Effect of degree of infection on peroxidase activity. TNV inocula were diluted to give different numbers of lesions on the primary leaves of a number of seedlings. Extracts were made 4 days after inoculation of 0.6 g of discs respectively containing 1, 5, 10 and 20 lesions/disc. Controls were extracts from 0.6 g of discs cut from leaves 'inoculated' with distilled water and from the non-necrotic areas of virus-inoculated leaves. Peroxidase activity increased with lesion number (Table) and any increase seemed to depend upon the presence of virus in an active state in the leaf cells. Approximately the same differences in relative activity were found between results on leaf area basis and fresh weight basis.

Peroxidase activity increase following infection. Aliquots of a concentrated TNV inoculum were rubbed on the primary leaves of seedlings at varying times before extraction of the enzyme. The relative peroxidase activity was greater the longer the period of virus infection but there was a detectable increase at 24 and 48 h after inoculation although no lesions could be seen at these stages (Figure 2).

With TMV infection of *N. glutinosa*, YAMAGUCHI and HIRAI[7] suggest that the increase in respiration is related to the necrotic process, but other workers[2,8] found that respiration increases before the appearance of local lesions. The latter would appear to be true for TNV infected *P. vulgaris*, i.e. respiration increases during the period of virus synthesis. The increase in peroxidase activity also began before there was any visible sign of lesions but further work is required to establish whether this increase is involved in the glycolytic pathway. An increase in polyphenoloxidase activity has been found in a number of necrotic virus infections in *N. glutinosa* and *Datura stramonium*[8] but I found very little activity of this enzyme in either healthy or infected French bean seedlings. One of the roles of peroxidase is to catalyse the oxidation of phenolic substances to quinones in the presence of hydrogen peroxide[9]. It is possible that the necrotic reaction in TNV-infected *P. vulgaris* is a result of such activity. It may be that peroxidase is connected with the hexose monophosphate shunt in that it may oxidise aromatic compounds such as coumarin and polyphenols produced in this pathway.

Zusammenfassung. Atmungsgeschwindigkeit und Peroxidasetätigkeit mit Tabak-Nekrose-Virus eingespritzter Primärblätter des *Phaseolus vulgaris* L. nehmen bei 26 °C vor dem Erscheinen der lokalen Wunden zu, und zwar proportional zur Wundenzahl und ebenso in Abhängigkeit vom Abstand der Periode vom Zeitpunkt der Einspritzung an.

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Eye of the Cockle, *Cardium edule*: Anatomical and Physiological Investigations

The cockle responds defensively to shadow, the reflex consisting of siphon withdrawal and shell closure. The sense organs which probably mediate the reflex are a series of about 60 small eyes at the apices of tentacles, which arise from around the base of each siphon.

The structure of these eyes has been described by light microscopists[1]. Each eye consists of a cup of reflecting material enclosing 12–20 receptor cells. Nerve fibres arising from these cells leave the eye in a single bundle at the lowest point of the reflector cup (Figure 1). This nerve runs down the tentacle, and joins with those from other tentacles in the external pallial nerve. A semi-circle of brown pigment runs round the side of the eye nearest to the siphon.

Eyes were fixed for electron microscopy as in our previous study on *Pecten*[2]. The receptor cells are irregularly arranged, some in the centre and some in contact with the walls of the reflector cup. They are unusual in possessing large numbers of cilia (Figure 2). We estimate that there are of the order of 100 cilia per cell. The cilia have a 9 + 0 filament content and have basal bodies but no roots. Over much of the cell the cilia form an intertwining tangle, but in the part of the cell nearest to the reflector they are often flattened, with many cilia forming a regular parallel array. No synaptic structures have been seen in the eye, and so we presume that the optic nerve fibres arise directly from the receptors and that there are no synaptic interactions between the cells.

The only important optical structure in the eye is the reflector. It is not possible that the 'lens', an ill-defined region of soft tissue overlying the receptors, exerts any significant convergent effect on the incident light over so short a distance. A crude image formed by reflection is visible when the eye is viewed from the apex of the tentacle. This image, located by optical construction, does lie in the region occupied by the receptors. However, as each receptor also occupies regions of the eye-cup other

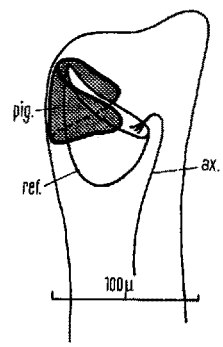

Fig. 1. Drawing of an eye of *Cardium edule* from living material, showing the reflector cup (ref.), which encloses the receptor, the brown pigment band (pig.), and the optic nerve (ax). The siphon would be to the left.

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than the focal plane, it is not clear how much directional discrimination this image provides. The reflector itself consists of a system of thin (0.1 μ) parallel-sided plates, each separated from the next below by a layer of cytoplasm of similar thickness. Such a structure indicates that this is an interference reflector, and the properties of such systems have been discussed elsewhere[3].

The response of the visual afferent nerve fibres were investigated by recording from groups of fibres in the external pallial nerve near the base of the siphons (nerve cut centrally). The recordings were made with fine platinum hook electrodes, connected via an A.C. preamplifier to an oscilloscope. The siphons were illuminated with the collimated beam from a small filament lamp equipped with a shutter.

The only visual responses recorded were 'off' responses (Figure 3). During darkness there was usually a weak resting discharge, which ceased upon illumination, and resumed at a temporarily increased rate at the end of illumination (the 'off' response). The longer the period of illumination, and the greater the light intensity during this period, the more intense was the burst of activity at off', and the shorter its latency. The latency of the first spike varied from 300 msec at threshold (1 sec exposure to 10^3 lumens/m^2) down to less than 10 msec (after 1 min at the same intensity). After illumination for 1 min or more at this intensity, responses were produced to dimming of less than 10%.

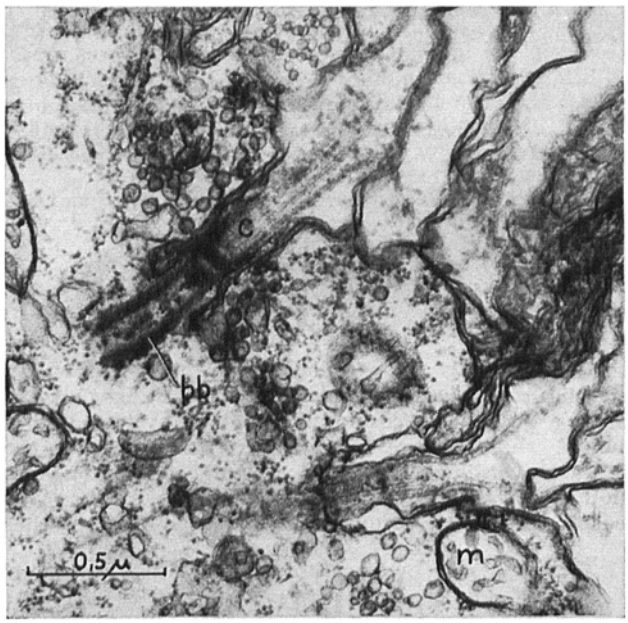

Fig. 2. Electron micrograph of a radial section through the periphery of one of the presumed photo-receptor cells to show 2 of its cilia (c); bb, basal body; m, mitochondrion. Fixed osmium (buffered with veronal acetate to pH 7.4), stained with lead citrate.

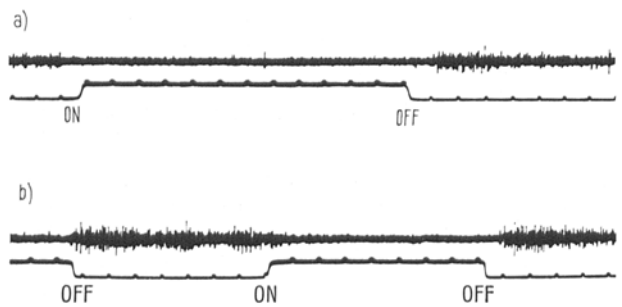

Fig. 3. Responses from a bundle of fibres in the external pallial nerve, containing axons from the eyes. (a) Response to 'on'-going light (10^3 lumens/m^2) after 5 min in the dark. (b) Response to 'off'-going light after 5 min exposure to 10^3 lumens/m^2. Time pips every 100 ms. Average spike height about 50 μV.

In many respects the eyes of *Cardium edule* resemble those of the scallop, *Pecten maximus*. *Pecten* has however 2 sorts of receptor cells in separate layers.

(1) In both eyes an image is formed by reflection[4], and the structure of the reflector is similar in both[3].

(2) The structure of the receptors in *Cardium* resembles that of the distal cells of *Pecten*[2], where the receptor surface consists of an array of flattened cilia, similar to that just described. This kind of arrangement is most unusual in invertebrate photoreceptors, most of which have microvilli as their receptive surface, as for example in the proximal cells of *Pecten*[2,5]. Ciliary arrays have also been reported in the eyes of a gastropod, *Onchidium verruculatum*[6] and an annelid, *Branchiomma vesiculosum*[7]. None of these conform to EAKIN's[8] evolutionary classification of photoreceptors into rhabdomeric (annelid) and ciliary (echinoderm) lines.

(3) In both *Cardium* and the distal cells of *Pecten* activity in the primary nerves occurs only in response to decrease in light intensity[9]. This is taken to mean that the direct effect of light on these cells is inhibitory. Such 'primary inhibition' is known to occur in *Aplysia* ganglion cells[10] and in the pallial nerve of a lamellibranch *Spisula*[11]. In other systems, such as the insect dorsal ocellus[12] and in barnacles[13], 'off' responses are produced by synaptic inhibition of second order cells, but as far as we know in *Cardium* there is no synaptic region between the receptors and the point of recording.

We suggest that there is a functional connection between the ciliary structure of these receptors and the generation of 'off' responses[14].

Zusammenfassung. Die Tentakelaugen der Muschel *Cardium edule* enthalten nur eine Rezeptorenzellart, welche derjenigen der sogenannten distalen Zellen bei den Augen der verwandten Muschel *Pecten maximus* entsprechen.

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[14] We should like to thank Prof. J. A. B. GRAY, Prof. A. F. HUXLEY and Prof. J. Z. YOUNG for encouragement and advice, and Mr. S. WATERMAN for photographic assistance.