CILIOEXCITATORY AND CILIOINHIBITORY PROCESSES INITIATED BY LIGHT ON TWO IDENTIFIABLE NEURONAL PIGMENTS IN MYTILUS EDULIS

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SUMMARY

Two neuronal chromoproteins (red-haemoprotein and yellow-carotenoprotein) initiate changes in ciliary activity on the gill of Mytilus. Photic, electrical and chemical stimulation of these chromoproteins correlate with fluctuations in ionic (calcium and iron) deposits in gill bulk tissue samples. Microspectrophotometric measurements of chromoprotein spectral band patterns reveal two or three absorption maxima for carotenoprotein and haemoprotein respectively. Under aerobic conditions, cilioinhibition and cilioexcitation results after activation of carotenoprotein or haemoprotein respectively. Carotenoproteins are only engaged during gradual anoxia. Immediate anoxia renders both chromoproteins inoperative. Changes in ciliary activity initiated by photic stimulation of neuronal chromoproteins are abolished by denervation of the gill. It is postulated that photoactivation and/or release of specific neurotransmitters lead to transformations in chromoproteins with subsequent neuronal regulation of gill ciliary activity.

INTRODUCTION

Recent experiments performed in different pigment cells permitted the recognition of bioelectrical reactivity to light as a widespread cellular property. In nerve cells it has been established that inhibitory processes as well as excitatory ones may be triggered by radiations of different wavelengths (Arvanitaki & Chalazonitis, 1947, 1949a, b, c, d, 1950, 1958a, b, 1960, 1961; Bullock, 1956; Bullock & Diecke, 1956; Bullock & Fox, 1957; Chalazonitis, 1954; Hisano, Tateda & Kuwabara, 1972). Simultaneous intracellular recordings in many photoactivated nerve cells revealed patterns of activity in which excitation in a given cell is accompanied by inhibition in the immediately neighbouring ones, recalling the 'contrasting effects' already known to occur in different receptor organs, in the retina (Hartline, 1938, 1941, 1949; Hartline, Wagner & Ratliff, 1956; Granit, 1947, 1950, 1952; Kuffler, 1953, 1958, 1959; Hartline, Wagner & Ratliff, 1956) in the auditory system (Galambos & Davis, 1944) and, in cerebral patterns of activity (Mountcastle, 1957). Obviously the interest lies in the action of light being analysed in single cells, by methods permitting time resolution as well as site resolution of the events.
In this study we investigate the cilioexcitatory and cilioinhibitory process initiated by light on two identifiable neuronal pigments (see also Paparo & Murphy, 1976). The approach to such problems at the cellular level holds interest for many reasons. For instance, it is hoped that the knowledge of how light energy specifically trapped by known molecules located in known cellular sites leads to electrical work might provide an insight into mechanisms of importance in general neurophysiology. Photons absorbed by suitable cellular molecules serve to activate or trigger a system to which these molecules are functionally coupled, and which partially converts the absorbed energy into electrical work. But this system is activated just as well by any other stimulus, whether it be electrical, chemical or mechanical, whose sites and mechanism of action are, however, less specific than that of the photic stimulus.

It is proposed that this common cellular photosensitivity might be basically compared to that which is at the origin of the performance of a functional photoreceptor cell. In this study we have concentrated on the three following aspects.

(a) The cytostructural aspect involving the identification at the electron microscopic level of the cellular location of the molecules that absorb the light energy, in other words, the site of action of the stimulus.

(b) The biophysical aspect, involving monochromatic activations of the intracellular pigments, and allowing some information on the probable biophysical and biochemical transitions initiated by the light.

(c) The bioelemental aspect, involving the identification of ionic changes initiated by light using elemental analytical techniques at the level of the organelle in one or both of these pigmented cytosomes.

**Methods and Materials**

**Measurement of ciliary activity, electrical stimulation and drug perfusion in anaerobic conditions.** All experiments were performed on the mussel *Mytilus edulis*, purchased from Woods Hole Biological Supply Company and kept for 1–2 weeks in artificial sea water (Rila Marine Mix) in an Instant Ocean Aquarium (temperature 17 °C, pH 7.5–8.0, density 1.025). Before each experiment mussels of 4.8–5.2 cm length were placed in finger bowls of the same sea water, the posterior adductors were cut, and each gill with its branchial nerve, visceral ganglion, and a piece of adductor muscle for support was isolated. Gills measured 3.6–3.9 cm in length along the gill axis. This ganglion/nerve/gill preparation was pinned to rubber mats glued in the bottom of a Petri dish containing sea water, and the dish was placed in a holder fastened to the adjustable stage of a microscope.

A field was selected for observation by measuring 1.4–1.5 cm in an anterior direction from the visceral ganglion. A field of view contained about 50 filaments which were grouped for ease of observation into three vertical columns. The rate of ciliary beating in beats per second was estimated by synchronizing the rate of flashing of a calibrated, stroboscopic light used in place of the substage lamp with the rate of beating of the cilia. Synchronization was achieved when the metachronal wave appeared to stand still. Measurements were made from dorsal to ventral border, and from left to right across the field, giving 12 sets of measurements.

When nerve stimulation was part of the experiment the mounted preparation was
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Further dissected under a four-power magnifying lens to expose the nerve, and the concentric electrode was placed at its point of emerge from the visceral ganglion, just before it passes posteriorly into the gill axis. Asimulator supplied electrical pulses of the following characteristics: 0.1 V, 2–50 biphasic pulses s⁻¹, 2 msec pulse duration. The stimulus was applied for 3 min and the effect was observed during that period and the following 27 min or whatever time was required for ciliary beating to return to a constant rate.

Drugs were added by replacing the content of the perfusions dish with the desired concentration of the drug in sea water at 21 °C, and continuing to perfuse with the drug solution. Drugs were washed out by repeating the procedure with sea water. The following drugs were used: 5-hydroxytryptamine (5-HT, serotonin) as creatinine sulphate, and 3,4-dihydroxyphenylethylamine (DA, dopamine; as hydrochloride) and ethylene glycol-bio (aminoethyl ether)-N₁,N₂ tetraacetic acid (EGTA). The standard error (s.E.) of measurement of rate of beating was small compared to the magnitude of experimental effects. In a preliminary experiment, untreated gills of five animals were examined hourly over a 9 h period following the usual 2 h equilibration period.

When achieving anaerobic conditions as part of the experiment, animals were placed into a vessel containing eight times more sea water than the body weight of the animal. The surface of the water was then covered with a paraffin-oil layer of 1–2 cm thickness. Under such circumstances oxygen content of the water was used up and a total anoxia commenced within 48 h. The animals were considered as living up to the moment when they ceased to react with movement upon influence of mechanical stimulation. This procedure follows that of Zo. Nagy & Ermini (1972).

Pigment distribution, cellular localization, and extraction. Colour of the visceral, pedal and cerebral ganglia and nerves of Mytilus edulis were examined in living preparations. Cellular localization of red and yellow pigments were examined in squash preparations and cryostat sections of fresh ganglia and nerves.

Extractions of nerve haem-protein were performed by leaching it from the visceral ganglion and branchial nerve by dilute salt solutions. Preliminary leachings of the nerves for 1 h at 250 °C in 0.01 M potassium phosphate buffer, pH 7.4, removed a fraction of the haem-protein that is easily air-oxidized. Subsequent leaching for 1 h in 0.15 M potassium phosphate buffer, pH 7.4, extracted the bulk of the haem-protein in a form that probably resisted air-oxidation.

The extracted haem-protein derivatives were divided into four equal parts; each part is used to prepare a different haem-protein. These derivatives were (a) oxygenated and deoxygenated haem-protein, using pure gaseous oxygen or nitrogen, (b) oxidized and reduced chromoprotein, using potassium ferrimonoxide and (d) cyanide complex using the method of Wittenberg et al. (1965). Spectra were recorded using the microfluorometric method below.

The carotenoid pigment is soluble in acetone, protroleum ether and other organic solvents. The pigments were collected in the protroleum ether extract and are compared to the in situ measurement (465 and 480 nm).

Monochromatic activation of pigments. Microfluorometric measurements of pigments were carried out with a Leitz MP II system consisting of Orthroplan Large Field microscope, incident fluorescence, microfluorometry, and a photo-multiplier
with a measuring-beam interference-graduated filter. A coarse and fine combination scanning state permitted accurate localization and repeated measurement of neuronal pigments within a nerve cell.

**Elemental analysis of thick sections of ganglion nerve gill preparations.** Samples for X-ray analysis were fixed in 3% glutaraldehyde in 0.1 M sodium cacodylate, dehydrated through a graded series of alcohols through 100% and then into Freon TF. Critical point drying was carried out in a Bomar 900 EX CPD using Freon 13. Samples for freeze drying were immediately frozen in liquid nitrogen and freeze-dried in a Pearse Tissue Dryer. Samples were examined in a Cambridge Mark IIA fitted with an ORTEC 6200 EDS system. Spectra were collected for 100 s at an accelerating voltage of 20 kV on a 0-10 keV scale and read out on an x-y recorder. A minimum of 20 analyses were collected from each piece of gill epithelia to check for reproducibility. Various magnifications were used to check for homogeneity.

**RESULTS**

*Neuronal pigments.* Observations of living mussel ganglia show that the over-all colour of a particular ganglia is always the same in different specimens of *Mytilus edulis.* Ganglia are predominately red-orange. The cellular localization of *Mytilus* ganglia pigment was examined in squash preparations and two cryostat sections. Most pigments seem to localize in the neuronal cell bodies and proximal axon regions of nerve cells. It occurs within discrete granules in the neuronal cytoplasm which are usually of about 1-2 μm in diameter. Large numbers of these granules occur in any given neurone and are particularly abundant in the region of the perikaryon below the nucleus and adjacent to the main neuropil axon projection. The granules are of two basic colours: red and yellow. However, pedal neurones always contain some red granules but many granules which are of intermediate colour between red and yellow. It is presumed that the granules of intermediate colour contain a mixture of these two pigments. The uniformity of colour is interrupted by groups of white cells which are probably neurosecretory cells. These unpigmented regions will be used as 'blanks' for a typical absorption spectrum.

*Monochromatic activation of neuronal pigments.* The nerve cells of *Mytilus* ganglia share with other pigmented photoactivable cells the common importance cytostructural feature of localization of absorbing molecules in intracellular organelles (cytosomes) where they are tightly ordered on fine substructures. Among the intracellular pigments found in the neuronal somata: (a) haem-protein with three main absorption bands of λ 420, 540 and 580 nm (in the oxygenated state) and (b) caroteneprotein absorbing mainly at λ 465 and λ 490 nm (Figs. 1, 2).

The homogeneous red fractions obtained showed absorption maxima which indicated the presence of a haem-protein with usual α, β and γ peaks (Table 1). It was usually possible to reversibly oxygenate–deoxygenate the haem-protein but only for a limited number of cycles. Repeated passing of gaseous oxygen or nitrogen through the aqueous haem-protein solution produced a gradual breakdown of pigment and loss of colour in solution. This breakdown occurs more quickly at room temperature than at 0 °C. Time taken for complete oxygenation or deoxygenation is less at higher temperatures. The retention of two absorption maxima other than the γ peak in
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Fig. 1. Emission spectrum for red pigment. Each point is an average of 14 preparations, 12 points per reading for this and subsequent figures.

Fig. 2. Emission spectrum for yellow pigment.

deoxygenated haem (Table 1) is unusual in vertebrate haem-proteins but occurs in some vertebrate blood haem-proteins (see Wittenburg et al. 1965) and in the nerve haem-protein of Spisula (Strittmatter & Burch, 1963). The absorption maxima of other compounds of Mytilus haem-protein were listed in Table 1.

Monochromatic activation of neuronal pigments and lateral ciliary activity: aerobic conditions. There is a cilioinhibitory response to photoactivation of cytosomal caroten-
Table 1. Absorption maxima of extracted haem-protein derivatives from the visceral ganglion of the mussel, Mytilus edulis

<table>
<thead>
<tr>
<th>Form of haem-protein</th>
<th>Absorption maxima (nm)</th>
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<tbody>
<tr>
<td>Oxygenated (gaseous)</td>
<td>α: 580, β: 540, γ: 420</td>
</tr>
<tr>
<td>Oxidized (ferricyanide)</td>
<td>α: 580, β: 539-540, γ: 420</td>
</tr>
<tr>
<td>Deoxygenated (gaseous)</td>
<td>α: 572, β: 539, γ: 420</td>
</tr>
<tr>
<td>Reduced (dithionite)</td>
<td>α: 530-580, β: 533, γ: 420</td>
</tr>
<tr>
<td>Cyanide complex</td>
<td>α: 561, β: 533, γ: 440</td>
</tr>
<tr>
<td>Carbon monoxide complex</td>
<td>α: 575, β: 537, γ: 426</td>
</tr>
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Fig. 3. Average ciliary activity as a function of emission wavelength and intensity. Photic stimuli occur at the short wavelength (490 nm) prior to excitation at the longer wavelength (580 nm).

Oxidation of cytosomal pigments at 490 nm. The increase in ciliary activity is directly proportional to the emission intensity of the two spectral peaks which are found within this yellow pigment. Furthermore, the cilioexcitatory response to photoactivation of the cytosomal haem-pigments at 580 nm follows the same relationship to spectral peaks as stated above (Fig. 3). This relationship of changes in cilioactivity to photoactivation of spectral peaks of specific neuronal pigments still holds even when the photoactivation λ is reversed (Fig. 4). These responses to photoactivation of neuronal pigments can be abolished if the branchial nerve is cut prior to experimentation. Specific λ 580 nm elicit a gradual increase in the average rate of beating which reverts to a gradual decrease in average rate of beating following λ 490 nm exposure (Fig. 5). The perfusion of gill preparation with 10 mM EGTA abolished the cilioinhibitory effect of λ 490 nm. EGTA did not alter cilioexcitatory following λ 580 nm. Washing gill preparation with physiological solution restores the specific effect produced by both λ 490 and λ 580 nm (Figs. 5 and 6).
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Fig. 4. Same as Fig. 3 except that photoexcitation occurs at the longer wavelength prior to activation at the shorter wavelength.

Fig. 5. Average lateral ciliary activity function for specific excitatory (58 nm) and inhibitory (490 nm) processes. Ten mM EGTA is added to both the excitatory and inhibitory components.

Anaerobic conditions. Under gradual anoxic conditions there is no change in ciliary activity in response to photoactivation at 580 nm. However, even under anoxic conditions lasting 72 h, the cilioinhibitory response to photoactivation at 490 nm is still present with enhancement of the effect. If the gill preparation is immediately placed under anaerobic conditions, both photoactivation λ's (580 and 490 nm) elicit no change in ciliary activity with subsequent death to the organism resulting.
Fig. 6. Average ciliary activity as a function of time. Large arrow indicates the addition of the following agents (580 nm; 580 nm + 20 mM-Ca; 490 nm + 10 mM EGTA; 490 nm; 490 nm; 490 nm + 20 mM-Ca).

Fig. 7. Average ciliary activity as a function of time. Large arrow indicates electrical stimulation of the branchial nerve with low- (5 Hz) or high- (50 Hz) frequency biphasic pulses. Points A and B on the graphs indicate the removal of specimens for microfluorometric analysis of red and yellow pigments for 5 and 50 Hz stimulation (inserts A1–A3 and B1–B3, respectively).
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Fig. 8. Average ciliary activity as a function of time. Gill preparations are perfused with 10⁻⁶ M 5-HT or 10⁻⁶ M DA. The points A–H indicate removal of specimens for microfluorometry (Figs. 9, 10) and elemental analysis (Fig. 11) of bulk tissue samples.

Fig. 9. Excitation and a function of wavelength. Microfluorometric analysis of pigments taken from specimens A–H in Fig. 8.
Electrical stimulation of the branchial nerve–photo-analysis of neuronal pigments. Low-frequency electrical stimulation (5 Hz) produced a significant increase in the average rate of beating. Monochromatic analysis of neuronal pigments, at this time, indicates a significant increase in the \( \lambda \) 54 nm over \( \lambda \) 580 nm band (Fig. 7, insert A). The carotene-protein did not show alterations in either \( \lambda \) bands (Fig. 7, insert A).

High-frequency electrical stimulation (50 Hz) decreased the average rate of beating. Monochromatic analysis showed an alteration in the \( \lambda \) bands for haem-protein (occurrence of \( \lambda \) bands of 420 equal to 580 nm; elimination of \( \lambda \) band for 540 nm) and for carotene-protein (occurrence of \( \lambda \) 490 nm, and new band of \( \lambda \) 510 nm and elimination of the \( \lambda \) 465 band). See Fig. 7, inserts B₁ and B₂, respectively.

The effect of 5-HT and DA on the photo- and elemental analysis of neuronal pigments in bulk samples. The control (Fig. 8, point A) contained neuronal pigments which display typical spectral bands (Fig. 9 A) and a presence of Ca ions and Fe ions after elemental analysis of bulk samples (Fig. 11 A).

Perfusion with \( 10^{-8} \) M 5-HT produced a rapid increase in the average rate of beating (Fig. 8, points B and C) with subsequent alteration of spectral bands for the haem-protein and decrease in the typical spectral bands for the carotene-protein (Fig. 9 B, C). Elemental analysis of bulk tissue at this time revealed significant decrease in the amount of Fe ions and elimination of Ca ions (Fig. 11 B, C).
Perfusion with $10^{-6}$ M DA decreased the average rate of beating over a 2 h and 0-5 h period (Fig. 8, points D–G inclusive). At this time, photoanalysis of the neuronal pigments indicated a typical spectral band pattern for the haem-protein with only a minor decrease in the λ 580 nm band in the last analysis (Figs. 9D and 10E–G inclusive). Subsequent elemental analysis of bulk tissue showed a gradual increase in the Ca ion and rapid increase in Fe ions until the last analysis in which the amounts of Ca ions were a little more than half the amount of Fe ions (Fig. 11 D–G inclusive).

Washing the gill preparation with physiological solution returned the average rate of beating to the basal rate observed in the control (compare Fig. 8A–H). Subsequent photoanalysis showed a typical spectral band pattern for the haem-protein and carotene-protein (compare Figs. 9A to 10H and Figs. 9A and 10 for red and yellow pigments, respectively). Also, the elemental analysis of bulk tissues revealed a return of the amounts of Ca ions and Fe ions to the control amounts (compare Fig. 11 A–H).
DISCUSSION

The haem-protein and carotene-protein present in high concentrations in the ganglia of the mussel *Mytilus edulis* are presumed to be responsible for the dual red-orange pigmentation of neurones in this species. The oxygenated form of nerve haemoglobins in three species of molluscs (*Lymnea*, 414, 539, 576 nm; *Spisula*, 414-5, 539, 573 nm; *Aplysia*, 418, 542, 579 nm) show similar absorption maxima as the haem-protein identified in the neurones of *Mytilus*. The generally suggested function for tissue haem-proteins in molluscs is as a facilitator of oxygen transfer to the tissues (discussed by Read, 1966), but in nervous systems other functions are possible.

The experiments described in this study show that the response of the *Mytilus* photoreceptor is composed of an excitatory and an inhibitory component as it relates to ciliary activity. The inhibitory process is initiated at a lower λ (threshold) and under anoxic conditions (Zo. Nagy & Ermini, 1972). That these two components are mediated by different photosensitive pigments is demonstrated by the finding that various parameters of the response to illumination are λ-specific – that is, for example, that a response to λ 580 nm is not elicited under anoxic conditions. Such specificity is not shown by systems in which a single photosensitive pigment is involved.

Since the inhibitory component of the response is especially prominent at short λ, it seemed reasonable to suppose that one pigment – having a relative prominent absorption as short λ’s – is involved in the mediation of inhibition, and another – absorbing more strongly at long λ’s – mediates excitation.

As a consequence of the experiments dealing with wavelength discharge patterns, it can be stated with considerable assurance (a) that the response of the receptor consists of an excitatory and inhibitory event, the latter having a lower threshold; (b) that these processes are mediated by separate pigments, located in the neurones since denervation abolishes effect on ciliary activity; and (c) that the absorption spectrum of the pigment mediating inhibition has its maxima at shorter λ’s than that mediating excitation. The problem of correlating these responses with known photosensitive pigments is obviously far from any solution, but a few specifications can be made. The spectral sensitivity function for the inhibitory process may be assumed to represent fairly well the absorption spectrum of the pigment mediating inhibition, since the process obviously has the greatest sensitivity and can be viewed without interference. This sensitivity function has a maximum at around 405 and 490 nm, and a general shape similar to the absorption spectra of some carotenoid proteins; it is clearly not consistent with those of haemoproteins, and it appears certain that the extractable haemoprotein from *Aplysia* nerve is not mediating this process. The probability that the pigment is a carotenoid protein is supported by the ubiquity of such pigments in photosensory processes, and by the findings of Arvanitaki & Chalazonitis (1949b) in *Aplysia* neurones. They showed that in the cells of young animals, where both carotenoids and haemoproteins are found, illumination in the region of absorption of the carotenoid produced inhibition of spontaneous activity, whereas the haemoprotein(s) appeared to mediate excitation. As in the present case, the thresholds for inhibition were lower than for excitation. If the inhibitory process in the *Mytilus* receptor is, indeed, produced through photoactivation of a carotenoid protein and subsequent loss of elements which have been sequestered (Bauer et al.
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1977) it is probably the same one from those found in Aplysia cells, which have absorption maxima at 482 and 447 nm (Arvanitaki & Chalazonitis, 1949b), and also from the rhodopsins of cephalopod molluscs, which have absorption maxima at 478 and 492 nm (Brown & Brown, 1958).

The pigment that mediates excitation is probably a haemoprotein. Microspectrophotometric measurements were performed on freshly isolated tissue, either nerves or ganglia, with low-power objectives; unpigmented regions of the tissue were used as 'blanks'. Typical absorption spectra obtained in this way are shown in Figs. 1 and 2; they indicate that the red pigment of Mytilus neurones is a haemoprotein. It is spectrally different from the more familiar vertebrate cytochromes, but does resemble cytochrome λ, a haemoprotein previously isolated from several molluscan tissues (Keilin, 1956).

The haem-protein present in Mytilus would appear to be similar to the oxygenated forms of nerve haemoglobins, in three species of molluscs where information exists (Lymnaea, λ 414, 539, 576 nm; Spisula, λ 414.5, 539, 573 nm; Aplysia, λ 418, 542, 579 nm). The oxidized and reduced Mytilus haem-protein resembles to some degree those of vertebrate cytochromes (Strittmatter & Burch, 1963) but are more similar to the haem-proteins which were identified in Helix pomatia by Keilin (1956), the so-called cytochrome λ and helicorubin, particularly the latter. Furthermore, the presence of a cytochrome system would support the finding of low anaerobic tolerance for the haem-protein.

The studies on bulk tissue samples have indicated that (a) the presence of 10 mM EGTA (a specific chelator of Ca ions) abolishes the cilioinhibitory response produced by photactivation of carotene-protein; (b) 10 mM EGTA does not effect the cilioexcitation elicited by photoactivation of haem-protein; (c) the cilioexcitatory effect of low frequency (5 Hz) electrical stimulation alters the spectral patterns for the haem-protein and leaves the spectral patterns of carotene-protein unaltered; (d) the cilioinhibition which follows high-frequency (50 Hz) stimulation alters the spectral peaks of both haem- and carotene-proteins; (e) the results observed above [(c) and (d)] can be abolished in the distal neurones if the branchial nerve is severed. Proximal neurones (those closest to the visceral ganglion) still show the change in spectral patterns of pigments; (f) the addition of 10⁻⁸ M 5-HT (a probable neurocilioexcitatory transmitter) alters the spectral pattern of only the haem-protein; (g) the addition of 10⁻⁸ M DA (a probable neurocilioinhibitory transmitter) alters the spectral patterns of both the haem- and carotene-protein with a subsequent translocation of Ca ions.

The value of the foregoing photoactivation data lies mainly in providing means for the analysis of a series of outstanding cellular reactions: among others, the cilioexcitatory and cilioinhibitory processes and the integrative processes taking place in sensory and central organs.

The interesting point is here twofold. First, to use injections of light as cellular stimuli: photons act specifically in definite cellular sites where they are absorbed and therefore act through specific pathways. Secondly, the fact that the reactions are fairly similar in the nerve cell and in the axon, which is free of synaptic structures, affords several sound conclusions in otherwise complex situations.

The cytosome may be visualized as membrane-bounded cytoplasmic islands, the internal contents of which are separate, in effect, from the lateral cell's cytoplasm.
Such islands could conceivably furnish an extension of the transport activity of the cell membrane to bring about even more sophisticated microscopic control of solute homeostasis in the cytoplasm. Since the cytosomal matrices do not communicate with the extracellular phase, ion accumulation by cytosomes could not rid the intact lateral cell permanently of any net amount of unwanted electrolytes. The cytosome could, however, serve as temporary ion-buffering systems capable of local and transient sequestration of certain ions to control microscopic disparities in solute composition of the cytoplasm.

Whatever the mechanisms of light action may prove to be, the introduction of light as a stimulus into the methods of comparative investigations might be consistently a useful tool in the analysis and extension of new concepts to the electrophysiological field.

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