Ultrastructural and Histochemical Observations on the Pigmented Eyes of the Oncomiracidium of *Entobdella soleae*, a Monogenean Skin Parasite of the Common Sole, *Solea solea*

Graham C. Kearn and Norman O. Baker

School of Biological Sciences, University of East Anglia, Norwich, NOR 88C, England

Received February 19, 1973

**Summary.** The pigmented eyes of the oncomiracidium of the monogenean skin parasite *Entobdella soleae* are rhabdometric in nature, the smaller anterior eyes each containing a single rhabdomere and the larger posterior eyes each containing two rhabdomeres. Each eye has a lens and a single cup-shaped pigment cell. There are differences in the arrangement of the microvilli in the rhabdomeres of the anterior and posterior eyes. Microvilli from each side of the anterior retinular cell are convergent. In each rhabdomere of the posterior eyes most of the microvilli lie parallel to each other but their direction is perpendicular to that of the microvilli of the adjacent posterior rhabdomere. The retinular cell leaves the eye between the pigment cell and the lens, and the retinular cell nucleus lies outside the eye. The dark-brown pigment in the pigment cell was identified as melanin. The lens contains carbohydrate and possibly protein but tests for fat were negative.

**Introduction**

Pigmented eyes are a conspicuous feature of the larvae of many digenean and monogenean (platyhelminth) parasites and in some monogeneans these pigmented eyes are retained by the adults. The dimensions of most of these eyes are such that the structural information which the light microscope can provide is limited and low power studies of thin sections using the electron microscope, notably by Isseroff (1964a, b), by Pond and Cable (1966) and by Isseroff and Cable (1968), have provided much information on the structure of the eyes of several digenean larvae (miracidia and cercariae). Similar ultrastructural studies of the pigmented photoreceptors of monogeneans are lacking in spite of increasing recent interest in the part played by light in the biology of monogeneans (see Lyons, 1972; Llewellyn, 1972; Kearn, 1973), although at the turn of the century histologists and light microscopists, notably Lang (1880), Hesse (1897) and André (1910), paid a good deal of attention to the eyes of juvenile and adult monogeneans.
Accounts of the kinds of eyes found in monogeneans and their number and distribution have been given by Bychowsky (1957) and in more detail by Baer and Euzet (1961) and by Llewellyn (1963). Llewellyn points out that the eyes possessed by free-swimming larvae (= oncomiracidia) of monogeneans are of two kinds, those with and those without permanent crystalline lenses. Droplets associated with the latter may serve as temporary lenses. Eyes with crystalline lenses always occur as two pairs and are found in the oncomiracidia of the so-called monopisthocotylean monogeneans and in some polypisthocotylean oncomiracidia. Eyes without permanent lenses are restricted to diclidophoridean polypisthocotyleans and most frequently occur as a single pair of eyes. A few oncomiracidia appear to have a single median eye but Bychowsky (1957) suggested that this may have arisen by fusion of a pair of eyes. Lyons (1972) has suggested that the possession of two pairs of eyes may be the primitive condition in monogeneans.

Nothing is known about the chemical nature of the pigment shielding the eyes of monogeneans or of the so-called permanent lenses associated with these eyes.

The monopisthocotylean monogenean skin parasite Entobdella soleae (van Beneden and Hesse, 1863) Johnston, 1929, was selected for an ultrastructural and histochemical study of the pigmented eyes because of the ready availability of the parasite for work on its behaviour in relation to light and because recent work (Kearn, 1973) has shown that light plays an important part in the biology of E. soleae. The four pigmented eyes are retained by the adult parasite but it is known that loss of the lenses takes place soon after the invasion of the host by the larva (see Kearn, 1971), so it was decided to begin by studying the intact eye of the freshly-hatched oncomiracidium.

Materials and Methods

Living adult specimens of the monogenean Entobdella soleae were collected from the skin of soles (Solea solea) kindly maintained by Mr. John Green at the Laboratory of the Marine Biological Association of the United Kingdom at Plymouth. The parasites were sent to Norwich and the eggs laid by them were harvested and incubated at 13°C. The ciliated free-swimming oncomiracidia begin to hatch after an incubation period of about 30 days. Some freshly hatched larvae were studied alive using phase contrast and oil immersion equipment; others were preserved at 4°C in 5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.2—7.4) for periods of about 2 hours, followed by washing and then post-fixation for 1 hour in 1% osmium tetroxide also in 0.1 M phosphate buffer. As there was some difficulty in locating the pigmented eyes after osmium fixation, this was omitted with later specimens. Following dehydration, the larvae were embedded in Araldite resin and thin sections were cut using an L. K. B. ultramicrotome and glass knives. The sections were mounted on coated grids and then double stained using uranyl acetate and lead citrate. The grids were examined using a Philips E. M. 200 microscope.
In an attempt to obtain information about the chemical composition of the eye pigment and of the lens of the larva, various histochemical tests were performed either on fresh unfixed material or on larvae preserved in 5% formaldehyde. It was found that the responses of the eyes to some of the histochemical reagents were stronger in squash preparations of fresh larvae than in whole unsquashed fresh or preserved specimens and furthermore, it was easier to handle squashed specimens, made to adhere to a slide by drying for a few seconds in air, than whole unattached larvae. In some of the tests the responses of frog skin melanin (thin sheets of skin containing the pigment) were compared with those of larval eye pigment. Details of the histochemical tests employed are given by Pearse (1968, 1972).

Observations with the Light Microscope

The oncomiracidium of Entobdella soleae has four pigmented eyes situated in the translucent head region (Figs. 1 and 2). The eyes lie anterior to the pharynx and close to the dorsal surface of the body. The two anterior eyes are noticeably smaller than the two posterior eyes (Figs. 1 and 2). Furthermore the anterior and posterior eyes are orientated differently; each anterior eye receives light from a postero-lateral direction and each posterior eye receives light from an antero-lateral direction. In view of these differences in size and in orientation between the anterior and posterior pigmented eyes it was of interest to determine whether these differences were reflected in structural dissimilarities.

Each eye has a cup-shaped mass of brown pigment granules. These granules are round or oval in shape and their average width is approximately 0.5 μm. A spherical translucent lens occupies the opening of each pigment cup; in the anterior eye the lens is about 6 μm in diameter and in the posterior eye the larger lens is about 10 μm in diameter. When excessive coverslip pressure is applied to the larva, sudden shattering of the lens occurs, most of the cracks running in a radial direction, demonstrating that the lens consists of rigid material. The arrangement of the pigment shield is such that only a limited cone of light, passing through the lens is admitted to the cavity enclosed by the pigment cup.

Twitching movements of the eyes were observed in the living oncomiracidium of E. soleae.

In view of reports by Zeller (1872, 1876) and by Hesse (1897) of red and blue material inside the pigment cups of the eyes of living specimens of Polystoma integerrimum from the frog Rana temporaria, a search was made for similar material associated with the oncomiracidial eyes of Entobdella soleae. No such material was found.

Histochemistry

The Pigment. The pigment granules of the eyes of the oncomiracidium of E. soleae and frog skin melanin were found to be resistant to treatment with dilute acid (1% acetic acid, 30% hydrochloric acid) and with dilute
Fig. 1. A freshly hatched oncomiracidium of *Entobdella soleae*. The ciliated epidermal cells have been lost as a result of partial flattening by a coverslip. Fig. 2. The pigmented eyes of a freshly hatched oncomiracidium. Fig. 3. Electron micrograph of a section through a posterior eye.
alkali (10% sodium hydroxide exposed for more than 24 hours) but pigment from both sources dissolved slowly in normal sodium hydroxide. The eye pigment and frog skin melanin were bleached by a variety of agents and the speed of bleaching of the eye pigment and that of the frog skin pigment were closely similar. The two pigments were bleached after treatment for 15 hours with 0.1% potassium permanganate followed by immersion in 1% oxalic acid for 1 minute. The eye pigment was bleached after treatment for only 15 minutes in a mixture of chromic acid and calcium chloride; the frog skin pigment resisted for a little longer but had lost all its colour after 1 1/2 hours. A 10% solution of hydrogen peroxide bleached both the eye pigment and the skin melanin in less than 5 hours but both pigments resisted treatment with a mixture of potassium chlorate and hydrochloric acid. After 3 1/2 days in this solution the eye pigment was still visible but significantly paler than in untreated controls; no lightening of colour was observed in the frog skin pigment. After treatment in 35.5% peracetic acid for between 6 and 16 hours the eye pigment and the skin melanin lost all their colour.

Using squash preparations of unfixed larvae the eye pigment gave a strong positive response with Schmorl's test (see Pearse, 1968, 1972 for details of this and other tests). The Gomori hexamine-silver test for melanin was also conducted on squash preparations with controls omitting the hexamine-silver treatment. Comparison between test specimens and controls showed a significant blackening of the pigment under test. Lillie's ferrous iron technique for melanin did not give a strong response either with whole preserved larvae or with fresh squash preparations. Some green coloration was observed around the pigment cups in some of the test specimens. Perl's test for ferric iron and Millon's test for tyrosine were negative.

The Lens. The lens dissolves in a few minutes in 0.1% sodium hydroxide and in glacial acetic acid but less rapidly in 1% acetic acid or in 0.1% sulphuric acid. The lenses were no longer visible after immersion for less than 24 hours in a 2% solution of formaldehyde (pH 4); 0.5% cetyl pyridinium chloride in 4% aqueous formaldehyde, recommended by Curran (1964) for the precipitation of acid mucopolysaccharide material, failed to preserve the lenses. In contrast the lenses were still clearly visible after immersion for more than 48 hours in buffered 5% glutaraldehyde (pH 7.2-7.4), and the lenses survived the following consecutive treatments: 3 1/2 hours in cold acetone, 2 1/2 hours in hot acetone, 1 hour in hot ether and 1 hour in a hot mixture of chloroform and methanol. (The hot solutions were maintained at 50°C with constant agitation.) Lenses were apparently unaffected by overnight treatment in a mixture of chloroform and methanol at room temperature followed by treatment in a hot (50°C) chloroform and methanol mixture for 3 hours.
Fig. 4. Electron micrograph of a section through an anterior eye (left) and a posterior eye (right). The point where the anterior retinular cell leaves the eye is shown by the large arrow. Fig. 5. Electron micrograph of a section through the anterior eye
Because of the ready solubility of the lenses in dilute acids and alkalis and in formalin many histochemical tests were impracticable. Prolonged treatment with the reagents in the PAS technique led to dissolution of the lenses but on several occasions when the treatment times were not excessive not all of the lens dissolved and lens remnants were observed to be strongly PAS positive, staining more intensely than the rest of the larval body.

The lenses failed to stain with Sudan Black B following brief immersion in 70% ethanol.

**Ultrastructure**

Low power studies with the electron microscope revealed that in addition to the pigment cup and the lens which can be plainly seen with the light microscope, large retinular cells are also associated with the eyes (Figs. 3 and 4). Much of the retinular cell, including the nucleus, lies outside the eye and communicates by means of a narrow neck with a smaller segment of the retinular cell lying between the lens and the pigment cup (Fig. 4). The inner segment of each retinular cell gives rise to a closely packed array of microvilli, collectively termed the rhabdomere (Figs. 4, 6 and 7).

Each pigment cup consists of a single cell (Fig. 3). The cytoplasm of each pigment cell is packed with round or oval pigment granules and the pigment cell nucleus lies centrally near the convex outer surface of the cell, distal to the pigment-laden cytoplasm. Electron-translucent vesicles about the same size as the pigment granules are often encountered between the pigment cell nucleus and the bulk of the cytoplasm of the pigment cell. However, no vesicles were found showing the patterned internal structure characteristic of premelanosomes. No mitochondria were seen in the pigment cell.

Each small anterior eye contains only one rhabdomere (Figs. 4 and 5) whereas the larger posterior eyes each contain two rhabdomeres (Fig. 3). Each rhabdomere consists of closely packed microvilli (Figs. 6 and 7). In transverse sections of the rhabdomere these microvilli are seen to be variable in diameter, ranging from about 25 to 50 nm (average about 35 nm). Many of them have club-shaped distal ends. Most of the microvilli are seen to be packed in an irregular fashion when viewed in transverse section, but some of them are arranged in relatively straight double rows (Fig. 6). Numerous round bodies with an average diameter of 100 nm are found in association with the roots of the microvilli but it is not clear whether these are cytoplasmic vesicles or whether they are the roots of microvilli seen in transverse section. In the posterior eye the majority of the microvilli of each rhabdomere run parallel to each other but the microvilli of adjacent rhabdomeres in the posterior eye are
approximately perpendicular to each other. The microvilli of the single rhabdomere of the anterior eye of *E. soleae* are less precisely arranged parallel to each other than those of the posterior rhabdomere, and there is a tendency for the microvilli from opposite sides of each anterior retinular cell to converge, so that where the tips of the microvilli meet, approximately in the middle of each rhabdomere, there is a discontinuity in sections which is characteristic of the anterior eye (Fig. 5).

In the posterior eye there are no pigment granules between the two rhabdomeres. Consequently the rhabdomeres are close together but not fused (Fig. 3).

The cytoplasm of that part of each retinular cell lying within the eye contains abundant mitochondria (Fig. 7) sometimes closely packed. In contrast the external segment of the retinular cell contains relatively few, sparsely distributed mitochondria (Fig. 4). The internal segment of the retinular cell communicates with that part of the cell lying outside the eye by a narrow neck of cytoplasm which leaves the eye between the pigment cell and the lens and may possibly penetrate the edge of the pigment cell (Figs. 4 and 7). In the posterior eyes the retinular cell exits lie on opposite sides of the lens. At the point where the retinular cell leaves the eye the adjacent membranes of pigment cell and retinular cell are thickened and form an electron-dense, desmosome-like structure (Fig. 7). The external segment of the retinular cell, which contains the nucleus, a few mitochondria and electron translucent vesicles of various sizes, is relatively large compared with the rest of the eye (Fig. 4). Regions where the cell membrane is tucked into the cytoplasm were occasionally observed, the intucked membranes being closely apposed. Some evidence of neurotubules was found in the retinular cell cytoplasm lying within the pigment cup but neurotubules were not observed in the retinular cell cytoplasm outside the eye. No synaptic vesicles or other evidence of synaptic connections were found in the retinular cell.

In the oncomiracidium of *E. soleae* the lens is a distinct body enclosed by one or more membranes, lacking a nucleus and mitochondria and revealing little evidence of an orderly arrangement of material such as might be expected in a crystalline structure (Fig. 3). In section the peripheral region of the lens usually appears more electron-dense than the central region where high resolution pictures reveal irregularly arranged short branching fibres. It is probable that the peripheral region is also made up of these short fibres but because it is usually more closely packed it appears granular. In some sections both front and rear lenses on each side appear to be enclosed by a common membrane.

Processes apparently joining the outer surface of the eye appear to be muscular in nature (Fig. 5).
Discussion

There is a resemblance between the pigmented larval eyes of *Entobdella soleae* and those of digenean miracidia (see Isseroff, 1964a, b; Isseroff and Cable, 1968; Brooker, 1972) and cercariae (see Pond and Cable, 1966). In the larvae of these parasitic platyhelminths the eyes are made up of...
1–5 rhabdomeres shielded by cells containing pigment granules. According to Hesse (1897) eyes of comparable complexity are found in rhabdocoeels of the genus *Derostoma* and in some triclads, and he regards these as primitive eyes compared with those of other free-living triclads and polyclads in which the pigment cup is large and sometimes multicellular enclosing many retinular cells (Hesse counted 32 in eye sections of *Dendrocoelum lacteum*, and Röhlich and Török, 1961, report 100–150 in *Dugesia lugubris*).

In *E. soleae* each eye has a single cup-shaped pigment cell. The difference in size of the anterior and posterior eyes is related to the number of rhabdomeres enclosed by each pigment cup: each small anterior eye contains a single rhabdomere whereas each of the larger posterior eyes contains two rhabdomeres. Junctional complexes resembling desmosomes may serve to bind the retinular cell to the pigment cell at the point where the retinular cell emerges from the pigment cup and similar structures are met with in corresponding locations in the pigment-ed eyes of digenean miracidia (Isseroff and Cable, 1968).

The functional significance of the fact that the parallel microvilli of each rhabdomere in the posterior eyes of *E. soleae* lie at right angles to the microvilli of the adjacent rhabdomere is unknown. A similar arrangement of microvilli has been observed in the adjacent lateral rhabdomeres of the miracidial eye-spots of *Fasciola hepatica* by Isseroff and Cable (1968), and contrasts sharply with, on the one hand, radiating microvilli, seen for example in the posterior median miracidial rhabdomere of *F. hepatica*, and the interdigitation of microvilli observed by Pond and Cable (1966) in the lateral cercarial eyes of *Macravestibulum exersum*. Mutually perpendicular microvilli have been observed in the rhabdomeric eyes of many molluscs and arthropods (Wolken, 1971). The anterior eyes of *E. soleae* show an arrangement of microvilli which is different from any so far mentioned; there is an apparent convergence of microvilli from opposite sides of the same retinular cell so that a well-defined line appears in sections at the point where the tips of the microvilli meet. In *E. soleae* the diameters of the microvilli (25–50 nm) fall within the range of sizes (20–100 nm) given for the microvilli of *Dendrocoelum lacteum* by Röhlich and Török (1961), but appear to be a little smaller than those of digenean miracidia (60 nm in *Heronimus chelydrae*, see Isseroff and Cable, 1968). The significance of the closely packed double rows of microvilli observed in transverse sections of parts of the rhabdomere of *E. soleae* is obscure, and this, and the structure of the microvilli, requires more intensive high resolution study. Röhlich and Török (1961) gave evidence that vacuoles near the bases of the microvilli of *D. lacteum* may supply photoreceptive sites in the microvilli; bodies which may be cytoplasmic vesicles have been found in a similar position in *E. soleae*. 
In the retinular cells of *E. soleae* the mitochondria are most abundant close to the bases of the microvilli but these mitochondria are not closely apposed as observed by Brookor (1972) in the miracidium of *Diplostomum spathaceum*.

All previous studies on the pigmented eyes of monogeneans were made using the light microscope. André (1910) found that each adult eye of *Polystoma integerrimum* consists of a pigment cup (produced by a pigment cup cell) and a sensory cell. The latter is differentiated into two parts, the "Stiftchenkappe" or organ of perception (presumably equivalent to the rhabdomere), lying against the concave inner surface of the pigment cup, and an efferent nerve. According to André the pigment granules are located in a distinct cytoplasmic zone, which may be enclosed by a membrane, inside the pigment cell, so that there is an extensive area of pigment cell cytoplasm devoid of pigment granules. In the oncomiracidium of *E. soleae* there is no such zonation of the pigment cell cytoplasm and most of the pigment cell contains pigment granules.

Hesse (1897) reported only one sensory cell in the eyes of the monogeneans *Tristomum molae* (= *Capsala martinieri*), *T. papillosum* and *Polystoma integerrimum*. André (1910) noted that the posterior eyes of adult specimens of *P. integerrimum* are larger than the anterior eyes but he records only one sensory cell associated with each of the four eyes. Ozaki (1935) working with another adult polystome, *Diplorchis ranae*, observed a single nucleus associated with each anterior eye and two nuclei associated with each posterior eye; he pointed out that this was an indication that the sensory part of each anterior eye originated from one cell and that of the posterior eye from two cells.

The eye-cup pigment of *E. soleae* readily reduces ferricyanide to ferrocyanide (the so-called Schmorl method, see Pearse, 1972), a property which is shared by melanin, argentaffin granules and lipofuscin. However, the rapid bleaching of the eye-cup pigment in various oxidising agents is good evidence that the pigment is melanin, particularly the rapid decolorisation in peracetic acid, regarded by Pearse as the best of the bleaching methods for melanin. According to Pearse (1972) any pigment which is resistant to most solvents, except those that disrupt the tissue, is soluble in normal sodium hydroxide, is bleached in less than 48h by strong oxidising agents and reduces appropriate silver solutions is almost always melanin. The pigment spots of *E. soleae* failed to stain blue with Perl’s method for ferric iron and it is therefore unlikely that the eye spots contain any appreciable amount of haemosiderin, which has been reported in the eye spots of some nematodes (Bollerup and Burr, 1971). Nadakal (1960) found that the eye-spots of three species of digenean cercariae consist of melanin and tests for iron on these eye-spots also
proved negative. In *E. soleae* a few electron-translucent vesicles were found in the pigment cell cytoplasm near the nucleus. Similar vesicles were observed in the miracidium of *Allocreadium lobatum* by Isseroff and Cable (1968) and large vesicles close to the pigment cell nucleus in the miracidium of *Spiroorchis* sp. were regarded as nascent pigment granules. However, in *E. soleae*, no premelanosomes were found and the pigment cell gave a negative response to Millon's test for tyrosine, the precursor of melanin. There is therefore no evidence that the pigment cells of the oncomiracidium of *E. soleae* are manufacturing melanin.

In the oncomiracidium of *Capsula martinieri* there are extensive tracts of body pigment of unknown function as well as eye spot pigment (see Kearn, 1963a). These body pigment tracts link up with the pigment cups of the eyes and may be chemically the same; the preliminary tests performed on the pigment at the time were inconclusive but were not inconsistent with the possibility that the pigment is melanin.

There have been conflicting opinions on whether or not lenses are present in the eyes of monogeneans. Zeller (1872) failed to find a lens in the oncomiracidium of *Polystoma integerrimum* but later he became convinced of the existence of a lens while studying adult eyes (Zeller, 1876). On the other hand Hesse (1897) and André (1910) both failed to find a lens in the adult eye of *P. integerrimum* and they believed that earlier observers had confused the nuclei of the sensory cells with lenses. Lang (1880) reported a light refracting body in the adult eye of *Tristoma molae* (= *Capsula martinieri*) but Hesse (1897) pointed out that Lang's light refracting body was almost certainly the sensory (retinular) cell. According to Heath (1902) a lens is present in the adult eye of *Epidella* (= *Entobdella*) *squamula*. Llewellyn (1957) identified oil droplets close to the eyes of diclidophoroidean monogeneans and suggested that they may function as lenses.

There have been few reports of lenses in the eyes of other platyhelminths. Pond and Cable (1966) found a lens associated with the median eye of the cercaria of *Macravestibulum eversum*; this lens was described as a single cell with an eccentric nucleus, a few peripheral mitochondria and clear refractile cytoplasm. Nothing was found inside the lens of *E. soleae* to indicate that the lens is cellular in nature. In some sections there is evidence that the front and rear lenses on each side of the body in *E. soleae* are enclosed within a common membrane but the significance of this and the origin of the lenses will not be clear until studies are made of the embryonic development of the eyes.

There is no doubt that discrete spherical lenses are present in the oncomiracidial eyes of *E. soleae* but it has proved difficult to determine the chemical nature of these lenses by means of standard histochemical tests because of the ready solubility of these bodies in acidic and alkaline
solutions and in formalin. The physical and chemical properties of the lenses do not permit more than a few generalized comments on the lens material. The few tests that have been made indicate that there is no appreciable amount of oil or fat in the lenses in contrast with the oily lens-like droplets associated with the eyes of some polyopisthocotylinean monogeneans (see Llewellyn, 1957). The lenses of *E. soleae* proved to be insoluble in fat solvents and failed to stain with Sudan Black B. Furthermore, compression with a coverslip shows that the lenses are solid bodies. There is little evidence from electron microscopy of an orderly crystalline arrangement of material in the lens. In most sectioned lenses the peripheral region appears more closely packed than the centre of the lens and the lens substance under high resolution consists of granules or short irregularly branching fibres. However, the lens is so unstable that it may well undergo substantial changes during preparation for electron microscopy. The diameter of the lens is certainly smaller after glutaraldehyde preservation, and the space between the lens and the surrounding membranes seen in electron micrographs (Fig. 3) may be the result of contraction of the lens material or of dissolution of the outer region of the lens. The PAS positive responses of lens remnants point to carbohydrate as a component of the lens material but the failure of cetyl pyridinium chloride to preserve the lenses indicates the absence of acid mucopolysaccharide. It was not possible to demonstrate unequivocally the presence of a protein component in the lens but the success of glutaraldehyde as a lens preservative makes it likely that there is such a component which depends for its stability on the more efficient crosslinking ability of glutaraldehyde (see Pearse, 1968).

In *E. soleae* and in *Trochopus pini* it has been observed that the lenses are absorbed soon after the oncomiracidia attach themselves to their respective hosts (Kearn, 1971). Thus the lenses have short lives, appearing in the embryo of *E. soleae* some time after the 18th day of incubation at about 14°C (see Kearn, 1963b) and in all probability persisting for little more than one week. The instability of the lenses is such that presumably only a small change in their micro-environment would be necessary to remove them after the larvae alight on the host.

Zeller (1872, 1876) and Hesse (1897) reported that the concavity of the pigment cup in living specimens of *Polystoma integerrimum* contains brightly coloured material. According to Hesse the pigment cup has a double lining, a red layer closely applied to the pigment cup and an inner blue layer. Hesse also found that although the red layer is conspicuous when the parasite is freshly taken from the bladder of the host frog the red colour fades upon prolonged exposure to light; this bleaching process is reminiscent of the photochemical change undergone by the visual purple in the vertebrate eye. A careful examination of the oncomiracidial
eyes of *E. soleae* has so far failed to reveal coloured material, but because of the orientation of the eyes (the eyes point laterally and on a slide larvae almost always come to lie with the dorsal or ventral surface uppermost), and because of the envelope of pigment granules, observation of the lining of the pigment cup is not easy in the living animal. It is possible that colours observed by Hesse might be interference colours produced by regularly arranged microvilli.

The eyes of *E. soleae* are orientated in a characteristic manner; the larger posterior eyes point in an antero-lateral direction and the smaller anterior eyes in a postero-lateral direction. This arrangement is shared with most other monogeneans possessing two pairs of pigmented eyes and as Lyons (1972) has pointed out this may permit simultaneous comparison between light intensities striking each eye or set of eyes, allowing rapid realignment of the swimming, non-spiralling larva by keeping the amount of light received on each side of the head constant. Many monogenean larvae show directional sensitivity to light for at least part of their free-swimming lives (see Lyons, 1972); it is not yet known whether the larva of *E. soleae* orientates itself with respect to light but behavioural studies of the larvae are underway at present.

Eye movements have been recorded in monogeneans. Zeller (1872) and André (1910) working on *Polystoma integerrimum* and Maclaren (1904) working on *Diplectanum aequans* observed eye movements but they believed that they were the result of muscular contractions in the neighbouring tissues. Lang (1880) believed that jerky eye movements of *Tristomum molae* (= *Capsalad martinieri*) were produced by dorso-ventral muscle fibres which he called eye muscles, and Heath (1902) described independent eye movements in *Epidella (= Entobdella) squamula* which at times took place “with the rapidity of the pulse beat”. Lyons (1972) reports eye movements in the oncomiracidium of *Entobdella soleae*. In the present study similar twitching movements of the oncomiracidial eyes have been observed and the electron microscope has revealed the presence of fibres, resembling muscle cells, which appear to join the outer surface of the eye. It is not easy to understand what purpose such small rapid eye movements would serve; the cone of light acceptance is so large compared with the small movements of the eyes that changes in the illumination of the retinular cells during eye movement would seem to be small.

It has been shown recently that light also plays a part as a cue for an endogenous circadian hatching rhythm in the eggs of *E. soleae* and may have also a parallel role as a direct hatching stimulus (Kearn, 1973). It is possible then that the pigmented receptors of *E. soleae* may act as recorders of the length of the day or the night. There is, however, an alternative candidate for this role because Lyons (1972) has discovered unpigmented
organs in the transparent head region of the oncomiracidium of *E. soleae* which resemble the unpigmented photoreceptors of some molluscs.

**Acknowledgements.** We are grateful to Dr. K. M. Lyons for suggestions and for helpful discussion.

**Key to Lettering of Figs. 1—7**

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>ae</td>
<td>anterior eye</td>
</tr>
<tr>
<td>ar</td>
<td>retinular cell of anterior eye</td>
</tr>
<tr>
<td>d</td>
<td>desmosome-like thickenings</td>
</tr>
<tr>
<td>e</td>
<td>eye</td>
</tr>
<tr>
<td>ex</td>
<td>retinular cell exit from posterior eye</td>
</tr>
<tr>
<td>f</td>
<td>possible muscle fibre</td>
</tr>
<tr>
<td>h</td>
<td>haptor</td>
</tr>
<tr>
<td>l</td>
<td>lens</td>
</tr>
<tr>
<td>m</td>
<td>mitochondrion</td>
</tr>
<tr>
<td>np</td>
<td>nucleus of pigment cell</td>
</tr>
<tr>
<td>nr</td>
<td>nucleus of the retinular cell</td>
</tr>
<tr>
<td>pc</td>
<td>pigment cell</td>
</tr>
<tr>
<td>pr</td>
<td>retinular cell of posterior eye</td>
</tr>
<tr>
<td>pe</td>
<td>posterior eye</td>
</tr>
<tr>
<td>rh</td>
<td>rhabdomere</td>
</tr>
</tbody>
</table>

**References**


