J. mar. biol. Ass. U.K. (1963) 43, 749–766
Printed in Great Britain

FEEDING IN SOME MONOGENEAN SKIN PARASITES: *ENTOBDELLA SOLEAE* ON *SOLEA SOLEA* AND *ACANTHOCOTYLE* SP. ON *RAIA CLAVATA*

By G. C. Kearn

Department of Zoology and Comparative Physiology, The University, Birmingham 15*

(Plate 1 and Text-figs. 1 and 2)

Most monogeneans inhabit the gills or the skin of marine and freshwater fishes. The Polyopisthocotylea, one of the two sub-orders of the Monogenea, includes mostly gill parasites which have been shown by Llewellyn (1954) to feed on the blood of their hosts. Parasites belonging to the other sub-order, the Monopisthocotylea, inhabit a wider variety of habitats including the skin and gills of their hosts and very little is known of their food. Goto (1895) examined a number of polyopisthocotylineans and monopisthocotylineans and stated (p. 180), without supporting evidence, that the majority of them fed on the slime of their hosts, although some were able also to extract blood. Heath (1902) considered that mucus formed the food of the monopisthocotylinean skin-parasite *Entobdella squamula* (Heath), and Folda (1928) was of the opinion that a gill-parasitic monopisthocotylinean, *Megalocotyle marginata* Folda, was also a mucus feeder. Maccallum (1927) reported that *Benedenia melleni* (MacCallum) damaged the cornea of its hosts. Histochemical examination by Llewellyn (1954) of the gut contents of two skin parasites, *Leptocotyle minor* (Monticelli) and *Acanthocotyle* sp., revealed no evidence of a blood-feeding diet, and led him to suggest that they fed on eroded host tissue or on mucus or were merely commensals. Bychowsky (1957, p. 81) put forward the view, unaccompanied by evidence, that some monopisthocotylineans fed mostly on mucus secretions and epithelial cells of the skin of the host, whereas others preferred blood.

In addition to these conflicting views on the nature of the food of monopisthocotylineans no account is available of the way in which these parasites feed. Bychowsky (1957, p. 81) was of the opinion that feeding was accomplished either by the attachment of the anterior end of the parasite or, less often, by scraping motions. He considered that the protrusible 'pharynx', which contained gland cells, was responsible for feeding, but his remarks are unaccompanied by examples or by supporting evidence.

Entobdella soleae (van Beneden & Hesse, 1863) Johnston, 1929, a skin

---

* Present address: School of Biological Sciences, The University of East Anglia, Norwich.

parasite of the common sole, and *Acanthocotyle* sp. from the skin of *Raia clavata* L. are relatively abundant at Plymouth, and because of this were selected as good vehicles for the investigation of feeding problems.

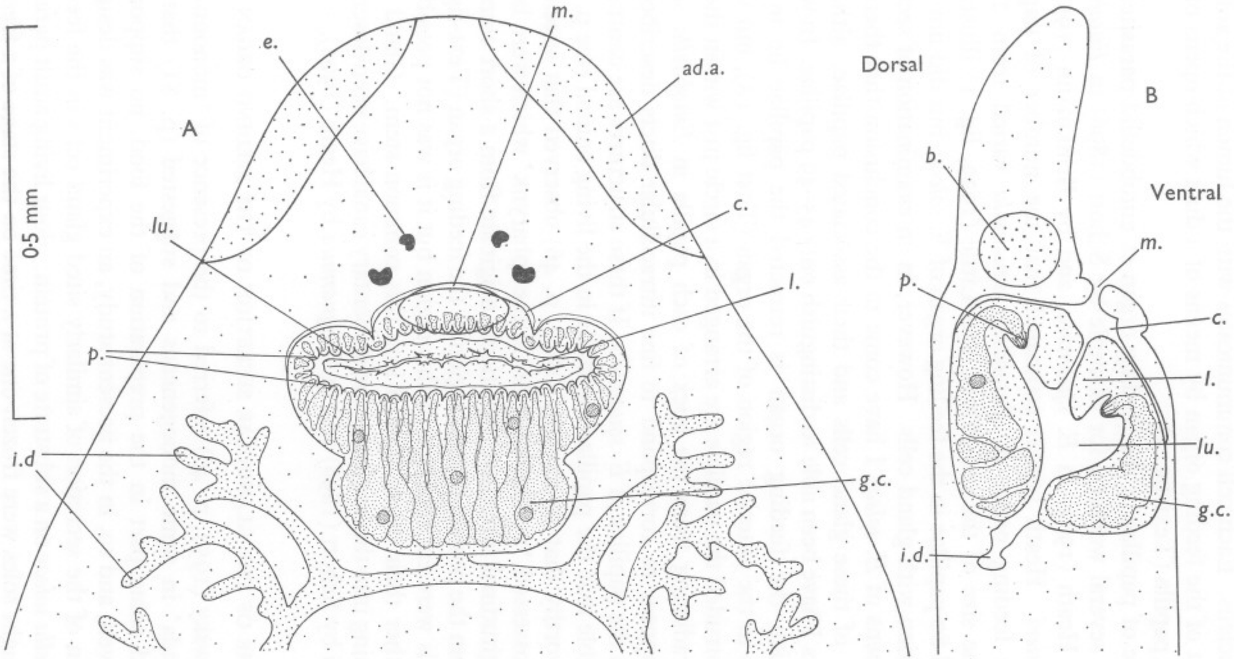
THE FEEDING OF *ENTOBDELLA SOLEAE* ON THE SKIN OF *SOLEA SOLEA*

THE MORPHOLOGY OF THE FEEDING APPARATUS

The feeding apparatus of *Entobdella soleae* differs considerably from that of blood-feeding polyopisthocotylineans. In these blood feeders the mouth is terminal and is either provided with an oral sucker or leads into a buccal cavity containing a pair of suckers; the buccal cavity in turn communicates with a muscular pharynx. In *E. soleae*, however, the mouth is not terminal but is ventrally situated and the feeding apparatus consists of a conspicuous glandular organ which occupies most of the 'head region' of the parasite (Text-fig. 1 A). This glandular organ has been referred to as a 'pharynx' but is not comparable in structure or in function (see below) with the muscular pharynx of blood-feeding polyopisthocotylineans, and the name 'feeding organ' will be used for it. In the head region of *E. soleae* two antero-lateral adhesive areas (Text-fig. 1 A), which are capable of attaching the anterior end of the parasite to the skin of the fish (see Kearn, 1963*b*), may also be associated with feeding.

Several authors have commented on the protrusible nature of the feeding organs of entobdellid parasites. Schott (1896) noted that the feeding organ was protruded in one of his specimens of *Entobdella hippoglossi* (Müller), and Brinkmann (1952, fig. 39) published a photograph of a sagittal section through the protruding feeding organ of *Capsala martinieri* Bosc. Bychowsky (1957, p. 81) noted that the 'pharynx' of many monogeneans could be protruded in a similar way to the pharynx of turbellarians, and the feeding organs of some of the specimens of *Benedenia posterocolpa* Hargis examined by Hargis (1955) were in the protruded position.

The feeding organ of *Entobdella soleae* (van Beneden & Hesse) consists of two regions, an anterior protrusible part and a posterior non-protrusible part. In most specimens examined the anterior part of the feeding organ was in the retracted position and was accommodated in a cavity communicating with the outside by the mouth (Text-fig. 1 A, and B). The fibrous lip of the retracted feeding organ is tucked inside the lumen of the organ as shown in Text-fig. 1. I have found a few specimens of *E. soleae* which had been preserved with the lip of the feeding organ partially everted and protruding through the mouth, and in one specimen of *E. hippoglossi* in my possession and in a specimen of *E. diadema* (Monticelli) given to me by Dr J. Llewellyn the lip was fully everted and protruding through the widely open mouth (Pl. 1, figs. 1, 2). The posterior part of the feeding organ of *E. soleae* is separated from the surrounding parenchyma by a thin basement membrane. The wall of the posterior region



Text-fig. 1. The morphology of the feeding apparatus of *Entobdella soleae*. (A) The retracted feeding organ as seen in dorsal or ventral view; (B) A median sagittal section through the retracted feeding organ. *ad.a.*, Anterior adhesive area; *b.*, 'brain'; *c.*, cavity accommodating feeding organ; *e.*, eye; *g.c.*, gland cell; *i.d.*, intestinal diverticulum; *l.*, lip, tucked inside lumen of feeding organ; *lu.*, lumen of feeding organ; *m.*, mouth; *p.*, papilla.

accommodates a number of 'giant gland cells' (Text-fig. 1), each containing a conspicuous nucleus. Each cell communicates with the lumen of the anterior protrusible part of the feeding organ by means of a duct which opens on the apex of a large papilla (Text-fig. 1).

The presence of papillae in the feeding organ of entobdellid parasites has been noted by several workers, for example by Schott (1896) in *Entobdella hippoglossi*, by Heath (1902) in *E. squamula*, and by Brinkmann (1952) in *Capsala martinieri*. Heath (1902) estimated that the number of papillae present in the feeding organ of *Entobdella squamula* varied from 25–50 according to the size of the individual, and Little (1929, fig. 1) illustrated between 70 and 80 papillae in the feeding organ of *E. soleae*, but did not refer to their association with gland cells. However, in an examination of sections of adult specimens of *E. soleae* I have come to the conclusion that there are only about 60 of these gland cells and their associated papillae, although in whole mounts I have been able to distinguish only 45–49 papillae. In whole mounts in which the feeding organ is retracted the papillae lie in two transverse rows in the anterior region of the organ (Text-fig. 1A), but when the organ is protruded the papillae are arranged in a circle just within the lip.

Fine fibres radiating from the apex of each papilla in *Entobdella soleae* (Text-fig. 1B) appear to correspond to fine intracellular ducts described by Heath (1902) in the papillae of *E. squamula*. If these structures are contractile fibres it is possible that the papillae are labile in the living animal (see p. 757) and it is noteworthy that Bychowsky (1957, p. 46) observed that species of *Gyrodactylus* possessed 'pyramidal cells' in the 'pharynx' which were labile.

The two longitudinal intestinal diverticula originate from a short common stem arising from the postero-dorsal region of the feeding organ (Text-fig. 1). A few gland cells were encountered in this region but it is was not possible to determine whether their ducts opened into this common stem. Gland cells with ducts opening into this region of the alimentary canal have been described in *E. hippoglossi* by Schott (1896) and in *E. squamula* by Heath (1902).

THE NATURE OF THE GLANDULAR SECRETION OF THE FEEDING ORGAN

Though Bychowsky (1957, p. 46) referred to the presence of 'mononuclear pharyngeal glands' in some monogeneans and suggested (p. 81) that the secretion played some part in the preparation of the food, no supporting evidence was given, and so, in the present study, an experiment was designed to test the action of the secretion of similarly sited gland cells in the feeding organ of *Entobdella soleae* on a substrate of protein. Four living adult parasites from freshly caught soles were frozen one at a time on the stage of a freezing microtome, and facial sections through each parasite's feeding organ were cut by hand with a razor blade. These sections were then placed so that the cut surfaces of the gland cells were in contact with the gelatin (i.e. 'proteinaceous') coating of a piece of photographic film which had been blackened by exposure to light followed by development and fixing. For purposes of comparison frozen facial sections through the non-glandular oral sucker and pharynx of two specimens of a blood-feeding polyopisthocotylinean, *Hexabothrium appendiculatum* (Kuhn) from the gills of *Scyliorhinus canicula* (L.), were also cut and placed adjacent to the sections of *Entobdella soleae*. Then the gelatin film was covered with a filter paper soaked in sea water and enclosed in a Petri dish to prevent drying, and the sections were left undisturbed for 12 h.

At the end of this period the sections were gently removed from the film and the gelatin was examined. That part of the gelatin layer which had been in contact with the cut surface of the feeding organ of each of the four specimens of *E. soleae* had been dissolved, whereas the parts of the film which had been in contact with the cut ends of the intestinal diverticula (see p. 758) and other exposed tissues such as the haptor were undamaged. When the liquid products of digestion were gently washed away a well-defined hole in the gelatin film showed that the feeding organ had liquefied all the gelatin beneath it. The exposed muscular tissue of the oral sucker and pharynx of each of the two specimens of *Hexabothrium appendiculatum* had not affected the gelatin. This experiment indicates clearly that the contents of the giant gland cells of the feeding organ of a skin parasite, *Entobdella soleae*, contain a proteolytic substance, whereas the non-glandular oral sucker and pharynx of a blood feeder, *Hexabothrium appendiculatum*, are unable to digest protein. Similar experiments in which frozen sections of the feeding organ of *Entobdella soleae* were placed on an agar film impregnated with starch gave no indication that the feeding organ was capable of digesting starch.

THE MODE OF FEEDING

During some experimental work (Kearn, 1962, 1963*a*) involving observations on many living specimens of *Entobdella soleae* attached to the host, the feeding organ was never seen in the protruded position and the behaviour pattern of these parasites gave me no reason to believe that feeding had taken place. The 'scraping' movements of some monogeneans which were thought to be associated with feeding by Bychowsky (1957) (see p. 749), appear to be identical with the undulating movements which occur in *E. soleae* and which have been shown elsewhere (Kearn, 1962) to be associated with breathing.

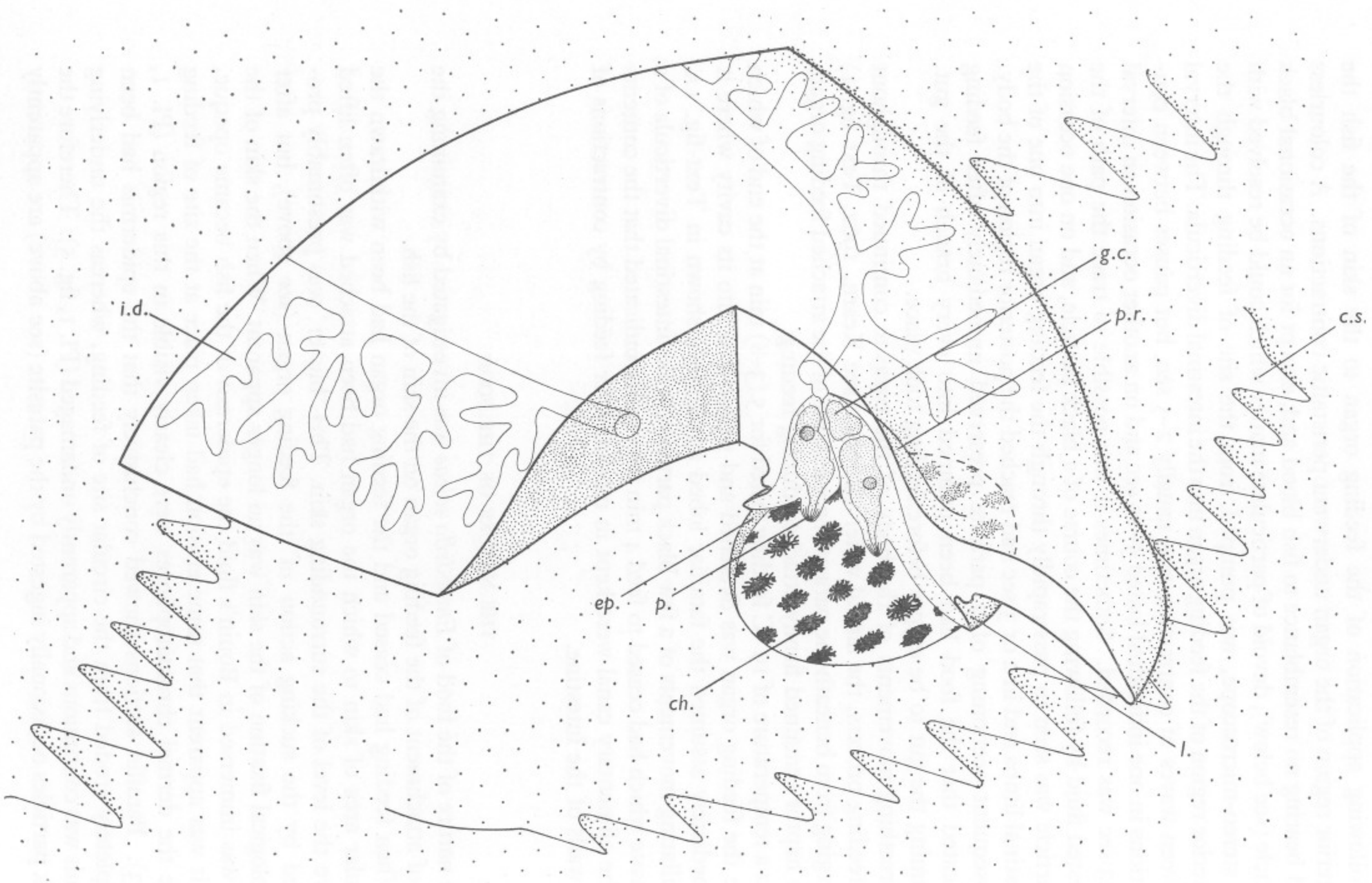
An attempt was made to induce some parasites to feed on host tissue by first separating them from the host for some time; these starved parasites were then placed on the fish and kept under constant observation with a stereo-microscope. Freshly killed soles were used for this purpose since it was difficult to prevent the movements of a living fish during the observation period. This procedure was found to be a successful way of inducing parasites to feed, and the act of feeding described below was witnessed on several occasions. It was found practicable to starve parasites for about 24 h, and after such starving feeding commenced within 30 min. of replacement on the host at 19° C. Some parasites were starved for $2\frac{1}{2}$–$3\frac{1}{2}$ days and then placed on a sole but although each parasite was observed for an hour feeding did not take place. Many parasites starved for more than $3\frac{1}{2}$ days became moribund and unable to attach themselves effectively by means of the haptor, and this condition was followed within a few hours by death.

Adult parasites live on the lower surfaces of soles (Kearn, 1963*a*), and although parasites can readily be induced to feed on this surface of the fish it is difficult to observe the behaviour of a whitish parasite feeding on unpigmented white skin. In order to overcome this difficulty starved parasites were placed on the upper surface of the host, where feeding took place in the same way as on the lower surface but with the advantage that the behaviour of the parasite could easily be seen against the dark background of pigmented skin. The details of the act of feeding described below are therefore based mainly on observations made on seven parasites induced to feed on the upper surfaces of soles.

After being placed on the dead fish each starved parasite attached itself by means of the haptor and performed breathing movements (see p. 753), interrupted occasionally by locomotion for short distances or by searching activity involving movement of the body from side to side. This behaviour pattern is characteristic of parasites attached to freshly caught soles.

At the commencement of feeding the anterior region of the feeding organ was protruded through the mouth and applied to the surface of the skin of the host so as to enclose a circular area (Text-fig. 2). It was not possible to determine whether the antero-lateral adhesive areas of the parasite were used for preliminary attachment of the head region to the skin of the fish, but when the feeding organ had gained attachment the adhesive areas appeared to be free from the fish. These adhesive areas therefore appear to play little or no part in feeding and are concerned mainly with the attachment of the anterior end of the parasite during locomotory movements (Kearn, 1963*a*).

The sites at which feeding took place were usually located on the exposed outer surface of one of the host's ctenoid scales as illustrated in Text-fig. 2. Occasionally the feeding organ enclosed some of the spines at the posterior border of a scale, thus overlapping one of the scales lying posterior to it (see Pl. 1, fig. 3), or the organ overlapped an adjacent lateral scale. When the feeding organ was applied to the skin of the host the papillae were arranged in a circle close to the skin of the fish (see Text-fig. 2), and the cavity enclosed between the papilla-bearing surface of the feeding organ and the circular area of fish skin was sealed peripherally by the lip of the feeding organ. Because of the intimate contact between the papilla-bearing surface of the feeding organ and the fish's skin and the transparency of the parasite, the dermal pigment cells of the skin could be seen through the tissues of the parasite in the region of the feeding organ.



Text-fig. 2. Diagrammatic reconstruction of the anterior region of a specimen of *Entobdella soleae* in the act of feeding. A sector of the head region of the parasite has been cut away to display the anatomy of the feeding organ. *ch.*, Chromatophore in dermis exposed by erosion of the overlying epidermis; *c.s.*, ctenoid scale; *ep.*, epidermis; *g.c.*, gland cell; *i.d.*, intestinal diverticulum; *l.*, lip of feeding organ; *p.*, papilla; *p.r.*, posterior region of feeding organ.

Following application of the feeding organ to the skin of the fish the posterior region of the organ underwent peristaltic contractions. A colourless fluid bearing no resemblance to fish blood and, except for an occasional black particle (see below), devoid of particulate matter which could be resolved with the stereo-microscope, was pumped from the site of feeding through the posterior region of the feeding organ into the intestinal diverticula. The interval between waves of contraction was usually 2–5 sec, but pauses between contractions in one individual lasted 5–10 sec and on another occasion an interval of 23 sec was recorded. Sometimes it was possible to trace the path of the ingested fluid by following the course of a black particle, and on one occasion a particle was seen to pass rapidly through the feeding organ into one of the intestinal limbs and in 2 or 3 sec had reached the posterior region of the body. Subsequent sectioning of a parasite preserved immediately after feeding indicated that the food had been pumped into every branch of the gut, assuming the gut to be empty before feeding took place.

Breathing movements of the body of the parasite continued throughout the feeding process, the lateral water currents (see Kearn, 1962, Text-fig. 1) emerging from beneath the parasite on each side of the attached feeding organ. The haptor remained firmly attached during feeding.

At a temperature of 19° C feeding lasted for 5 (3–8) min at the end of which time the feeding organ was detached and withdrawn into its cavity where it immediately assumed the familiar lobed appearance shown in Text-fig. 1 A. Oscillating movements of a few black particles in the intestinal diverticula of a parasite which had ceased to feed 4 min previously indicated that the contents of the alimentary canal were kept in motion after feeding by contractions of the walls of the intestine.

THE NATURE OF THE FOOD

The nature of the food of *Entobdella soleae* was investigated by examining the site of attachment of the feeding organ on the skin of the fish.

When feeding had ceased and the feeding organ had been withdrawn the circular area of skin to which the organ had been attached was often lifted above the level of the surrounding skin. This 'blister' was presumably produced by the sucking action of the feeding organ (see above), but after histological fixation of the skin was no longer apparent. When the skin of the fish was immersed in Bouin's fluid the epidermis of the fish became opaque, but it was apparent that some erosion had taken place at the site of feeding since the dermal chromatophores were clearly visible in this region (Pl. 1, fig. 3). Paraffin sections showed conclusively that the epidermis had been completely eroded from the circular site of feeding, whereas the underlying dermis was continuous and apparently undamaged (Pl. 1, fig. 5). Therefore the black particles occasionally ingested by the parasite (see above) are apparently not derived from the dermal chromatophores and may come from the host epidermis. Adult parasites from 2–3 mm in length produced lesions varying in diameter from 480–600 μ.

It can therefore be concluded that *Entobdella soleae* feeds on the epidermis of its teleost host. In addition mucus on the outer surface of the epidermis, as well as mucus cells (Pl. 1, fig. 5) within the epidermis itself, must also be ingested. Since the blood vessels lie in the dermis of the sole and do not occur in the superficial epidermis, the parasite does not ingest blood.

It seemed possible that in a search of an infested sole, preferably preserved in Bouin's fluid, feeding wounds would be found on the skin of the fish. On examination of a Bouin-preserved sole, with more than 100 parasites distributed over its upper and lower surfaces, previously removed from a tank at the Plymouth Laboratory, 17 circular skin lesions were found on the upper surface and 4 on the lower surface. It is possible that other wounds on the lower surface were overlooked because of the optical difficulties referred to above (p. 754). All of these lesions, except 2 on the upper surface of the fish, were found close to attached parasites and no more than one lesion was found in the vicinity of each parasite. Histologically these wounds were indistinguishable from the feeding wounds produced experimentally and the diameter of wounds associated with parasites of 1–2 mm in length on the heavily infested sole varied from 360–480 μ, whereas those in the vicinity of parasites from 2–3 mm in length varied from 420–660 μ in diameter. The shape, size and histological structure of these wounds and their proximity to attached parasites indicates that these lesions are sites where the neighbouring parasites have fed. A possible reason for the unusual distribution of feeding wounds on the skin of this fish is given on p. 763.

DIGESTION, ABSORPTION AND EGESTION

The proteolytic secretion produced by the gland cells of the feeding organ of *Entobdella soleae* appears to be responsible for the chemical erosion *in situ* of the epidermis of the common sole. In addition to *E. soleae*, strigeid metacercariae are thought to prepare their food by extracorporeal digestion using the holdfast organ and pseudosuckers (see Lee, 1962). The secretion of the feeding organ of *E. soleae* is poured out through the papillae on to the circular patch of host skin enclosed by the protruded feeding organ, and is prevented from escaping into the ambient sea water or from being diluted by an influx of sea water by the peripheral lip. The function of the papillae is difficult to comprehend, but the widespread occurrence of these structures in the feeding organs of entobdellids suggests that they play an important part in feeding. It has already been seen (p. 752) that these papillae may be labile.

An outline of the processes of erosion and digestion was obtained by histological examination of host epidermal cells at the edge of the wound and of the gut contents of a parasite which had been preserved shortly after the cessation of feeding. Host epidermal cells at the edge of the wound were undergoing pyknosis (a form of pathological degeneration in which each nucleus in the tissue involved contracts to an intensely staining mass), a condition apparently resulting from exposure to the proteolytic secretion of the feeding organ. The pyknotic cells had apparently lost their power to adhere to adjacent cells and many were found free in the lesion. Epidermal cells were not recognized in the finely granular gut contents of a recently fed parasite (Pl. 1, fig. 4) indicating that digestion had reached an advanced state. Perhaps the continuous epithelium of flat cells lining the intestinal diverticula of *Entobdella soleae* (Pl. 1, fig. 4) are concerned with absorption rather than with digestion.

The elaboration of the simple ring-gut of the oncomiracidium of *Entobdella soleae* into a branched system of intestinal diverticula has been described elsewhere (Kearn, 1963*b*, figs. 3, 4). The gut diverticula appear to compensate for the lack of a vascular system by carrying digested food rapidly to most regions of the body (see p. 756).

The elimination of gut contents, presumably containing indigestible remnants, through the feeding organ and mouth of the parasite, was observed on five occasions in living specimens of *Entobdella soleae*. Egestion was preceded on all occasions by a few contractions of the retracted feeding organ. In one parasite just before egestion took place, it was possible to see that the gut caeca, visible just posterior to their junction with the feeding organ, were undergoing peristaltic contractions. These contractions may be produced by fibres beneath the gut epithelium and not by movements of body tissues around the intestinal diverticula. The material egested consisted of liquid containing large numbers of white, roughly spherical cells, each about 19 μ in diameter. It seemed probable that these cells were derived from the intestinal epithelium and it is worth noting that Llewellyn (1954) observed the evacuation of gut epithelial cells through the mouth in blood-feeding polyopisthocotylineans and found that these egested cells were perfectly spherical.

OBSERVATIONS ON THE FOOD OF OTHER ENTOBDELLID SKIN AND GILL PARASITES

The family Capsalidae, as defined by Bychowsky (1957), embraces a number of genera of parasites including *Entobdella*, some of which are skin parasites and others gill parasites. A preliminary examination of whole mounts and sections of *Entobdella hippoglossi*, *E. diadema*, *E. bumpusii* (Linton), *Benedenia melleni* and *Capsala martinieri* from the skin of various fishes, and *Trochopus* sp. and *Nitzschia sturionis* (Abildg.) from the gills of their hosts, showed that in spite of their different habitats all these parasites possess a conspicuous feeding organ containing prominent gland cells and papillae. This indicates that all these parasites may feed in a similar way to *Entobdella soleae* on the epidermis of their hosts.

On examination of whole mounts and sections of Bouin-fixed and deep-frozen specimens of *Capsala martinieri* from the skin of a sun-fish landed at Plymouth, black pigment granules were seen lying free in the lumen of the intestinal diverticula. So far as it was possible to tell the gut epithelial cells were free of pigment granules but tracts of granules were seen in the parenchyma in the neighbourhood of the gut caeca. This pigment was particularly abundant in and around the finer gut branches. In view of the fact that Llewellyn (1954) has identified a similar black pigment, haematin, derived from a diet of blood in the gut lumen and intestinal cells of polyopisthocotylineans, it was desirable to establish the nature of the gut pigment in *C. martinieri*, the feeding apparatus of which points to a diet of epidermis. It was therefore decided to apply the pyridine haemochromogen test for haematin (see Llewellyn, 1954) to the gut pigment of *C. martinieri*.

Six small pieces of tissue containing abundant pigment were cut from two deep-frozen specimens of *Capsala martinieri* and treated with warm freshly made Takayama's solution. No change in the pigment in any of the tissue samples was seen and attempts to identify pyridine haemochromogen by its absorption band with a Hartridge reversion spectroscope were unsuccessful. No crystals with the characteristic shape and colour of pyridine haemochromogen were seen even after treatment with the warm solution for 30 min. In contrast a sample of human blood and a formalin-fixed specimen of a blood-feeding polyopisthocotylinean, *Diclidophora merlangi* (Kuhn), which contained abundant black pigment in the alimentary canal, when treated with the same solution gave the characteristic absorption band of wavelength 558 mμ of pyridine haemochromogen and yielded salmon pink crystals of this substance. Therefore it is probable that the gut pigment of *Capsala martinieri* is not haematin and is not derived from the blood of the host, and its nature and origin await further investigation, but it is perhaps worth noting that the freshly hatched oncomiracidium is also extensively pigmented (Kearn, 1963*c*). There appears to be little doubt that *C. martinieri* feeds on the epidermis of its host.

It has been seen above that the feeding organs of *Nitzschia sturionis* and *Trochopus* sp., which are gill-parasitic entobdellids, are morphologically similar to the feeding organ of an epidermis feeder, *Entobdella soleae*. This suggests that although *Nitzschia* and *Trochopus* are gill inhabitants they do not feed on blood but upon epidermal tissue within the gill chamber. However, Bauer (1958) made reference to observations by Russian workers to the effect that *Nitzschia sturionis* was capable of extracting 0·5 c.c. of blood per day from the gills of its host, a sturgeon, and Goto (1895, p. 180) commented on the occurrence of a red fluid, which he regarded as blood, in the alimentary canal of many specimens of *Tristomum* (= *Capsaloides*) *sinuatum* (Goto), collected from the gills of *Histiophorus* sp. During a visit to the Laboratoire Arago, Banyuls sur mer, France, I examined 17 living specimens of a species of *Trochopus* while still attached to the gills of the host *Trigla* sp., but the gut contents were colourless and devoid of any black pigment which might indicate a blood-feeding diet. Subsequent spectroscopic examination of a Bouin-fixed parasite after immersion in warm Takayama's solution failed to establish the presence of pyridine haemochromogen. Therefore, it seems very probable that *Trochopus*, although a gill parasite, is nevertheless an epidermis feeder.

THE FEEDING OF *ACANTHOCOTYLE* SP. ON THE SKIN OF *RAIA CLAVATA*

Entobdella soleae, *Capsala martinieri* and *Trochopus* sp. are epidermis feeders of teleost fishes, but several monopisthocotylineans, including *Entobdella diadema* and *E. bumpusii*, inhabit the skin of elasmobranch fishes. The question therefore arose as to whether skin parasites of elasmobranch fishes were capable of digesting the epidermis of their host in the same way that the epidermis of teleost fishes is digested by skin parasites. Sting-rays, the hosts of *E. diadema*, are rarely landed at Plymouth, but another skin parasitic monogenean, *Acanthocotyle* sp., the feeding apparatus of which is very similar to that of an entobdellid parasite, is relatively abundant on the skin of species of *Raia*.

The feeding organ of *Acanthocotyle borealis* Brinkmann from the skin of *Raia radiata* Donovan has been described by Brinkmann (1939, p. 39), who found that the organ contains 16 conspicuous gland cells each opening into the lumen of the organ on a papilla. I have also been able to identify 16 gland cells and papillae in sections of a specimen of a species of *Acanthocotyle* from *Raia clavata*. Brinkmann also referred to the presence of gland cells associated with the junction of the feeding organ and the intestinal caeca (see p. 752).

In the light of experience with *Entobdella soleae*, an attempt was made to induce a living specimen of *Acanthocotyle* from the lower surface of *Raia clavata* to feed on its host. The parasite was separated from its host for a period of 30 h, at the end of which the fish was killed and the parasite replaced on its lower surface and observed with a stereo-microscope. Eight minutes after replacement on the skin of the host the parasite attached itself by means of the feeding organ in a similar way to *Entobdella soleae* (p. 754), and thus enclosed a circular area of skin. Feeding lasted for only 30 sec and movements within the attached feeding organ were not discernible. When the organ was detached the epidermis of the ray was rendered opaque by immersion in Bouin's fluid and it was apparent that a great deal of epidermis had been removed from the skin encompassed by the feeding organ. This was confirmed by histological sectioning of the lesion, which measured about 400 μ in diameter, but the incomplete erosion of epidermal cells around the edge of the wound and the large numbers of epidermal cells with pyknotic nuclei and weakly staining cytoplasm (see p. 758) indicated that feeding was incomplete when the feeding organ was detached. It is worthy of note that the pathological changes undergone by elasmobranch and teleost epidermal cells (see p. 758), exposed to the proteolytic fluid from the feeding organs of monogenean skin parasites, are very similar.

The specimen of *Acanthocotyle* was preserved in Bouin's fluid immediately after the feeding organ became detached, and was subsequently sectioned so that the contents of the gut could be examined. The unbranched lateral diverticula were lined by a continuous shallow epithelium similar to that of *Entobdella soleae* and contained finely granular contents, among which no host epidermal cells could be identified.

Thus *Acanthocotyle*, and probably *Entobdella diadema*, *E. bumpusii* and other entobdellid skin parasites of elasmobranch fishes, feed on epidermis in a manner which closely resembles the mode of feeding of *E. soleae* on the skin of a teleost fish.

THE FOOD AND FEEDING OF OTHER MONOPISTHOCOTYLINEANS

A brief survey of other skin- and gill-parasitic monopisthocotylineans reveals considerable variation in the morphology of their feeding apparatus, suggesting that not all monopisthocotylineans feed in the same way. However, the available evidence suggests that most if not all monopisthocotylineans are epidermis feeders. Llewellyn (1954) found no evidence of a diet of blood in specimens of *Leptocotyle minor* from the skin of *Scyliorhinus canicula* or in specimens of *Calicotyle kroyeri* Diesing from the cloaca of various species of *Raia*, and I have observed no pigment in the gut of preserved specimens of *Calceostoma calceostoma* (Wagener) from the skin of *Argyrosomus regium* (Asso). Numbers of living specimens of a species of *Gyrodactylus* were collected from the skin and gills of a goldfish but no gut pigment was found and the gut contents were colourless. Mr J. E. Paling (personal communication) has likewise seen no red fluid or black pigment in the alimentary canal of living specimens of *Diplectanum aequans* (Wagener) from the gills of bass, *Morone labrax* (L.). On the other hand, Dr J. Llewellyn (personal communication) has observed red fluid on a few occasions in the alimentary canal of living specimens of *Amphibdella torpedinis* Chatin on the gills of *Torpedo marmorata* Risso, whereas living specimens of *Amphibdelloides maccallumi* (Johnston & Tiegs) from the gills of *Torpedo nobiliana* Bonaparte were seen to have colourless gut contents. Four preserved specimens of *Amphibdella torpedinis*, in which Dr Llewellyn had observed red fluid, were given to me, but when examined spectroscopically after treatment with warm Takayama's solution (see p. 759) the gut contents of only one of these parasites gave the absorption band of pyridine haemochromogen of wavelength 558 mμ. In contrast five similarly treated specimens of *Amphibdelloides maccallumi* gave no absorption band. Further investigation may show that *Amphibdella torpedinis* may feed, at least in part, on the blood flowing in the gill capillaries of the host, whereas *Amphibdelloides maccallumi* is probably an epidermis feeder.

DISCUSSION

Entobdella soleae, a monogenean from the skin of a teleost fish, and *Acanthocotyle* sp., a skin parasite from an elasmobranch fish, have been shown to erode the epidermis of their (unrelated) hosts by means of a proteolytic secretion produced by conspicuous gland cells in the feeding organ. The evidence suggests that the majority of other monopisthocotylineans living on the skin and gills of their hosts are also epidermis feeders, although the variation in the morphology of their feeding apparatus indicates a variety of feeding methods. Some gill-parasitic monopisthocotylineans however, including *Amphibdella torpedinis*, may feed on the blood of their hosts.

Llewellyn (1957) suggested that host specificity in the Monogenea may be controlled by blood-borne antibodies, but this now seems unlikely in the Monopisthocotylea since some if not all of these parasites feed on epidermis which is not vascularized.

In order to understand the evolution of ectoparasitism it will be useful to consider some of the properties of fish epidermis. If the relationship between an ectoparasite and its host is not to be detrimental to host or parasite, the host tissue used by the parasite for food must be accessible and easily eroded by the parasite, but at the same time the host must be able to withstand and repair the damage to its tissues. The epidermal layer of teleost skin (and probably the epidermis of elasmobranchs as well) possesses all these qualities. The superficial position of the epidermis and its lack of external keratinization (Burgess, 1956) renders it easily accessible and vulnerable to attack by the parasite. In addition there is evidence that teleost epidermis has considerable powers of regeneration after damage. Burgess (personal communication) found that the apparent growth rate of stickleback (*Gasterosteus aculeatus* L.) epidermis was about 0·5 mm per day, and Berlin (1951) found that the epidermis of a loach (*Misgurnus fossilis* L.) re-covered experimental skin wounds, varying in area from 5×5 to 10×10 mm, in 24–36 h. Berlin found that this process was primarily due to the emigration of undamaged epidermal cells from the edge of the wound across the surface of the lesion, forming a thin covering epithelium (see Berlin, 1951, fig. 1). A true regenerative process, involving mitoses of the epidermal cells covering the wound, began 3–4 days after wounding and resulted in the return of the epidermis to its original thickness. In contrast with the regeneration of the epidermis Berlin found that the regeneration of dermal tissues was a much longer process, which was not completed until more than 4 months after wounding.

Thus an epidermis feeder has the advantage that when an area of epidermis is eaten it is soon replaced by regeneration, and in addition the ill-effects on the host are minimized since the feeding wounds are rapidly healed. The dermis on the other hand regenerates slowly and the parasite would gain little advantage by attempting to erode it.

In view of the rapid rate of regeneration of fish epidermis and the frequent movements made by *Entobdella soleae* from place to place on the skin of the host (Kearn, 1963*a*), it is probable that each of the 19 feeding wounds, found close to attached parasites on the skin of the heavily infested sole (p. 757), had been freshly inflicted by the nearby attached parasite, perhaps within the 24 h period prior to the death of the host. It seems likely that the parasites which produced the two feeding lesions found in unpopulated parts of the upper surface of the fish moved to other parts of the sole before the fish was preserved. The rare occurrence of feeding wounds in unpopulated parts of the fish suggests that the epidermis removed from the wound is replaced possibly even before the parasite that produced the wound has moved away. A suggestion made elsewhere (Kearn, 1963*a*), that these movements made by *E. soleae* from place to place on the host are necessary because the parasite uses up the food in its immediate vicinity, now seems unlikely in view of the regenerative powers of fish epidermis. Perhaps locomotion in *E. soleae* is more closely associated with the reproductive habits of the parasite.

It is worthwhile at this point to consider the probable role of fish epidermis in the evolution of the kind of ectoparasitism displayed by the Monogenea. It is not difficult to imagine a rhabdocoel-like animal, living epizoically on an ancestor of the fishes, adopting the habit of feeding on the great expanse of host epidermis, which was nutritious and accessible and which when destroyed was so rapidly replaced by regeneration. Epidermis covers not only the surface of fishes but also lines their buccal and gill cavities and the cloaca of elasmobranchs. It is conceivable that some skin-inhabiting epidermis feeders, in search of areas of epidermis unpopulated by other skin grazers, became either facultative gill parasites like *Gyrodactylus* sp. or obligatory gill parasites like *Trochopus* sp. and perhaps *Diplectanum aequans*. In the same way other skin parasites colonized the cloaca of elasmobranchs giving rise to such monogeneans as *Calicotyle kroyeri*, if indeed this parasite is an epidermis feeder. An alternative food material was available to those epidermis feeders which took to living permanently in the gill cavity. Here blood capillaries, which in the skin are usually deep in the dermis and therefore inaccessible, run close to the surface in gill tissue. In addition to the accessibility of blood in the gills, a parasite feeding on blood would be assured of a continuous supply at one spot, and presumably at an early stage in the evolution of the Monogenea some gill-inhabiting parasites gave up feeding on epidermis and became blood feeders. This early off-shoot gave rise to the modern blood-feeding polyopisthocotylineans, with their adaptations to living a sedentary life in the gill-ventilating current of the host (see Llewellyn, 1956). However, the habit of feeding on blood may have been acquired more than once by epidermis-feeding monogeneans living in the host's gill cavity, since there is some evidence that the food of *Amphibdella torpedinis* and some other gill-parasitic monopisthocotylineans is blood.

It is likely that the favourable qualities of fish epidermis as a food for a potential skin parasite have made the evolution of skin parasitism in fishes possible and it has been seen that the available evidence supports the view that most monogenean skin parasites feed on epidermis. There are also a number of rhynchobdellid leeches and crustacean parasites living attached to the skin of fishes, and unless these parasites have an effective means of penetrating the dermis and reaching the blood capillaries, it seems likely that they are also epidermis feeders. The absence of hard skeletal parts in the proboscis of rhynchobdellid leeches and reports of the occurrence of albuminous fluid with no resemblance to blood in the oesophageal diverticula of some species (see Mann, 1962, p. 42), are more consistent with a diet of epidermis than with one of blood. In addition Bauer (1958) refers to small round wounds produced by the leech *Acanthobdella peledina* Grube on the skin of salmonid fishes.

I would like to record once again my thanks to the Director and Staff of the Plymouth Laboratory for laboratory facilities and particularly to Mr J. E. Green for invaluable co-operation. I am also grateful to Dr J. Llewellyn for helpful discussion. The work was conducted during the tenure of a Fishery Research Training Grant from the Development Commission.

SUMMARY

Entobdella soleae, a monogenean skin parasite of the common sole was induced to feed on its host. The feeding organ (= 'pharynx') is protruded during feeding and encloses a circular area of host skin. The gland cells in the feeding organ produce a proteolytic secretion which digests the epidermis of the fish. The liquid food is then pumped by the feeding organ into the intestinal diverticula, where it is absorbed.

Acanthocotyle sp. from the skin of an elasmobranch fish, *Raia clavata*, feeds on epidermis in the same way as *Entobdella soleae*. Observations on other skin- and gill-parasitic monopisthocotylineans indicate that the majority of them are epidermis feeders, although there is evidence that some gill parasites, for example *Amphibdella torpedinis*, may feed on blood.

The evolution of parasitism in the Monogenea is tentatively attributed to the outstanding qualities of fish epidermis as a food for a potential ectoparasite and in particular to its rapid rate of regeneration.

REFERENCES

BAUER, O. N., 1958. Relationships between host fishes and their parasites. In *Parasitology of Fishes*, pp. 84–103. Leningrad: University Press. (English translation by Z. Kabata, 1961. London: Oliver and Boyd.)

BERLIN, L. B., 1951. Compensatory regeneration of the epidermis of the groundling. *C.R. Acad. Sci. U.R.S.S.*, Vol. 80, pp. 245–48. (In Russian.)

BRINKMANN, A., 1939. Contribution to our knowledge of the monogenetic trematodes. *Bergens Mus. Årb., naturv. R.*, 1939, Nr. 1, 117 pp.

BRINKMANN, A., 1952. Fish trematodes from Norwegian waters. *Univ. Bergen. Årb., naturv. R.*, 1952, Nr. 1, 134 pp.

BURGESS, G. H. O., 1956. Absence of keratin in teleost specimens. *Nature, Lond.*, Vol. 178, pp. 93–4.

BYCHOWSKY, B. E., 1957. *Monogenetic Trematodes, their Classification and Phylogeny.* 509 pp. Moscow, Leningrad: Academy of Science, U.S.S.R. (English translation edited by W. J. Hargis, 1961. Washington: American Institute of Biological Sciences.)

FOLDA, F., 1928. *Megalocotyle marginata*, a new genus of ectoparasitic trematodes from the Rock Fish. *Publ. Puget Sd mar. (biol.) Sta.*, Vol. 6, pp. 195–206.

GOTO, S., 1895. Studies on the ectoparasitic trematodes of Japan. *J. Coll. Sci. Tokyo*, Vol. 8, pp. 1–273.

HARGIS, W. J., 1955. Monogenetic trematodes of Gulf of Mexico fishes. Part V. The superfamily Capsaloidea. *Trans. Amer. micr. Soc.*, Vol., 74, pp. 203–25.

HEATH, H., 1902. The anatomy of *Epibdella squamula* sp.nov. *Proc. Calif. Acad. Sci. (Zool.)*, Vol. 3, pp. 109–36.

KEARN, G. C., 1962. Breathing movements in *Entobdella soleae* (Trematoda, Monogenea) from the skin of the common sole. *J. mar. biol. Ass. U.K.* Vol. 42, pp. 93–104.

—— 1963*a*. The life cycle of the monogenean *Entobdella soleae*, a skin parasite of the common sole. *Parasitology*, Vol. 53, pp. 253–63.

—— 1963*b*. The egg, oncomiracidium and larval development of *Entobdella soleae*, a monogenean skin parasite of the common sole. *Parasitology*, Vol. 53, pp. 435–47.

—— 1963*c*. The oncomiracidium of *Capsala martinieri*, a monogenean parasite of the sunfish (*Mola mola*). *Parasitology*, Vol. 53, pp. 449–53.

LEE, D. L., 1962. Studies on the function of the pseudosuckers and holdfast organ of *Diplostomum phoxini* Faust (Strigeida, Trematoda). *Parasitology*, Vol. 52, pp. 103–12.

LITTLE, P. A., 1929. The anatomy and histology of *Phyllonella soleae* Ben. & Hesse, an ectoparasitic trematode of the sole, *Solea vulgaris* Quensel. *Parasitology*, Vol. 21, pp. 324–37.

LLEWELLYN, J., 1954. Observations on the food and the gut pigment of the Polyopisthocotylea (Trematoda: Monogenea). *Parasitology*, Vol. 44, pp. 428–37.

—— 1956. The host-specificity, micro-ecology, adhesive attitudes, and comparative morphology of some trematode gill parasites. *J. mar. biol. Ass. U.K.*, Vol. 35, pp. 113–27.

—— 1957. Host specificity in monogenetic trematodes. In: *First symposium on host specificity among parasites of vertebrates*, pp. 191–212. Neuchâtel.

MACCALLUM, G. A., 1927. A new ectoparasitic trematode, *Epibdella melleni* sp.nov. *Zoopathologica*, Vol. 1, pp. 291–300.

MANN, K. H., 1962. *Leeches*. 201 pp. London: Pergamon Press.

SCHOTT, H., 1896. *Einige Bemerkungen über den Bau von* Epibdella hippoglossi *O. F. Müller*, pp. 255–65. Uppsala: Zoologische Studien, Festschrift Wilhelm Lilljborg.

EXPLANATION OF PLATE I

Feeding in *Entobdella*

Fig. 1. Ventral view of the head region of *Entobdella diadema* with the feeding organ protruded. (From a photograph taken by Dr J. Llewellyn.)

Fig. 2. Median sagittal section of the protruded feeding organ of the specimen of *Entobdella diadema* in Fig. 1: *l.*, lip; *p.*, papilla; *g.c.*, gland cells in the non-protrusible region of the organ.

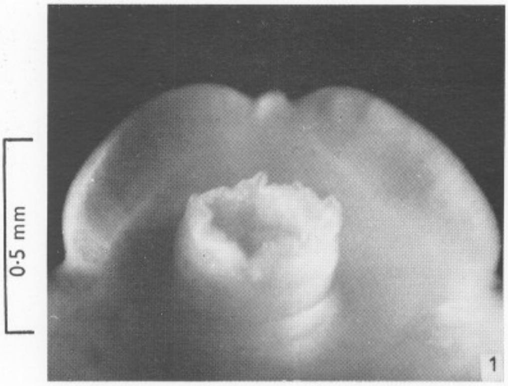
Fig. 3. A feeding wound (*AB*) inflicted by the adjacent attached specimen of *Entobdella soleae* on the skin of the upper surface of a sole. The skin was preserved in Bouin's fluid immediately after feeding had ceased in order to render the epidermis of the fish opaque. This treatment caused the parasite to contract and separated the head region of the animal from the wound. *h.*, Haptor.

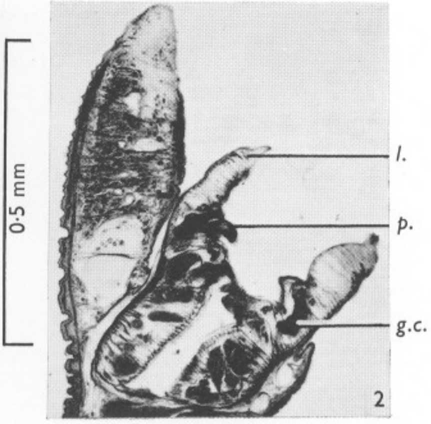
Fig. 4. A section through the intestinal diverticula of the recently fed parasite in Fig. 3: *e.c.*, epithelial cell of intestine; *i.f.*, ingested food; *v.f.*, vitelline follicle.

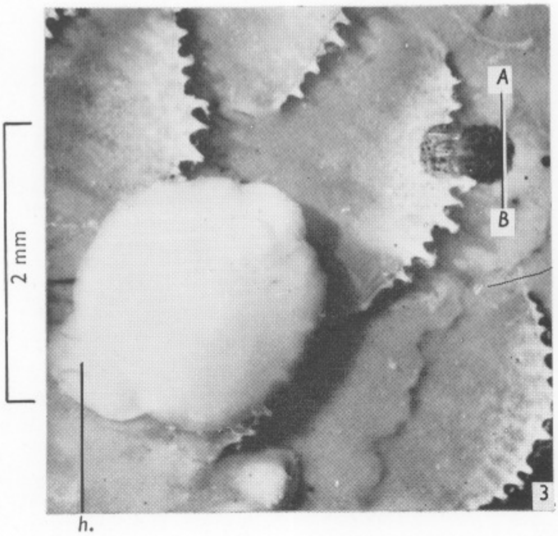
Fig. 5. A section (*AB*) through the feeding wound *AB* in Fig. 3: *ch.*, chromatophore; *d.*, dermis; *ep.*, epidermis; *m.c.*, mucus cell.

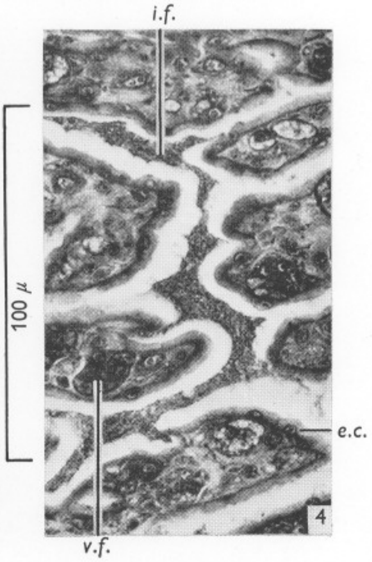
ADDENDUM

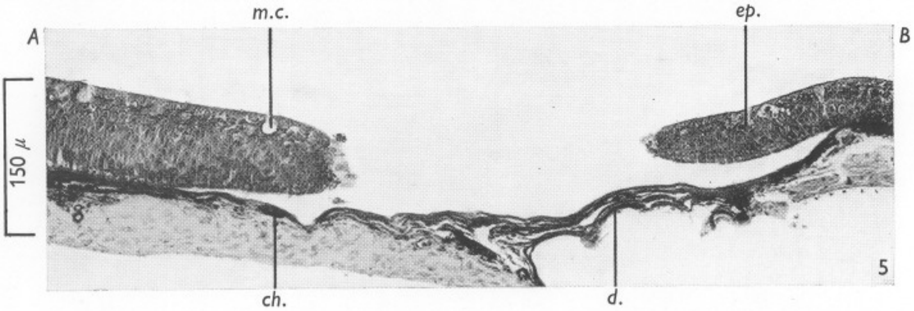
Since writing this paper I have observed red gut contents in a single living specimen of *Amphibdella flavolineata* MacCallum attached to the parietal gill mucosa of *Torpedo nobiliana*. The parasite was preserved in alcohol and later treated with Takayama's solution (see p. 759). A prominent absorption band at 558 mμ, characteristic of pyridine haemochromogen, indicated that the parasite had ingested blood from the host fish.


0·5 mm
1


0·5 mm
l.
p.
g.c.
2


2 mm
A
B
h.
3


i.f.
100 μ
e.c.
v.f.
4


A
m.c.
ep.
B
150 μ
ch.
d.
5