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Mechanism of Polarized Light Perception

Receptor potentials in a crab eye support a proposed two-channel intraretinal system of wide occurrence.

Talbot H. Waterman and Kenneth W. Horch

Electroretinographic evidence recently obtained by us supports a specific explanation of the way in which polarized light is perceived by the compound eye of decapod crustaceans. This evidence, derived mainly from selective adaptation in the eye of the crab *Cardisoma,* demonstrates the presence of a two-channel intraretinal analyzer whose components are perpendicular and oriented vertically and horizontally. Previously obtained information about the behavior of many arthropods in linearly polarized light indicates that this mechanism probably lies within a single ommatidium. Furthermore, extensive evidence from light and electron-microscope studies points cogently to the rhabdom as the site of the initial molecular events. Finally, recent spectrophotometric evidence supports the basic assumption that the visual-pigment molecules involved must play the dual role of photon absorbers and dichroic analyzers.

These various kinds of data, reviewed below, converge to support a relatively simple two-channel model of the analyzer which detects the *e*-vector orientation of polarized light entering compound eyes. So far our new electrophysiological data have been limited to receptor potentials in a few species of decapod crustaceans. Yet the overall congruence of electron-microscopic and other evidence is sufficiently detailed to allow the prediction that the model elaborated in this article will be applicable to many, if not most, of the intraocular polarized-light analyzers in other crustaceans, in cephalopod mollusks, and perhaps even in insects and arachnids.

Dr. Waterman is professor of biology and Mr. Horch is a graduate student in biology at Yale University, New Haven, Connecticut.

Sensitivity to Polarized Light

Altogether, more than 90 species of animals, mostly arthropods but also some cephalopods, have been clearly shown to possess the ability to detect linearly polarized light and determine its plane of polarization (*1*). In a number of arthropods and several cephalopods (*2*) behavioral experiments have proved that this visual capability depends on an intraretinal mechanism.

Crucial support for this conclusion in arthropods came from the demonstration that phototaxis, or oriented responses to light-intensity patterns, can be radically changed without alteration of polarotaxis, or orientation to the *e*-vector (*3*). This independence of the two orientation mechanisms has been established in the aquatic beetle *Bidessus* and the small freshwater crustacean *Daphnia* (*4*). Such discrimination could not occur if the polarization analyzer were either (i) extraocular or (ii) intraocular yet extraretinal, because the animal would confound intensity and polarization. Similarly, *e*-vector determination must be retinal in the honeybee (*5*) and in the wood ant *Formica rufa* (*6*) because, in these insects, perception of polarization is independent of the intensity and direction of the light. This independence, again, is evidence that polarized-light analysis involves utilization of information channels separate from those for pattern vision.

Most experimental and morphological data support Johannes Müller's 1826 hypothesis that, in the mosaic vision of compound eyes, each ommatidium makes just a unit contribution to image perception (*7*). This hypothesis, taken together with the apparent independence of pattern and *e*-vector discrimination, implies that the elementary mechanism of polarization detection lies within a single ommatidium. In fact, evidence from intracellular receptor potentials demonstrates directly that single retinular cells can act as analyzer elements.

More information about the nature of this analyzer may be derived from the fine structure of the retinula, or, more specifically, the rhabdom. This axial organelle is made up of component parts (rhabdomeres), one contributed by each of the usually seven or eight photoreceptor cells of a single ommatidium. All recent evidence points to the rhabdom as the only likely site for the compound eye's visual pigment; hence it must be the locus of the primary photochemical reactions induced by photon absorption. Clues for identification of an intraretinal polarization analyzer would therefore be expected in rhabdom fine structure and its molecular architecture.

Rhabdom Fine Structure

For many years it has been known that the rhabdom of the decapod crustacean is a fusiform or rod-shaped structure, often squarish in cross section, made up of a number of alternating plate-like elements (*8*) (Fig. 1). There are about 350 rhabdom layers

in *Cardisoma*, many more than are drawn in the diagram. These layers in decapods generally were found long ago to be composed of three or four sectors, each contributed by one of the surrounding seven regular retinular cells (*9–11*). One of these cells is larger than the rest and occupies one whole side of the four-sided rhabdom; pairs of the smaller cells occupy each of the other three sides. All seven retinular cells contribute rhabdomeres of characteristic size and regular location.

Rhabdomere elements in alternate layers of plates are produced by cells located on opposite sides of the rhabdom. If the rhabdom plates are numbered consecutively from the distal end and the retinular cells are numbered in order around the optic axis, with the large cell as number 1, rhabdom plates 1, 3, 5, . . . are thus parts of cells 1, 4, and 5, while plates 2, 4, 6, . . . comprise parts of cells 2, 3, 6, and 7 (Fig. 1, B and C). Cell number 1 is responsible for producing half of each rhabdom plate in its series; all the rest form only one quarter of a plate layer.

The 19th-century histologists, as long ago as Schultze (1868), recognized that the rhabdomeres were striated in appearance and apparently consisted of parallel fibers extending from the retinular cells toward the ommatidial axis and lying perpendicular to that axis (*9–11*). Electron microscopy has demonstrated that the regions where these fibers, or *Stiftchensäume*, were observed are in fact filled with closely parallel fine villi, or tubular projections, 500 to 1000 angstroms in diameter. They are membranous structures, continuous with the lipoprotein plasma membranes of their respective retinular cells.

Such ordered arrays of microvilli are characteristic of the rhabdoms of all arthropods and cephalopod mollusks studied since Fernández-Morán in 1956 first reported evidence for them in a dipteran eye (*12*); during the next year a number of other workers correctly recognized the nature and relations of these fine photoreceptor villi (*13*). Max Schultze's (*9*) 98-year-old explicit analogy of the elaborately structured rhabdom of insects and crustaceans with the outer segment of vertebrate rods and cones seems almost clairvoyantly apropos today! Current work in our laboratory and elsewhere has clearly shown the occurrence of a dual system of microvilli oriented at 90° to one another in the rhabdoms of all the decapod crustaceans studied (*14, 15*).

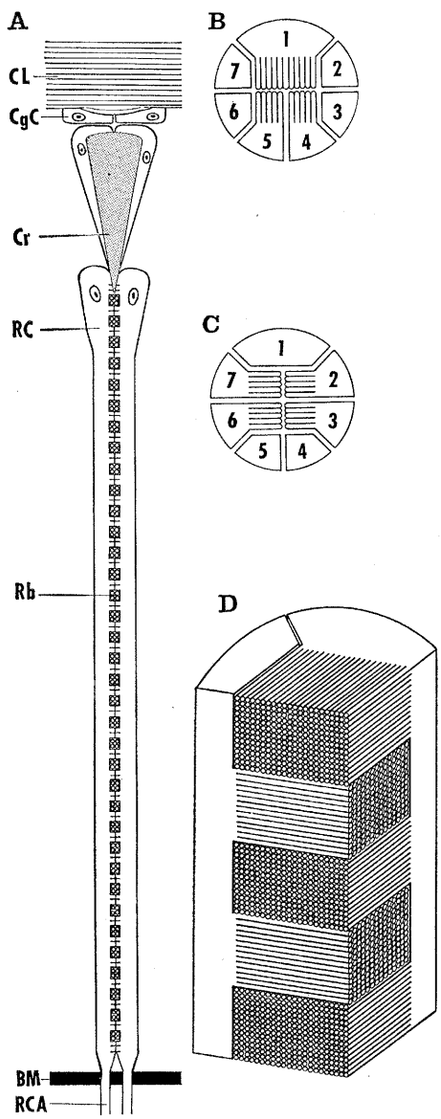

Fig. 1 (left). Structure of a typical crab ommatidium, morphological unit of the compound eye. (A) Diagrammatic longitudinal section through the optic axis. Facet diameters in this crab eye are about 20 microns. (B) Diagrammatic cross section of the retinula showing, within the rhabdom, one of the two alternating types of microvillus layer having three rhabdomere components contributed respectively by retinular cells 1, 4, and 5. (C) Similar section through the other type of layer, in which cells 2, 3, 6, and 7 form rhabdomeres. (D) Stereodiagram of part of a rhabdom quarter, showing the characteristic regular close packing of the straight parallel microvilli in each layer and their perpendicular orientation in alternate layers. (*BM*) Basilar membrane; (*CgC*) corneagenous cell; (*CL*) corneal lens; (*Cr*) crystalline cone; (*Rb*) rhabdom; (*RC*) retinular cell; (*RCA*) retinular cell axon. [From Eguchi and Waterman (*15*)]

Hypotheses Concerning Mechanism

The presence of two such distinct sets of differently oriented microvilli, considered together with some of the earlier data, clearly suggests that the rhabdom could be a two-channel polarization analyzer. In fact, a model based on two sets of perpendicularly oriented receptor microvilli was developed by one of us (T.H.W.) in 1964 (*16*). An essentially similar model had been suggested for insects by Jander (*17*), but his model was based on a four-channel system, for which there seems to be little direct experimental support.

The evidence which favors our two-channel model is both electrical and optical. The sensitivity of single retinular cells to *e*-vector orientation has been electrophysiologically demonstrated in flies, as has the apparent coincidence between the direction of rhabdomere microvilli and maximum receptor potentials. Thus, intracellular recordings from retinular cells in compound eyes of dipteran insects have shown that rotating the polarization planes of a stimulating light modulates the amplitude of the receptor potentials, with a period of 180° (*18*). Furthermore, extracellular recordings from retinas of the flies *Musca* and *Calliphora* show maximum responses to transverse illumination when the *e*-vector of the stimulating light is parallel to the regularly arranged microvilli (*19*).

The most likely optical basis for these findings is that the known dichroism of the visual-pigment molecules is responsible for the differential sensitivity to linearly polarized light (*20*). For the molecular dichroism of a pigment like rhodopsin to appear on the rhabdomere scale, a paracrystalline arrangement of the photopigment molecules must be postulated. By analogy with the situation known for the outer segment of the rods of vertebrates (*21*), such a regular molecular pattern in the rhabdom appears quite likely. In fact, recent microspectrophotometric examination of dipteran rhabdomeres along their normal optic axis has directly demonstrated their postulated dichroism (*22*), which had been observed earlier in cephalopod rhabdoms (*23*). That this polarization-analyzing property of the insect microvillus system depends specifically on the visual pigment is attested by the dichroism's action spectrum and by the disappearance of dichroism on retinal bleaching (*22*).

Selective Adaptation Experiments

These various facts and hypotheses relating to individual retinular cells, coupled with the recent successful selective adaptation of the isolated *Octopus* retina by K. Tasaki (*24*), convinced us that a careful electrophysiological test, in crustacean compound eyes, of the two-channel-analyzer hypothesis was highly desirable.

The experimental strategy we used was derived from the generally recognized principle that, if more than one type of receptor cell is present in a sense organ, differential adaptation should distinguish the different types (*24, 25*). Specifically, for a two-channel polarization analyzer with axes of maximum absorption normal to one another, sharp differential adaptation of the two channels should occur when the *e*-vector orientation of the stimulus coincided with one or the other of these major axes. Adaptation when the *e*-vector was at 45° to these two most effective directions should affect both channels equally and thus yield no differential effect. Both of these expectations were clearly fulfilled in our experiments on the giant land crab *Cardisoma guanhumi* Latreille.

The experimental setup for our specific method is shown schematically in Fig. 2. Two linearly polarized light sources were used. One (L_F) provided brief flashes lasting about 10 microseconds, at intensities that could be varied between 800 and 13,000 lumens per square meter and at a rate of ten flashes per second; the *e*-vector of this stimulus was established by a polarizer (P_R) rotated at about 65° per second. In this manner a series of linearly polarized test stimuli differing progressively from one another by 6° to 7° were presented in a horizontal beam illuminating a large anterolateral area of the crab eye.

The second stimulating source (L_A) was a steady light which was used to adapt the eye to fixed intensity and *e*-vector orientation. This adapting light, with available intensities of 60 and 325 lumens per square meter, was used to illuminate the same retinal area from a direction approximating that of the flashing stimulus. For a given adaptation session the polarizer for this beam (P_A) was fixed, but it could be rotated, between measurements, to any desired *e*-vector orientation.

Eyes were studied *in situ* in healthy live crabs, the eyes having been solidly fixed in their normal upright position by imbedding the proximal external segment of the eyestalk in dental acrylic. The whole crab was clamped firmly in the position it usually assumes when standing on a horizontal surface, and was so placed that the two stimulating light beams converged on the desired retinal area.

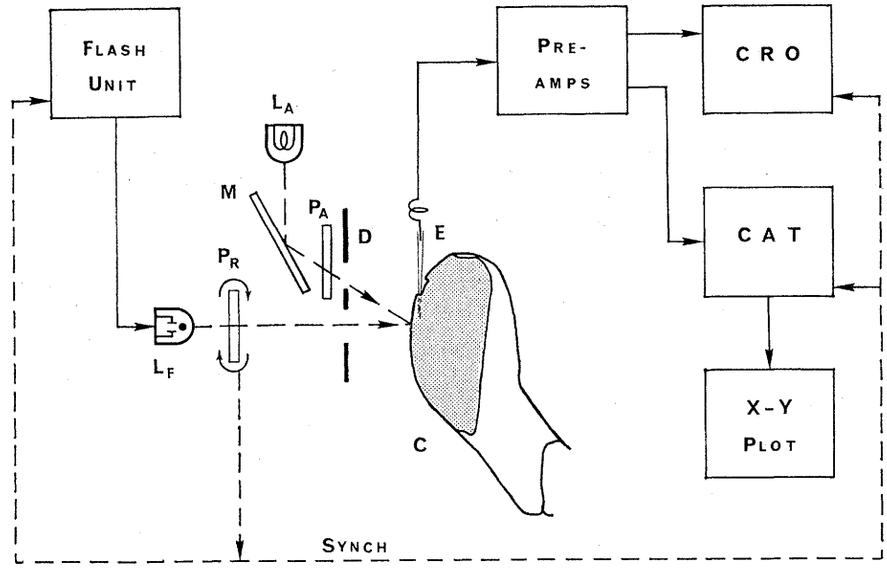

Fig. 2. Experimental setup for studying selective adaptation to polarized light. Two linearly polarized light sources were used. One (L_F) produced 10-microsecond flashes, at a rate of ten per second, polarized by a dichroic polarizer (P_R) rotating with a period of 5 to 6 seconds; the other (L_A) was a steady adapting light polarized (P_A) in a plane held fixed for a given test. These two sources illuminated the same large anterolateral retinal area from directions about 15° apart (this angle is exaggerated in the diagram). Receptor potentials were picked up with a coarse capillary electrode placed extracellularly in the retinal layer. The sweeps of the recording systems and the flash cycling were synchronized (connections labeled *SYNCH*) at the instant the rotating polarizer was vertical (0°). (*C*) Crab eyestalk; (*CAT*) average transient computer; (*CRO*) double-beam oscilloscope; (*D*) fixed diaphragm; (*E*) recording electrode; (*M*) mirror.

In our experiments, glass capillary electrodes filled with 3*N* potassium chloride with a resistance of about 2 megohms were used to pick up extracellular electrical responses. The electrode tip was placed in the retinal layer by being passed tangentially into the eye through a hole cut in the cornea (Fig. 2). The resulting electroretinograms (ERG's) were amplified and then displayed either on an oscilloscope, where they could be photographed, or on an X-Y plotter after processing by an average transient computer (CAT 400B), which added series of responses in sequences of sweeps. In typical plots for one condition, ten sweeps, each covering 270° of test light rotation, were summed.

The flash control unit, the oscilloscope, and the computer were all synchronized with the rotating polaroid so that successive sweeps were accurately superimposed. A photodiode placed in the stimulus beam and having a fixed linear polarizer covering its face was monitored on the second beam of the oscilloscope, to show the directions of the *e*-vector in the test flashes (Fig. 3). Because of the critically short duration of the electrophysiological responses, the test flash frequency used had to be carefully chosen to minimize recording artifacts due to the finite durations of the sampling and digitizing intervals.

Evidence for Two Channels

With this setup the responses of the *Cardisoma* eye could be recorded for series of test flashes under a variety of stimulus conditions. Exploratory tests showed that when the overall state of adaptation and the intensities of the two stimulating lights were appropriate, specific directions of polarization of the adapting light produced significant modulation of the amplitude of the electroretinograms, with a period of 180°. More extensive tests showed that, with the adapting light on and polarized in a horizontal plane (90°, 270°), maximum responses to the test flashes were obtained when the flashes were polarized vertically (0°,

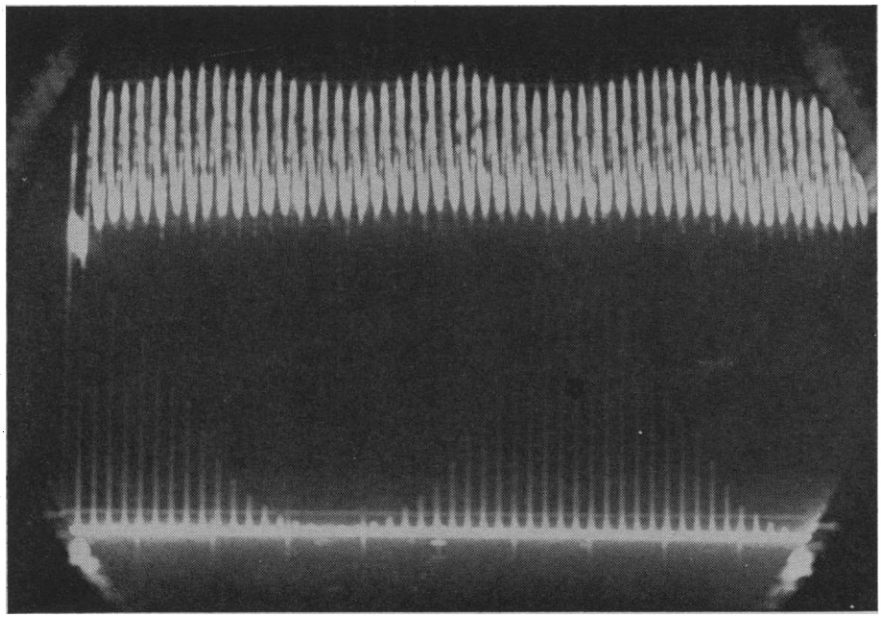

Fig. 3. Oscillograph sweep, showing responses of the two polarized-light-analyzing channels in the *Cardisoma* eye moderately dark-adapted to unpolarized light. The upper trace shows receptor potentials in response to the polarized flashes. In the lower trace the *e*-vector directions of the stimuli were monitored by a photodiode having a linear polarizer with its transmitting axis vertical. Amplitude modulation with a period of 90° is clearly evident in the retinal responses. The probable reasons for the 45°, 135° positions of the maxima are discussed in the text. Time marks in the lower trace, 500 milliseconds. Coordinates as in Fig. 4.

180°), hence, at 90° to the plane of polarization of the adapting stimulus. Minimum responses resulted from flashes polarized in the same plane as the adapting light (Fig. 4A).

When the adapting light was polarized obliquely at 45° to the vertical, no modulation with a period of 180° was evident in the responses to the flashes (Fig. 4B). Finally, vertical polarization (0°, 180°) of the adapting light evoked minimum responses to test flashes in this plane, and maximum electroretinogram amplitudes in response to flashes at right angles (90°, 270°) (Fig. 4C). These results clearly suggest the presence of a dual *e*-vector analyzer with its two components oriented at 90°.

While the amplitudes of responses to flashes presented when the adapting light was polarized obliquely at 45° to the vertical do not show any significant periodicity, a different pattern of electroretinogram amplitudes appeared when no polarized adapting light was present. In particular, when the preparation was moderately dark-adapted, quite strong modulation of the response amplitudes appeared, with a 90° period (Fig. 3). This effect diminished as successive flashes changed the degree of adaptation, so that after ten sweeps had been summed, the modulation appeared marginal. However, if only the first few sweeps were processed, the effect was still clear.

This modulation with a 90° period certainly suggests an integrated response of the two perpendicular analyzers in the absence of an adapting light. The finding that the maximum-response amplitudes were at 45° and 135° may at first seem surprising, but this does in fact match the behavior of the simple trigonometric function we propose as a model of the analyzer mechanism.

In general, equations of the type $y = f(\cos 2\theta)$ describe the response amplitudes of Fig. 4, A and C. Note that since there is classically a linear relation (within reasonable limits) between generator potential amplitudes, such as our measured electroretinograms, and the resulting spike frequencies for primary afferent axons, equations of this type and the equations given below should be valid not only for the observed responses but also for first-order centripetal visual messages.

The trigonometric functions needed for modeling the polarization analyzer more specifically should take into account other likely features of the system. To begin with, the energy absorbed by a dichroic polarizer per unit time is proportional to $\cos^2\theta$, and for a second unit, with its major axis perpendicular to that of the first, absorption will vary as $\sin^2\theta$, where θ is the angle between the *e*-vector direction and the major dichroic axis of the vertical channel. Secondly, some account needs to be taken of the relation between stimulus intensity and the amplitude of physiological response. In the absence thus far of the requisite data for the *Cardisoma* eye, we may assume the classic logarithmic transform. Then the differential response of the two-channel system to the flashes would be

$$R = \log\left\{\left[\frac{(\sin^2\theta + a)}{a}\right]\left[\frac{(\cos^2\theta + b)}{b}\right]\right\}$$

where a and b represent adapting light intensities. If there were no adapting light, $a = b = 1$ and

$$R = \log\left[(\sin^2\theta + 1)\,(\cos^2\theta + 1)\right]$$

which has a period of 90° with maxima at 45° and 135°, as in the electroretinograms shown in Fig. 3. When there is a horizontal adapting light of intensity a, then

$$R = \log\left\{\left[\frac{(\sin^2\theta + a)}{a}\right]\left[(\cos^2\theta + 1)\right]\right\}$$

The response would then have a period of 180° with maxima at 0° and 180° and minima at 90° and 270°, as in Fig. 4A.

A further correlation of this model with our *Cardisoma* data can be made if the degree of polarized light discrimination is plotted as a function of adapting light orientation (Fig. 5). Discrimination was calculated as relative amplitude modulation

$$\frac{R_{max} - R_{min}}{R_{max} + R_{min}}$$

where R is the amplitude of response to a given stimulus flash. The value of a, corresponding to an average discrimination to adaptation in the horizontal plane of 0.26, was 2; the reasonableness of this value can be tested in further experiments.

Note that the discrimination observed was a periodic function, with maxima at 0° and 90° as well as minima at ± 45°. The curve seems more cycloidal than sinusoidal since the minima appear as cusps. The 90° period of this function is consistent with the symmetry of the *e*-vector, which yields

functions of 2θ, coupled with the presence of two perpendicular analyzers which would halve the period again.

In addition, Fig. 5 suggests that selective adaptation in the vertical plane (0°) apparently had a weaker effect than adaptation in the horizontal plane (90°). This in turn implies an asymmetry in the two channels such that one of them makes a larger contribution to the output than the other. This may be the result of the different numbers of retinular cells contributing to the two components, but other explanations are possible. For example, in a dipteran insect there is known to be differential wavelength sensitivity in the various retinular cells of the same ommatidium (*22*). In addition, the wavelength most effective for polarotaxis, at least in *Daphnia*, is quite distinct from that reported most effective for phototaxis (*26*). The interaction of these undoubtedly related phenomena remains to be studied.

In assessing the significance of our selective adaptation experiments in *Cardisoma*, three features of the data should be emphasized. These are important in establishing the presence of distinct channels in the analyzer system.

1) When the direction of the stimulus *e*-vector was altered, the location of the maxima and minima in the response curve did not change concomitantly. When it did change there was one discrete 90° phase shift (Fig. 4). This implies that the analyzer elements were oriented in two particular directions and not just selectively affected as parts of a randomly or radially oriented absorbing system.

2) The same view is also supported by the systematic changes in *e*-vector discrimination resulting from alteration of the adapting *e*-vector (Fig. 5); in a uniformly oriented receptor system such changes would not be expected.

3) Finally, the appearance of detectable peaks in response amplitude with *e*-vectors at 45° and 135°, when the whole eye was moderately dark-adapted (Fig. 3), would be most reasonably explained by the assumption of two perpendicularly oriented analyzer systems.

These considerations directly support the notion that the polarization analyzer is definitely channeled and, more specifically, that in *Cardisoma* it depends on an intraretinal two-channel mechanism with its perpendicular directions of maximum absorption vertical and horizontal relative to the eyestalk's normal spatial orientation.

In our experiments we are not only studying *Cardisoma* but are beginning to use the same approach in studies of other decapod crustaceans. Current observations on crayfish eyes and on eyes of the lobster *Homarus* demonstrate that a two-channel polarization analyzer is also present in the superposition eyes of these macruran decapods. Hence the apposition eye of the crab is not unique in its mechanism of *e*-vector perception.

The data so far obtained with superposition eyes of these other decapod crustaceans show a considerably weaker degree of polarized light discrimina-

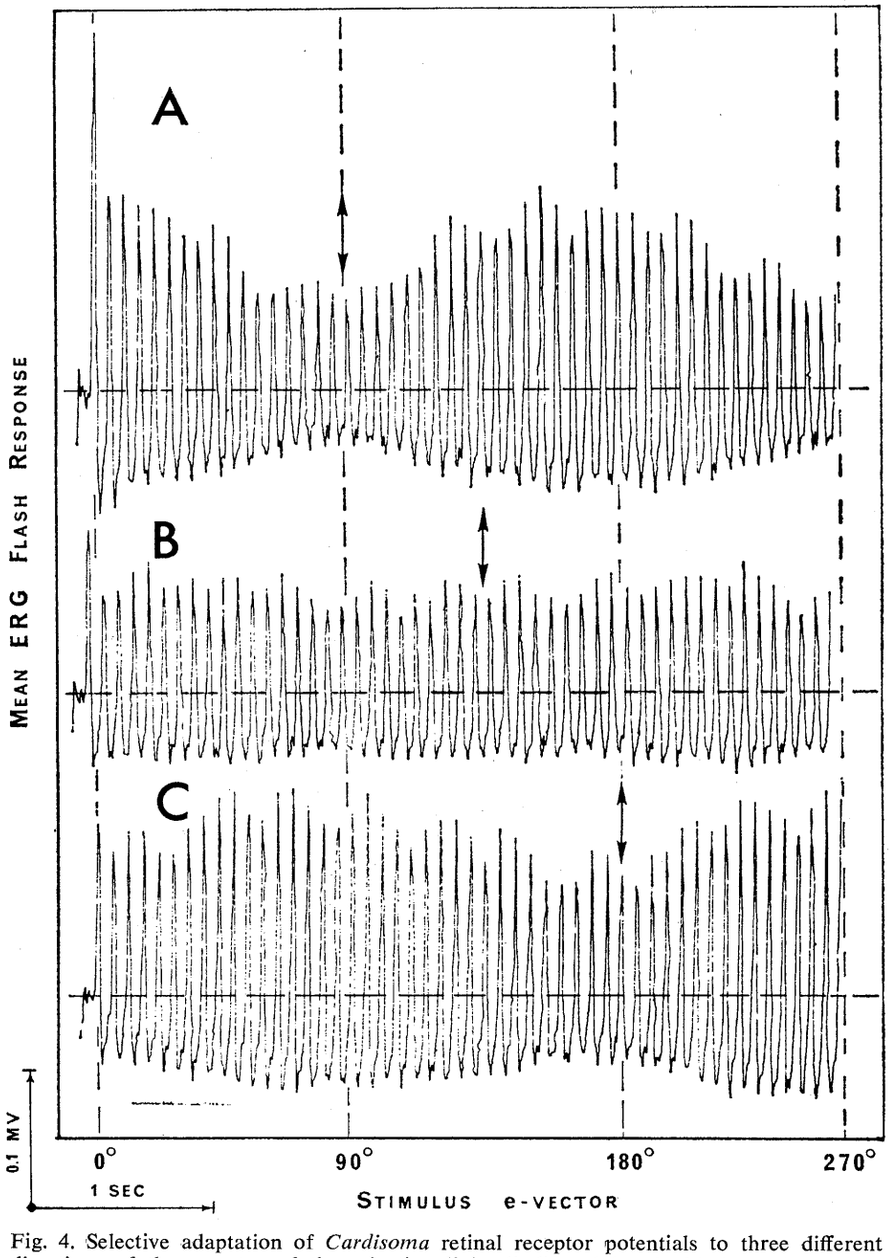

Fig. 4. Selective adaptation of *Cardisoma* retinal receptor potentials to three different directions of the *e*-vector of the adapting light (indicated by double-headed arrows). Each of the three records is the sum of ten sweeps accumulated with an on-line computer and recorded by an X-Y plotter. (Ordinate) Amplitude of receptor potential; (abscissa) both time and *e*-vector direction of the test flash stimuli. The modulation of response amplitude with a period of 180° indicates the presence of two receptor channels differentially sensitive to polarized light and oriented at right angles. (A) Adapting light horizontally polarized (90°, 270°); minimum responses to flashes at about 90° and 270°, maximum responses near 0° and 180°. (B) Adapting light polarized at 135° and 315°; there is no significant modulation with a 90° period. The same negative result was obtained with adaptation at 45° and 225°. (C) Adapting-light *e*-vector at 0° and 180°; minimum responses to flashes around 0° and 180°; maximum responses near 90° and 270°. The crab's normal vertical direction was 0°.

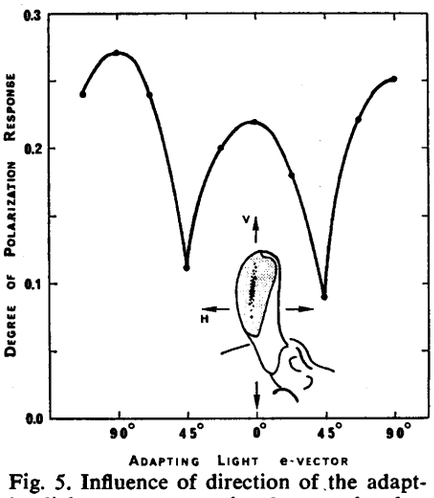

Fig. 5. Influence of direction of the adapting-light *e*-vector on the degree of polarized light sensitivity in *Cardisoma*. The ordinate was calculated from records like those of Fig. 4, by determining $(R_{max} - R_{min})/(R_{max} + R_{min})$. Maximum sensitivity lies in the planes of the two sets of microvilli in the rhabdoms of the eye. These directions correspond to the horizontal (90°) and vertical (0°) planes of the crab's normal spatial orientation.

tion than was found for *Cardisoma* (Fig. 5). Nevertheless, the orientation of the two analyzer components in the superposition eyes are vertical and horizontal, as in the crab. The asymmetry of the degrees of discrimination with adaptation at 0° and 90° seems even more marked in the crayfish and lobster than in the crab.

Clearly a more sophisticated understanding of this receptor mechanism in decapod crustaceans will require quantitative data on the effects of stimulus intensity and of visual adaptation on the system's responses. It will also be highly desirable to make intracellular recordings from one channel, or preferably both channels, in a single ommatidium (*18*).

When the electroretinographic data were found to indicate vertical and horizontal directions for the two tubular systems hypothesized to constitute the dichroic elements of the polarization analyzer, knowledge about the actual microvillus directions in the retina became essential. Externally it is obvious that the ommatidia in the *Cardisoma* eye are aligned in remarkably regular rows which are approximately horizontal when the eyestalk is in its normal erect position (Fig. 6). Two related questions need to be asked about the fine-structure geometry in relation to eye and whole-animal coordinate systems. First, what is the orientation, relative to these coordinates of retinular cells and microvilli within a single ommatidium? Second, how do these relations compare in neighboring or remote parts of the retina?

Let us answer the second question first. Tangential electron micrographs which cut cross sections through the retinulas in anterolateral regions of the *Cardisoma* retina have been found by our collaborator Eisuke Eguchi to show nearly identical orientation of the seven retinular cells and their two sets of microvilli over areas containing at least six to ten ommatidia; more extensive correlations have not yet been studied. Comparably uniform retinular patterns, at least over local areas, have also been demonstrated for the cladoceran *Leptodora* (*27*), for the crayfish *Astacus* (*11*), for a variety of insects (*28*), and for cephalopods (*29*).

In *Cardisoma* the retinular components show a remarkably simple relationship to the horizontal rows of ommatidia (Fig. 6) and hence to the crab's horizon. The large asymmetrical cell (number 1 in Fig. 1, B and C) lies in a horizontal plane paralleling these ommatidial rows. The rhabdom layers to which cell number 1 and the two retinular cells on the opposite side of the rhabdom contribute have their microvilli oriented horizontally (Fig. 7). The microvilli of retinular cells in the alternate rhabdom layers are, of course, vertical in orientation. This parallels the situation observed by G. H. Parker in the crayfish, where the "fibers" in the rhabdom plates to which the large retinular cell contributes were seen in the light microscope to lie in the horizontal plane relative to the animal's normal spatial orientation, and the alternating layers, to have their fine structure vertically oriented (*11*). Electron micrographs have recently shown this to be true also for the closely similar eye of the lobster *Homarus* (*30*).

In cephalopods, too, the vertical and horizontal axes of the animal's normal position in space correspond

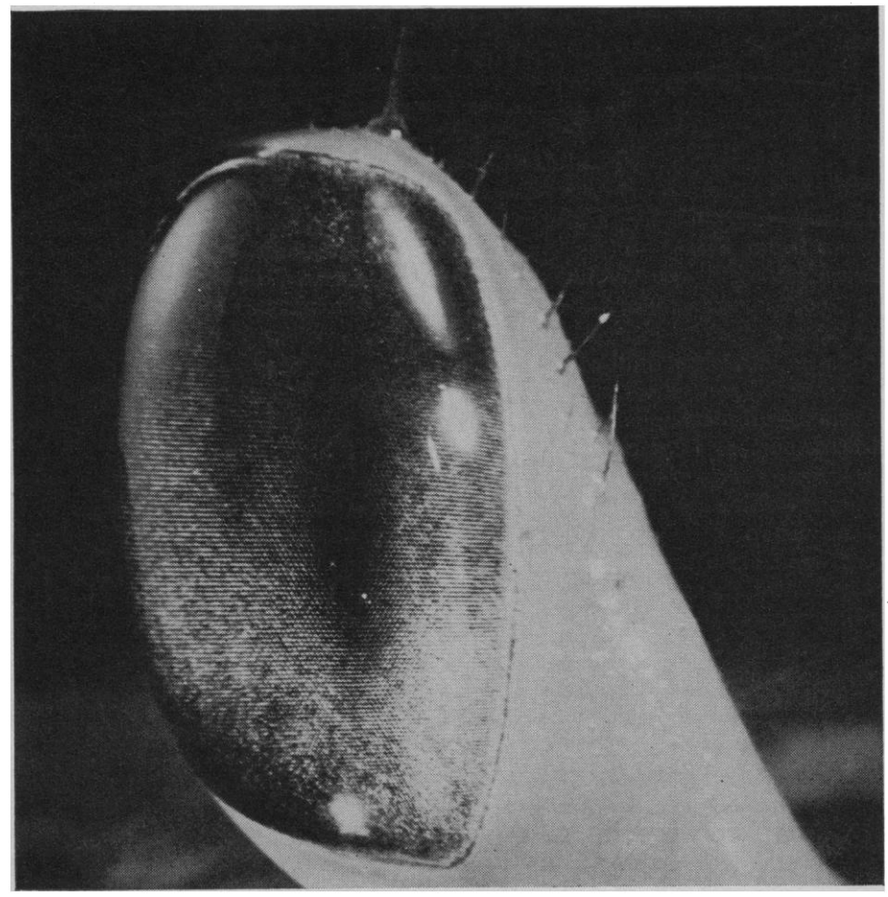

Fig. 6. Anterior view of the *Cardisoma* compound eye. The minute hexagonal facets (about 20 microns in diameter) are regularly arranged in horizontal rows which parallel the direction of one set of rhabdom microvilli. Where the line of sight is close to the optic axes of the ommatidia, the pseudopupil appears as a black, vertically elongated ellipse; its eccentricity arises from the marked "astigmatism" of the retina in this region. The diameter of the eyestalk at the middle of the eye was 4 to 5 millimeters in this adult female crab, which had a carapace length of 63 millimeters.

with the two orientations of the microvilli in the retina (*29*) and also with the two directions of maximum selective adaptation to linearly polarized light found by Tasaki (*24*).

Thus the electroretinographic data for *Cardisoma*, the crayfish, and *Octopus* are congruent with the fine-structural detail and with the hypothesis given above for polarized light analysis. The presence of the same sort of two-channel polarization analyzer in animal groups as phylogenetically remote as crustaceans and cephalopods is remarkable, especially in view of the marked divergence in gross structure of compound and camera eyes.

The widespread occurrence of such a two-component afferent system for polarized light perception provides an interesting example of the kind of sensory input hypothesized by Mittelstaedt in developing his bicomponent modulation theory for animal orientation (*31*). According to this theory the angle between an animal's body axis in a given plane and the direction of the relevant sensory cue for orientation is split into two components, one a function of the angle's sine, the other of its cosine.

Whether in these crustacean visual systems the sinusoidal turning tendency, which is the motor output controlling polarotaxis (*16, 17*), originates through processing of the two-channel sensory input in the manner further postulated by Mittelstaedt is not known. In any case the information processing of polarized light and other visual input signals in the decapod eyestalk is a particularly interesting problem. Extensive channeling and integration of signals have been shown to occur between the retina and the optic nerve, which are connected by a series of four complex ganglia (*32*). This elaborate eyestalk nervous system also receives a wide range of efferent information from the other eyestalk and the rest of the animal (*33*), but little is known as yet about the influence of the efferent outflow on the reception and processing of visual data (*34*).

Status of Earlier Four-Channel Model

While the evidence now available seems overwhelmingly to favor a two-channel analyzer for decapod crustaceans and cephalopods, the most reasonable earlier model proposed to account for polarized light perception was a four-channel one. Such an analyzer was hypothesized for the honeybee eye more than 15 years ago by Autrum and Stumpf (*35*) and supported by von Frisch (*36*).

Four channels in the analyzer were probably postulated because the retinula of *Apis* has eight regular photoreceptor cells each with a rhabdomere. Since the radial symmetry of the retinula would establish four pairs of diammetrically opposed cells, one might expect four corresponding analyzer channels. However, electron micrographs have shown that the rhabdom microvilli of the honeybee have in fact only two, mutually perpendicular directions (*37*). Hence, this fine-structural evidence, unknown to Autrum and von Frisch when they proposed the four-channel model, dictates a two-channel analyzer. No electrophysiological evidence for a four-channel system has yet been presented, and the one reported attempt at selective adaptation to polarized light in the honeybee failed to demonstrate any such discrimination (*25*). Since there is unquestionably a specific intraretinal analyzer in the bee eye, and since selective adaptation in decapod crustaceans does yield evidence of such a mechanism, a reexamination of the insect case seems an important experiment for the future.

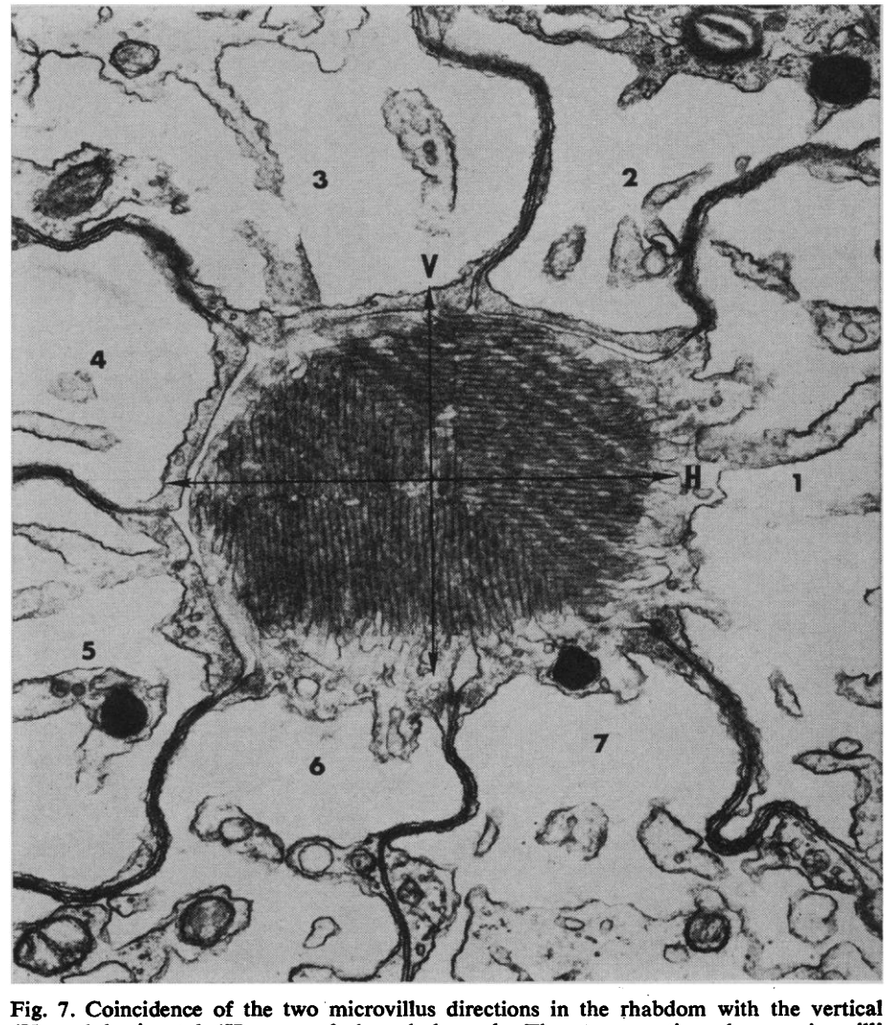

Fig. 7. Coincidence of the two microvillus directions in the rhabdom with the vertical (*V*) and horizontal (*H*) axes of the whole crab. The cross section shows microvilli and the axial parts of the seven retinular cells (numbering begins with the large cell at right) which contribute corresponding rhabdomeres. The section is slightly oblique so that parts of two microvillus layers are shown. In the sector showing vertical villi their abutting ends may be seen approximately along the midline where rhabdomeres originating from opposite cells 3 and 6 meet. The microvilli average about 700 angstroms in diameter. Around the rhabdom is a well-developed system of vacuoles traversed by narrow cytoplasmic bridges in some of which cell membranes and desmosomes may be seen. The three large black bodies are pigment granules in retinular cell cytoplasm. For this electron micrograph tissue was doubly fixed in glutaraldehyde and osmic acid, then stained in lead citrate and uranyl acetate. (× 11,000) [Courtesy Eisuke Eguchi]

Comparative study of rhabdom fine structure in a wide range of forms indicates that in certain crustacean and insect species more than two microvillus directions occur in a single ommatidium (*38*). This might imply that an analyzer of more than two channels is present or, if microvilli within a single rhabdomere have different orientations, that sensitivity to polarized light is weak or even absent. Obviously the further study of such cases, particularly from electrophysiological and behavioral points of view, will be an important test of the generality of the conclusions reached here.

Summary

As background for a report on our current selective adaptation experiments in decapod crustaceans, the various facts and hypotheses generally relevant to intraretinal sensitivity to polarized light in arthropods as well as cephalopods have been marshaled. On the basis of this review, the following working hypotheses have been made.

1) One ommatidium in the compound eye is the functional unit in image perception but contains in its component retinular cells subunits which can work independently in detecting other visual parameters, such as polarization.

2) Single retinular cells do respond differentially to light polarized in various planes.

3) Light sensitivity, including *e*-vector detection, is localized in the rhabdomeres, which comprise closely packed arrays of microvilli protruding axially from retinular cells; the dichroism of the photopigment molecules, which are contained within the microvilli, provides the molecular basis of *e*-vector detection.

4) The visual pigment molecules have their major dichroic axis aligned predominantly parallel to the long axis of the microvillus containing them; typically all microvilli in a single rhabdomere are closely parallel to one another, thus comprising at the cellular level a unit dichroic analyzer with maximum optical density to photons vibrating in the direction parallel to these microvillous protrusions.

5) In most decapod crustaceans, in cephalopods, and in some insects the microvilli in all rhabdomeres of a retinula are oriented in only two directions, perpendicular to each other. Therefore, *e*-vector perception must depend at the retinular level on a two-channel system consisting of a pair of dichroic analyzers with their major transmitting axes fixed at a 90° angle determined by the two directions of microvillus orientation.

Our new results on selective adaptation in the eye of *Cardisoma* provide direct experimental evidence for such a two-channel analyzer in which the pair of functional units have their maximum sensitivity to polarization in the same retinal directions as the rhabdom microvilli observed in electron micrographs. In turn, these directions correspond with the vertical and horizontal axes of the animal's normal spatial orientation. In *e*-vector detection the seven retinular cells of a single decapod ommatidium thus form two operational subgroups of four and three cells, respectively (*39*). The correspondence of the electrophysiological evidence for a dual polarization analyzer with the perpendicular directions shown by the microvilli in a single rhabdom strengthens the idea that one ommatidium is enough for detecting *e*-vector orientation.

On this evidence we may conclude that the model developed above for a two-channel polarization analyzer effectively accounts for the relevant spectrophotometric, fine-structural, electrophysiological, and behavioral data currently available for a considerable number of arthropods and cephalopods.

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39. *Note added in proof.* Experiments we carried out while this article was in press provide a new line of evidence supporting our hypothesis for the two-channel polarization analyzer. In the spider crab *Libinia* 6 hours of selective adaption to moderate intensities of vertically or horizontally polarized light induce quantitatively significant differences in the fine structure of retinular cells 1, 4, and 5 compared with 2, 3, 6, and 7 (E. Eguchi and T. H. Waterman, in preparation).
40. We thank the following colleagues: Professor Robert Galambos for the loan of the CAT 400B and X-Y plotter for use in these experiments; Dr. Eisuke Eguchi for essential collaboration on rhabdom fine structure: Dr. Charles E. Lane and Miss M. Elaine Pringle for providing *Cardisoma*; Dr. Timothy H. Goldsmith for critical reading of an early version of the manuscript; and Mrs. Mabelita Campbell for general assistance, particularly in preparing the paper for publication. This study was aided by U.S. Public Health Service grant No. 03076 and by U.S. Air Force grant No. 1064. A preliminary account of this work was presented in November 1965 at the International Symposium on Information Processing in Sight Sensory Systems, at the California Institute of Technology.

From Enzymatic Adaptation to Allosteric Transitions

Jacques Monod

One day, almost exactly 25 years ago—it was at the beginning of the bleak winter of 1940—I entered André Lwoff's office at the Pasteur Institute. I wanted to discuss with him some of the rather surprising observations I had recently made.

I was working then at the old Sorbonne, in an ancient laboratory that opened on a gallery full of stuffed monkeys. Demobilized in August in the Free Zone after the disaster of 1940, I had succeeded in locating my family living in the Northern Zone and had resumed my work with desperate eagerness. I interrupted work from time to time only to help circulate the first clandestine tracts. I wanted to complete as quickly as possible my doctoral dissertation, which, under the strongly biometric influence of Georges Teissier, I had devoted to the study of the kinetics of bacterial growth. Having determined the constants of growth in the presence of different carbohydrates, it occurred to me that it would be interesting to determine the same constants in paired mixtures of carbohydrates. From the first experiment on, I noticed that, whereas the growth was kinetically normal in the presence of certain mixtures (that is, it exhibited a single exponential phase), two complete growth cycles could be observed in other carbohydrate mixtures, these cycles consisting of two exponential phases separated by a complete cessation of growth (Fig. 1).

Lwoff, after considering this strange result for a moment, said to me, "That could have something to do with enzyme adaptation."

"Enzyme adaptation? Never heard of it!" I said.

Lwoff's only reply was to give me a copy of the then recent work of Marjorie Stephenson, in which a chapter summarized with great insight the still few studies concerning this phenomenon, which had been discovered by Duclaux at the end of the last century. Studied by Dienert and by Went as early as 1901 and then by Euler and Josephson, it was more or less rediscovered by Karström, who should be credited with giving it a name and attracting attention to its existence. Marjorie Stephenson and her students Yudkin and Gale had published several papers on this subject before 1940. [See (*1*) for a bibliography of papers published prior to 1940.]

Lwoff's intuition was correct. The phenomenon of "diauxy" that I had discovered was indeed closely related to enzyme adaptation, as my experiments, included in the second part of my doctoral dissertation, soon convinced me. It was actually a case of the "glucose effect" discovered by Dienert as early as 1900, today better known as "catabolic repression" from the studies of Magasanik (*2*).

The die was cast. Since that day in December 1940, all my scientific activity has been devoted to the study of this phenomenon. During the Occupation, working, at times secretly, in Lwoff's laboratory, where I was warmly received, I succeeded in carrying out some experiments that were very significant for me. I proved, for example, that agents that uncouple oxidative phosphorylation, such as 2,4-dinitrophenol, completely inhibit adaptation to lactose or other carbohydrates (*3*). This suggested that "adaptation" implied an expenditure of chemical potential and therefore probably involved the true synthesis of an enzyme. With Alice Andureau, I sought to discover the still quite obscure relations between this phenomenon and the one Massini, Lewis, and others had discovered: the appearance and selection of "spontaneous" mutants (see *1*). Using a strain of *Escherichia coli mutabile* (to which we had given the initials ML because it had been isolated from André Lwoff's intestinal tract), we showed that an apparently spontaneous mutation was allowing these originally "lactose-negative" bacteria to become "lactose-positive." However, we proved that the original strain (Lac^-) and the mutant strain (Lac^+) did not differ from each other by the presence of a specific enzyme system, but rather by the ability to produce this system in the presence of lactose. In other

The author is head of the Department of Biochemistry of the Pasteur Institute, Paris, France. This article is the lecture he delivered in Stockholm, Sweden, 11 December 1965, when he received the Nobel Prize in Physiology or Medicine, which he shared with François Jacob and André Lwoff. It is published here with the permission of the Nobel Foundation and will also be included in the complete volumes of Nobel Lectures in English published by the Elsevier Publishing Company, Amsterdam and New York. It was translated from French by François Kertesz.