axis of the microvillus.

So far the spectrophotometric data for arthropods (*Calliphora* and *Orconectes*) give no evidence for preferential alignment of this sort; however, one might well anticipate its occurrence in other species of arthropods or cephalopods known to be highly sensitive to polarized light. Thus some intracellular electrophysiological evidence (*Carcinus*, Shaw, 1966; *Octopus*, Tomita, 1968; *Procambarus*, Waterman and Fernández, 1968, unpublished data) suggests dichroic ratios greater than 2, but so far their possible dependence on secondary factors has not been eliminated.

B. *Comparison with Vertebrate Outer Segments*

Certain similarities and differences in the molecular as well as the fine structural geometry of crustacean rhabdoms as compared with vertebrate outer segments have important implications in the present context. Thus the available data are consistent with models that assume three common characteristics for both types of receptor organelle: (*a*) the photopigments are either part of, or directly attached to, the receptor membranes; (*b*) the major axis of absorption of the chromophores lies parallel to the surface of the membrane; (*c*) this axis is randomly oriented within this surface.[3] As a result, one of the three rotational degrees of freedom available to molecules in solution or otherwise quite randomly oriented is essentially eliminated in both types of receptor organelle.

In rod outer segments where the chromophores are coplanar with membrane discs which are in turn normal to the incident light, the probability of absorption by a given number of receptor molecules is increased by 50% for

[3] In this section it will be assumed for simplicity that the chromophores are strictly coplanar with the membranes.

both linearly polarized light and unpolarized light over the three-dimensional random orientation (see Commoner and Lipkin, 1949). Furthermore, the absorption of polarized light is independent of the orientation of the *e*-vector for light traversing the organelle parallel to its long axis, as is normal in the living eye, but is markedly dichroic (R_A's reported from 3 to 6) when irradiation is transverse, as discovered by Schmidt (1934, 1935, 1938) and more recently studied in detail by Denton (1959), Liebman (1962), and Wald, Brown, and Gibbons (1962, 1963).

In contrast, the chromophores of a rhabdomere are everywhere perpendicular to the radii of the microvilli, whose long axes (z) are in turn perpendicular to the incident light (Fig. 8). As a result, the probability of absorption by a given number of chromophores is increased over a three-dimensional random orientation by 12.5% for unpolarized light, increased by 50% for light polarized parallel to the axes of the microvilli, and decreased by 25% for light polarized perpendicular to these axes.

C. *Dichroism and the Perception of Polarized Light*

The model we have proposed predicts that a given retinular cell *in situ* will absorb linearly polarized light preferentially, depending on $\cos^2 \psi$ where ψ is the angle between the axes of the microvilli and the *e*-vector of normally incident illumination (Fig. 8). This is consistent with the data on selective adaptation of the ERG (Waterman and Horch, 1966) and of cytoplasmic organelles in the receptor cells (Eguchi and Waterman, 1967, 1968).

The presence of dichroism, however, does not prove that discrimination of *e*-vector is operationally significant for the species concerned. Nor is the literal dichroic ratio in the retina likely to be the sole determiner of the sensitivity to plane of polarization shown at various higher levels in the system, including behavioral output, for contrast between channels might be sharpened by lateral inhibition in the optic pathway.

Thus several cases of physiologically determined response ratios greater than 2 have been observed in individual receptor cells (Shaw, 1966; Tomita, 1968; Waterman and Fernández, in preparation). Behavioral response ratios greater than 2 have also been reported for polarotactic responses of *Mysidium* and particularly *Daphnia*, which shows very strong polarized light orientation (Jander and Waterman, 1960; Waterman, 1960). Response ratios less than 2 are even more numerous in the literature. Moreover, in species with color vision there are probably interactions between the channels for polarized light perception and those for color (von Frisch, 1965; Waterman, 1966 *a*, *b*; Horridge, 1967). Further analysis of such visual information channeling and its relevance to the behavioral output are promising areas for continuing research.

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