A STUDY OF THE STAGES IN THE LIFE CYCLES OF TREMATODES IN RELATION TO THE FAUNA OF THE LOCAL FRESHWATERS.

A thesis submitted for the degree of
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INCOMPLETE LIFE CYCLES (Cont'd)

SUMMARY

Digenetic trematodes are parasites possessing complex life histories. The egg hatches in water giving rise to a miracidium which penetrates into a mollusc. Development occurs, and each individual infection results in the production of many free swimming cercariae. Depending on the type of life history the cercaria may penetrate into its final host and produce more eggs. Alternatively it may encyst on vegetation, or on, or in other animals - second intermediate hosts and their remain inactive until the second intermediate host is eaten by the final host either accidently or as food, whence the encysted stage grows into the adult. These encysted and adult stages are located in many organs of a wide variety of animals. Moreover each individual trematode stage is specific being associated with a single or closely related group of hosts. It is not surprising therefore, that many life cycles are imperfectly known, as are records of the occurrence of the various stages, and the success or otherwise of life cycles under natural conditions. This is the problem which has been investigated, eight species of trematodes having been studied, the life cycles of four being completed. Of the four completed life cycles, two, namely those of Phyllodistomum folium and Dolichosaccus rastellus were hitherto incorrectly known. Close attention has been given to the ecological aspects of the problem, and this has shown that in spite of the general instability of the trematode population in the area under survey, several species were highly successful in completing their life cycles.

GENERAL INTRODUCTION.

Little work on fresh water larval trematodes and their life histories has been recorded in this country compared with similar work accomplished in America and on the Continent. Thomas was the first British worker and his papers of 1881, 1882, and 1883 revealed the life cycle of Fasciola hepatica Linnaeus, 1758. No further work on fresh water larval trematodes was recorded in this country until Walton (1918) included details of the infection and production of cercariae of Fasciola hepatica in Limnaea truncatula (Muller, 1774) in his study of liver rot in sheep and the bionomics of Limnaea truncatula. This work was followed by that of Hesse (1923) who gave descriptions of two cercariae from Scotland.

Brown in a series of papers (1926, 1927, 1931, 1933) showed that the larval trematodes of this country were as extensive as those on the Continent, and recorded several new species. He did not attempt to complete the life cycles of the cercariae experimentally as did Harper (1929, 1931). Thomas's observations on the cercaria and redia of Fasciola hepatica were supplemented by those of Wright (1927, 1928) who also described two new cercariae from North Wales, whilst some of the larval trematodes of South Wales were described by Rees (1932) who also studied their biology (1931), together with attempts at elucidating the life histories of other trematodes (1933). An outbreak of dermatitis led to Taylor & Baylis (1930) publishing details of observations and experiments on Cercaria ocellata La Valette, 1855 and Cercaria X Baylis, 1930.

In the following years interest in fresh water larval trematodes subsided until Vickers (1940) gave a detailed description of Cercaria macrocerca Filippi, 1854. Recently more work has been published on the bionomics of Limnaea truncatula and the development of Fasciola hepatica in it and other snail hosts by Kendall (1949a, 1949b, 1950) Kendall & McCullough (1951), whilst similar work has been recorded by Roberts (1950).

Knowledge of the marine larval trematodes of our coast is more complete, this being largely due to intensive study by a few workers notably Lebour, Nicoll, Rothschild, Rees and Cole.

Work on the Continent and in America has been much more extensive where Looss, Lühe (1909) and L. Szidat in Germany, Dubois (1929) in Switzerland, Wesenburg-Lund (1934) in Denmark, Mathias, Brumpt, Joyeux and Baer in France, Sewell (1922) in India; Faust in South Africa, China and America; and Cort and Stunkard also in America have been particularly prominent. Even so, much work remains to be accomplished particularly that which is concerned with the completion of life histories together with descriptions of the various stages.

It is this problem which has been studied in the present work, not only experimentally, but also from the ecological aspect. Altogether ten cercariae have been found, of which eight are described. The life cycles of four of these have been completed, the attempts to elucidate the life cycles of the other four being only partially successful.

AREA UNDER SURVEY.

The area that has been under survey lies to the west and south of Sheffield, although the collecting areas that have been most closely studied are situated within the City Boundary. This area as can be seen from Fig.1 may be divided into three main geological regions; the Coal measures situated in the east, the Millstone grits in the centre, and the Carboniferous limestone in the west.

The area is drained by three river systems; the Derwent which lies between the Carboniferous limestone and the Millstone grits and which flows south, the Don which drains the north-east region; and the Rother which drains the south-east. The Derwent rises at a considerable height where peat overlays the Millstone grits, and in the main there is no significant volume of water from the limestone area until the Wye joins it at Great Rowsley. The tributaries of the Don also rise on peat, whilst those of the Rother rise mainly on the Coal measures. Consequently most of the water to be found in the area can be seen to drain from peat, particularly as this region

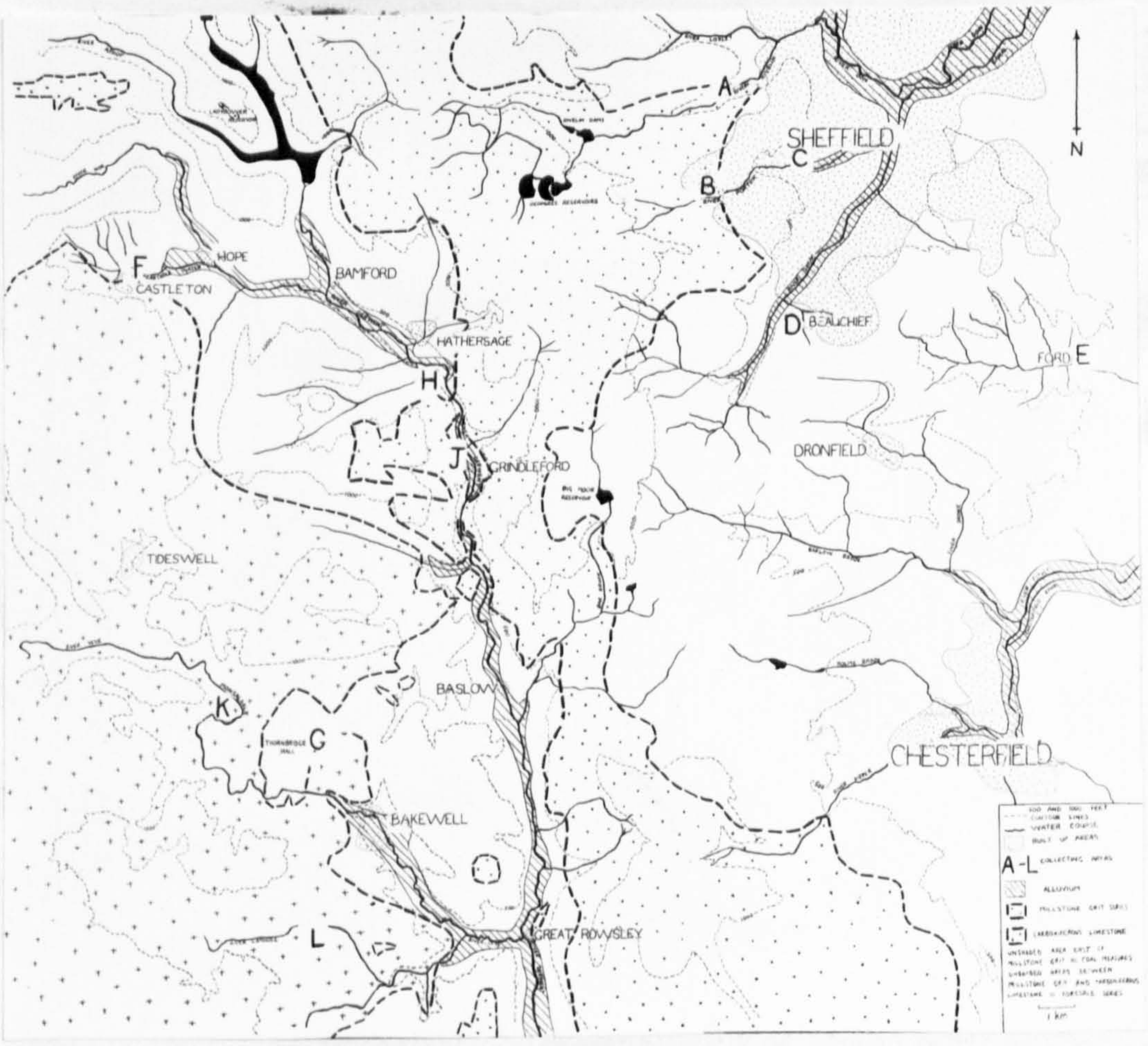

Fig.1. Map showing the area under survey.

has a much heavier rainfall owing to its high altitude.

The valley bottoms of all these rivers consist of alluvium, whilst a considerable part of the Derwent valley is formed of Yoredale series-a mixture of grits, sandstones, shales and limestone.

Farmland in the area is restricted to a narrow belt between the industrial areas of Sheffield and Chesterfield on the one hand, and the peat which is found at a height of about 1000 feet on the other, and to the region west of the Derwent.

As has already been mentioned, the collecting areas which were most closely studied were those within the City

Boundary. The small pond in the Botanical Gardens (Fig.1,C) is described later, as are the three dams at Beauchief (Fig.1,D), and those at Ford (Fig.1,E). Fig. 2 shows the collecting area of B (Fig.1) enlarged, and it can be seen to consist of a series of dams, six of which were visited regularly. The occurrence of such a large number of dams in such a short length of river is

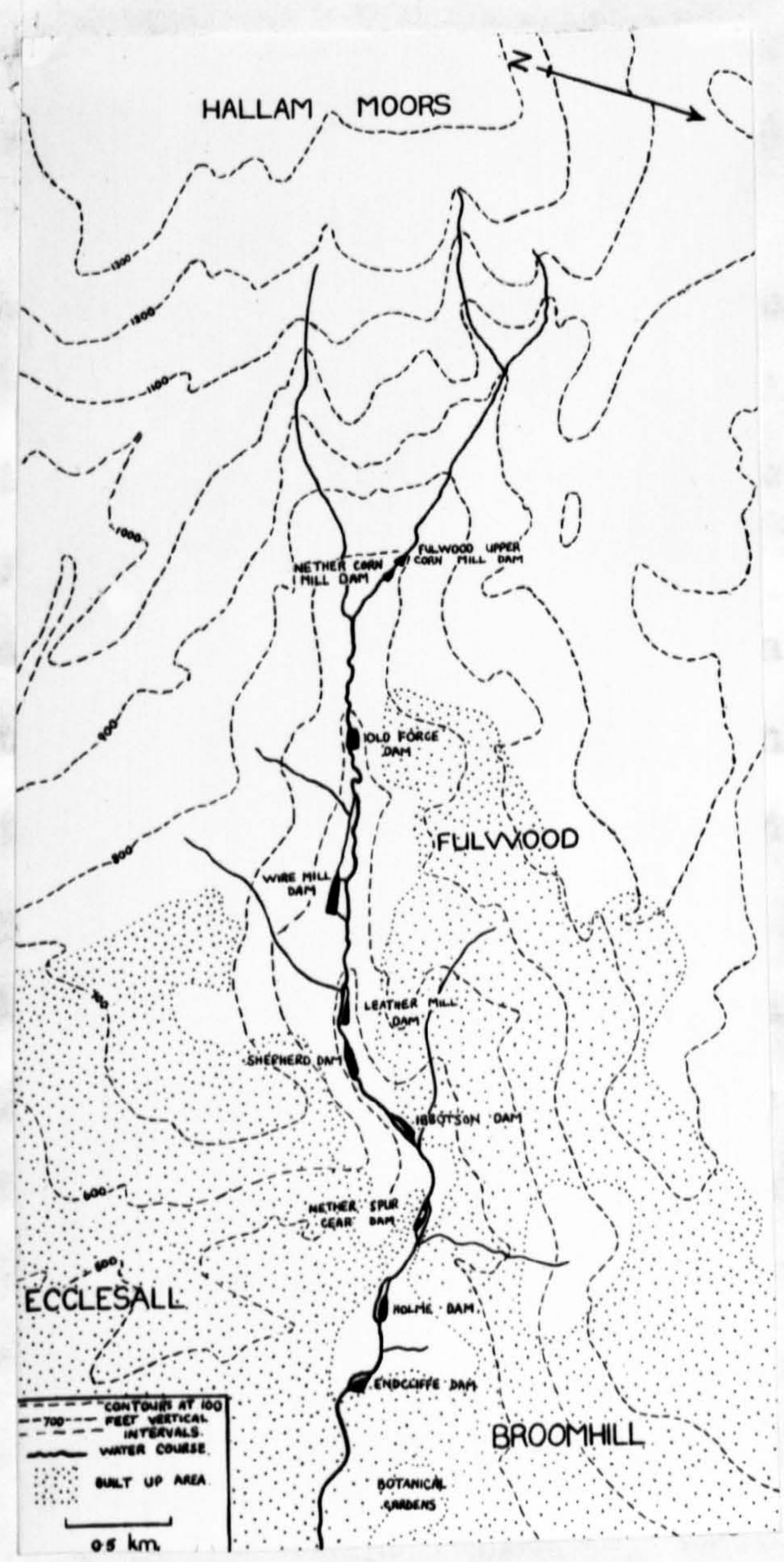

Fig. 2. The valley of the Porter river.

unusual, but as W. T. Miller (1949) remarks " greater use of available water power was made in this area than in any other part of the Kingdom". The impounded water was used to drive water wheels in connection with the cutlery industry, all the dams being built before 1800. With the invention of the steam engine the water wheels fell into disuse, as did the dams themselves. The Porter valley is now a public park, and recently several of the dam walls have been repaired so that the dams are not all in the same condition. Nevertheless they all present similar ecological conditions, all are silted up to varying degrees having a bottom of mud and decaying leaves-much of the valley supporting deciduous trees. Due to their construction the dams maintain a constant depth of water at all seasons or the year, thus in summer nearly all the water in the river is diverted into each dam, so maintaining its level, whilst in winter much of the greater volume of water is directed downstream, by-passing the dam. Under extremes of drought and heavy rain this constancy of the water level may however, be disturbed.

These systems of dams are common on all the tributaries of the Don. Other dams visited include these on the Rivelin (Fig.1, A) whilst the dams at Ford and Beauchief are undoubtedly of similar origin.

The aquatic flora of the area as a whole is poor, the moorland streams not supporting any higher plants. The vegetation of the dams on the Rivelin and Loxley Rivers is dominated by *Equisetum fluviatile* Linnaeus, 1758 particularly where heavy silting has occurred. In the dams on the Porter *Elodea canadensis* Michx. occurs and spread considerably during 1949 and 1950. The three dams at Beauchief and those at Ford have a varied plant community both streams rising on the Coal measures, the fauna too in both these habitats is likewise much richer. The river Derwent possesses a stony bottom, and is devoid of higher plants, whilst the most prominent plant of the limestone rivers is *Ranunculus aquatilus* Linnaeus, 1758 emend.

DISTRIBUTION OF MOLLUSCAN AND OTHER HOSTS.

Boycott (1936) records that molluscs are rarely found in peaty water, and this has been found to be the case in the present work. However, once the water is off the peat molluscs begin to appear, and whilst no deliberate attempt has been made to locate all the species of freshwater molluscs in the area, a number have been found and investigated for their trematode fauna. The following is a list of those found with remarks on their distribution and infections. As agreement regarding the nomenclature of the mollusca has not been reached, the lead of Macan (1949) using the names given by Ellis (1926) in his hand book has been adopted.

Ancylastrum fluviatile (Müller,1774) common in the rivers, frequently the only species found as in parts of the River Derwent. Never found parasitised.

Hydrobia jenkinsi Smith,1889 the most common and widely distributed mollusc in the area, frequently found in isolated stretches of water. As reported by Rees (1932) this species was never found parasitised.

Limnaea pereger (Müller,1774) common, found in all the collecting areas visited, growth small in rivers compared with dams. The most important snail in the area parasitologically nine of the ten larval trematodes found, utilising it as the intermediate host.

Limnaea stagnalis (Linnaeus,1758) very local in its distribution undoubedly introduced into the areas from which it has been obtained, namely, the Botanical Gardens (Fig.2) and Wire Mill Dam (Fig.2). During 1949, 1950, and 1951 it spread downstream from Wire Mill Dam to Leather Mill and Shepherd Dams (Fig.2) although the numbers remained small. A single specimen was obtained in a collection from the Round Dam (Fig.1.A). Susceptible to infection, but to a less extent than Limnaea pereger.

Limnaea palustris (Müller,1774) obtained on one occasion only from a dew-pond near the River Lathkill (Fig.1,L) not found parasitised.

Planorbis corneus (Linnaeus, 1758) Two specimens found on one visit to the Botanical Gardens, undoubtedly introduced into the pond. They were not found to be parasitised.

Planorbis albus Müller, 1774 very common in both rivers and dams. Never found parasitised.

Planorbis planorbis (Linnaeus, 1758) limited in its distribution. Found in the ponds of Thornbridge Hall (Fig.1,G) and in the Botanical Gardens. Never found naturally infected with cercarial stages, but was found to serve experimentally as a host of the metacercaria of Echinoparyphium recurvatum

(Linstow, 1873) Luhe, 1909

Sphaerium corneum (Linnaeus, 1758) limited in its distribution, only being found in dams and ponds where the bottom deposit was mud, although by no means confined to it. Abundant in Wire Mill Dam, and also occurred in the other dams on the Porter River, in the Round Dam, Botanical Gardens, and at Ford (Fig. 1,E). Is the intermediate host for Cercaria macrocerca and also harbours the metacercaria of E. recurvatum.

Anodonta cygnea Linnaeus, 1758 very local in its distribution. Found at Ibbotson Dam (Fig.2) and at the first dam at Ford. The presence of glochidia were observed at Wire Mill Dam, although no adults recovered. Never found infected with larval trematodes.

Pisidium spp.Pfeiffer,restricted to localities where there is a bottom deposit of fine mud, but more widespread than Sphaerium corneum. Never found parasitised in spite of it being the known intermediate host of an adult fluke which was collected.

Next in importance to the molluscs as intermediate hosts were insects. Whilst no general collection of them was made, it was obvious that suitable insects capable of acting as intermediate hosts to a particular cercaria were present in all the habitats where that cercaria occurred, as in no case did an attempt to find such an insect fail.

The three spined stickleback Gasterosteus aculeatus Linnaeus, 1758 also acts as an intermediate host. It was widely distributed, being found in all the localities where the corresponding cercarial stage was found except Peakshole Water (Fig.1,F).

The oligochaete Chaetogaster crystallinus Vejd, and the leeches Herpobdella atomaria Carena, 1820 and Helobdella stagnalis Linnaeus, 1758 also act as intermediate hosts. Their occurrence however, was spasmodic, not co-inciding in most cases with the appearance of the appropriate cercaria.

The distribution of two of the three definitive hosts that is frog (Rana temporaria temporaria Linnaeus, 1758) and mallard* (Anas *platyrhyncha platyrhyncha* Linnaeus, 1758) was localised but regular, whilst the three spined stickleback which was the other definitive host as previously stated was widespread in its distribution.

METHODS.

The material used in connection with the work consisted primarily of snails, which were collected and brought into the laboratory. They were then divided into small groups of three or four, or, if the infection rate was high, isolated individually and placed into small finger bowls partly filled with tap water (which was changed weekly) together with a sprig of *Elodea canadensis*. The finger bowls were placed in groups and covered with sheet glass to prevent the snails escaping, but suitably wedged to allow ventilation. The finger bowls were examined frequently for the presence of mature cercariae. If any cercariae were observed in a finger bowl containing several snails, these snails were immediately isolated in order to determine which snail was liberating cercariae. Any dead snails observed were dissected to determine the presence of immature infections, as were snails which had been maintained for a long time in the laboratory without liberating cercariae. In this way a supply of cercariae was readily available for much of the year. This method of obtaining cercariae is essentially the same as that advocated by Cort (1922) and Rees (1932). It was found to be advantageous to place the finger bowls on black paper, so that with the aid of a strong light the cercariae could easily be seen with the naked eye.

The gastropod molluscs treated in this way lived quite well, their length of life depending on their size and the nature of any infection, but the method was found unsuitable for the lamellibranch molluscs. Groups of these were dissected immediately after collection, whilst the water in which the remainder were placed was changed daily. In this way a sufficient number of

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*Mallard was the suspected natural host where duck (*Anas*) was experimentally infected.

cercariae were produced before the molluscs eventually died.

It has been found necessary to refer to the number of Limnaea pereger in certain habitats, and, in order to give some idea of the density of this snail, series of ten minute collections were made from time to time, and the following terminology used to express the results.

absent - no molluscs found.
rare - up to 2 specimens obtained.
occasional - 2 - 10 specimens obtained.
frequent - 10 - 50 specimens obtained.
abundant - more than 50 specimens obtained.

Previous workers on larval trematodes are all in agreement that the living cercaria provides the best means of studying its structure, particularly the excretory system. Stunkard (1930) analysed the methods used recording that neutral red was particularly useful as an intra-vitam stain. In the present work other intra-vitam stains were tried, but none proved as useful as neutral red which itself was found to be of little value when studying Xiphidiocercariae. The study of the living cercaria was facilitated by using one drop of horse serum and one drop of water as mounting fluid as described by Archibald & Marshall (1931)

Fixed and stained cercariae were used only in the study of the genital systems as these were not usually seen in living specimens. After trying various fixatives and stains the following two methods were employed.

A large number of cercaria were mounted in water under a coverslip and were gradually squashed by withdrawing the water with the aid of absorbent paper. This continued until the cuticle of each cercaria was on the point of disintegrating when 10% formalin as advocated by Harper (1929) as a suitable fixative was pipetted on to the slide and drawn under the coverslip. The whole process was manipulated so that the cercaria were kept under constant pressure until dead when the coverslip was floated off with excess fixative. It was found that by this method a number of cercariae remained attached to either the slide or the coverslip as was found by

Wright (1927) where they were stained in acetic alum carmine, dehydrated and mounted.

The second method used was that described by Archibald & Marshall (1931) using lactophenol and borax carmine. It did not give as good results as the other method, but was found to be useful in that it was quick, easy to manipulate, and could be used with large numbers of cercariae.

Sporocysts, rediae and cysts were studied and measured only from living material, with the exception of the location of cysts in second intermediate hosts. Adults were studied mainly from stained permanent preparations and only fixed adults were used for measurements.

Observations and measurements of cercariae always refer to living mature cercariae which had been shed by snails except where otherwise stated. The measurements of cercariae were obtained by squashing them under the coverslip just sufficient to prevent rapid motion so that accurate readings could be taken. All the measurements are in millimetres and at least ten specimens were measured for each reading. Where ranges of measurements are given, they are the smallest and largest of the specimens which were taken at random, with the exception of adults. In their case the smallest and largest specimens containing eggs (which was taken as signifying that the specimens were adults) were used to denote the range of size. In certain instances measurements are given of stages after a particular type of treatment, this is to facilitate comparison with previous workers' results who utilised such a treatment. All the diagrams of cercariae and adults are ventral views, with the exception of those of Notocotylus attenuatus(Rudolphi, 1809) Kossack,1911, where for clarity dorsal views are shown.

It is well known that the digenetic trematoda have stages in a large variety of other animals, consequently any animals encountered when collecting molluscs were taken back to the laboratory either for dissection in search of trematode stages, or for possible experimental infection. Certain visits were also made to habitats in search of particular animals required for specific purposes. In this way a knowledge of life cycles in the field was acquired. Various developmental stages of digenetic trematodes were found which could not be related to any of the cercarial forms being studied, such stages were preserved for possible future use.

Three methods are usually available in the determination of the life cycle of any digenetic trematode.

Eggs from a known adult are hatched and a variety of laboratory bred snails are placed in contact with the miracidia, successful infections produce the stages of that fluke as far as the mature cercaria.

By studying the comparative morphology, various stages in a life cycle may be related.

Naturally infected snails are collected, and allowed to produce cercariae from which adult stages are reared.

Undoubtedly the best way of determining the life cycle of any trematode is by combining all three methods, this however, involves the use of laboratory cultures of snails and eggs which may require much attention. Also it is often more difficult to find the adult than the cercarial stage, and the structure of the miracidium offers less evidence than the cercaria as to the next probable host. In view of this it was decided to attempt the completion of the life cycles of the cercariae obtained, by using a combination of the last two methods.

In completing life cycles, certain precautions have to be taken in order to prevent incorrect relationships being formulated. Accordingly the following precautions were taken. Animals which were to be infected were reared in the laboratory where possible, so that there was no risk of them already being infected (see pages 21 and 42). If this proved impossible such animals were taken from habitats where there had been no occurance of the trematode concerned (see page 69) In cases where a trematode was widespread animals used were brought into the laboratory, a number dissected and the percentage infection and stage of development of the trematode was determined. The faeces of the animals in both these last two groups were examined at intervals over a period of not less than one month for the presence of eggs.

Infection of the definitive hosts was frequently made in two doses, the interval between doses depending on the development rate of the fluke as noted from previous experiments, or from the results of other workers. If this method was adopted it was found that the flukes obtained from the host could be grouped into two sizes, thus offering further proof as to the correctness of the life cycle. The four life cycles were first completed in a series of experiments in 1949 and were repeated with the same results in 1950, when both naturally and experimentally infected material was used.

COMPLETED LIFE CYCLES.

(i) Notocotylus attenuatus. (Rudolphi,1809) Kossack, 1911.

The life history of N.attenuatus has previously been studied by Joyeux (1922), Mathias (1930a) L.& U,Szidat (1933) and Yamaguti (1939). The measurements of the cercaria given here compare favourably with those given by Mathias (1930) and L.& U.Szidat (1933). The life cycle and measurements given by Joyeux are unlike other descriptions, and it must be concluded, as was noted by Mathias (1930) and Rothschild (1938) that Joyeux described the life history of a closely related species, but different from N. attenuatus.

The measurements of the cercaria given by Yamaguti (1939) are smaller than those listed here, and closely resemble the measurements given by U.Szidat (1935) for Cercaria imbricata Looss, 1893. This cercaria was shown to develop into an adult-Notocotylus imbricatus U.Szidat, 1935 which appears to be almost indistinguishable in the adult stage from N.attenuatus. Further the descriptions of the redial stages are in close agreement, it seems likely therefore, that Yamaguti described the life history of N.imbricatus and not N. attenuatus.

(a) Redia.

Measurements (mm.)

Length.	0.348 - 2.15
Breadth.	0.145 - 0.4
Diameter of Pharynx	0.07 - 0.11

The rediae occur in the liver and gonad of <u>Limnaea pereger</u>. They are cylindrical in shape, rounded anteriorly and tapering posteriorly, and are without lappets or birth pore. Young rediae are colourless, but in older specimens the presence of yellowish brown particles in the intestine is apparent. This completely masks the presence of rust coloured granules which are found scattered in the cuticle. Occasionally rediae are found which have transverse constrictions, these constrictions as has been suggested by Harper (1929) for the redia of <u>Notocotylus seineti</u> Fuhrmann, 1919 are due to the resistance offered by the liver to the movement

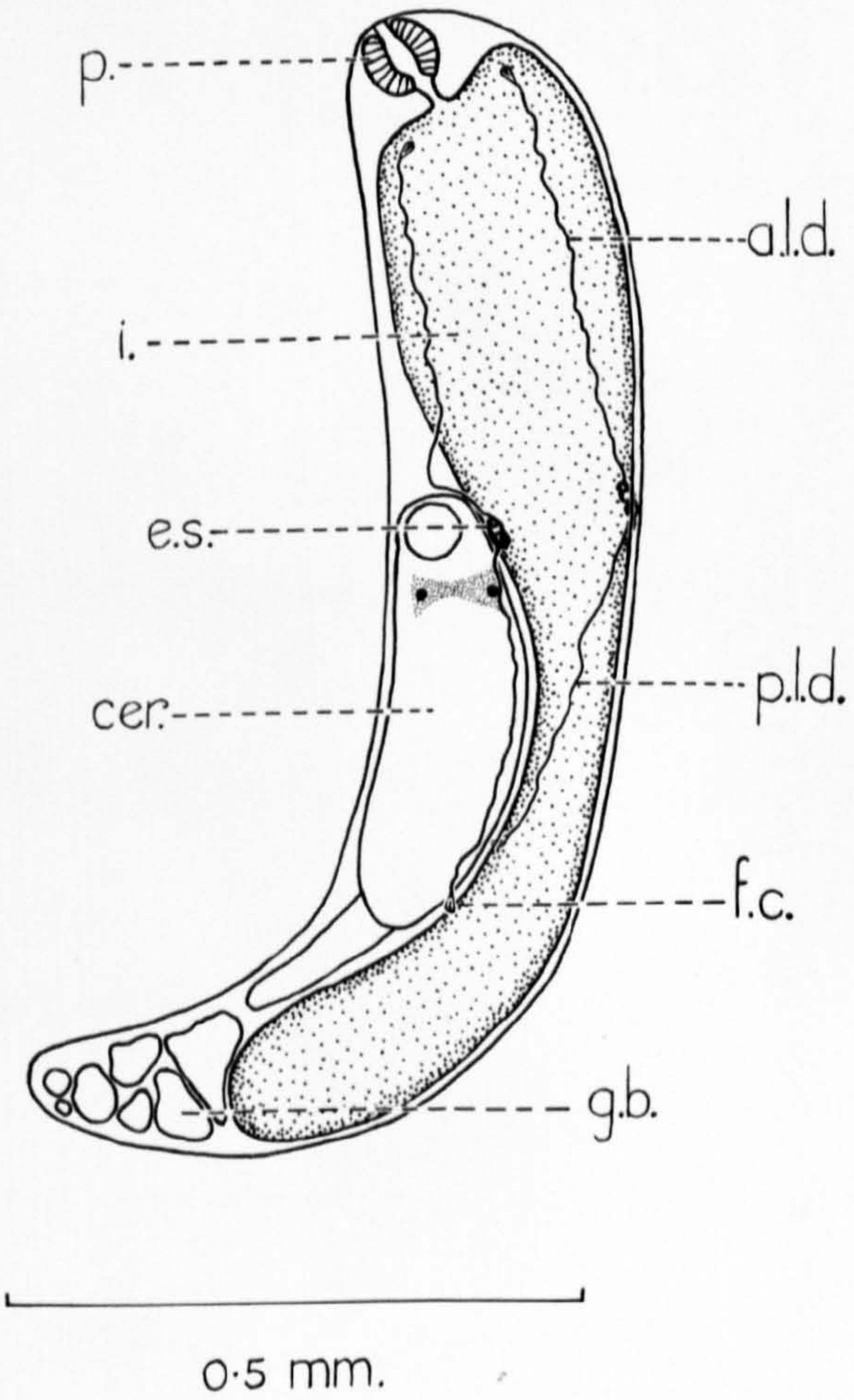


Fig. 3. Redia of <u>Notocotylus attenuatus</u>.

of the rediae. The rediae possess considerable powers of contraction and elongation, these being more pronounced in older rediae.

The mouth leads into a prominent pharynx (Fig. 3, p.) This opens into a long sac-like intestine (Fig. 3,i) which almost reaches the posterior end of the redia. In older rediae the intestine is not so conspicuous since it is pushed to one side by developing cercariae. Rediae usually contain one or two developing cercariae (of two eyespot stage) rarely three, and on one occasion four were observed. The area of proliferation is located at the posterior end of the redia.

The excretory system of the **redia** is very similar to Cercaria spatula Faust, 1919 and Cercaria ephemera Lebour, 1907 (nec Nitzsch) as described by Rothschild (1935) and consists of two antero-and postero-lateral ducts (Fig.3,a.l.d.,p.l.d.) each of which terminates in a single flame cell. The antero-and postero-lateral ducts of each side join together, and at this point considerable coiling with dilation of the ducts occurs, so that an excretory sinus (Fig.3,e.s.) is formed. Each excretory sinus has its own excretory pore. Usually the antero-lateral duct is longer than the postero-lateral duct. The excretory system thus consists of four flame cells, and although observation of flame cells in older rediae is difficult no increase in flame cell number has been detected.

(b) Cercaria.

Measurements. (mm.)

	Living.		Fixation by Heat.	
	Range.	Average.	Range.	Average.
Body Length	0.49 - 0.63	0.534	0.46 - 0.67	0.57
Body Breadth	0.165 - 0.21	0.19	0.143 - 0.198	0.16
Tail Length	0.58 - 0.81	0.65	0.52 - 0.63	0.60
Oral Sucker	0.049 - 0.066	0.058	0.044 - 0.055	0.051
Diameter of Eyespots.	0.15 - 0.25		0.165 - 0.22	
Diameter of Excretory Vesicle.		0.05		0.04

The cercaria is light brown in colour, positively phototactic and has considerable powers of contraction and extension. In an extended condition the cercaria is pointed anteriorly and rounded posteriorly, whilst when contracted it is almost circular in outline, a condition which is found prior to encystment. The tail is simple, muscular, longer than the body and during swimming movements is bent ventrally beneath the body. There is no ventral sucker but along the posterior edge of the body a pair of dorsal locomotor pockets (Fig.4,d.l.p.) are found, and these are used in creeping. Anteriorly the three dorsal eyespots are prominent, the median eyespot (Fig. 4,m.es.) is paler and smaller than the lateral eyespots (Fig.4,l.es.) and has a clear patch in its centre. A group of fine sensory hairs are set in the cuticle immediately in front of the oral sucker, two of these hairs (Fig.4,s.h.) being more prominent than the rest.

The mouth is surrounded by the oral sucker (Fig.4,o.s.) and leads directly into the oesophagus, there being no pharynx. The oesophagus (Fig.4,oes.) bifurcates immediately behind the transverse excretory canal into two intestinal caeca (Fig.4,c.) which pass backwards almost reaching the posterior end of the body. The caeca are partly obscured by the main lateral excretory canals which lie dorsal to them.

The excretory system of Monostome cercariae consists of two main lateral excretory canals which are united anteriorly by a transverse canal, and which discharge posteriorly into a median excretory bladder. These main lateral excretory canals contain excretory granules, are easily observed, and consequently have been described for most Monostome cercariae. The remainder of the system however, is imperfectly known since the body is rendered opaque by the large amount of cystogenous material present. Faust (1918) suggested the system for Cercaria robusta Faust, 1918 and later (1919) described the system of Cercaria spatula Faust, 1919. Horsfall, (1930) described that of Cercaria infracaudata Horsfall, 1930 and Rothschild (1935) that of Cercaria ephemera. Rees (1932) observed a single flame cell on either side of the oral sucker in Cercaria monostomi Linstow, 1896.

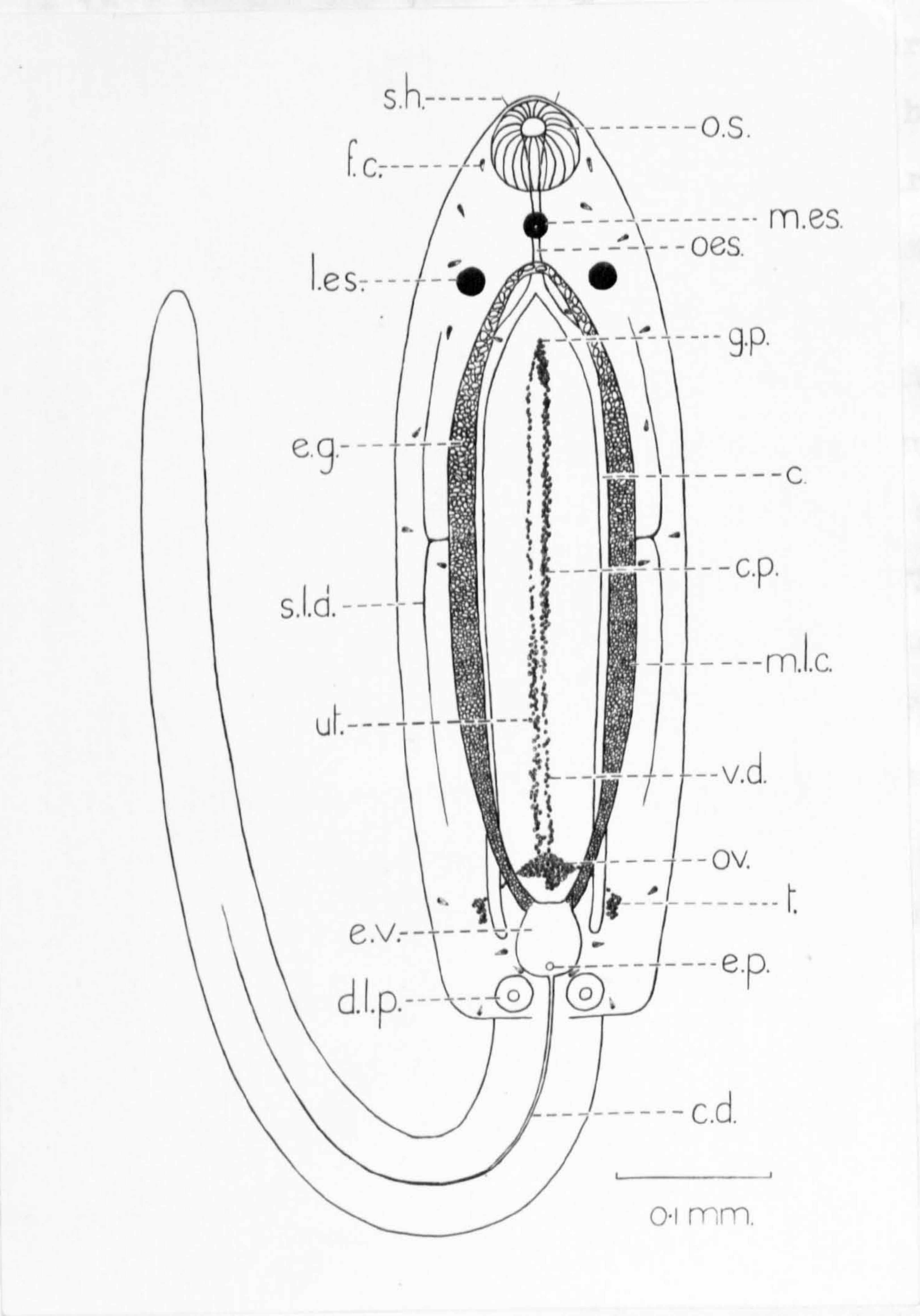


Fig. 4. The Cercaria of Notocotylus attenuatus.

The excretory system of the cercaria of Notocotylus attenuatus is similar to that of Cercaria spatula and consists of a spherical excretory vesicle (Fig.4,e.v.) located along the posterior edge of the body between the dorsal locomotor pockets. Anteriorly the vesicle gives off two main lateral canals (Fig.4, m.l.c.) which run forward almost parallel, and then unite in a position between the median eyespot and the bifurcation of the intestine. These canals contain excretory granules (Fig.4,eg.) which vary in size and number according to their position. Anteriorly the granules are large becoming progressively smaller posteriorly, until the mid-way position is reached, behind this they remain of a uniform small size. Occasionally cercariae were found in which the excretory granules were less numerous and sometimes absent in the region situated near the mid-way position. In this region the main lateral canal receives a duct which immediately divides into anterior and posterior secondary lateral branches (Fig.4,s.l.d). Flame cells have been observed but it has been impossible to trace their finer connections or elucidate their exact number, owing to the presence of the cystogenous cells which are packed with large numbers of rhabditiform granules. These cells extend throughout the body except in the region of the oral sucker. Twelve flame cells on each side of the body have been observed, all except one pair being lateral to the main excretory canals. The excretory vesicle which receives a duct from the tail (Fig.4,c.d.) opens to the exterior by a dorsal pore (Fig.4,e.p.) situated near the posterior border of the excretory vesicle.

In the living cercaria the only part of the genital system which is visible is the ovary (Fig.4,ov.) situated just in front of the excretory vesicle. A study of stained preparations shows that the system is more extensive than this. From the ovary which consists of a mass of small cells with prominent nuclei there passes anteriorly two columns of cells, at first hardly distinguishable as two seperate columns, but later quite distinct with the right column wider than its left counterpart. The left column is the rudimentary uterus (Fig.4,ut.) and the right the vas deferens (Fig.4,v.d.) - its terminal portion being the cirrus pouch (Fig.4,c.p.) The two columns end together just behind the bifurcation of the oesophagus. On each side of the body lateral to the intestinal caecum at the level of the excretory vesicle is a further mass of cells, smaller than the ovary, and these cells constitute the testis (Fig.4,t.)

As in other Monostomes, the cercariae leave the rediae before they are mature. At this stage the cercariae of *N.attenuatus* has developed the two lateral eyespots with an irregular mass of brown pigment between them. During the cercaria's free existence within the liver, the median eyespot condenses, and the brown pigment is further developed being found along two dorsal, two lateral, and two ventral lines as was found in Cercaria helvetica I Dubois, 1929 and Cercaria monostomi by Dubois (1929). Later however, the brown pigment is found distributed more or less evenly over the entire surface of the body except for the region near the oral sucker.

The existence of large cells - (" les cellules geantes" of Dubois, 1929) in Monostome cercariae has been reported in Cercaria monostomi and Cercaria helvetica I by Dubois (1929). Cercaria imbricata by Wesenburg-Lund (1934) and similar cells have been noted by Faust (1917,1919) in Cercaria pellucida Faust, 1917, Cercaria konadensis Faust, 1917 and Cercaria spatula who considered them to be part of the vitellaria. Dubois (1929) also noted the presence of six pairs of "les cellules glandulaires caudales" in Cercaria monostomi and Cercaria helvetica I as did Faust (1919) for Cercaria spatula and Cort (1915) for Cercaria urbanensis Cort,1914.

Similar cells to these have been seen in the cercaria of N. attenuatus during its development in the liver. They are arranged as in Cercaria monostomi as reported by Dubois (1929) that is in four longitudinal rows, four cells in the lateral rows and six in the median rows, making twenty cells in all, the anterior cell of each row being found at the same level or slightly in front of the lateral eyespots. The cells are very conspicuous at a certain stage of the cercaria's development in the liver, for at this time the remainder of the body is filled with opaque cystogenous granules, the cells themselves remaining transparent. In the tail six pairs of caudal cells were observed, the three anterior pairs being larger than the remaining three pairs. No sign of either type of cell was seen in mature cercariae either living or stained preparations.

(c) Cyst.

The mode of encystment of Monostome cercariae has been described by Cort (1915) for Cercariae urbanensis,Harper (1929) for the cercariae of Notocotylus seineti ,Rees (1932) for Cercaria monostomi and by Wesenburg-Lund (1934) for Cercaria ephemera Nitzch,1807. The free life of the cercaria of Notocotylus attenuatus is short, and since it is positively phototactic the cercaria swims to the lighter side of the containing vessel and there encysts. The cercaria will encyst on water plants, and on the shell of Limnaea pereger.Harper (1929) records that the cercaria of Notocotylus seineti shows a preference for non-infected snails, no such preference was observed with the cercaria of Notocotylus attenuatus although only a few snail hosts have been collected, and hence observations have been limited.

The process of encystment is similar to that described by Rees(1932) for Cercaria monostomi, the cercaria attaches itself to the substratum by the oral sucker, contracts, becoming circular in shape, and cystogenous material is then secreted over the entire surface, except in the region of the oral sucker. This period lasts from one to two minutes, and during this time the tail which is vigorously lashed from side to side is gradually nipped off. The cercaria then begins rotating movements inside the cyst, as it does so the tail finally breaks free from the body. It frequently remains attached to the cystogenous material on the outside of the cyst wall for varying times, (three to thirty minutes) before finally breaking completely free.

Co-inciding with the rotation of the cercaria, excretory granules are extruded through the excretory pore. This process continues as long as the cercaria maintains its rotating action - a few granules being extruded at intervals. Observation of any single granule shows that it dissolves in the cystogenous fluid that is still being secreted. Two distinct layers to the cyst wall have been observed, although Yamaguti (1939) distinguished five. The outer layer (Fig.5,o.g.l.) is finely granular and is secreted during the initial period when the cercaria is at rest. It varies in thickness in different cysts having a maximum thickness of 0.011 mm. The formation of the inner layer (Fig.5,i.l.l.) which is laminated co-incides with the rotation of the cercaria and the extrusion of the excretory granules.

The extrusion of excretory granules appears to be an active process, since by varying the pressure of the coverslip on a cercaria during the initial period the diameter of the cyst can be greatly increased. If the pressure is then decreased the volume of the cyst becomes much larger than that of the contained cercaria, yet under these conditions extrusion of excretory particles still occurred. Although no cystogenous material is secreted in the region of the oral sucker in the initial period, the pore which is formed becomes closed, due to the flow of cystogenous fluid to this region during the latter part of the initial period. This original point of adherence to the substratum by the oral sucker is marked by a notch (Fig.5,o.p.a.) in the outer layer. In normal encystments no excretory granules have been observed to pass out of the cyst during its formation, so that the extrusion of granules cannot be said to be directly excretory in function. Consequently the extrusion must be regarded as being cystogenous in function. The process however, does serve to remove excretory materials from the body. It may be recalled that the insect removes some excretory material in an analogous manner when it moults.

The cyst (Fig.5,) is circular in outline, and its upper surface is convex in side view. It has a diameter of 0.187 - 0.21 mm. (average 0.196 mm.) excluding the thickness of the outer granular layer which is observed only in newly formed cysts. If this layer is included the diameter of the cyst is increased to 0.21 - 0.23 mm.

A large number of excretory granules remain in the main lateral canals after the process of encystment, but they are much less numerous than in the mature cercaria. The three eyespots lose their distinct character after encystment, the pigment gradually becoming diffuse and the metacercaria becomes darker in colour. This dark brown pigment becomes so intense that it completely obscures details of the genital system in stained excysted metacercariae.

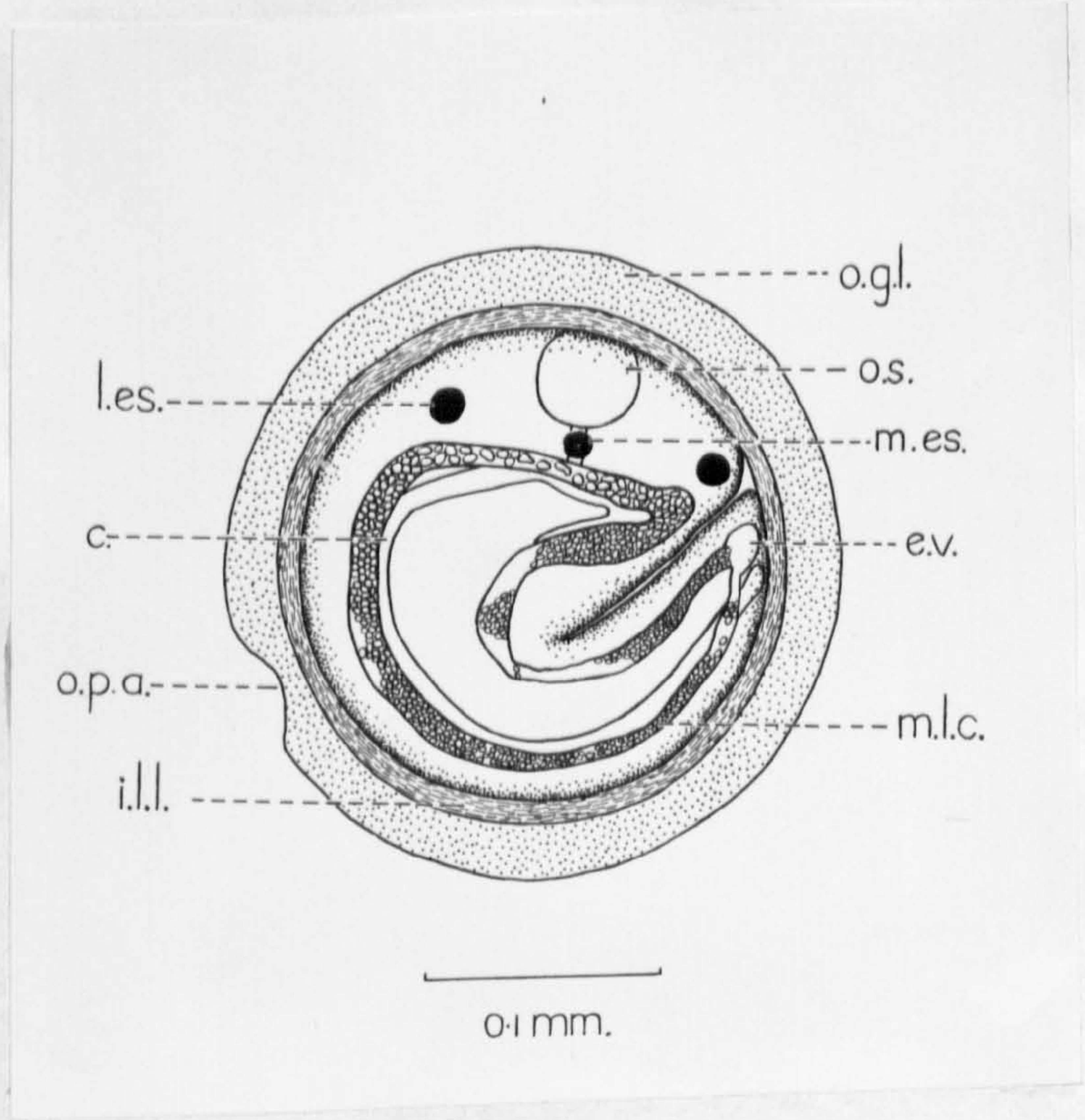


Fig. 5. Cyst of Notocotylus attenuatus.

(d) Juvenile and Adult.

In order to complete the life cycle, cysts were fed to laboratory reared ducklings which were killed at varying intervals after infection. As a consequence individuals aged 1,7,14,27 and 31 days old were collected and examined. The adults are located in the caeca; although one specimen was found in the rectum it was in a moribund condition, suggesting that the rectum is not a normal location of the parasite.

It was found that seven day old individuals are sexually mature and contain eggs, although their uteri were not gravid. At this stage however, they are only about one half the length of fully grown individuals, but even at this stage they have the full complement of ventral glands.

Measurements (mm.) of Fixed Sexually Mature Specimens.

Length.	1.9 - 4.6
Breadth.	0.48 - 1.1
Oral Sucker.	0.64 - 1.4
Vagina.	half length of cirrus pouch.
Eggs.	0.02 - 0.022 x 0.01 - 0.011
Number of Ventral Glands	lateral 15 - 16 median 14 - 15

The adult of Notocotylus attenuatus is elongate,

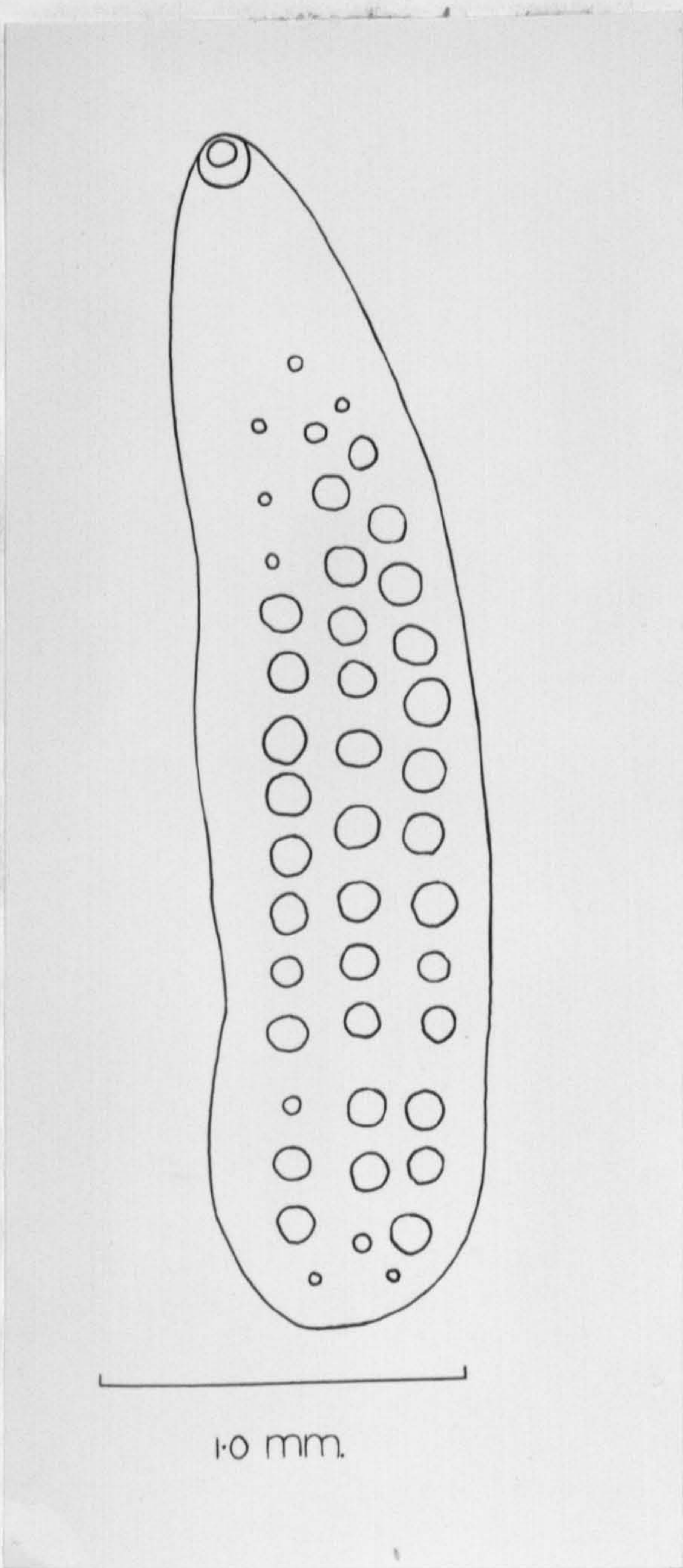


Fig.6, The ventral glands of the adult of Notocotylus attenuatus.

pointed anteriorly and rounded posteriorly. The concave ventral surface is covered with fine spines, and bears three rows of **orange** coloured glands. (Fig.6.)

The mouth surrounded by a sub-terminal oral sucker (Fig.7,o.s.) leads into a short oesophagus (Fig.7,oes.) which bifurcates immediately in front of the genital pore into long intestinal caeca (Fig.7,c.) These are irregular in diameter and extend to the posterior margins of the testes. The testes (Fig.7,t.) are situated lateral to the caeca, each is smooth on its internal face and lobed on the external face. The ovary (Fig.7,ov.) slightly lobed and smaller than the testes is situated between the intestinal caeca immediately anterior to the excretory vesicle, which has a prominent dorsal

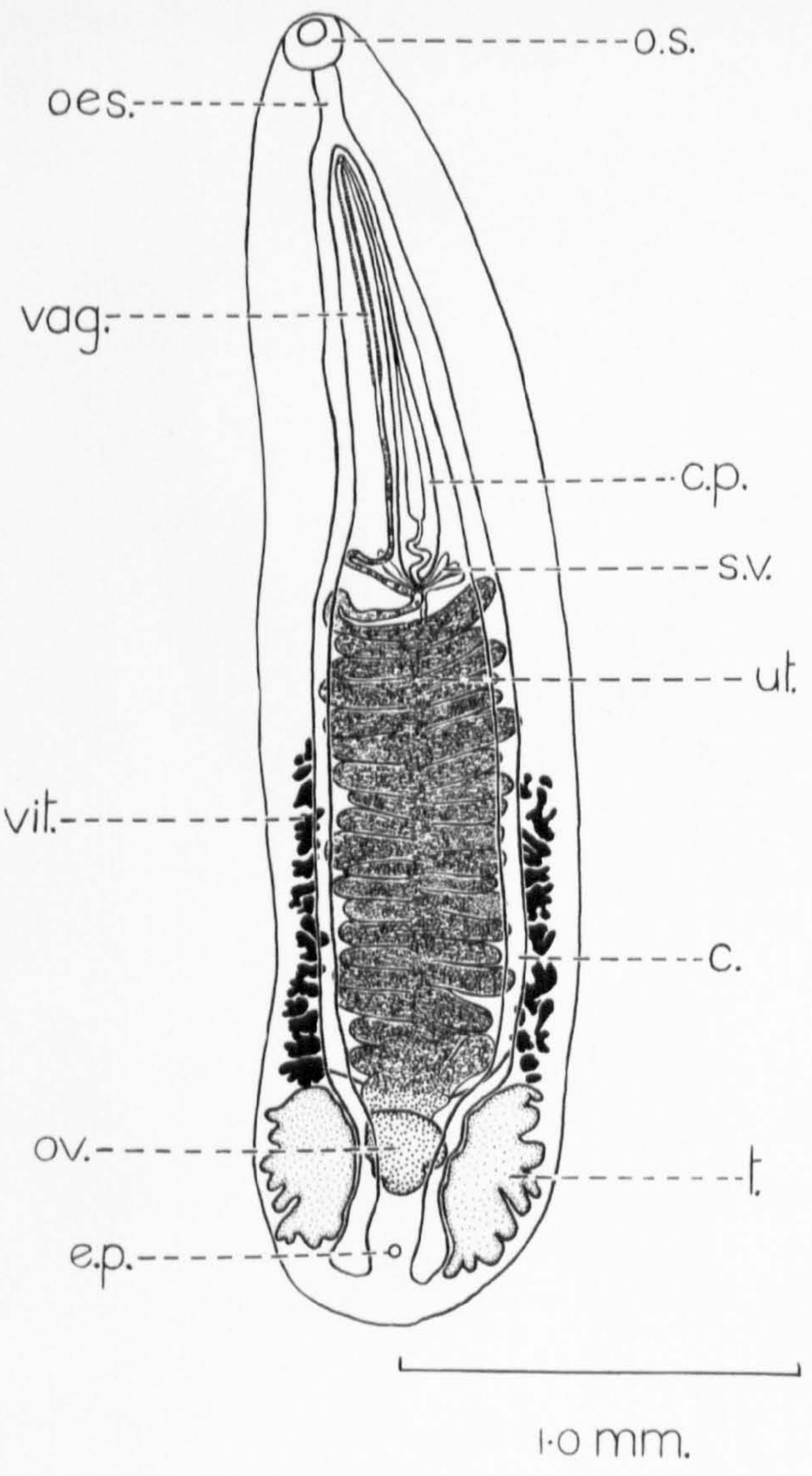


Fig. 7, Adult of Notocotylus attenuatus.

excretory pore (Fig. 7,e.p.) The vitellaria (Fig.7,vit.) consisting of lobed follicles are lateral to the caeca and extend from the anterior border of the testes to just behind the mid-body. Position of vitellaria - length of body divided by forward extent of the follicles 2.2 - 2.6 with a mean of 2.35. The uterus (Fig.7,ut.) consists of an ascending limb, intensely folded and occupies all the space between the ovary, the base of the cirrus pouch and the intestinal caeca. It then runs forward parallel with the cirrus pouch and terminates in a muscular vagina. (Fig. 7,vag.) The cirrus pouch (Fig.7,c.p.) is elongate and the seminal vesicle (Fig.7,s.v.) is convoluted. The eggs which are numerous possess long filaments at either pole.

(e) Occurrence.

Cercariae of N. attenuatus have been found in four localities.

In all cases the host has been Limnaea pereger. The localities and number of infected snails are given in Tables 1,5,6,7, and 11. At three of the localities namely, Ford - second Dam, Ibbotson Dam, and Holme Dam, mallard (Anas platyrhyncha platyrhyncha) have been observed. It has not been possible to examine any of these birds. The appearance of the cercarial stage is spasmodic, therefore, it seems that the infection of the mallard cannot be great.

Discussion.

Identification of Monostome cercariae is difficult and Rothschild (1938) listed the characters which she found useful in distinguishing species. As far as possible a description of these characters has been included here. A further character which has been found useful is that of size of the eyespots. The description of the cercaria of Notocotylus seineti given by Harper (1929) shows that it is similar to the cercaria of N. attenuatus but it was found that in the latter species the eyespots are about twice the size of those in the former species.

Baylis (1928) suggested that in the genus Notocotylus Diesing, 1839 the number of ventral glands in the adults increased in direct proportion to length. L.& U.Szidat (1933) found that the full compliment of glands were present in young specimens and this has been found to be the case in individuals examined in the present work. Even so, the number of ventral glands as was pointed out by Baylis (1936) "must be regarded as an unsatisfactory systemic character" Herber (1942) listed the characters which he utilised for the differentiation of species and he also found that the number of ventral glands " is of somewhat limited value".

(ii) Echinoparyphium recurvatum, (Linstow,1873) Lühe,1909.

The life history of Echinoparyphium recurvatum has been previously studied in this country by Harper (1929) who found the snail host to be Valvata piscinalis (Müller, (1874) whilst Rasin (1923) Bittner (1925) Mathias (1926, 1927) and Dinulesco (1936,1939) have described its development on the Continent. From the results of these workers it is evident that a variety of molluscs may act as intermediate hosts both for the cercaria and metacercarial stages.

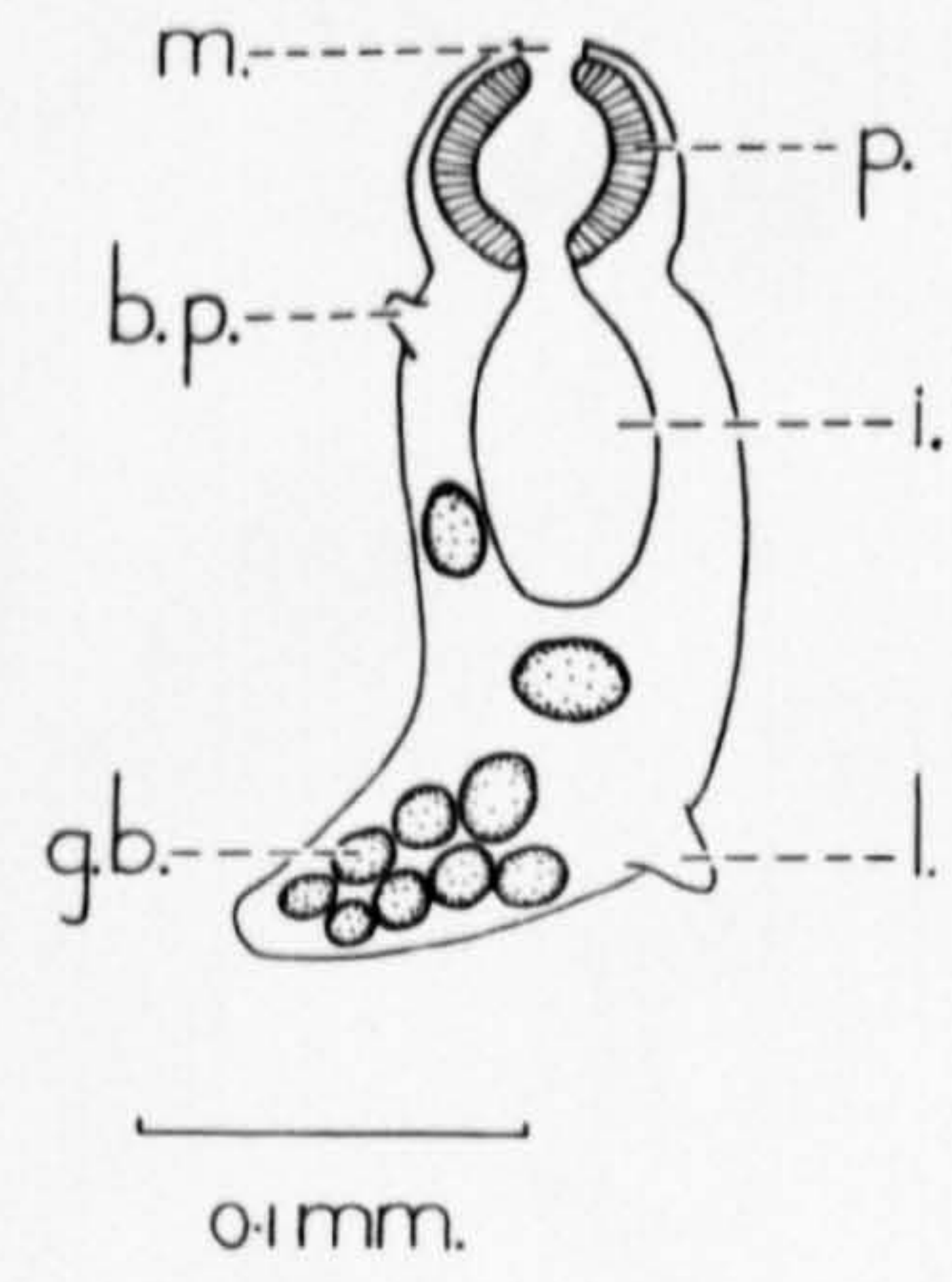
The structure of the cercaria has also been studied by Wesenburg-Lund (1934), whilst in America McCoy (1928) has described the life history of the closely related species <u>Echinoparyphium flexum</u> (Linton, 1892)

The measurements of the cercaria as given by Harper (1929) are smaller than those given by Wesenburg-Lund (1934) and those listed here. Disagreement also exists regarding the detailed anatomy of the cercaria. The descriptions and measurements of the rediae are however, in complete agreement.

(a) Redia.

	Measurements. (mm.)
Length.	0.348 - 2.4
Breadth.	0.075 - 0.232
Diameter of Pharynx.	0.06 - 0.132
Length of gut up to	0.2

The rediae occur in the liver and gonad of <u>Limnaea pereger</u>, and in a heavily infected specimen they may extend into the foot of the mollusc. They are rounded anteriorly, tapering posteriorly and possess birth pore and lappets. The rediae are transparent and mobile when young, but in older rediae the power of movement is lost, whilst they become yellow in colour, except in the region of the pharynx where the yellow pigment is but slightly developed. The mouth (Fig.9,m.) leads into a prominent pharynx (Fig.9,p.) posterior to which is a short intestinal sac (Fig.9,i.) This intestinal sac which is prominent in young rediae does not increase in length proportional to the increase in body length (Figs.8 and 9). The paired lappets (Fig.9,l.) are very variable in form, almost disappearing in some old rediae. The birth pore (Fig.9,b.p.) which lies posterior to the collar (Fig.9,co.) is constant in its position relative to the two lappets being situated in the plane midway between them.



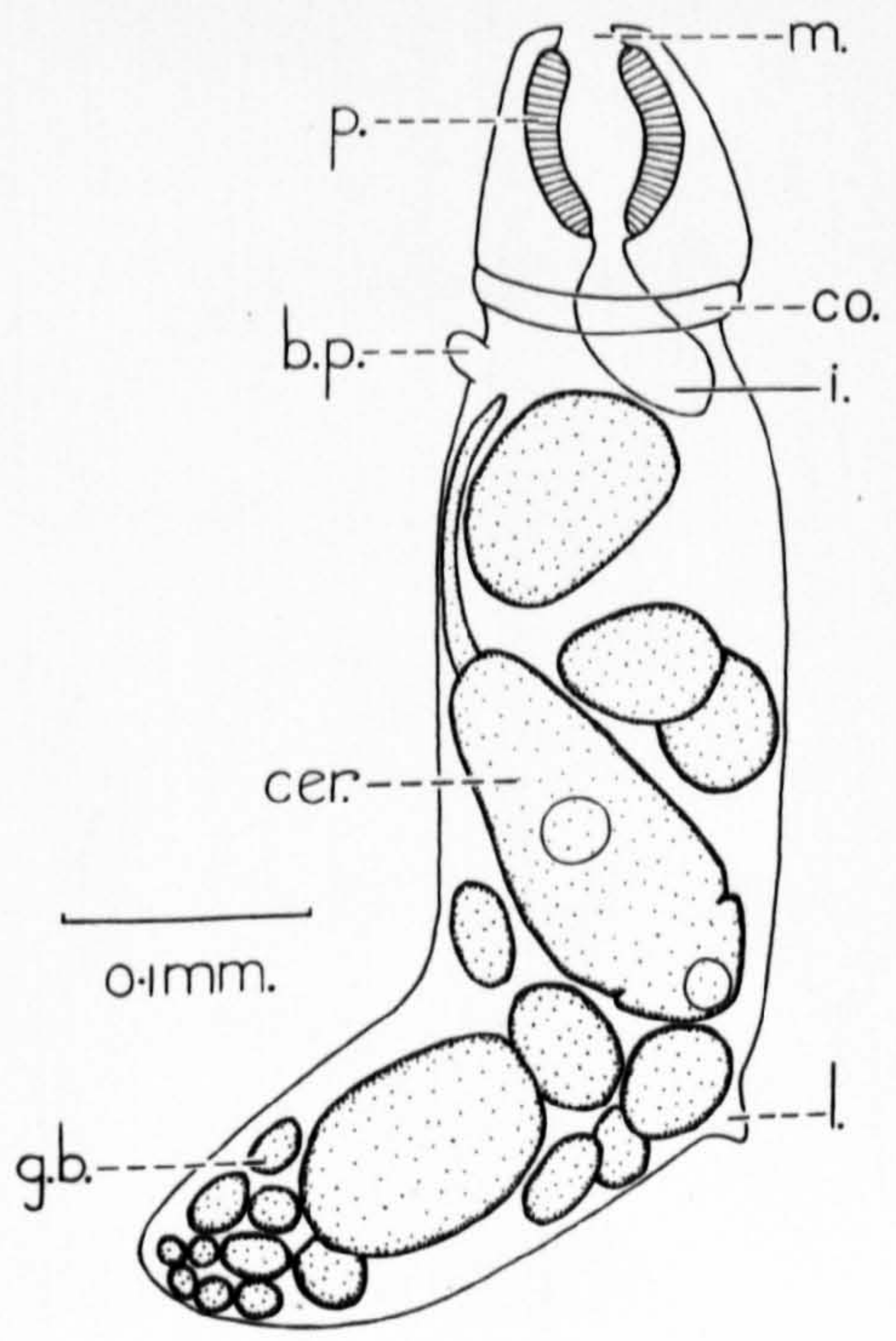


Fig. 8, Young Redia of <u>Echinoparyphium recurvatum.</u>

Fig. 9, Older Redia of <u>Echinoparyphium recurvatum</u>.

Occasionally rediae were found with transverse constrictions as have already been described for <u>Notocotylus attenuatus</u> (page 13) and by Harper (1929) for <u>Echinoparyphium recurvatum</u>. The germinal area is always located at the posterior end of the redia, and up to eight recognisable cercariae have been observed in one redia, as compared with six counted by Harper (1929) and "rarely more than six" reported by Wesenburg-Lund (1934). On three occasions encysted cercariae have been found inside a redia (one cyst in each case) an occurrence which is not uncommon in Echinostome development.

(b) Cercaria

Measurements (mm.)

		Extended	Contracted
Body	Length	0.58	0.232
	Breadth		0.197
Tail	Length	0.464	
	Breadth	0.050	

Oral Sucker		0.055
Ventral Sucker		0.066 x 0.077
Excretory Vesicle		0.044 x 0.044
Pharynx		0.022
Collar Spines	Aboral	0.012
	Oral	0.011
	Corner	0.016

The cercaria appears white to the naked eye, is oval in shape and the cuticle is spinated as far back as the ventral sucker. Anteriorly there is a 'head collar' (Fig.10,h.c.) on which are borne forty five backwardly projecting spines. These spines which at this stage are difficult to see, are arranged alternately in two rows, oral (Fig.10,o.sp.) and aboral (Fig.10,a,sp,) the spines in the aboral row being the larger. The collar is incomplete ventrally and where it terminates on either side of the body it bears four large corner spines (Fig.10,c.sp.)

The ventral sucker (Fig.10,v.s.) larger and much more powerful than the oral is situated just inside the posterior half of the body, which except in the region of the oral sucker is packed with cystogenous cells. The tail is simple, muscular, and when extended is about the same length as the body.

The mouth surrounded by the oral sucker (Fig.10,o.s.) leads into a small prepharynx (Fig.10,p.p.) This opens into a globular pharynx (Fig.10,p.) followed by a long oesophagus (Fig.10,oes.) The oesophagus bifurcates immediately anterior to the ventral sucker into two intestinal caeca (Fig.10,c.) which terminate at the level of the excretory vesicle. The intestine and oesophagus appear solid and are difficult to distinguish from the mass of cystogenous cells.

The excretory system consists of a prominent excretory vesicle (Fig.10,e.v.) situated at the posterior end of the body, and it may be divided for descriptive purposes into two parts; a posterior cubical part, and an anterior bilobed part, each lobe giving rise to a main lateral canal (Fig.10,m.l.c.) Each main lateral canal follows a sinous course anteriorly, lateral to the ventral sucker, and extends

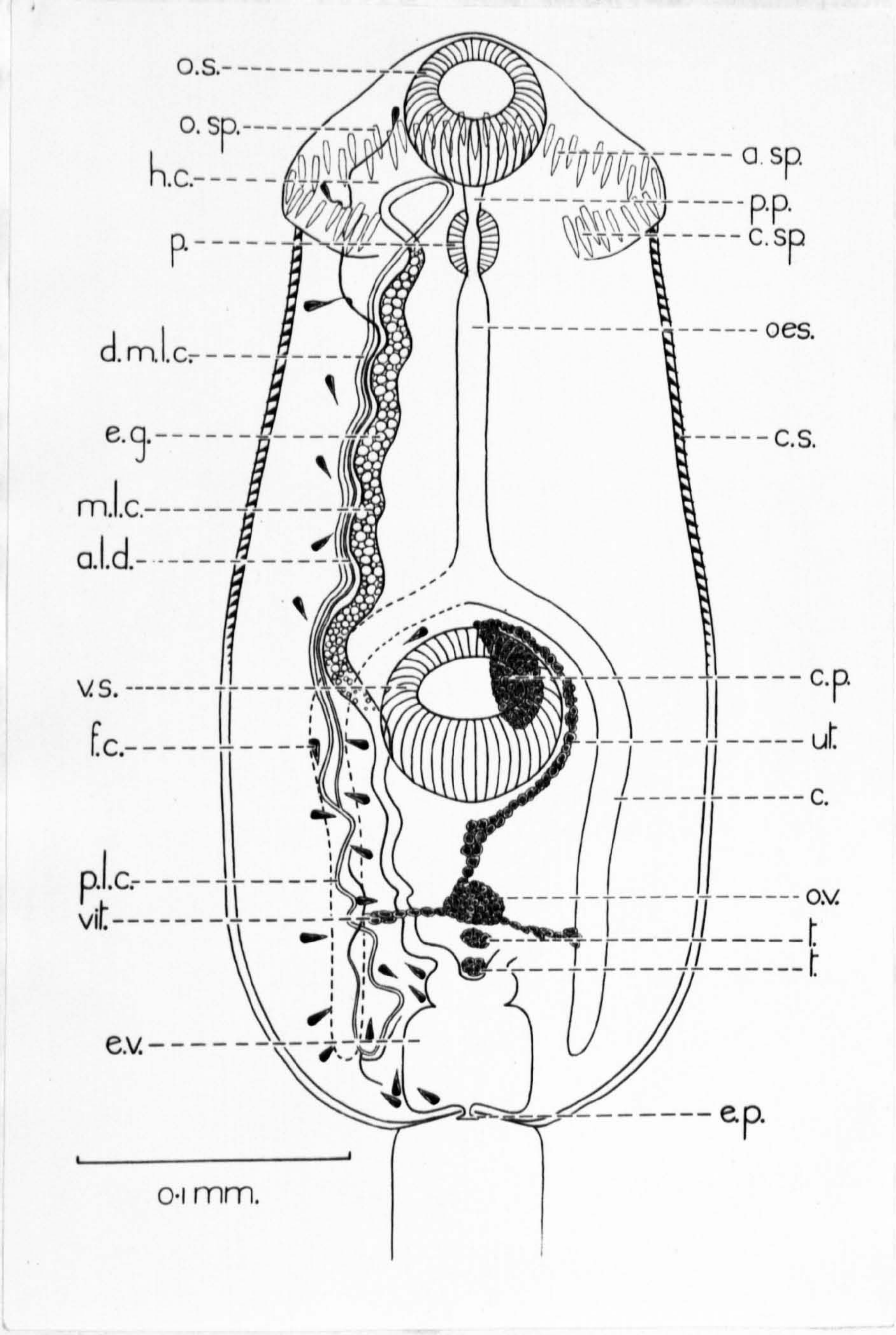


Fig.10, The Cercaria of Echinoparyphium recurvatum.

as far forwards as the posterior edge of the oral sucker where it loops back and as a descending lateral canal (Fig.10,d.m.l.c.) extends posteriorly parallel with the main lateral canal until it reaches the level of the posterior edge of the ventral sucker. Here the descending lateral canal divides into two, giving rise to an antero- and a postero-lateral duct (Fig.10,a.l.d.,p.l.d.) The ascending portion of the main lateral canal from the level of the ventral sucker to the collar is of large diameter and contains excretory granules (Fig.10,e.g.)

Each antero-lateral duct extends forwards lateral to, and parallel with, the main lateral **canal**, and terminates in a single flame cell, which is situated at the side of the oral sucker. It also receives the capillaries of at least seven other flame cells, the precise junctions not being observed.

Each postero-lateral duct extends as far backwards as the termination of the intestinal caeca where it gives off a branch which immediately divides into two. It then turns and leads forwards extending almost as far as the junction of the antero- and postero-lateral ducts with the descending lateral canal. In the posterior half of the body there are sixteen flame cells on each side, thus making twenty four for each side, and a complete total of forty eight. The excretory vesicle opens by an excretory pore (Fig.10,e.p.) into the caudal pocket.

The above description of the excretory system agrees with the observations of Wesenburg-Lund (1934) on Echinoparyphium recurvatum and McCoy (1928) on Echinoparyphium flexum, although both these workers only studied the course of the main vessels. It does not agree with the findings of Harper (1929) who found that the descending portion of the main lateral canal extends to the posterior end of the body and then returns to the anterior end.

All three workers show an excretory tube in the tail and according to Wesenburg-Lund and McCoy,the tube forks part way down the tail into two lateral branches which open to the exterior. A similar structure to this has been observed in the present work, but it appeared to consist of connective tissue. Observation failed to show any excretory function, even at the time when the vesicle was contracting. It is interesting to record the remarkable similarity between the excretory system of the cercaria of Echinoparyphium recurvatum as given here with that of the cercaria of Echinostomum revolutum (Frolich,1802) Looss, 1899 recorded by Johnson (1920).

The genital system present in the cercaria consists of a large mass of cells situated midway between the ventral sucker and the excretory vesicle which is the anlage of the ovary (Fig.10,ov.) together with the seminal receptacle. The vitelline ducts (Fig.10,vit.) are present as a string of cells which extend laterally from the posterior edge of this large mass of cells. Another string of cells - the anlage of the uterus (Fig.10,ut.) extends anteriorly from this same mass and passes to the left of the ventral sucker, where it joins the cirrus pouch (Fig.10,c.p.) which is present as a group of cells extending backwards dorsal to the left of the ventral sucker. The two testes (Fig.10,t.) are represented by two small groups of cells, one behind the other in the mid-line immediately anterior to the excretory vesicle.

The cercariae are liberated during the day and have a free life of less than twenty four hours, spending most of this time swimming round the bottom of the containing vessel. When swimming the anterior half of the body is bent ventralwards towards the tail, and the cercaria swims body forwards, usually with the ventral side uppermost. The tail during swimming is kept nearly perpendicular to its point of attachment with the body. At intervals swimming activity ceases, the body elongates, and the oral sucker is applied to the substratum. Such movement facilitates the location of the second intermediate host.

(c) Cyst.

The cercaria encysts in molluscs as was also found by Harper (1929) Mathias (1926) and Dinulesco (1939). It would not encyst in tadpoles as recorded by Bittner (1925) Metacercariae have been obtained experimentally from Planorbis planorbis and both experimentally and naturally from Limnaea pereger and Sphaerium corneum. In heavily infected molluscs it was observed that there **were** always a few cysts in which the fluke inside was dead; the proportion of these non-viable cysts was higher in S.corneum than in L.pereger particularly when the host had been kept some time in an aquarium. It seems likely therefore, that while S.corneum can act as a host for the metacercaria, it is not as favourable as L. pereger.

In the localities where the cercaria was found L. pereger is undoubtedly the most important host, the cysts being located in the tissues surrounding the mantle cavity and occasionally in the liver.

Much work on the encystment and viability of the metacercaria of Echinoparyphium recurvatum is given by Dinulesco (1939). No attempt to reproduce his results has been made here.

The cyst itself is spherical or nearly so, with a diameter of 0.158 mm. (0.137 - 0.193 mm.) compared with Harper's measurement of 0.11 - 0.13 mm. - although his reading neglected the thickness of the cyst wall which is given separately as 0.015 mm. The cyst wall consists of two layers, an outer transparent layer (Fig.11, o.t.l.) and an inner slightly laminated layer (Fig.11, i.l.l.) which is very resistant.

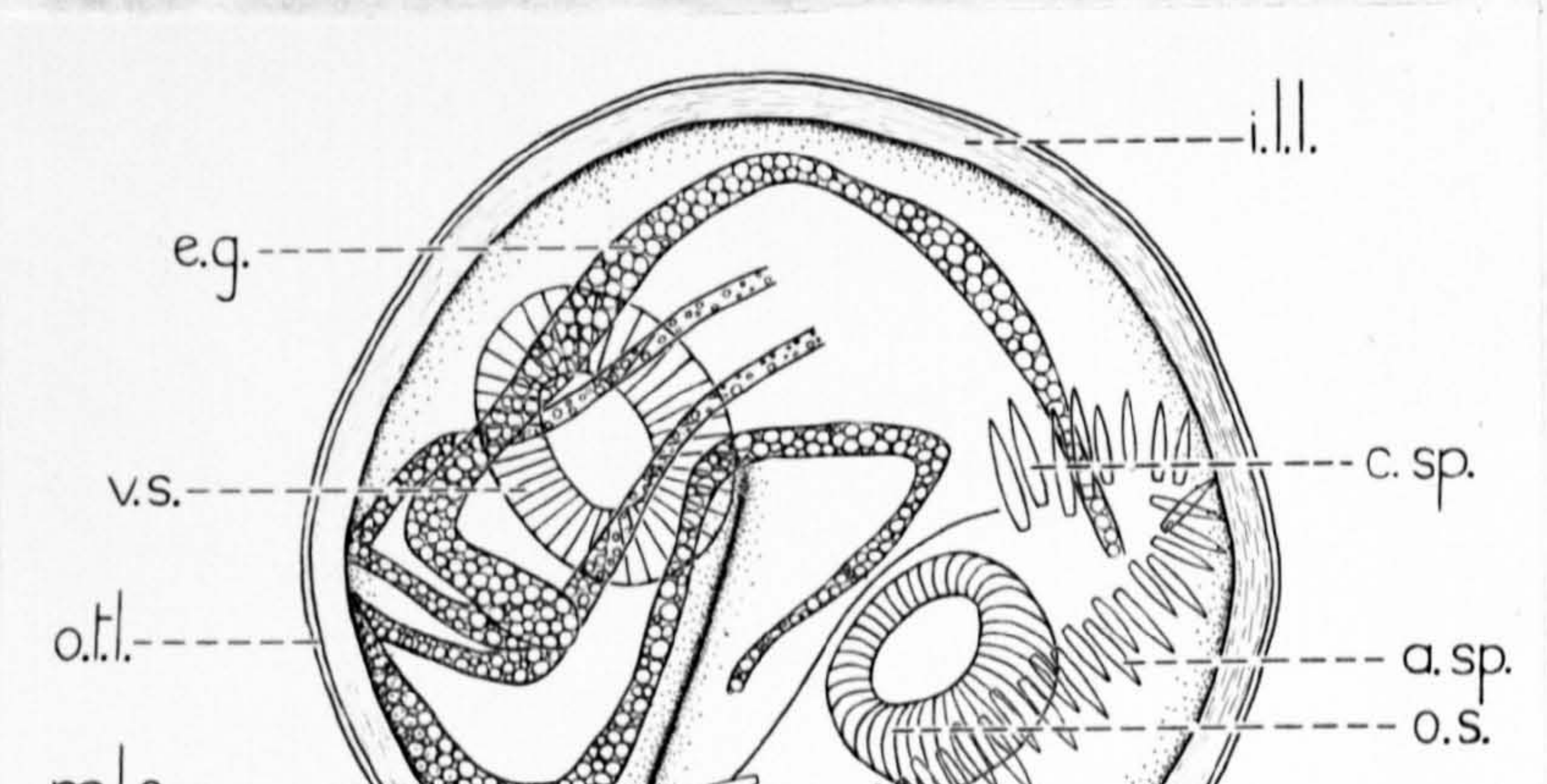


Fig.11, The Cyst of Echinoparyphium recurvatum

The contained metacercaria is not active, and is considerably folded up inside the cyst, this folding being much greater in extent than that observed in other metacercariae. Considerable changes occur in the metacercaria, the spines on the 'head collar' enlarge, become prominent and easy to count, whilst the cuticular spines likewise increase in size.

Measurements of the collar spines in seventeen day-old metacercariae gave the following readings.

Oral Spines	0.013 - 0.014 mm.
Aboral Spines	0.015 - 0.018 mm.
Corner Spines	0.020 - 0.022 mm.

The excretory granules which in the cercaria occur only in the main lateral canal anterior to the ventral sucker now extend throughout the entire length of this canal, several small excretory granules usually being seen in the excretory vesicle. Little further development of the genital system was observed in the few metacercariae that were successfully removed from their cysts.

Dinulesco (1939) found that the cercaria encysted in the same molluscan host as that in which it developed, and not in other individuals of the same species. Whilst this does occur to a great extent, it is evident from dissections that cercariae do encyst in molluscs of the same species which contain no rediae. This is to be expected since the cercaria is a good swimmer, a fact which was found to be generally true of all Echinostome cercariae by Wesenburg-Lund (1934)

(d) Juvenile and Adult.

The adult stage was obtained experimentally from ducks by Harper (1929) and Mathias (1926) and from mice by Dinulesco (1939). In addition it has been reported from a number of other birds. Disagreement exists as to the location of the adult, Mathias reporting it in the anterior part of the intestine, whilst Harper (1929) gives its location as the large intestine.

In the present work the life cycle has been completed experimentally in duck and pigeon (Columba livia Gmelin) and in all cases the adult was located in the anterior part of the intestine. In the pigeon they were concentrated in the distal loop of the duodenum, only a few being found in front of, and behind this region.

Dissection of birds, 1,3,10,14 and 37 days after infection shows that the flukes develop quickly, ten day old flukes being mature, the rate of development being similar to that of Notocotylus attenuatus (see page 21) and Echinoparyphium flexum McCoy (1928) and compares favourably with the time given by Mathias (1926) and Harper (1929) for the same species. Like Notocotylus attenuatus growth continues for some time after the fluke is sexually mature. This can be seen from the range of measurements given below.

Measurements of Fixed Sexually Mature Specimens.(mm.)

Length.	2.4 - 4.5
Breadth.	0.40 - 0.66
Oral Sucker.	0.07 - 0.14
Ventral Sucker.	0.25 - 0.40
Cirrus Pouch.	0.29 - 0.12 - 0.174
Collar.	0.29 - 0.37
Pharynx.	0.077 - 0.098 x 0.066 - 0.087
Eggs.	0.088 - 0.121 x 0.060 - 0.066
Oral Spines.	0.046 - 0.060
Aboral Spines.	0.051 - 0.068
Corner Spines.	0.068 - 0.077

The adult of Echinoparyphium recurvatum is white in colour and exhibits a remarkable bending over of the narrower anterior third of the body, on the stouter hind-body. Because of this flexure it proved difficult to obtain good permanent preparations. Prolonged **vigorous** shaking during fixation succeeded in relaxing the body although many of the collar spines were lost in the process.

The cuticle is spinous as far back as the ventral sucker which is large and powerful and occupies the position of fulcrum in relation to the anterior flexure. The collar is less prominent than in the immature stages, this being due to the great development that occurs in the hind-body. The forty five spines which the collar bears have the same relationship that is present in the cercarial and metacercarial stages, although the corner spines appear

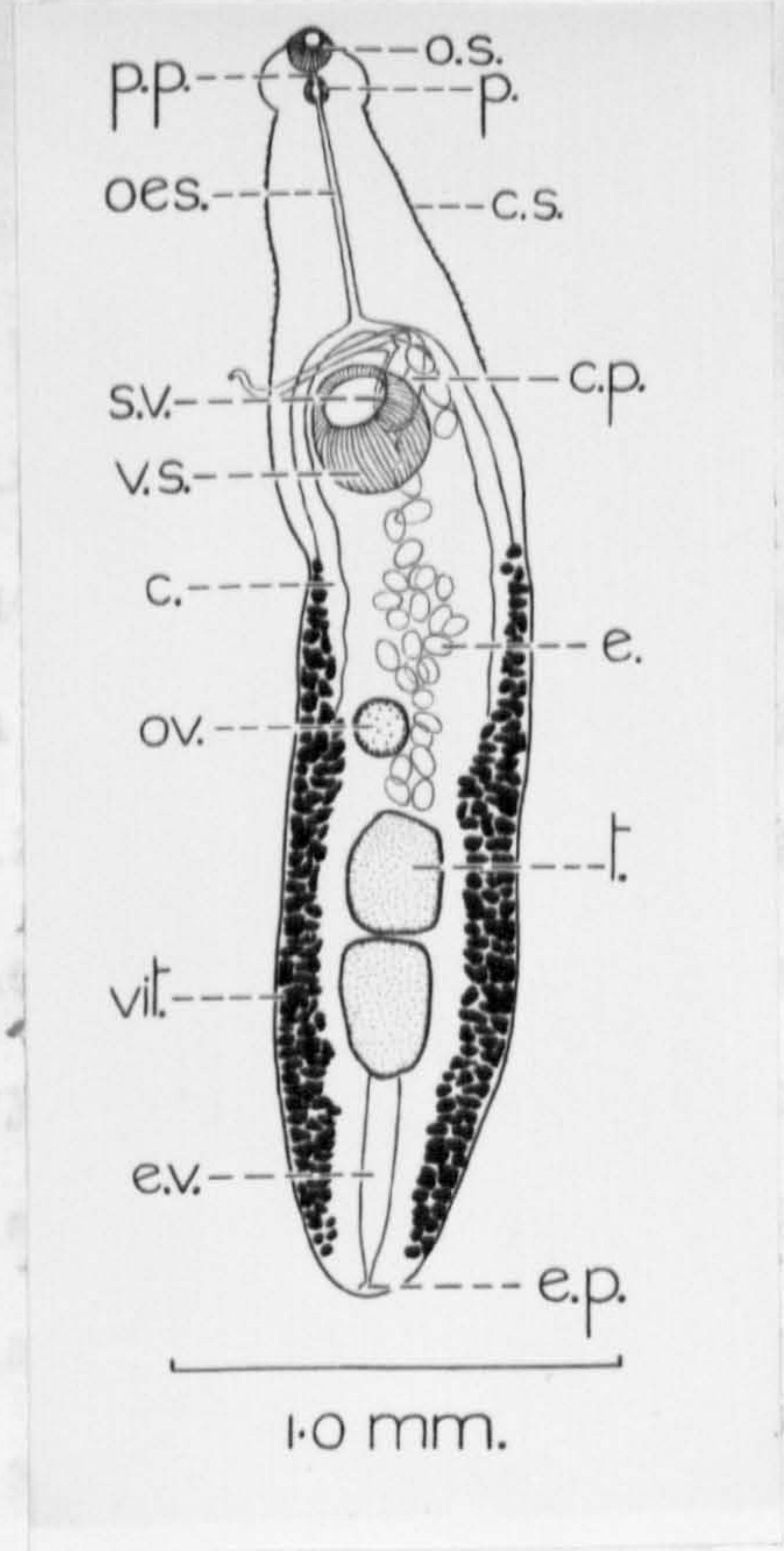


Fig. 12, Adult of Echinoparyphium recurvatum.

crowded together in the adult. It will be seen from the measurements that differences in the size of the various types of spine are not great. This is due primarily to the fact that the measurements are of large as well as small adults. There is however, a further factor which has been observed, namely, that the size of all the oral and all the aboral spines in any given specimen are not constant. The greatest difference in size is to be found in the mid-line, laterally the sizes tend to co-incide, those of the oral increasing and those of the aboral decreasing in size.

The mouth is surrounded by a small oral sucker (Fig.12,o.s.) and leads almost directly into the pharynx, (Fig.12,p.) the prepharynx (Fig. 12,pp.) usually being indistinguishable. As in the cercaria there is a long oesophagus (Fig.12,oes.) which divides immediately in front of the ventral sucker into long intestinal caeca (Fig.12,c.)

The ovary (Fig.12,ov.) is small and round and situated slightly on the right of the mid-line, well behind the ventral sucker. Postero-medial to it is the large seminal receptacle which lies immediately in front of two large oval testes (Fig.12,t.) situated behind the other in the mid-line.

The laterally placed vitelline glands (Fig.12,vit.) extend from the posterior end of the body to just behind the ventral sucker, follicles occurring both above and below the intestinal caeca. The uterus containing a few large eggs consists of an ascending portion only, and passes to the left of the ventral sucker to open at the genital pore. The large cirrus pouch (Fig.12,c.p.) containing a large seminal vesicle (Fig.12,s.v.) pars prostatica, and cirrus is also situated to the left, dorsal to the ventral sucker.

The excretory system of the adult was not studied, but the large tubular excretory vesicle (Fig.12,e.v.) lying dorsal to the testes is evident, whilst branching outgrowths from the main lateral canals were seen in one day old specimens.

(e) Occurrence.

Cercariae of *Echinoparyphium recurvatum* have been found in all the dams on the River Porter where the snail host *Limnaea pereger* occurred; at Ford and at Monsaldale. The infection rate of the snails (see Tables, 1, 2, 3, 4, 5, 6, 7, 12, 13 and 14) varied considerably in different localities and from season to season. This is to be expected since the definitive host is a bird, the suspected species being mallard, which is known to be a host of *Echinoparyphium recurvatum*. In the Porter valley these birds were always to be found at Nether Spur Wear and Holme Dams. Their constant occurrence here no doubt accounts for the relatively high infection rate of snails obtained from Holme Dam. Occasionally mallard were seen on the other Dams of the Porter River, particularly during the breeding season, when they tended to separate in pairs. These infrequent visits would account for the low irregular infection rates of the snails in these Dams.

At Holme Dam the infection rate was at its highest during September and October 1949, a time when there are few snails containing the parthenita of other species. This high infection rate remained at a relatively high level throughout the winter, decreased in spring, followed by a marked rise in September and October. These infected snails however, were of the daughter generation.

The survey at Ford included the number of snails containing cysts of Echinoparyphium recurvatum as well as those infected with redial stages (Table 7). It was found that the incidence of cysts was much greater than rediae and that the presence of cysts cause no apparent harm to the snail host Limnaea pereger. The state of the Dam during 1949 facilitated the cercaria locating the second intermediate host, since in the summer of that year the water flow became almost non-existent. Newly hatched snails were abundant during the following autumn.

(f) Discussion.

The extent of the differences in the structure and life history of Echinoparyphium recurvatum as reported by Harper (1929) and given here, has already been listed, and it must be concluded that superficially the two descriptions are not compatible. Analysis of the disagreements however, reveals that the differences may be partially reconciled. Measurements of the cercaria given by Harper (1929) are all proportionately smaller; measurements of the cercaria under varying pressures would give similar differences.

Although the difference between the two descriptions of the excretory system is great, such a difference can be caused by incorrect determination of one branch, namely, the antero-lateral duct. Disagreement regarding the number of flame cells indeed, regarding the whole excretory system in Echinostome cercariae is inevitable, owing to the extreme difficulty of elucidating large numbers of flame cells and capillaries amidst dense cystogenous cells. Thus the only irreconcilable difference is that regarding the location of the adult in the definitive host. Both Linstow (1873) and Mathias (1926) give the location as the intestine, as is given here.

The description of the redia, cercaria, cyst, and intermediate host of Cercaria Z Rees, 1932 shows a remarkable similarity with those of Echinoparyphium recurvatum as included here, even with regard to the detailed structure of the excretory system. It is obvious that the two species are closely related, and may well be synonymous.

(iii) Dolichosaccus rastellus (Olsson, 1876) Travassos, 1930.

The life history of Dolichosaccus rastellus was first described by Linstow (1884, 1887, 1896). The cercaria was reported as Cercaria limnaeae ovatae Linstow, 1884, and was shown to encyst in species of Trichoptera. From frogs infected with these cysts the adult was obtained. Joyeux & Baer (1927) also studied the life history obtaining cysts in different species of tadpoles, but not in any other animals. Adult frogs were infected by eating tadpoles containing encysted cercariae. Cercaria limnaeae ovatae was described by Wesenburg-Lund (1934) who showed that cercaria readily penetrated and encysted in corethra larvae.

(a) Sporocyst.

Measurements (mm.)

0.986 x 0.232 - 2.49 x 0.377

The sporocysts occur in the liver of Limnaea pereger and Limnaea stagnalis, and are white in colour when young, but become faintly yellow as they mature. The cuticle also becomes covered with scattered patches of black pigment as has been reported by Rees (1932) for Cercaria cambrensis I Wright, 1927. They are elongate, cylindrical in shape and are bluntly rounded at both ends. No powers of movement except that caused by contained cercariae, and no birth pores have been observed in the sporocysts which form a compact tangled mass with the liver tissue. Consequently the sporocysts in heavy infections are difficult to separate, always being twisted round each other. As many as twenty five recognisable cercariae in addition to many germ balls have been counted in mature sporocysts. Maturing cercariae may be found in all parts of the sporocyst.

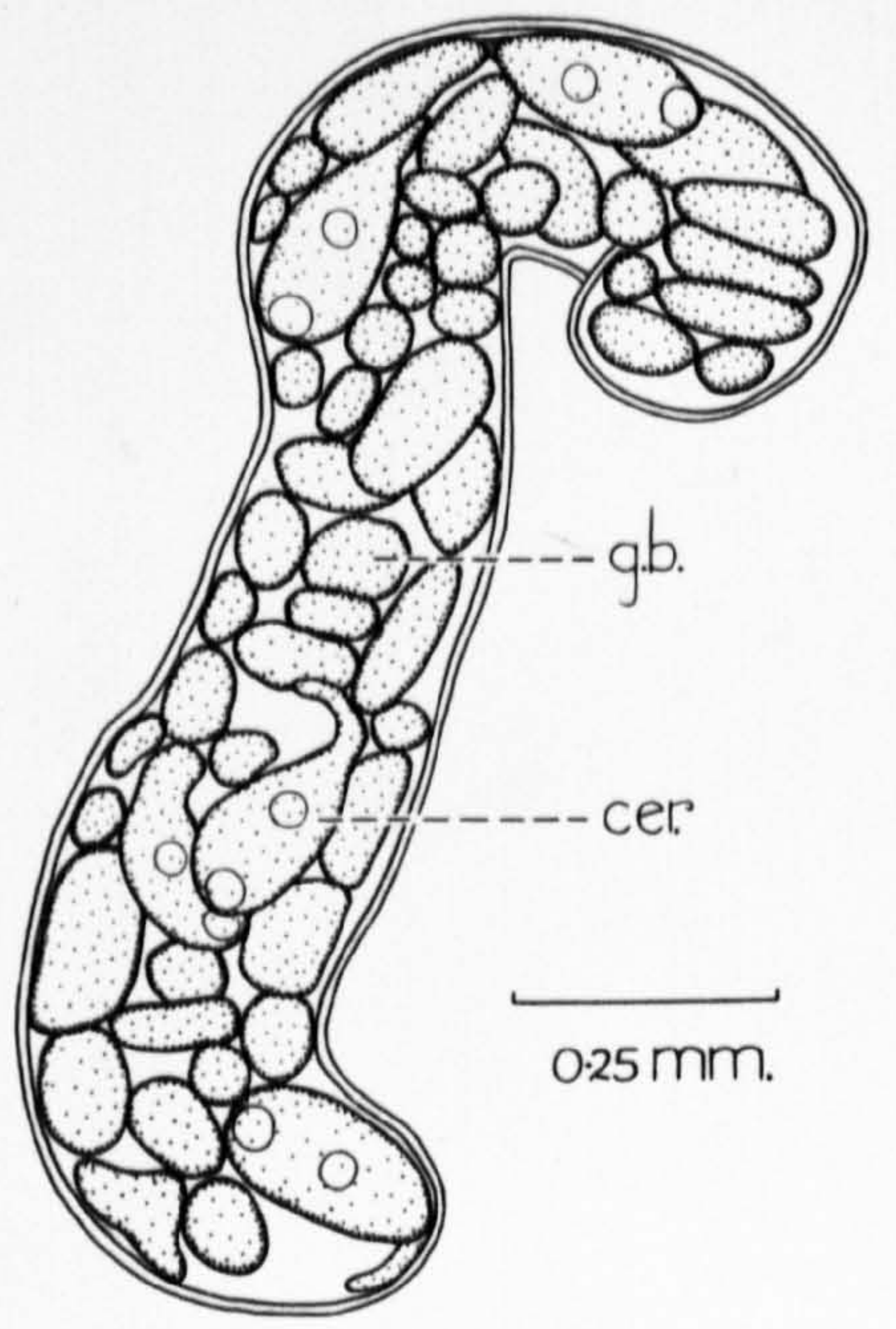


Fig. 13, Sporocyst of Dolichosaccus rastellus.

(b) Cercaria.

Measurements (mm.)

	Extended	Contracted.
Body Length.	0.488	0.232
Breadth.	0.094	0.197
Tail Length.	0.348	0.13
Oral Sucker.	0.071 - 0.084	
Ventral Sucker.	0.068 - 0.077	
Stylet.	0.033	

The cercaria is oval in shape, very contractile, having its maximum breadth immediately anterior to the ventral sucker, which is situated just behind the middle of the body. The oral sucker (Fig.14,o.s.) is larger than the ventral sucker (Fig. 14,v.s.) although during creeping movements the ventral sucker attains the same size as the oral when it is applied to the substratum. The cuticle is covered with transverse rows of small backwardly directed spines (Fig. 14,c.s.) producing a striated appearance. These spines become smaller posteriorly and cannot be seen in the hind quarter of the body. In addition to these small spines there are found ten or eleven pairs of long sensory hairs (Fig.14,s.h.) similar to those that have been decribed by Rees (1932) for Cercaria cambrensis I and Cercaria cambrensis III Rees, 1932 and by Sinitsîn (1905) for Cercaria secunda Sinitsîn, 1905 and Cercaria gibba Sinitsîn, 1906, nec Filippi, 1854 = Cercaria tenuispina Lühe, 1909. The cuticle which covers the body is intucked posteriorly to form a postero-ventral caudal pocket in which the simple tail is set. The cuticle of the caudal pocket is slightly thickened and on its lateral margins bears cuticular spines. (Fig.14,c.s.) which are directed into the base of the tail.

A stylet (Fig. 14,s.) measuring 0.033 x 0.005 mm. is inserted into the dorsal lip of the oral sucker. It is pointed anteriorly and bears a ring-like thickening of diameter 0.008 mm. one third of the length from the anterior tip. Behind this

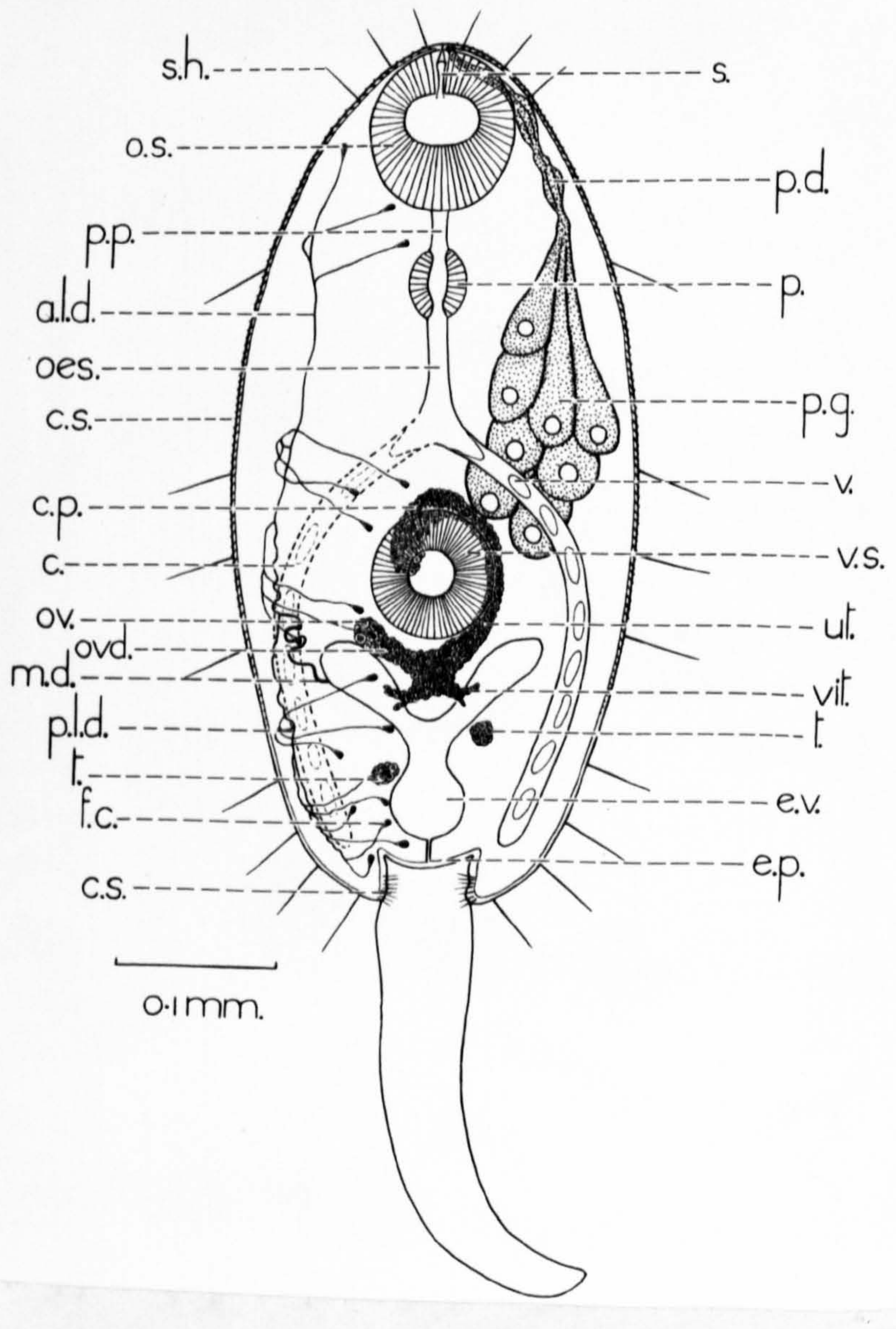


Fig. 14, The Cercaria of Dolichosaccus rastellus.

thickening the stylet becomes slender until the posterior end is reached, when a further small thickening is evident. The stylet is hollow and this cavity is not sealed off at the posterior end.

The sub-terminal mouth is surrounded by the oral sucker and leads into a short but distinct prepharynx (Fig.14,p.p.) There is a prominent pharynx(Fig.14,p.) and the oesphagus (Fig. 14,oes.) bifurcates a short distance in front of the ventral sucker into long intestinal caeca (Fig. 14,c.) which extend almost to the posterior end of the body. The caeca in the cercaria have no lumen except for some five to eight vacuoles. According to Joyeux & Baer (1927) and Wesenburg-Lund (1934) the intestinal caeca are much shorter than this, although Lühe (1909) records them as reaching the posterior end of the body.

The penetration gland cells (Fig.14,p.g.) are found in the dorsal part of the body, antero-lateral to the ventral sucker,most of them lying lateral to the oesophagus. They consist of at least eight pairs of cells and their ducts run forward to open at the tip of the stylet. Lühe (1909) made no mention of the penetration gland cells, whilst Wesenburg-Lund (1934) reported that "there are at all events five penetration glands". Joyeux & Baer (1927) give the number as four pairs.

The excretory vesicle (Fig.14,e.v.) is bicornuate, the two anterior arms extending almost to the level of the posterior edge of the ventral sucker. The vesicle opens by a narrow duct into the dorsal part of the caudal pocket. From the posterior edge of each anterior arm a coiled main collecting duct (Fig.14,m.d.) extends to the level of the ventral sucker where it divides into antero-lateral and postero-lateral ducts. (Fig.14,a.l.d.,p.l.d.) Each of these ducts receives capillaries from three sets of three flame cells. Thus there are eighteen flame cells on each side of the body, and the flame cell formula is $2\left[(3+3+3)+(3+3+3)\right] = 36$. Of these thirty six cells, twenty four are located behind the ventral sucker, thus, an adequate excretory system is present to serve the genital tadpoles of *Rana temporaria temporaria* and *Bufo bufo bufo* (Linnaeus,1758). The cercaria has been observed to penetrate into the above named insects, the cysts being most frequently found attached to the malpighian tubules and hepatic caeca, and occasionally to the fat body. Not all the insects are penetrated with the same facility. It was found that Sialis lutaria was the easiest to infect and *Phryganea grandis* the most difficult.

The cercariae do not show any taxis towards an insect when it is introduced into the containing vessel, but merely make chance contact. Once contact has been made the cercariae creep over the body of the insect and attempt to penetrate between the segments of the exoskeleton. This is not easily accomplished in any of the named insects. Success is more assured however, if penetration is attempted in groups, the occurrence of which was frequently observed experimentally.

Cercariae have not been observed to penetrate into tadpoles, although many have been observed creeping on them. This is in marked contrast to the findings of Joyeux & Baer (1928) who describe the cercariae as actively penetrating into tadpoles. Infection of tadpoles occurs by cercariae creeping into the mouth, or being sucked into the mouth with the respiratory current. The region of encystment is influenced to some extent by the stage of development of the tadpole. This has been closely studied in tadpoles *Rana temporaria temporaria* and it has been found that in young tadpoles in which the mouth has just formed, the majority of cysts are recovered from the soft tissues surrounding the buccal cavity. In older tadpoles most of the cysts are found in the lungs, whilst in tadpoles infected immediately before metamorphosis the cysts are again found in the tissues surrounding the buccal cavity. Cysts have been found in other parts of the body - attached to the mesentery, on the gut wall, on the surface of the kidneys, and in the soft tissues around the heart. The occurrence of these cysts - always few in number - appears to be independent of the stage of development reached by the tadpole.

organs when they develop.

No part of the genital system is visible in the living cercaria, but stained specimens show that it is well developed. The ovary (Fig.14,ov.) is situated on the right immediately behind the posterior border of the ventral sucker, and is attached to a median mass of cells (Fig.14,ovd.) which represents the oviduct. Posteriorly the median mass of cells is elaborated into two lateral projections on either side (Fig.14,vit.) these are the anlage of the vitelline glands. The uterus (Fig.14,ut.) leads forwards from the median mass of cells round the left border of the ventral sucker. Anterior to the ventral sucker it is connected to the anlage of the cirrus pouch.(Fig.14,c.p.) which extends posteriorly above the right side of the ventral sucker, and terminates at the level of the centre of the ventral sucker. Two testes (Fig.14,t.) are present, each consisting of a small group of cells, the anterior one situated to the left of the excretory vesicle, the posterior one to the right.

The cercariae are liberated during the hours of darkness and have a free life of up to sixty four hours. Each swims with a jerky motion as has been reported by Cort (1915) for *Cercaria isocotylea* Cort,1915, and spends most of its time swimming up to the surface of the water, it then ceases to swim and sinks to the bottom of the containing vessel. In this respect it is like many Furcocercariae, but the cercaria of *Dolichosaccus rastellus* sinks at a much faster rate. After some forty two hours it appears incapable of swimming upwards, but continues to swim round the bottom of the vessel. This is followed by creeping, decaudation and finally death. Frequently dead cercariae have been found in which decaudation had not taken place.

(c) Cyst.

Cysts have been obtained experimentally from *Anabolia nervosa* (Curtis,1834) *Sialis lutaria* (Linnaeus,1758) and *Phryganea grandis* Linnaeus,1758 and both experimentally and naturally from *Limnophilus rhombicus* (Linnaeus,1758) and

Photomicrograph of Transverse Section of Frog Tadpole Showing Six Cysts In the Lungs and one Cercaria in the Oesphagus.

In the developing tadpole at the time when the mouth opens to the exterior, it is found that a group of cells known as the oesphageal plug seals off the oesophagus immediately anterior to the position of the lung buds (Marshall, 1893). At this stage the lungs are very small and access to them is prevented by the oesophageal plug, consequently most of the cercariae encyst in the region round the mouth. Later this plug breaks down so that access to the lungs which are now much larger is not prevented, and it is during this period that the cercariae encyst in the lungs. It was found that tadpoles naturally infected acquire most of their infection at this time. Development of the lungs continues and before metamorphosis they become functional. To do so they must have an effective closing mechanism and it is this which is responsible for the change in location of the cysts back to the tissues surrounding the buccal cavity. The

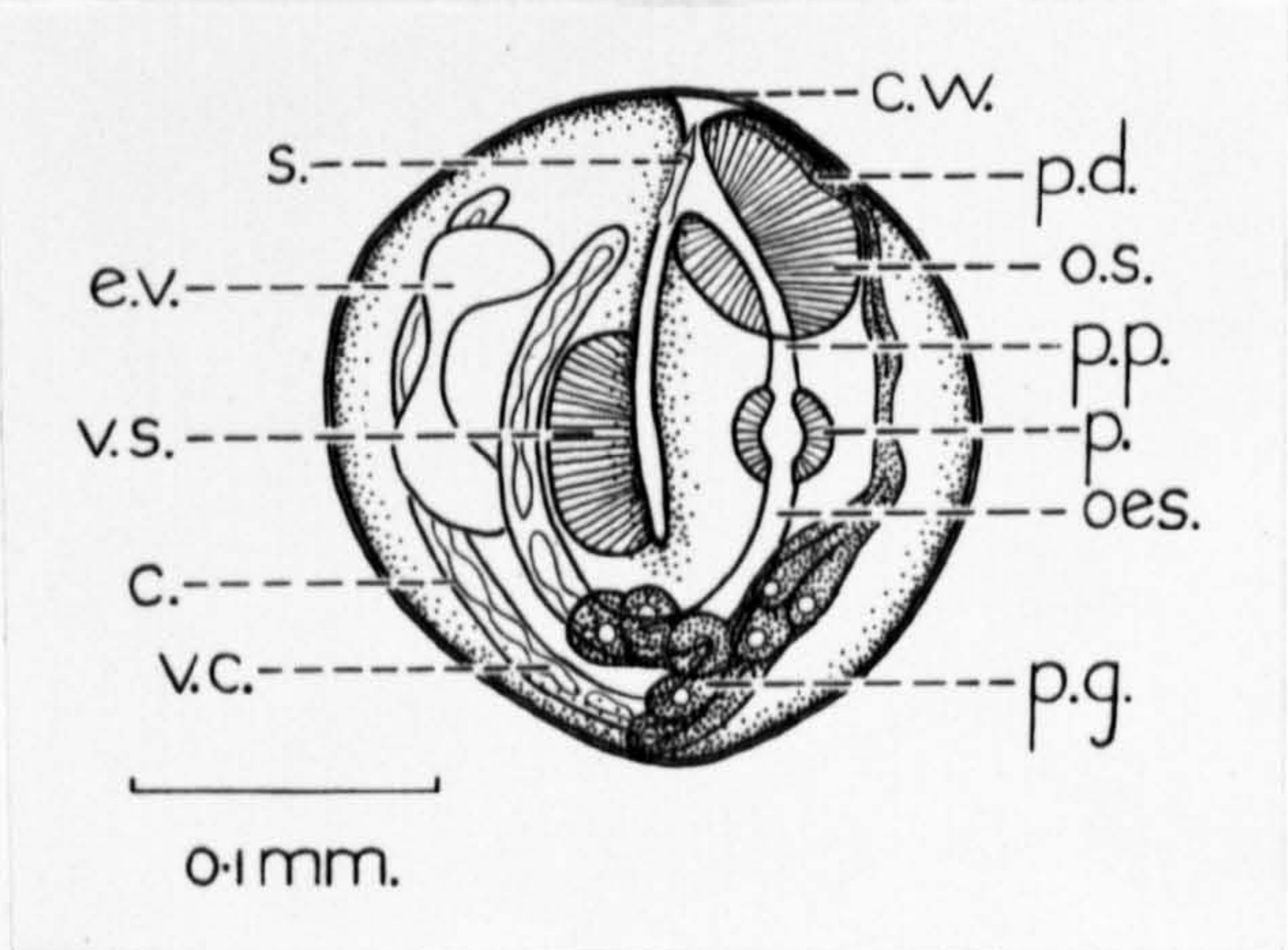


Fig. 15, Cyst of Dolichosaccus rastellus.

cyst is spherical or nearly so, and has a diameter of 0.18 - 0.22 mm. the cyst wall being transparent, thin and very easily ruptured. The stylet is shed after encystment and is always found free inside the cyst. Changes which occur to the metacercaria depend on whether encystment has occurred in an insect or tadpole. Little change occurs to the metacercaria in the former, but in the latter the vacuoles in the intestinal caeca enlarge and coalesce, as is shown in Fig. 15, a difference occurring five hours after encystment.

In all metacercariae excretory granules develop in the excretory vesicle, which in cysts of some age is the most striking feature. Little development of the genital system occurs.

(d) Juvenile and Adult.

The use of an insect as an intermediate host by a metacercaria which has frog as its definitive host is readily understandable, but the use of tadpoles as such is unusual. Joyneux & Baer (1927) concluded that frogs are infected by eating tadpoles containing metacercariae, a conclusion which Dawes (1946) finds surprising. In the experiments close attention was therefore, made to the part played by tadpoles in the life history, and it was found that tadpoles do not act as intermediate hosts, but that they are the definitive hosts, thus, the life cycle is shortened and the cercaria enters its final host directly.

Dissection of infected tadpoles of Rana temporaria temporaria shows that the encysted metacercariae excyst from four to eight days after infection, and pass down into the intestine via the oesphagus and stomach. The majority excyst six days after infection, whilst a few fail to excyst at all, and can be found later dead in the cyst. A similar fate occurs to those which encyst outside the digestive and respiratory tracts, those which excyst being unable to pass into the lumen of the gut.

Cercariae which enter tadpoles of Bufo bufo bufo behave in the same way, although experiments on toad tadpoles were not as extensive as those on frog tadpoles.

Examination of six day metacercariae from tadpoles did not reveal any great change in morphology, other than an increase in size of the intestinal caeca which by now, had a continuous lumen, together with a slight increase in the size of the testes.

Experiments with cysts of different ages taken from insects and tadpoles and fed to frogs reveal that the metacercaria is not immediately infective. Komiya (1939) found that metacercariae of Opisthioglyphe ranae (Frölich, 1791) Looss, 1907 are infective after fourteen days, those of Dolichosaccus rastellus are infective much earlier than this. 2,3,4,5, and 7 day old metacercariae taken from insect or tadpole are infective, whilst mature cercariae taken from both frog and insect were found to be non-infective, except on one occasion when two flukes were recovered from one frog.

It can be seen therefore, that metacercariae become infective between one and two days old.

It has also been found that mature cercariae cannot establish themselves directly in the tadpole and that encystment is necessary. This was shown by dissecting tadpoles at regular intervals during, and after infection. It was found that during infection some cercariae enter the stomach, but no free metacercariae were found in the guts of tadpoles examined one, two, and three days after infection. During this period only encysted metacercariae were found in the locations already given (page 42)

Compared with Notocotylus attenuatus and Echinoparyphium recurvatum, the rate of development is slow. On the other hand the rate is about the same as that of Opisthioglyphe ranae as reported by Looss (1894) and Brumpt (1945). Its rate of development was closely studied in tadpoles and young frogs of Rana temporaria temporaria where eggs were first seen in flukes three months old. The degenerating penetration gland cells and ducts remain visible in flukes up to six weeks old. The testes develop much more rapidly than the ovary, each enlarging towards the mid-line thereby, assuming the position they occupy in the adult. They are about twice the size of the ovary in three week old specimens and mature before the ovary, sperm being seen in the cirrus pouch of ten week old flukes.

In some six weeks old flukes located in frog tadpoles a yellow colouration was found to develop. Flukes lacking this yellow pigmentation developed more quickly. All flukes developing in toad tadpoles lacked this yellow pigmentation, and their rate of development was comparable with those in frog tadpoles lacking the pigment.

Dolichosaccus rastellus was first reported by Olsson in 1876 who gave Rana temporaria temporaria and Bufo bufo bufo as the hosts. Nicoll (1926) records it only as a parasite of Rana temporaria temporaria in England. Dawes (1946) likewise does not record it as present in Bufo bufo

bufo. It has been shown in the present work that toad tadpoles are infected with the same facility as frog tadpoles. Examination of over forty adult toads from Beauchief in 1950 revealed only one toad infected with a single specimen of Dolichosaccus rastellus. The percentage infection of adult frogs from the same area (see page 51) was much higher than this. Since flukes are present in metamorphosing tadpoles, and not in adult toads (except on rare occasions) it is evident that development is prevented, and that the host - parasite relationship is in some way not compatible. Only on four occasions under natural conditions were flukes observed to mature before the tadpole host metamorphosed. This occurred once in frog and three times in toads.

Measurements of Fixed Sexually Mature Specimens.(mm.)

Length.	0.93 - 3.9
Breadth.	0.38 - 0.87
Oral Sucker.	0.15 - 0.35
Ventral Sucker.	0.12 - 0.26
Pharynx.	0.081 - 0.087 x 0.17 - 0.20
Eggs.	0.040 - 0.049 x 0.020 - 0.024

The adult of Dolichosaccus rastellus is rounded anteriorly, tapers posteriorly, and is of a creamy brown colour. The cuticle is covered with transverse rows of backwardly directed spines' except for the region behind the posterior testis, where the spines were not observed. The oral sucker (Fig.16,o.s.) is larger than the ventral (Fig.16,v.s. which is situated about one quarter of the distance along the body. The mouth leads into a short prepharynx (Fig.16,p.p.) The oesophagus (Fig.16,oes,)is short and bifurcates about mid-way between the pharynx and the ventral sucker into long intestinal caeca (Fig.16,c.) which extend almost to the posterior end of the body.

The basic plan of the genital system is like that of the cercaria. The rounded ovary (Fig.16,ov.) is situated behind the ventral sucker on the right hand side of the body. The two testes (Fig.16,t.) each larger than the ovary, with the posterior one larger than the anterior, are found one behind the other in the third quarter of the body. They vary in their relative position to each other, the anterior testis being orientated to the left, and the posterior testis to the right. The uterus (Fig.16,ut.) occupies all the space between the ventral sucker and the anterior testis, and when gravid is also found lateral to the caeca. It leads forwards on the left side of the body to open at the genital pore (Fig.16,g.p.) which is situated slightly to the left of the median position immediately anterior to the ventral sucker. The cirrus pouch (Fig.16,c.p.) is elongate and extends behind the ventral sucker between a third to half its length, and

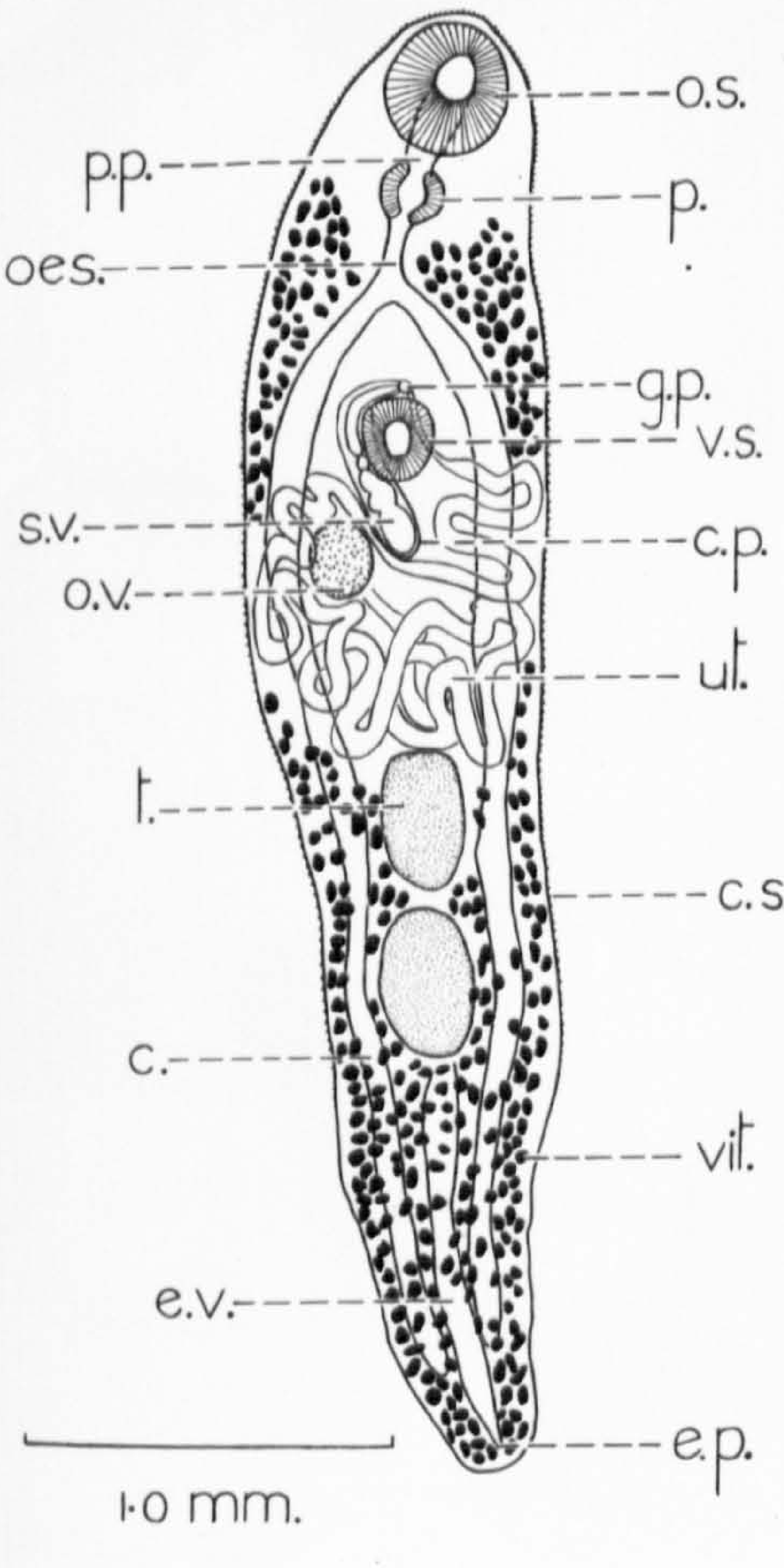


Fig. 16. Adult of Dolichosaccus rastellus.

contains the cirrus, pars prostatica and large seminal vesicle. The cirrus pouch is most frequently found dorsal to, and on the right of the ventral sucker with its posterior half lying between the ventral sucker and the ovary. Occasionally it is found immediately over and extending posteriorly to the left of the ventral sucker. The vitelline system (Fig.16,vit.) is extensive and is found throughout the body, from the level of the pharynx to the posterior end of the body. The follicles mainly occur dorsal and lateral to the caeca, but a few follicles are also found ventral to them.

The excretory vesicle (Fig.16,e.v.) is elongate, with two short anterior arms, a few small excretory granules are always present in the vesicle. The flame cell formula of the adults was not studied, but that of young flukes was found to be identical with that of the cercaria.

(e) Occurrence.

The cercaria was widespread in its distribution, having been found in the following localities. Ford Dam and stream, Beauchief, Round, Old Forge, Wire Mill, Leather Mill, Shepherd, Ibbotson, and Holme Dams, as well as the pond in the Botanical Gardens, (see Tables 1,2,3,4,5,6,7, 8,9,10,11,12,13, and 15). The degree of infection has been studied closely in three localities namely, the Botanical Gardens, Wire Mill Dam, and Beauchief Dams; in all three localities frogs and toads are found breeding in spring. Since each locality presented different problems, each will be described separately.

The pond in the Botanical gardens is small, some ten yards in diameter, and is not served by a natural stream, but by a tap which is turned on periodically. Breeding by frogs and toads is discouraged by the park keeper, who removes their progeny whenever possible. Further interference with the pond occurs, as it is periodically cleaned out. During the time that the pond was under survey <u>Limnaea pereger</u> disappeared, whilst the population of <u>Limnaea</u>

stagnalis was maintained. As can be seen from Tables 10 and 20 L. pereger was found to be infected with Dolichosaccus rastellus throughout the first six months of 1949, particularly during April, May and June. After August of that year no specimens of L. pereger were found. During this time L.stagnalis remained surprisingly free of infection. This absence of infection is more remarkable when the relative numbers of L. stagnalis and L.pereger are taken into consideration. From May 1949 until May 1950 no specimens of L. stagnalis containing stages of D. rastellus were collected. Thereafter infected specimens (all of the same generation) were found in each month until October 1950.

The locality at Beauchief consists of three distinct Dams, all about the same size, some twenty five yards in diameter. They are situated on a golf course, and are about six yards apart, all being served by the same stream, the water flowing from one to the other. The volume of water entering varies considerably. During one dry period the water flow was non existent. The outflow however, passes through the golf course, and has a width of three feet and a depth of two, this being sufficient to carry all the water. even during the wettest period. The walls separating the three dams are in varying states of repair, that between the first and second deteriorated greatly during 1949 and 1950, whilst that between the second and third dams had just been repaired before observations were started, the wall of the third dam was repaired during 1950.

Great changes were noticed in the populations of the three generations of Limnaea pereger that have been observed. According to Boycott (1936) L. pereger may get through two generations in one season during hot summers, but this has not been observed. Spawning begins at the end of March and continues until early summer, the newly hatched snails becoming noticeable during August and September. Growth at this period is rapid, and the snails soon reach adult size. These snails may be found throughout the winter and spring, but begin to die after spawning and no old snails are found after August.

The occurrence of the three generations of L.pereger that were observed is shown in the following table.

		First Dam.	Second Dam	Third Dam
1948	spawned	occasional	absent	frequent
1949	"	absent	frequent	occasional
1950	"	absent	abundant	rare

Frogs and toads spawned in all three dams during 1949 and 1950, particularly in the first and third dams. The following table gives the persentage infection of both frog and toad tadpoles at the time of metamorphosis.

	First Dam	Second Dam	Third Dam
1949	100	100	100
1950	0	100	100

The number of frogs breeding in the dams during 1950 was much less than in 1949, the reason for this being mainly attributable to the fact that some four hundred frogs were collected from the first dam in 1949. As a result there were relatively few tadpoles in the first and second dams in 1950. Fifty and fifty five adult frogs from the third dam were dissected in 1949 and 1950 and infection with Dolichosaccus rastellus was 38% and 73% respectively. The number of flukes in each host varied from one to nine, with an average of 5.1 flukes per infected adult. The rates of infection of frog tadpoles in the third dam was examined during 1950 and are given below.

April 27	5% nearly all metacercariae encysted, number of cysts varying from one to two.
May 24	100%
June 14	100% metacercariae nearly all excysted in the gut. Number of metacercariae four to ten average 5.6.

The figures show that during 1949 and 1950 the overall percentage of frogs parasitised by Dolichosaccus rastellus increased, but at the same time they demonstrate how unstable the population of D. rastellus is, as instanced by the drop in the infection rate of tadpoles at the first dam. They also show that in this locality the infection of frogs is by the direct life cycle, a conclusion which is further substantiated by the fact that out of one hundred and five frogs dissected, only one very young fluke was found, this no doubt entering the definitive host by utilising an insect.

The maximum infection of the snails (as is shown in Tables 8,9 and 15) occurred as it did at the Botanical Gardens during April, May and June, the August collections which consisted of very young snails never containing a single infected specimen.

Although three of the four mature specimens of D. rastellus taken from tadpoles (see page 47) were obtained from the third pond, it is most unlikely that this phenomenon causes any significant addition to the number of eggs found in any pond.

Wire Mill Dam showed a similar series of factors to those obtaining at the other two localities. Two additional observations were made however, firstly that no specimens of Limnaea stagnalis were found containing sporocysts of D. rastellus although Limnaea pereger occurred in far few numbers. The second observation arises out of the fact that some of the frog tadpoles failed to metamorphose in July 1950 and were collected up to November.

As at Beauchief tadpoles of Rana temporaria temporaria and Bufo bufo bufo were 100% infected in July. In those tadpoles which failed to metamorphose it was found that there was no further addition to the number of metacercariae after July, and that during August there was a loss of parasites, with the result that tadpoles collected during September, October, and November were completely free of flukes. The fact that no cercariae entered the tadpoles after July can be correlated with the absence of L. pereger during the latter months of 1950. All the flukes removed from the frog tadpoles during August possessed the yellow pigmentation mentioned on page 46 and none was mature. It appears therefore, that for normal development of the fluke metamorphosis of the host is required.

Again, (as shown in Table 2) the cercariae were prevalent during April, May and June, and combination of the infection records of all three localities showed that during April,May and June - the three months when tadpoles are present in the water, the degree of infection of the snails was 38% compared with 18% during the remainder of the year.

Examination of insects revealed that very few cercariae encyst in insects, and in the localities studied they played a negligible part in infecting the frogs. The swimming movements of the cercaria described on page 41 would be more likely to bring the cercaria into contact with tadpoles, than with the insects found to act as suitable intermediate hosts.

(f) Discussion.

The description of the life history and early stages of Dolichosaccus rastellus as given by Joyeux & Baer (1927) does not agree with those given by Linstow (1884, 1887, 1896) Wesenburg-Lund (1934) or those listed here, the greatest discrepancy being in the type of intermediate host. Joyeux & Baer never found the cercaria to encyst in insects, which contradicts the findings of the other workers. Joyeux & Baer (1927) believed that Linstow might have made an experimental error over this point, particularly as Linstow considered D. rastellus to be identical with Opisthioglyphe ranae. His drawing (Fig.2,plate II,page 115,1887) clearly indicates that Linstow studied D. rastellus. Further proof is to be seen in the accounts of the life history of Opisthioglyphe ranae as given by Sinitsîn (1905) and Brümpt (1945) both of whom found that O.ranae does not encyst in insects, but does penetrate into tadpoles. Further the dimensions of both cercariae are very similar, so that on measurements alone it is impossible to distinguish between them. Anatomical differences which occur between the cercariae of O. ranae and D. rastellus are not greater than the differences which occur within the cercaria of O.ranae according to the descriptions of that cercaria as given by Sinitsîn (1905) Komiya (1939) and Brumpt (1945). The presence of sensory hairs which were described by Sinitsîn (1905) and which were utilised by Joyeux & Baer (1927) in distinguishing between the two cercariae (since the cercaria of D. rastellus lacks them according to Joyeux & Baer) is entirely unreliable, as they were not seen by Brumpt (1945) and according to Komiya (1939) were fewer, and of a different structure than those described by Sinitsîn (1905). Also, the infection experiments of Joyeux & Baer (1927) which completed the life cycle in the definitive host are not conclusive. The frogs and one Bufo sp. were killed, four, eight, and fifteen days after infection, one Rana esculenta, Linnaeus, 1758, killed after fifteen days infection, contained large numbers of poorly developed flukes. Of three specimens of Rana esculenta and one Bufo sp. killed after eight days infection, one frog and one toad were free of parasites, one contained young trematodes which measured 0.4 x 0.16 mm. with genital organs well developed but without eggs, whilst the third frog contained well developed flukes 3 x 1 mm. with many eggs. Those frogs killed after four days contained a large number of young flukes which already had the anatomical structure of D. rastellus.

Comparison of the life history and stages of D.rastellus as given by Joyeux & Baer (1927) and those listed here, are therefore, of little value. Their findings are more in agreement with those given by Brumpt (1945) for Opisthioglyphe ranae than with any descriptions of corresponding stages of D. rastellus.

The anatomical feature which could be of greatest importance in distinguishing the cercariae of O. ranae and D. rastellus namely, the position and extent of the cirrus pouch is not figured by either Brumpt (1945) or Joyeux & Baer (1927), Sinitsîn (1905) shows that the anlage of the cirrus pouch is situated anterior to the ventral sucker, in the former, whilst in the latter (see page 41) it extends much more posterior relative to the position of the ventral sucker.

Only brief descriptions of the cercaria and sporocyst of D. rastellus are given by Linstow (1884) and Wesenburg-Lund (1934) and therefore, detailed comparison is impossible. There is however, general agreement regarding the dimensions of these stages.

The measurements of the adult agree closely with those given by Lühe (1909) and Travassos (1930) The latter worker also revised the genus, regarding Lecithopyge Perkins, 1928 as a synonym of Dolichosaccus Johnson, 1912. Their measurements of the minimum dimensions are larger than those listed here, but this is to be expected, since it has been possible to measure flukes which had only just reached maturity.

Perkins (1928) studying specimens of Dolichosaccus rastellus from different hosts and localities concluded that there existed several geographical races which were defined and named. The specimens which have been obtained during the present work are all D. rastellus subulatum.

Hall (1929) lists the second intermediate hosts of the family Plagiorchiidae Lühe, 1909, emend. Ward, 1917 and shows that they are of wide range. To his list can be added molluscs, an example of which occurs in the life history of Plagiorchis muris (Tanabe, 1922) as given by McMullen (1937a) and Opisthioglyphe ranae as given by Komiya (1939) and Brumpt (1945) and frogs, an example of which is Haplometrana utahensis (Olsen,1937) as given by Olsen (1937). Here the cercariae encysts in the skin which when cast is eaten by the frog. These second intermediate hosts are associated with a wide range of definitive hosts which in some way eat them as food.

The life cycle of Dolicosaccus rastellus which uses insects as the second intermediate host is of this type, but in addition there exists the more direct life cycle in which tadpoles become infected, the same individual ultimately becoming the definitive host. This is similar to the life cycle of Diplodiscus temperatus Stafford which was studied by Krull & Price (1932) who found that tadpoles may become infected by taking cercariae into the buccal cavity along with the respiratory current. The cercariae encyst during their passage along the oesophagus and excystment occurs in the intestine.

If the direct method of the life cycle was normal it would be expected that the cercaria would not require a period of encystment before beginning its development in the gut. It has been shown however, that the cercaria does require a period of encystment and so it must be concluded that this more direct life cycle is an adaptation of the more normal type of life cycle as found in the family *Plagiorchiidae*. Excystment in localities other than the gut is most unusual, and its cause has not been determined.

Unusual as the direct life cycle is, it has been shown to be far more successful in locating the final host than the normal life cycle. It is however, limited to a short period of the year, and occurs only once in the life of each frog, whilst the more normal life cycle is not so restricted.

(iv) *Phyllodistomum folium* (V.Olfers, 1817) Braun, 1899.

Baer (1827) described a cercaria under the name of *Distomum duplicatum*, Baer 1827, which developed in sporocysts found in species of *Anodonta*, Steenstrup (1842) considered that *Distomum duplicatum* was the larva of *Aspidogaster conchicola* Baer, 1827, whilst Wagener (1857) regarded it as the young form of *Azygia lucii* (Müller, 1776) Pagenstecher also in 1857 held that *Distomum duplicatum* was the larva of *Gorgodera cygnoides* (Zeder, 1800) but Cosmovici (1891) believed it developed into *Dicrocoelium dendriticum* (Rudolphi, 1819) Looss, 1899. From a study of the anatomy of the two forms Looss (1894) maintained that *Distomum duplicatum* was the larva of *Phyllodistomum folium*. In his description of the life history of *Phyllodistomum folium* Sinitsîn (1901) showed that the cercaria is not *Distomum duplicatum*. It is evident that his identification of *Phyllodistomum folium* was incorrect and Lühe (1909) agreed with the statement of Looss (1894) that *Cercaria duplicata* is the larva of *Phyllodistomum folium* and at the same time he showed that the *Phyllodistomum folium* of Sinitsîn was a new species and renamed it *Phyllodistomum macrocotyle* (Lühe, 1909) Odhner, 1911.

Under the name of Cercaria macrocerca, Filippi (1854) gave a description of a cercaria which was shown by Sinitsin (1907) to cover at least four species. Lühe (1909) retained the original name Cercaria macrocerca for a form which Sinitsin (1907) claimed to be the larva of Gorgoderina vitelliloba (Olsson, 1876), Vickers (1940) described Cercaria macrocerca in detail, his description agreeing closely with that of Lühe (1909).

The cercaria described below agrees closely with the descriptions of Cercaria macrocerca as given by Vickers (1940) and Lühe (1909), but it has been found to be the larva of Phyllodistomum folium and not Gorgoderina vitelliloba.

(a) Sporocyst.

Measurements. (mm.)

Up to 2.08 x 0.63.

The sporocysts occur between the gill lamellae of Sphaerium corneum. They are white in colour and vary in shape from long club-like to short sac-like structures. The sporocysts are attached to the internal surface of the gill lamellae by their narrower ends. The cuticle in young sporocysts is much wrinkled, and there is some power of movement, but as the sporocyst grows, the cuticle becomes stretched and the power of movement is lost. Young sporocysts are packed with germ balls (Fig.17,g.b.) and as these develop the wider free end of the sporocyst becomes further distended, so that nearly all the developing cercariae which may number up to thirteen are found at the free end of the sporocyst. No birth pore was observed.

The excretory system of the sporocyst consists of two antero-lateral and two postero-lateral ducts, (Fig.16,a.l.d., p.l.d.) The antero-lateral and the postero-lateral ducts of each side lead into an excretory sinus (Fig.17,e.s.) the excretory sinii of each side no not occur at the same level, one being more anterior in position than the other. This displacement is maintained in the case of the ducts and their flame cells. Vickers (1940) reports that each duct collects

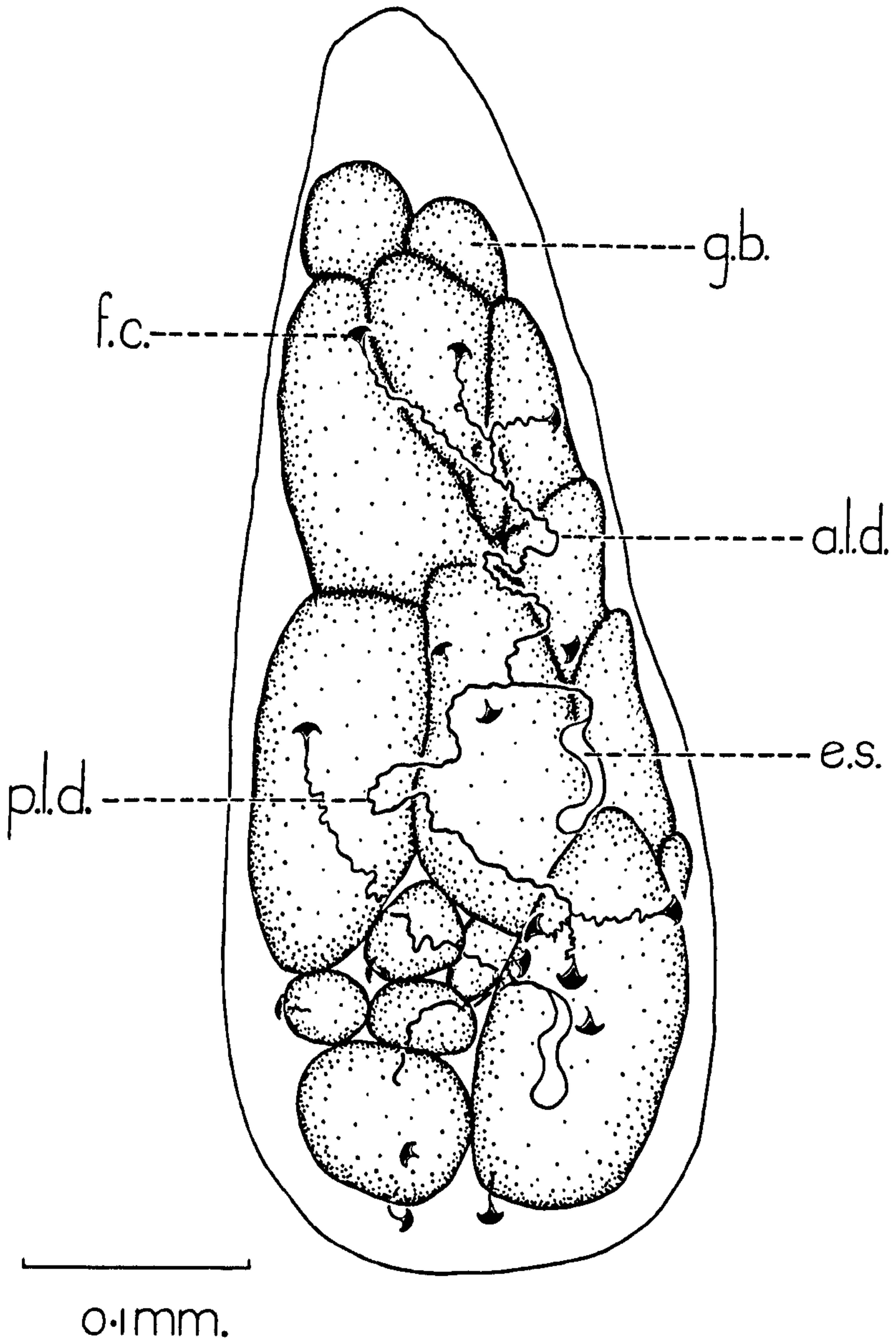


Fig. 17, Sporocyst of Phyllodistomum folium.

from five flame cells, but this condition has been observed only once during the present work. At other times only four flame cells could be found serving each duct. The flame cells (Fig.17,f.c.) are unlike the usual type and were first noted by Thiry (1860) for Cercaria macrocerca which Sinitsin (1905) showed to be a new species renaming it Cercaria gorgoderae pagenstecheri (Sinitsîn, 1905). They were also observed by Looss (1894), and Vickers (1940), includes a detailed description of them. Each flame cell consists of a sheet of fused cilia attached to an enlarged semi-circular basal plate. No increase in the number of flame cells was observed, but there was an increase in the size of the flame cells and in the diameter of the ducts with increasing age of the sporocyst.

The largest flame cell observed had a length across its base of 0.055 mm. which is in close agreement with the findings of Vickers (1940).

(b) Cercaria.

Measurements. (mm.)

Length of Proximal Swollen Globe.	0.406 - 0.464	
Breadth of Proximal Swollen Globe.	0.249 - 0.290	
Length of Elongated Distal Stem.	0.696 - 0.810	
Breadth of Elongated Distal Stem.	0.093 - 0.146	
	Extended	Contracted
Body Length.	0.522	0.26
Body Breadth.	0.116	0.174
Oral Sucker.	0.066 - 0.077	
Ventral Sucker.	0.066 x 0.088 - 0.099 x 0.077	
Stylet.	0.024 x 0.006	

Cercariae leave the mollusc at any time of the day or night, and have very limited powers of locomotion. Consquently, they never move far from the molluscan host. Some remain attached to the valves of <u>Sphaerium corneum</u> as was also noted by Wesenburg-Lund (1934). Although the cercaria has limited powers of locomotion it is nevertheless very active. The extreme tip of the tail can be withdrawn by muscular action, thus functioning as a sucker and attaching the animal to the substratum. With the aid of its powerful tail muscles the animal sways to and fro incessantly, and it is this characteristic movement which makes the somewhat transparent cercaria conspicuous in the vessel containing infected molluscs.

The most prominent feature about the cercaria (Fig. 18) is the tail. This is divided into two parts, a proximal swollen globe (Fig.18,p.s.g.) and a distal elongated stem (Fig.18,e.d.s.) The proximal swollen globe is further divisible into two parts, an anterior enclosing portion (Fig.18,ch.) which forms a chamber containing the body of the cercaria and a posterior cellular portion which contains a number of large cells.

The cuticle covering the body of the cercaria is continuous with that covering the tail, but the real connection between the body and tail is maintained by a pair of muscle bands. These muscle bands are attached to the ventral lip of the excretory pore from which they pass posteriorly down a short stalk (Fig. 18,st.) The pair of muscle bands (Fig.18,l.mu.) then diverge slightly as they pass through the cellular portion of the swollen globe, and the distal elongated stem. At this junction each longitudinal muscle band divides into three, and these pass posteriorly to the extreme top of the tail. Thus there are six longitudinal muscle bands in the distal elongated stem of the tail. Under the coverslip the cercaria always

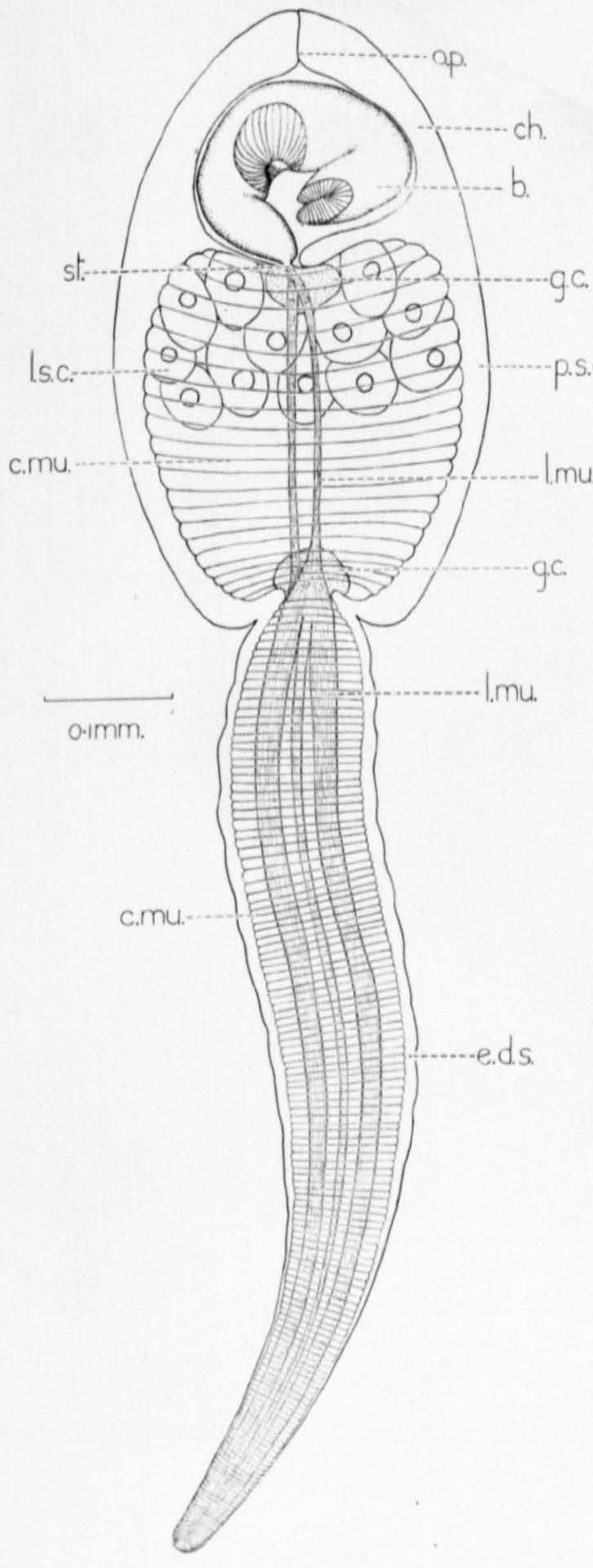


Fig. 18, Cercaria of Phyllodistomum folium.

appears as drawn in Fig.18, and in this position one set of three longitudinal muscle bands (Fig.18,l.mu.) lie above the other set. The median muscle band of each set is much smaller in diameter than the other two. Beneath the cuticle and outside the longitudinal muscle bands of both the distal elongated stem, and the cellular portion of the proximal swollen globe are found circular fibres (Fig.18,c.mu.) Down the centre, and extending the entire length of the distal elongated portion of the tail, is a fine non-muscular strand of elongated cells. This is in agreement with Vickers (1940), but not with Thiry (1860) or Wesenburg-Lund (1934) who believed this chain of cells to be an excretory canal.

In the cellular portion of the proximal swollen globe there are found three groups of cells in addition to the muscle cell bodies. The most prominent of these three groups of cells are the large cells (Fig.18,l.s.c.) found in the anterior half of the cellular portion. They vary in number from nine to fourteen and possess clear cytoplasm with large spherical nuclei. The other two groups of cells (Fig. 18,g.c.) are associated with the two longitudinal muscle bands, one group situated at the base of the stalk, and the other at the junction of the tail stem with the swollen globe. They both consist of round small cells and scattered amongst them are found ovoid muscle cell bodies mentioned earlier.

Under natural conditions the body of the cercaria does not emerge from the chamber until the cercaria is moribund. This is in contrast to the findings of Wesenburg-Lund (1934) who states "the bodies are often stretched out almost at full length from the opening of the first chamber, and they are able to pass out and in at will". The body may easily be squeezed out of the chamber with slight pressure, and it is then seen to be an elongated form, with the sides of the body almost parallel. The body has considerable powers of contraction and extension, these being more pronounced in the region anterior to the ventral sucker which is slightly larger than the oral, and situated just inside the posterior half of the body.

The cuticle is unarmed, and is similar to that of the sporocyst in that its surface is wrinkled, these wrinkles being more evident when the body is contracted. Down each side of the body is an irregular row of sensory papillae (Fig.19,s.p.) Six of these papillae together with a fringe of long thin spines (Fig.19,c.s.) are set round the opening of the ventral sucker (Fig.19,v.s.) Shorter more conical spines line the cavity of the ventral sucker and the ventral lip of the oral sucker.

A stylet (Fig.19,s.) is present and is embedded in the dorsal part of the oral sucker, lying at right angles to the axis of the body, with its tip pointing slightly backwards.

The mouth is surrounded by the oral sucker (Fig.19,o.s.) and as there is no pharynx, it leads directly into a sinous oesphagus (Fig. 19,oes.) which divides into two intestinal caeca just behind the mid-way position between the oral and ventral suckers. The intestinal caeca (Fig.19,c.) are of varying diameter and extend almost to the posterior end of the body.

Situated anterior to the ventral sucker and posterior to the bifurcation of the intestine are six pairs of penetration gland cells (Fig.19,p.g.) each having a prominent nucleus. From each of these cells a duct (Fig.19,p.d.) leads forwards dorsal to the intestinal caeca, and opens at the tip of the stylet. The penetration gland cells are most prominent in newly emerged cercariae. In older cercariae they lose their compact nature, and occupy a position antero-lateral to the ventral sucker.

There is a further group of cells found antero-lateral to the diverging intestinal caeca consisting of about six to eight cells on each side of the body. They have no ducts and Vickers (1940) suggests that they may be the true cystogenous cells.

Most of the region between the ventral sucker and the posterior end of the body is occupied by large columnar cells (Fig.19,c.e.v.) which surround the excretory vesicle (Fig.19,e.v.). These cells contain a large number of small clear granules and were suggested by Sinitsîn (1907) to be cystogenous in function. Vickers (1940) studied their formation in detail, concluding that "although positive evidence is lacking it seems most probable that these cells have an excretory function". In two occasions during the present investigation cercariae underwent encystment while being studied under the microscope. Observation of these cells and their granules did not reveal any active participation in the process of encystment. A few granules were observed floating in the cystic fluid, but they did not dissolve, nor were they incorporated into the cyst wall. This supports Vicker's suggestion, but it must be pointed out that both these cercariae were encysting under very abnormal conditions, and that only the initial stages of encystment were observed.

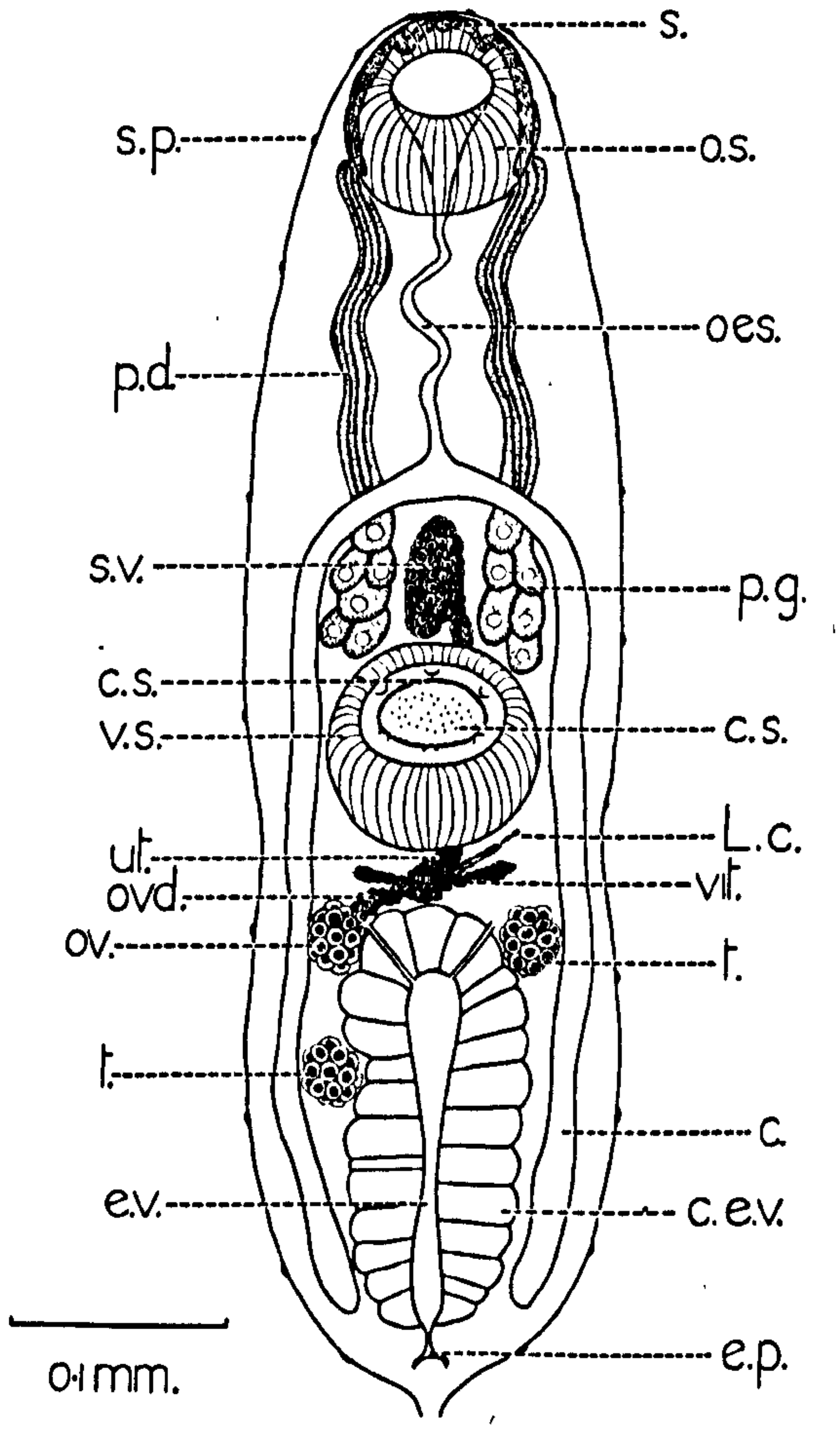


Fig. 19, Cercaria of Phyllodistomum folium.

The actual excretory vesicle itself is tubular in shape, and anteriorly receives two main collecting ducts. These two ducts (Fig.20,m.d.) run forward on either side of the ventral sucker, becoming intensely coiled as they do so, each bifurcating at a level posterior to the divergence of the intestinal caeca, into antero- and postero-lateral ducts. (Fig. 20,a.l.d.,p.l.d.) The antero-lateral duct runs forwards to the level of the oral sucker, where it terminates into the capillaries of three flame cells. Each postero-lateral duct extends backwards lateral to the intestinal caecum to the posterior end of the body. Eighteen flame cells were observed on each side of the body, but they are not arranged symmetrically.

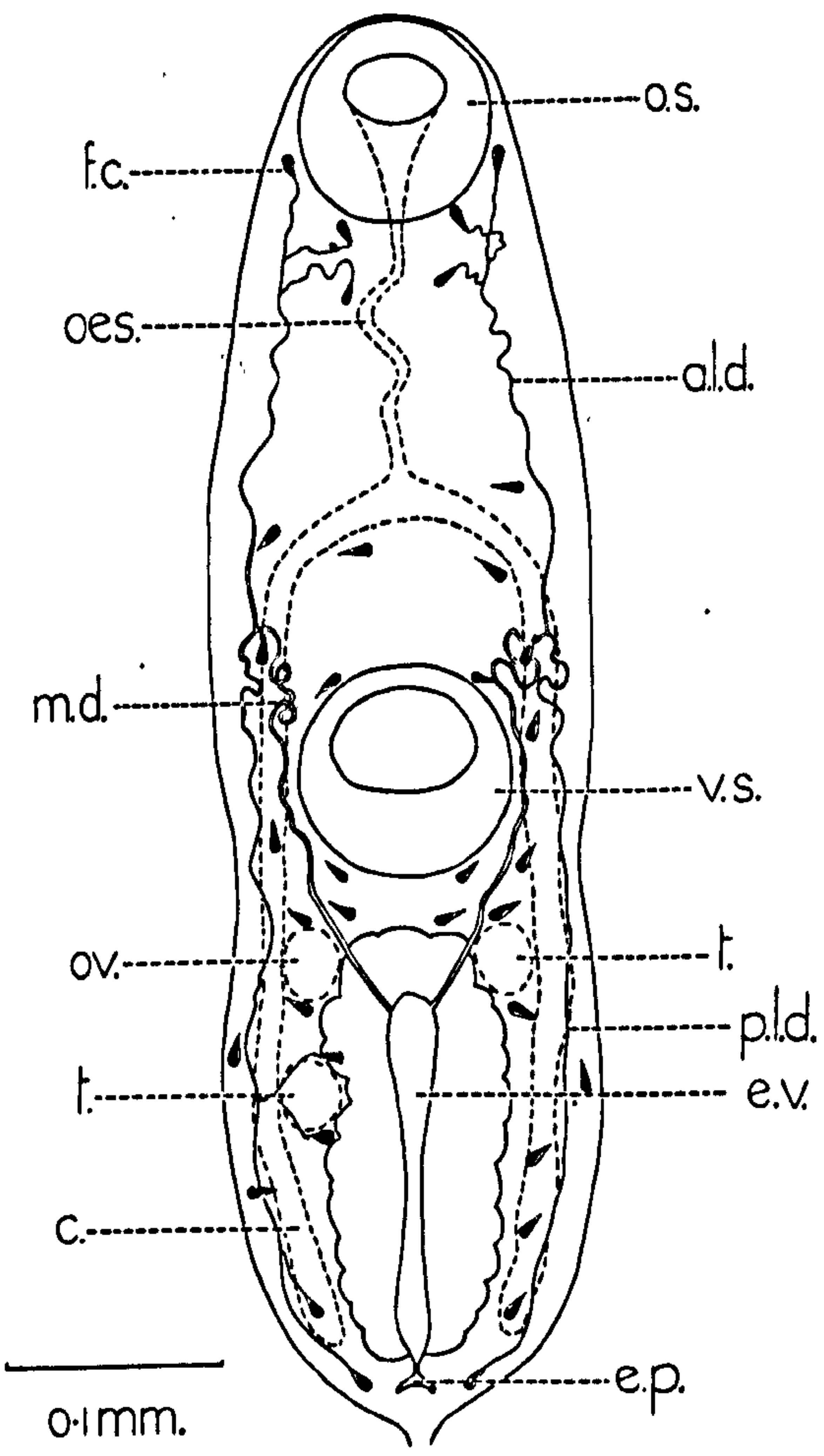


Fig.20, Excretory System of Cercaria of Phyllodistomum folium.

The genital system is well developed in the cercaria, the ovary and the two testes consisting of round cells with prominent nuclei, being easily seen in the living cercaria. Stained specimens revealed the remainder of the system. The ovary (Fig.19,ov.) is situated behind the ventral sucker on the right side of the body. From it leads the oviduct (Fig. 19,ovd.) which passes inwards to a median mass of cells immediately behind the ventral sucker. Extending laterally on either side of the median mass of cells are the vitelline glands. (Fig.19,vit.) Immediately posterior to the junction of the vitelline gland with the median mass of cells on the left side of the body, is found Laurer's canal (Fig.19,L.c.) which leads dorsally to a position lateral to the ventral sucker. The uterus (Fig.19,ut.) passes dorsally over the ventral sucker, and joins the large seminal vesicle (Fig.19,s.v.) which is found anterior to the ventral sucker in the median position between the penetration gland cells of each side. One of the testes (Fig. 19,t.) is situated behind the ovary lateral to the columnar cells surrounding the excretory vesicle, whilst the other is found in a similar position to the ovary, but on the other side of the body.

In a number of cercariae, instead of the ovary and one testis being found on the right side of the body, these organs were found on the left side. This condition was noted by Vickers (1940), but the percentage of specimens having the reversed arrangement was higher, (43.5%) than that found by Vickers (15%.) Both types of cercariae were found in the same sporocyst as was also found by Vickers (1940).

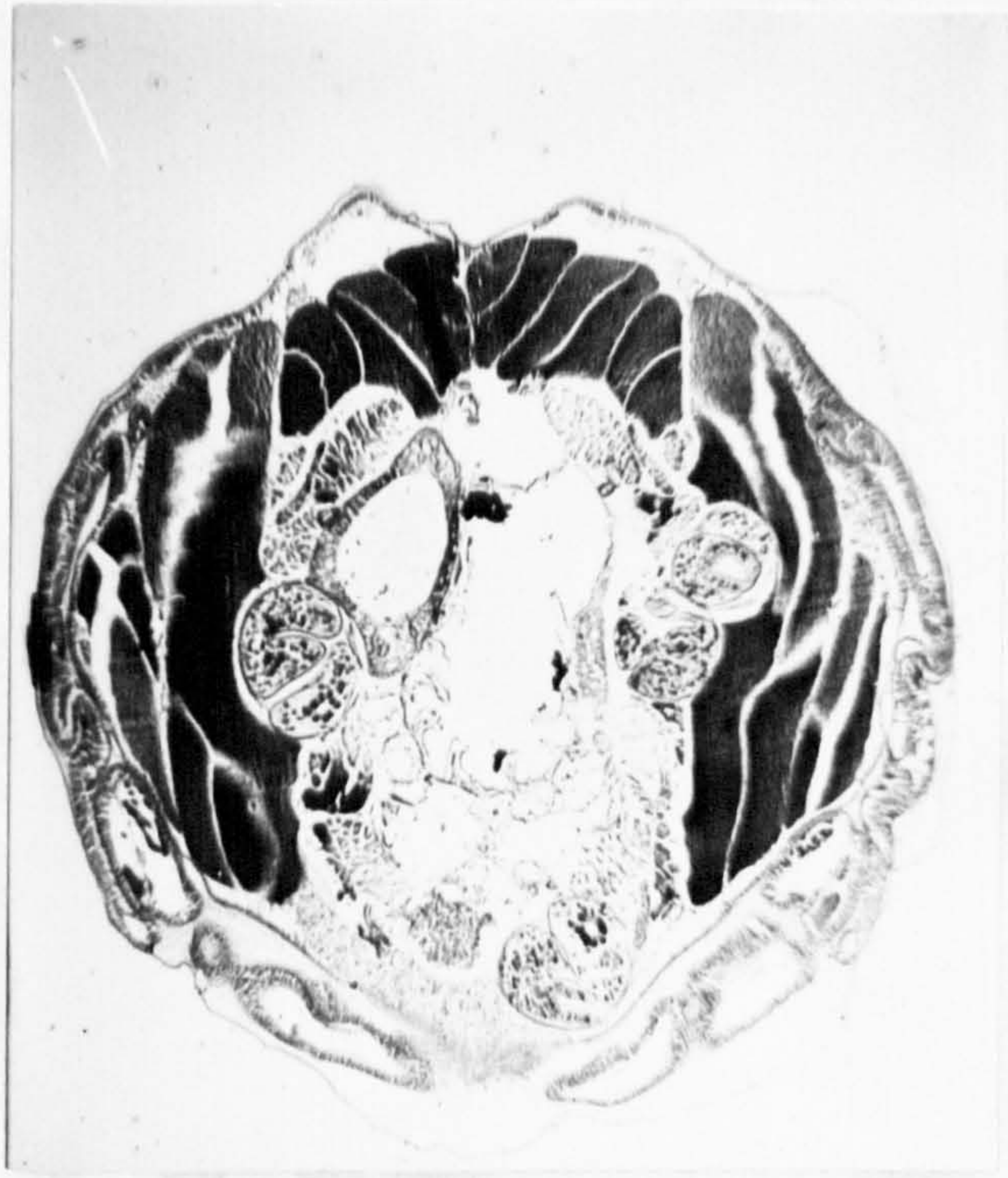
(c) Cyst.

Encysted stages of the cercaria have been obtained naturally and experimentally from larval chironomidae which included members of the following genera, Procladius Skuse, 1889, Psectrotanypus Kieffer, 1909, Tanypus Meigen,1803 and Chironomus, Meigen 1803, and experimentally from Chaetogaster crystallinus. On one occasion a cyst was found in the coelom of a tadpole of Rana temporaria temporaria. On two occasions cysts have been found in sporocysts, but their occurrence in these last two species is considered accidental. The use of an oligochaete as a second intermediate host is unusual, although Wallace (1941) found that the cercaria of Triganodistomum mutabile (Cort) was eaten by Chaetogaster limnaei K. Baer, the cercaria penetrating the gut wall and encysting in the body cavity.

The mode of entry of the cercaria into the second intermediate host is like that described by Sinitsin (1907) for the cercaria of Gorgodera loossi, Gordera pagenstecheri, Gorgodera varsoviensis Sinitsin, 1905, and Gorgoderina vitelliloba, although in the last species Sinitsin was unable to obtain cysts under natural conditions, and by Groves (1945) for Phyllodistomum solidum Rankin, 1937. The cercaria by its incessant oscillations appears to attract the chironomid which suddenly grabs at the cercaria and eats it. The body of the cercaria is protected to some extent from the action of the mandibles by the proximal globe which surrounds it. When the cercaria has been eaten the body breaks free and immediately attempts to penetrate the oesophageal wall. If it succeeds the cercaria encysts in the haemocoel, frequently attaching itself to muscles.

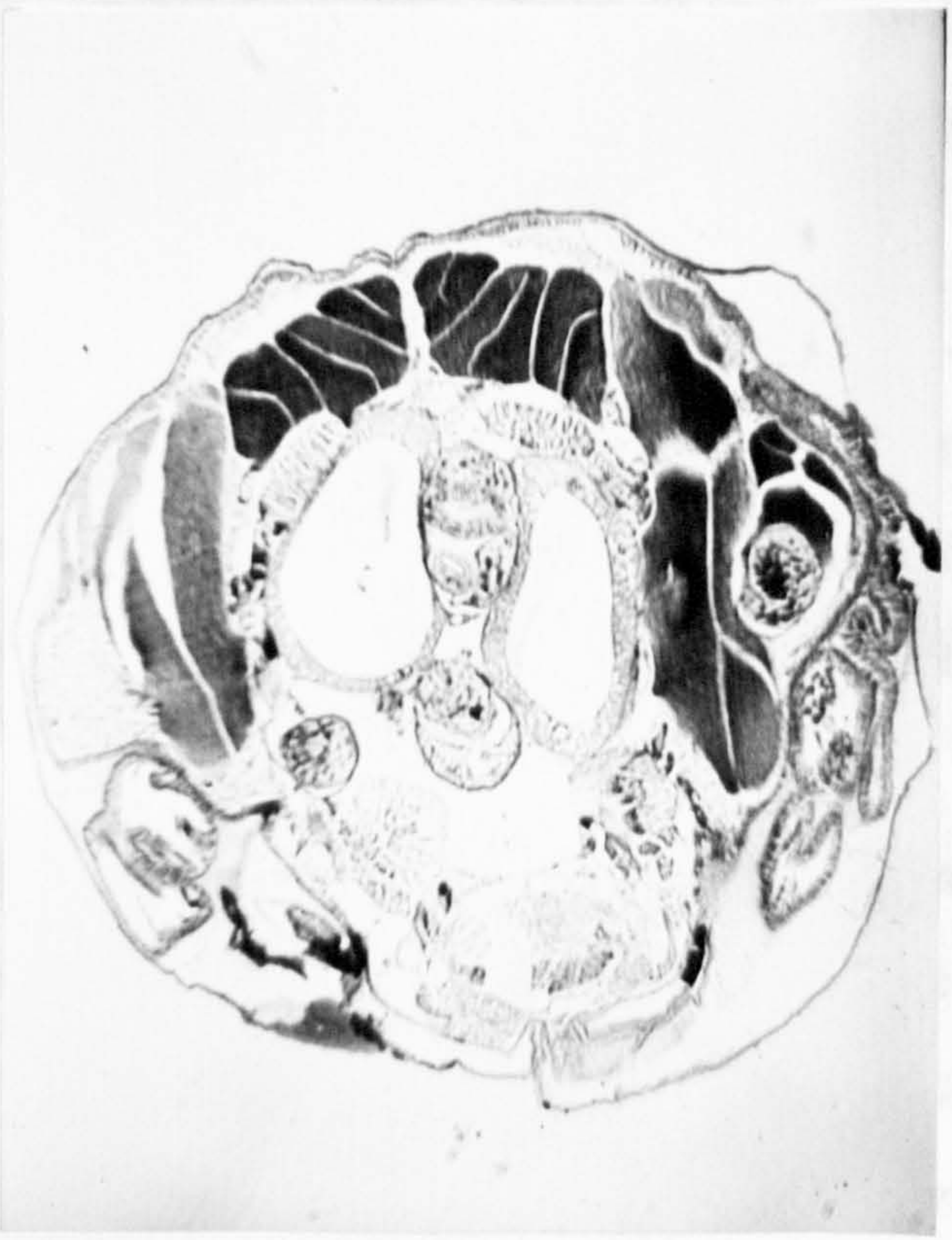
Of those cercaria eaten, only a few succeed in encysting as was found by Groves (1945) in the case of Phyllodistomum solidium. Some are partly eaten and then regurgitated, some are killed by the action of the mouth parts, whilst others fail to penetrate the oesophageal wall, and are passed into the mid gut, where they are digested. The number of cysts found in naturally infected chironomid larvae was never more than three in any individual, although in experimentally infected animals more cysts could be obtained - see photomicrographs, pages 67 and 68. All the cysts obtained have always been located in the thorax, except on one occasion when one cyst was observed in the head.

Chaetogaster crystallinus was easier to infect experimentally than the chironomid larvae,up to twenty cysts being obtained from one animal,the method of infection being exactly the same as that already described for the chironomidae.



Photomicrograph of Transverse Section of Thorax of

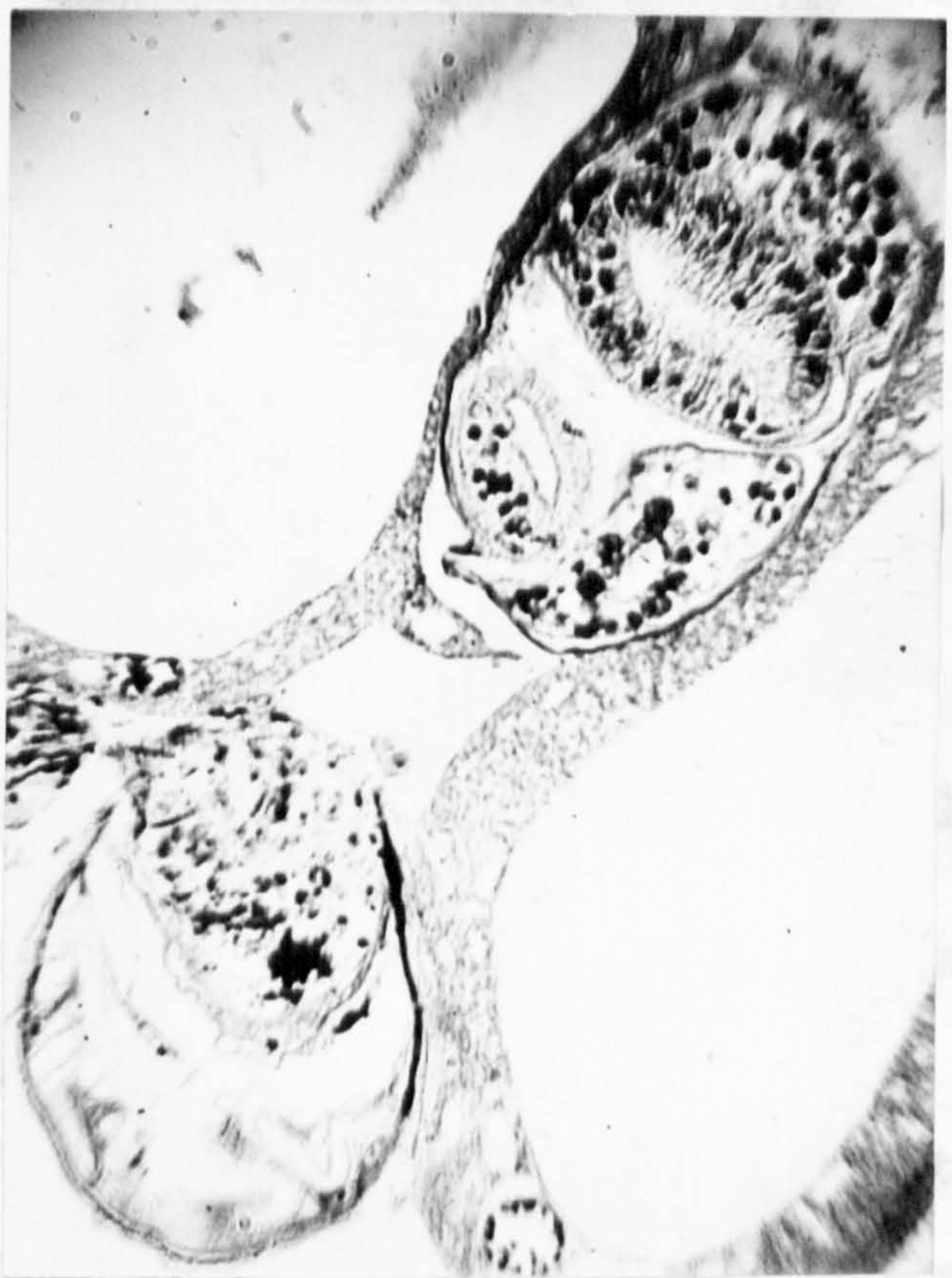
Psectrotanypus sp. Showing Four Cysts of Phyllodistomum folium.



Photomicrograph of Transverse Section of Thorax of

Psectrotanypus sp. Showing Three Cysts and a Cercaria

Penetrating through the Oesophageal Wall.



Photomicrograph of Transverse Section of Thorax of Psectrotanypus sp. showing the Cyst Between the Salivary Glands and the Cercaria Penetrating through the Oesophageal Wall under Higher Magnification.

The thin walled spherical cyst (Fig. 21) has a diameter of 0.18 - 0.22 mm. and the metacercaria inside is folded ventrally into a " U " shape. This folding may become more intense so that the two ends overlap as is seen in Fig. 21.

The stylet is shed when the cercaria encysts and is always to be found free inside the cyst. The genital system continues its development, the two testes and the ovary being clearly seen through the transparent cyst wall which is easily ruptured by slight pressure.

(d) Juvenile and Adult.

A survey of adult flukes found at the dam where the cercaria was obtained, revealed that Phyllodistomum folium was particularly abundant, whilst no specimens of Gorgoderina

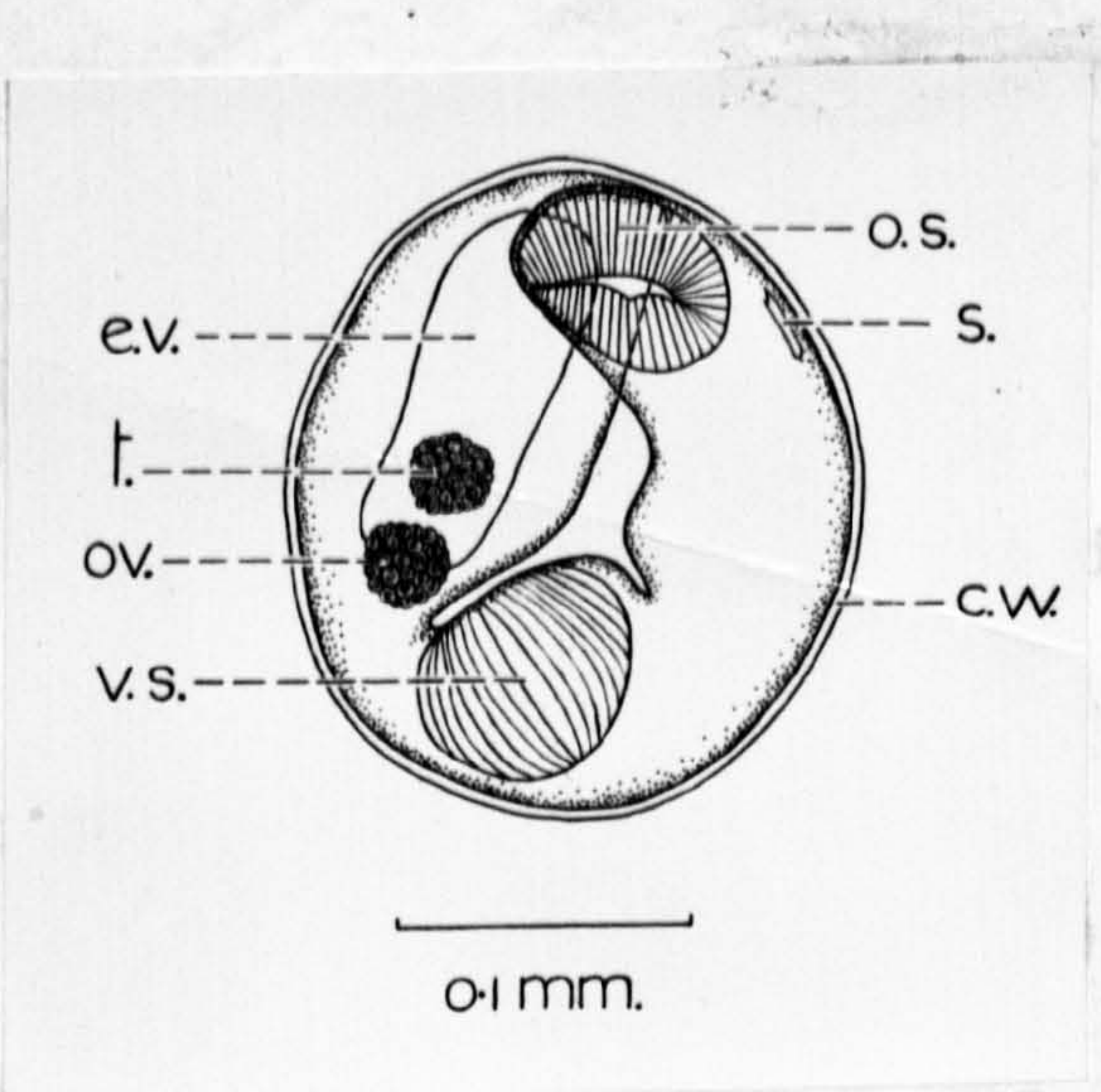


Fig. 21, Cyst of Phyllodistomum folium.

vitelliloba could be found. Comparison of immature specimens of Phyllodistomum folium with the cercaria revealed close similarity between the two. Consequently in attempts to obtain adult stages infected larvae and worms were fed to three spined sticklebacks (Gasterosteus aculeatus) which were obtained from the Beauchief Dams (a locality where the cercaria or adult was never found) and to frogs (Rana temporaria temporaria. The attempts to infect frogs were unsuccessful, but mature and immature specimens of Phyllodistomum folium were taken from the bladder and uterus of the sticklebacks. Eggs were first seen in twenty six day old flukes, although these eggs were not typical and contained only vitelline cells.

From the results of the experiments it is evident that only a few of the metacercariae succeed in establishing themselves in the definitive host. In one series of experiments, out of one hundred and three cysts fed to twelve sticklebacks, only twenty three adult flukes were obtained. It seems probable that the metacercariae are not immediately infective, but owing to the scarcity of infected intermediate hosts, the age at which metacercariae become infected was not determined. The passage of the excysted metacercariae from the gut to the final location was likewise not studied.

Attempts to infect sticklebacks and frogs with mature cercaria failed, even though small sticklebacks once they had eaten a cercaria, would readily eat large numbers. Large sticklebacks were fed specimens of *Sphaerium corneum* which contained mature infections, but here again no adult flukes were obtained.

Measurements of Fixed Sexually Mature Specimens, (mm.)

Length	0.580 - 1.020
Breadth of Forebody	0.174 - 0.320
Breadth of Hind Body	0.290 - 0.566
Diameter of Oral Sucker	0.112 - 0.157
Diameter of Ventral Sucker	0.139 - 0.203
Eggs when formed	0.038 - 0.044 x 0.022 - 0.023
Eggs containing active miracidium	0.058 - 0.074 x 0.035 - 0.046.

The adult of *Phyllodistomum folium* is creamy yellow in colour, and spatulate in shape, being broad behind the ventral sucker and tapering anteriorly. When living it is very variable in shape, particularly the region anterior to the ventral sucker which is capable of elongating to an enormous extent. When fixed this region becomes much contracted.

Like the cercaria the cuticle is unarmed, but down each side there is the irregular double row of sensory papillae (Figs. 22 and 23, s.p.), those in the region of the oral sucker being particularly prominent, as are the six which are set round the opening of the ventral sucker. The cuticular spines which are associated with the ventral and oral suckers in the cercaria were not seen in the adult.

The digestive system is like that of the cercaria, a sinuous oesphagus (Fig. 22 and 23, oes.) dividing about midway between the oral and ventral suckers into long intestinal caeca (Figs. 22 and 23, c.) These are more prominent than the caeca in the cercaria, since they contain food.

The degenerating penetration gland cells and ducts (Fig. 22, p.d.) can be seen in young specimens of *Phyllodistomum folium* and it is interesting to record that the number of ducts are more easily counted at this stage than in the cercaria, for, with the growth of the fluke the openings of the penetration ducts are separated as is shown in Fig.21. No detailed study has been made of the excretory system, but the main ducts and some flame cells were observed and were of the same arrangement as in the cercaria.

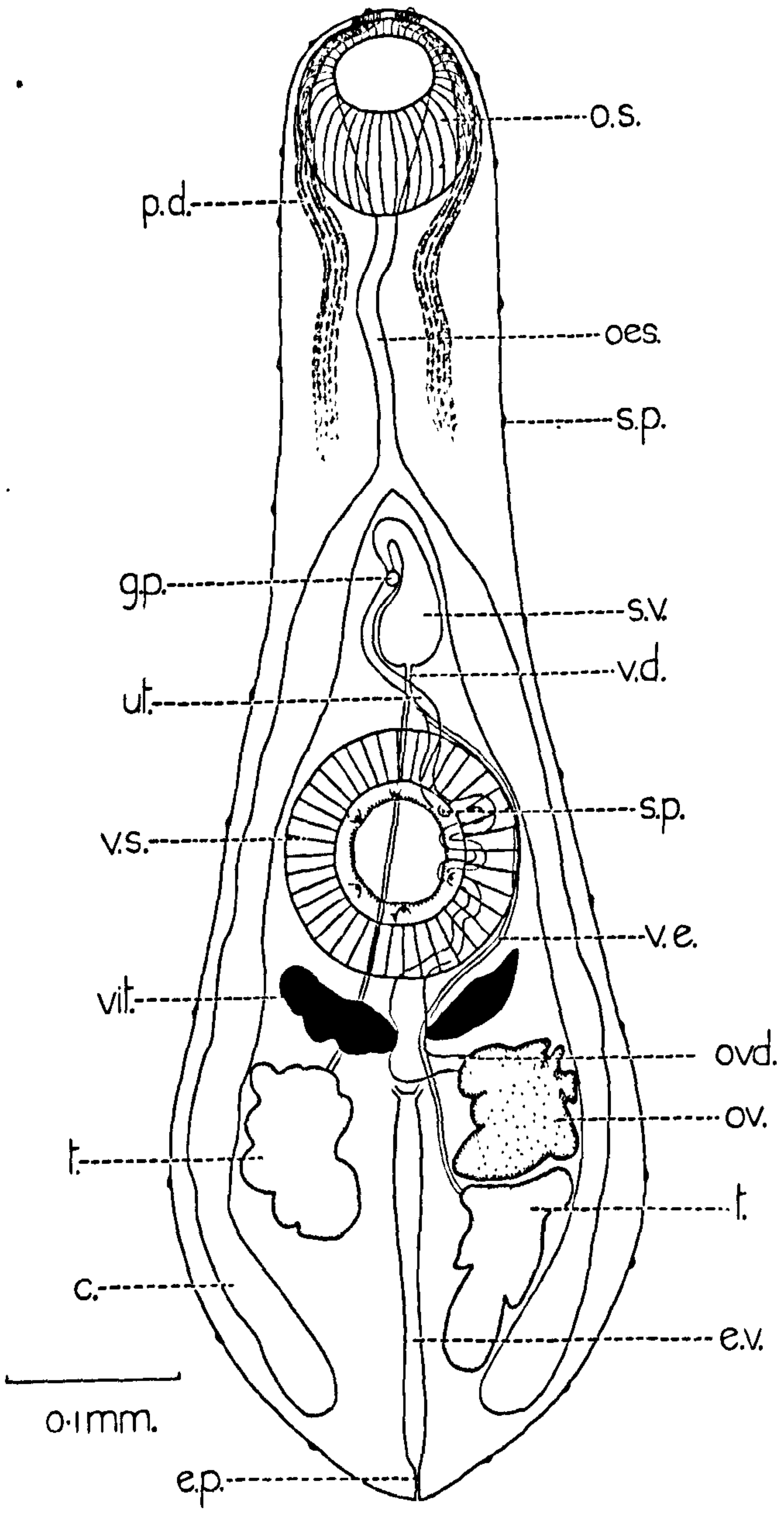


Fig. 22, Immature Specimen of Phyllodistomum folium.

The reproductive system is of the same plan as that in the cercaria, but now occupies most of the region behind the ventral sucker. In young specimens the ovary (Fig.22,ov.) and the two testes (Fig.22,t.) are lobed, with the testes larger in size than the ovary. In older specimens (Fig.23) the two testes remain larger than the ovary, all three being less lobed and more rounded. This is probably due to the confining influence of the uterus as it becomes distended with eggs. The uterus becomes profusely coiled in the posterior region, mainly between the caeca, but in gravid flukes part of the uterus may lie lateral to the caeca.

Each testis has a long vas efferens (Fig.22,v.e.) which passes dorsally over or round, the ventral sucker joining to form a short vas deferens (Fig.22,v.d.) which then leads into a large seminal vesicle (Fig.22,s.v.) immediately anterior to the ventral sucker. The compact vitelline glands (Fig. 23,vit.) are found immediately posterior to the ventral sucker. The eggs begin their

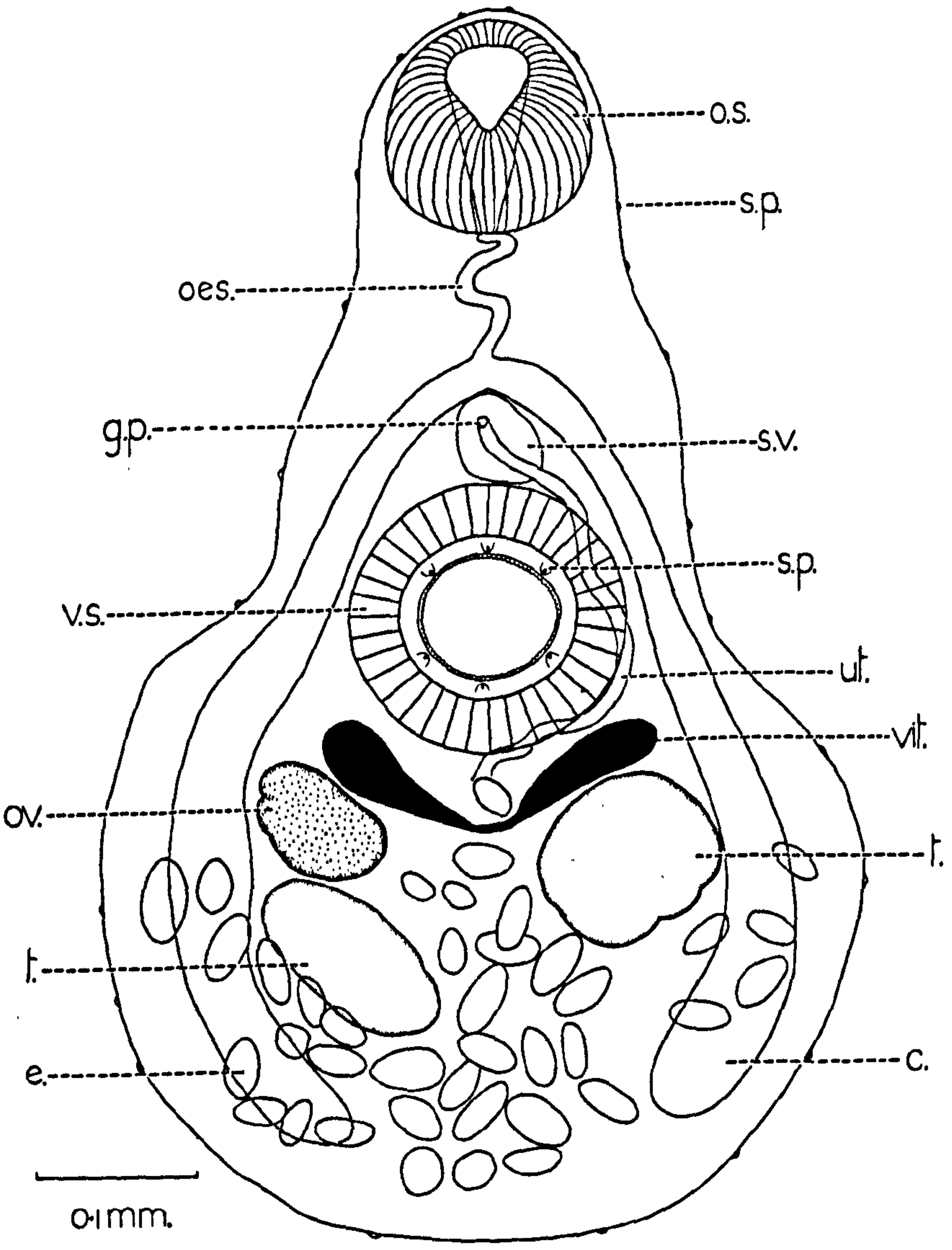


Fig.23, Adult of Phyllodistomum folium.

development immediately after formation, and are ready to hatch when they are expelled through the genital pore (Fig. 23,g.p.) which is situated just behind the bifurcation of the oesophagus. Some of the eggs produced, particularly those first formed are non-viable. They are easily observed since viable eggs increase in size as they develop. For this reason two sets of egg measurements are given, one being the size of eggs when just formed, the other the size of eggs containing active miracidia. The miracidia itself is pear shaped, lacking eyespots and possesses two flame cells.

Amphitypy has been reported for a number of species of the genus Phyllodistomum Braun, 1899 including Phyllodistomum solidum by Groves (1945) Phyllodistomum semotili Fischthal, 1942, Phyllodistomum notropidus (Fischthal 1942) Phyllodistomum nocornis (Fischthal, 1942) by Fischthal (1942) and the same has been found true of Phyllodistomum folium both in the adult and larval stages, the percentage of adults where amphitypy occurred being 39.3%.

(e) Occurrence.

Both adult and larval stages have been found in the following localities, Old Forge, Wire Mill, Ibbotson, Holme and Round Dams. The percentage infection has been studied only at Wire Mill Dam during 1950, when the following results were obtained.

Table showing percentage infection of Sphaerium corneum of eight mm. in length or over.

Feb.	Mar.	Apl.	May.	June.	July.	Aug.	Sept.	Oct.	Nov.	Dec.
23	44	34	50	73.3	63.3	40	27.5	16.6	13.3	16

Samples of chironomid larvae taken in June and August showed that 27% and 36% respectively were infected with cysts, the number of cysts varying from one to three.

Examination of sticklebacks in the Dam revealed that there was a 100% infection with Phyllodistomum folium throughout the year, except in newly hatched fish during June. By July these fish were 100% infected.

(f) Discussion.

All the measurements of the cercaria of *Phyllodistomum folium* agree fairly closely with those of *Cercaria macrocerca* as given by Vickers (1940) and Lühe (1909) with the exception of the length of the stylet. According to Vickers (1940) the stylet has a length of 0.017 mm. whilst Lühe (1909) gives it as 0.21 mm. (This is obviously a misprint and should read 0.021 mm.) as compared with 0.024 in the present work. Otherrdifferences which have not already been mentioned concern the presence of the median muscle bands in the tail, and the presence of non-muscular cells at the base of the stalk, neither of which are mentioned by Vickers (1940). These differences are not sufficient to prevent identification of the cercaria of *Phyllodistomum folium* as *Cercaria macrocerca*.

This means then that the existing accounts of two life cycles are incorrect, for as has been seen from the review of the literature, *Cercaria duplicata* was regarded as the larva of *Phyllodistomum folium* and *Cercaria macrocerca* as the larva of *Gorgoderina vitelliloba*. The review also shows that both life cycles have caused confusion to previous workers, and that neither of them has been completely proved experimentally.

An attempt was made to substantiate the experimental proof of the life histoy of *Phyllodistomum folium* by using ecological data. Infected *Sphaerium corneum* were quite common in the localities visited, and yet no frogs were found containing *Gorgoderina vitelliloba*.Correspondingly attempts to find specimens of *Anodonta* infected with *Cercaria duplicata* also failed. None were found at Wire Mill Dam, but fifteen individuals were collected from Ibbotson Dam during 1950, but none were infected. These results although of limited value augment the experimental proof of the life history.

The descriptions of the anatomy of *Cercaria duplicata* show that it is very similar in its basic structure to *Phyllodistomum folium*, yet there are features which suggest that the two are not identical species. Cercaria duplicata according to Lühe (1909) and Reuss (1903) has a length of about 2 mm. whilst the adult (ie.Phyllodistomum folium) according to Lühe (1909) is at the most 2 mm. in length. No mention of penetration gland cells is made by Reuss (1903) and yet the degenerating penetration gland cells and ducts are clearly visible in young specimens of Phyllodistomum folium. Further the main excretory ducts in the adult Phyllodistomum folium do not extend anterior to the bifurcation of the oesophagus, whilst Reuss (1903) shows them extending to a position midway between the oral sucker and the bifurcation of the oesophagus in Cercaria duplicata. Many species of the genus Phyllodistomum have been described but complete life histories of all of them are not known, it seems likely that Cercaria duplicata will ultimately prove to be the larva of one of these incompletely known species. The measurements of the adult and eggs compare favourably with those of Lühe (1909) and Zandt (1924). The length of the body is less than that given by either worker, but may be due to the fact that all the specimens were recovered from the three spined stickleback, whereas, Phyllodistomum folium is known to occur in larger hosts. The size of the uterus and bladder of the stickleback, together with the occurrence of a large number of flukes in one host, may have a restricting effect on size.

INCOMPLETE LIFE CYCLES.

(i) Trichobilharzia ocellata (La Val.1855) Brumpt,1931.

The life history of Trichobilharzia ocellata has been described by Brumpt (1931) its cercaria - Cercaria ocellata is well known, being first described by La Valette (1855) and has since been studied by Sinitsîn (1909) Dubois (1929) Mathias (1930 b) Taylor & Baylis (1930) Vogel (1930 a, 1930 b) Rees (1932) and Wesenburg-Lund (1934). It is considered to be identical with a larva described by H.M. Miller (1923, 1926, 1927) as Cercaria elvae, Miller, 1923.

In Britain the cercaria has previously been found in the Cardiff area where it caused dermatitis among bathers. The description of the cercaria included here agrees in most respects with the details already recorded, but its host in the area under review was found to be Limnaea pereger and not the more usual Limnaea stagnalis.

(a) Sporocyst.

Measurements (mm.)

Length up to 3.4

The long sporocysts occur in a tangled mass in the liver of Limnaea pereger and are pale yellow in colour. Each sporocyst consists of a series of ovoid swellings forming pockets, inside which the cercariae develop, there being no localised area of proliferation. The cercariae are at first folded up on themselves, but as they mature, they straighten out, giving the sporocyst a more uniform appearance. No birth pore was observed, the cercariae escaping by rupture of the sporocyst. In the cuticle of the sporocyst are found small spherical granules, these are less numerous in the distended portions of the sporocyst. These granules are undoubtedly responsible for the grey narrow regions, alternating with very broad transparent regions as described by Wesenburg-Lund (1934)

(b) Cercaria.

Measurements (mm.)

	Extended	Contracted
Body Length	0.406	0.19
Body Breadth	0.055	0.09
Tail Stem Length	0.564	0.29
Tail Stem Breadth	0.033	0.053
Length of Caudal Furca	0.174	0.29
Length of Anterior Organ	0.099	0.077
Breadth of Anterior Organ	0.055	0.066
Diameter of ventral Sucker	0.027 - 0.033	

Cercaria ocellata is faintly yellow in colour and possesses two eyespots. From the measurements it will be seen that it has considerable powers of elongation and contraction. In the normal condition however, the tail stem is much longer than either the body or the caudal furcae. The cuticle covering the body is finely striated, the striations being more dense in the anterior third of the body. The tail is similarly but less densely striated. The caudal furcae are provided with dorso-ventral fin folds. (Fig. 24,f.f.)

The body of the cercaria is elongate oval in shape, the anterior end being rounded. The anterior organ (Fig.25,a.o.) is large and pear shaped, its posterior part (Fig. 25,m.a.o.) being particularly muscular. The sac-like head gland (Figs.25 and 26,h.g.) is found in the dorsal half of the anterior organ. The ventral sucker (Fig.25,v.s.) is small but powerful, and situated just inside the posterior third of the body. It is provided with muscles and frequently protrudes from the ventral surface of the body. When it does this, the dorso-ventral measurement is greater than the lateral measurement, and a side view of the cercaria as is shown in Fig. 26 is obtained.

From the mouth (Figs. 25 and 26,m.) which is small and situated on the ventral side of the body, the long narrow oesophagus (Fig.25,oes.) extends posteriorly through the anterior organ. As there is no pharynx the oesophagus still consisting of a narrow tube continues, until it divides into two short intestinal caeca (Fig.25,b.c.) about half way between the posterior margin of the anterior organ, and the anterior margin of the ventral sucker.

The penetration gland cells are very prominent and occupy most of the posterior half of the body. There are five pairs of gland cells which are of two types; the anterior two pairs (Figs.25 and 26,p.g.) situated at the level of the ventral sucker are coarsely granular in content with large nuclei, and the posterior three pairs (Figs.25 and 26,h.p.g.) found behind them, which are finely granular, with slightly smaller nuclei. The nuclei of all five pairs of cells are situated in the posterior part of their respective cells. The penetration ducts (Figs.25 and 26,p.d.) (one from each cell) lead forward and pass median to the lateral eyespots. (Fig.25,l.es.) situated anterior to the

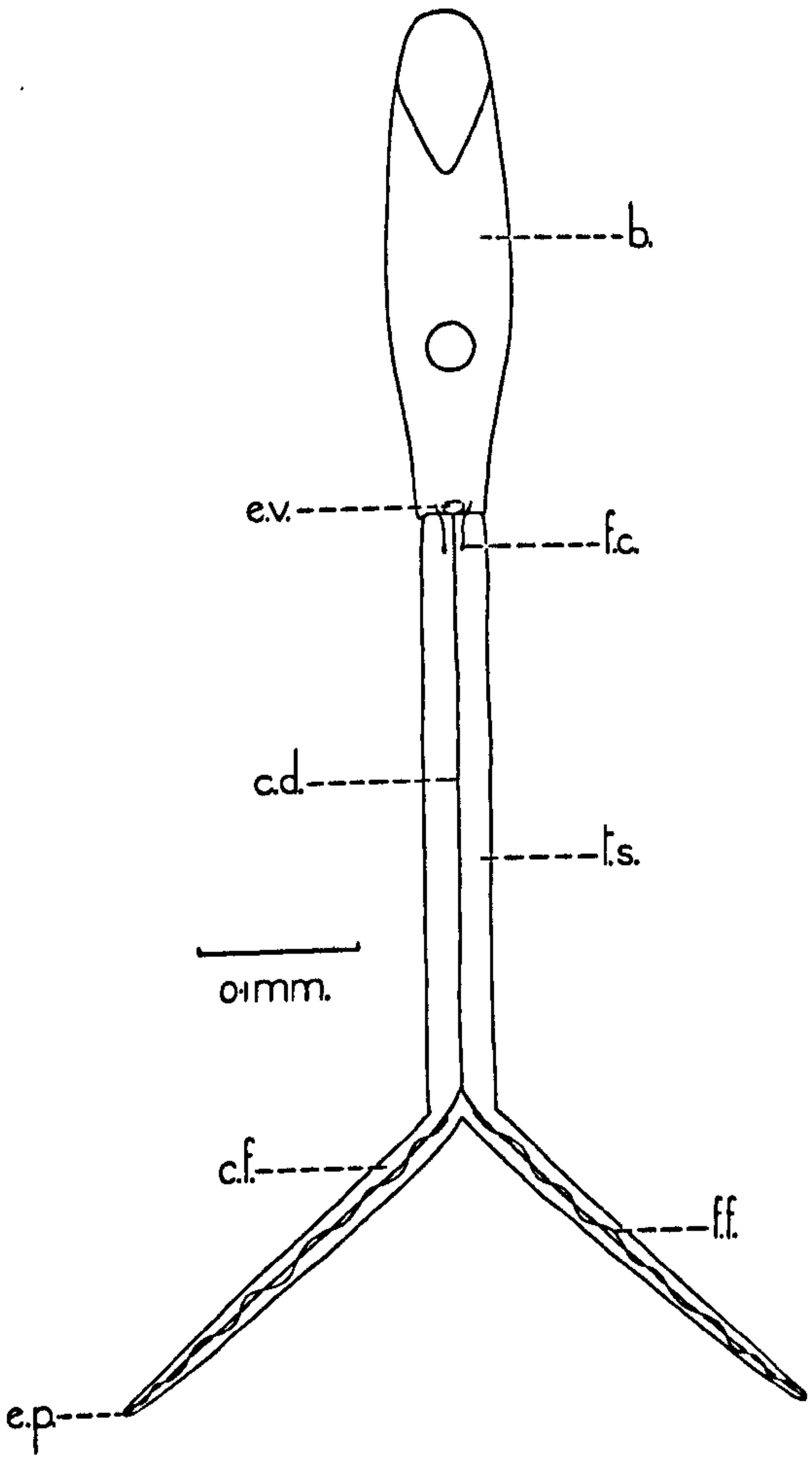


Fig. 24, Cercaria ocellata.

bifurcation of the oesophagus. The ducts of each side then diverge until they reach the level of the anterior organ, where they turn inwards entering immediately anterior to the muscular portion of the anterior organ. The ducts then follow a somewhat sinuous course through the anterior organ, opening anteriorly, lateral and ventral to the head gland. Associated with the openings of the ducts are triangular cuticular spines (Fig.25,c.s.) It was difficult to observe their precise relationship with the ducts, but it is quite probable that each duct is capped by one of these spines, which thereby, function as papillae through which the secretion of the penetration gland cells is passed. The musculature of the ventral sucker was found to be slightly different from that given by Rees (1932) and consists of antero-lateral, lateral, and postero-lateral sets of muscles (Figs. 25 and 26 a.l.m.,lm.,p.l.m.) which extend dorsally, lateral to the penetration gland cells, and are attached to the dorso-lateral part of the cuticle; and a median posterior set of muscles (Figs.25 and 26,m.p.m.) which pass between the penetration gland cells of either side, and are attached to the dorsal wall of the cuticle. In addition to the ventral sucker musculature there is a dorso-ventral set of muscles (Fig.26,d.v.m.) situated anterior to the ventral sucker, which is used during creeping movements, and probably when entering the final host.

From the excretory vesicle (Fig.25,e.v.) situated at the posterior end of the body there leads forward on each side a main collecting duct (Fig.25,m.d.) which extends to the level of the ventral sucker where it turns posteriorly for a short distance before it divides into antero- and postero-lateral ducts. (Fig.25,a.l.d.,p.l.d.) Each main collecting duct contains two ciliated areas (Fig.25,c.a.) situated near the junction of the antero- and postero-lateral ducts. Each antero-lateral duct extends to the level of the posterior border of the anterior organ where it terminates in a flame cell. It also receives the capillaries of two other flame cells. Each postero-lateral duct passes posteriorly until it reaches the level of the middle cell of the posterior group of penetration gland cells where it divides into four capillaries, three of which terminate in flame cells in the body, the remaining one in the tail. The exact nature of the junction of the four capillaries varied slightly in different cercariae, sometimes the capillaries would pair before joining the postero-lateral duct, whilst at other times all four would unite together. H.M.Miller (1927) also records variations in the same system. The differences he observed however, were much greater than those observed here. The flame cell formula for the excretory system of the cercaria agrees with that of previous workers, and is $2 \left[2 \times 3 \ (+ 1) \right] = 14$. A caudal duct (Fig.24,c.d.) leads posteriorly from the excretory vesicle down the tail stem (Fig.24,t.s.) dividing at the junction of tail stem and caudal furcae (Fig.24,c.f.) the two branches opening at the tips of

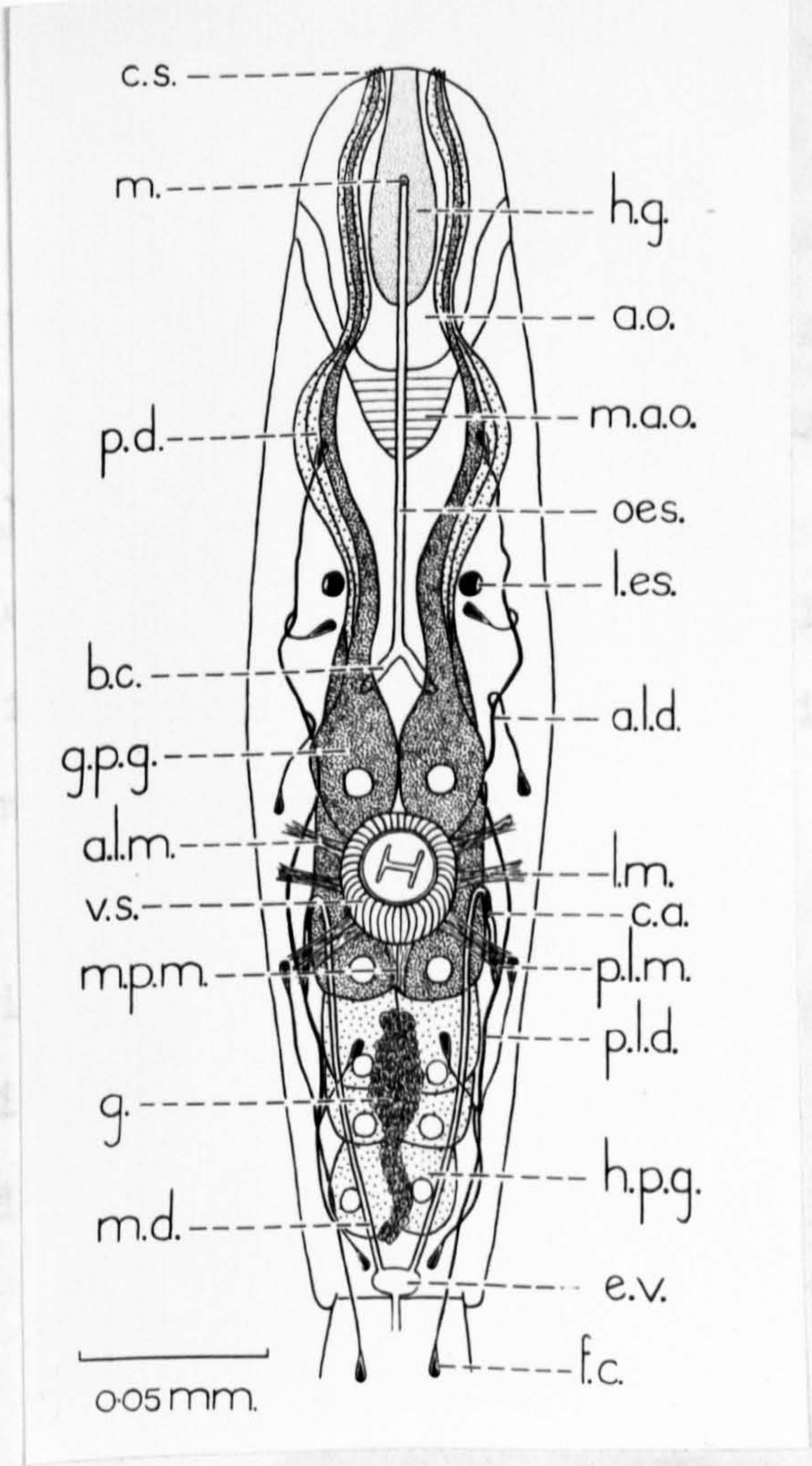


Fig. 25 Ventral View of Cercaria ocellata.

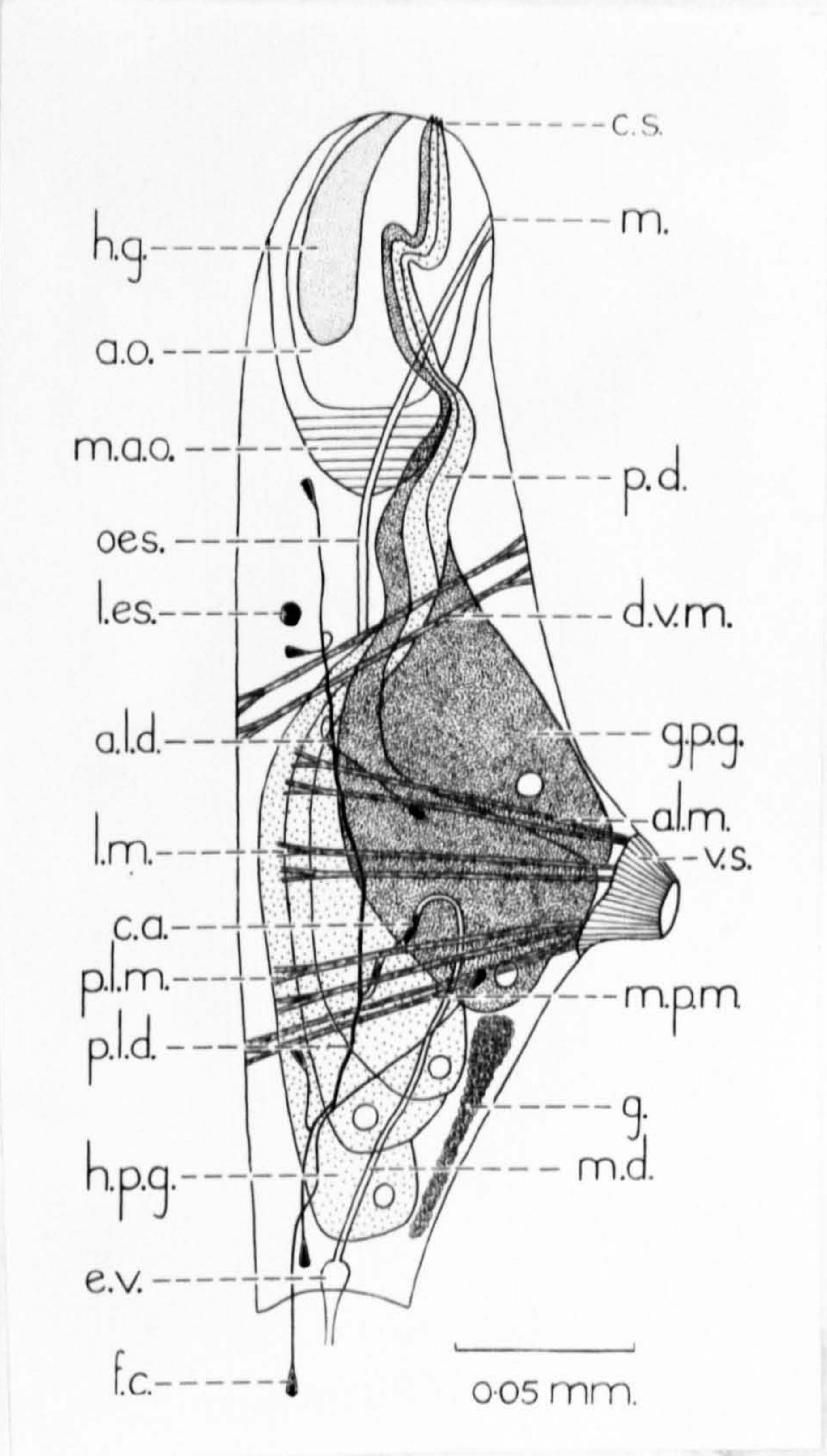


Fig. 26 Lateral View of Cercaria ocellata.

the caudal furcae through excretory pores (Fig.24,e.p.)

The behaviour of the cercaria which has been studied as frequently as its morphology is very characteristic. The cercaria is positively phototactic, and spends most of its free life, which may be up to seventy hours, attached to the illuminated side of the containing vessel with the aid of its ventral sucker. In creeping, the cercaria uses the anterior organ, the ventral sucker and its musculature, and the dorso-ventral musculature. The anterior organ is applied to the substratum and an adhesive secretion is poured on to it, the dorso-ventral muscles contract, arching the body, and the ventral sucker is protruded until it contacts the adhesive secretion, when the forebody elongates, and the anterior organ again applied to the substratum. This action can easily be utilised for penetration into the final host, as is shown by Wesenburg-Lund (1934, Plate XXI Fig.3) The origin of the excretion was not observed, but as the secretion is transparent it is possible that it is derived from the head gland.

Attempts to obtain the adult stage from duck were unsuccessful. Owing to the scarcity of cercariae only two ducks were used in the experiments, cercariae being administere by mouth and by placing the ducks in water containing cercariae. The ducks were killed five and six weeks after infection, and the mesenteric veins searched for flukes, but with no results.

Other workers too have been unsuccessful in infecting ducks, whilst Brumpt (1931) experienced difficulty, - it seems probable therefore, that duck is not a natural definitive host of Cercaria ocellata.

(c) Occurrence.

Cercaria ocellata was exceedingly rare in the area under survey, only four snails having been found containing stages of Cercaria ocellata. Two snails were obtained from Ibbotson Dam in April 1949 and two from Holme Dam, one in November 1949 and one in March 1950. It is interesting to record that one of these snails was doubly infected, containing rediae, and cercariae of Notocotylus attenuatus another rare cercaria of the area.

(d) Discussion.

Cercaria ocellata is a member of Lühe's Furcocercariae and on the basis of H.M.Miller's classification (1926) (see page 113) belongs to the apharyngeal brevifurcate distome cercariae. This division has received much attention, since the Schistosome cercariae are members of the same group. All these cercariae have no intermediate host penetrating directly into the definitive host. As has been shown by Vogel (1930 b) and Taylor & Baylis (1930) Cercaria ocellata will attempt penetration into the skin of man. It is not successful however, in establishing itself, but causes dermatitus. Penetration through the skin of a bird which is the normal vertebrate host would seem to be complicated by the presence of feathers. Under such conditions one would not expect penetration directly through the skin. It seems more probable that infection will occur via the mouth, the cercariae either entering directly when the bird is searching for food, or adhering to the feathers, and entering the mouth when the bird preens itself. Penetration into the tissues surrounding the mouth is undoubtedly easier than directly through the skin.

(ii) Cercaria hirsuta Miller, 1927.

Cercaria hirsuta has been described by H.M.Miller (1927) who obtained it from species of Planorbis in America. The cercaria described below does not agree entirely with the description of Miller, but the differences found are considered insufficient to justify naming it a new species.

(a) Sporocyst.

Measurements (mm.) Length up to 7.2

The sporocysts are long and white in colour and occur in the liver of Limnaea pereger. They are difficult to separate in their entirety from the liver, the sporocyst measuring 7.2 being incomplete. For most of their length the sporocysts are uniform in diameter, but constrictions do occur as would be expected with such long sporocysts. No birth pore was observed which is contrary to Miller's findings.

(b) Cercaria.

Measurements (mm.)

	Contracted	Extended
Body Length	0.110	0.390
Breadth	0.077	0.040
Tail Stem Length	0.144	
Breadth	0.044 - 0.055	

Caudal Furca	0.166 - 0.177
Ventral Sucker	0.022 x 0.027
Anterior Organ	0.053 - 0.40 x 0.033

At rest the body, tail stem and caudal furcae are much the same in length, but the body is frequently found extended which results in the tail stem appearing wider than the body. It is the region anterior to the ventral sucker which undergoes greatest elongation. The body is elongate with a rounded anterior end, the partly protrusible anterior organ (Fig.27,a.o.) being ovoid in shape. The ventral sucker (Fig.27,v.s.) which is situated just inside the posterior half of the body is small, and its inner free edge is beset with four alternating rows of cuticular spines (Fig.27,c.s.) Associated with the mouth on its dorsal lip are about ten anteriorly projecting spines (Fig.27,p.s.) Miller (1927) gives the number as about sixteen, behind which is a narrow region which is devoid of spines. Posterior to this region are found transverse rows of backwardly projecting spines. These are most numerous in the region of the anterior organ and extend as far back as the ventral sucker.

The mouth (Fig.27,m.) is sub-terminal and small. There is a distinct prepharynx (Fig. 27,p.p.) which opens into a globular pharynx(Fig.27,p.) this in turn leads immediately into a blind caecum (Fig.27,b.c.) which is divided unequally into two by a longitudinal septum. The shape of the caecum varies in different cercariae from a pear shaped to a spherical structure.

The whole alimentary system terminates mid-way between the posterior border of the anterior organ and the anterior border of the ventral sucker.

There are six pairs of granular penetration gland cells (Fig.27,p.g.) but the individual cell boundaries were not observed. The cells extend from the level of the excretory vesicle to the ventral sucker, the inner cells of each side meeting in the mid-line. The penetration gland cells merge

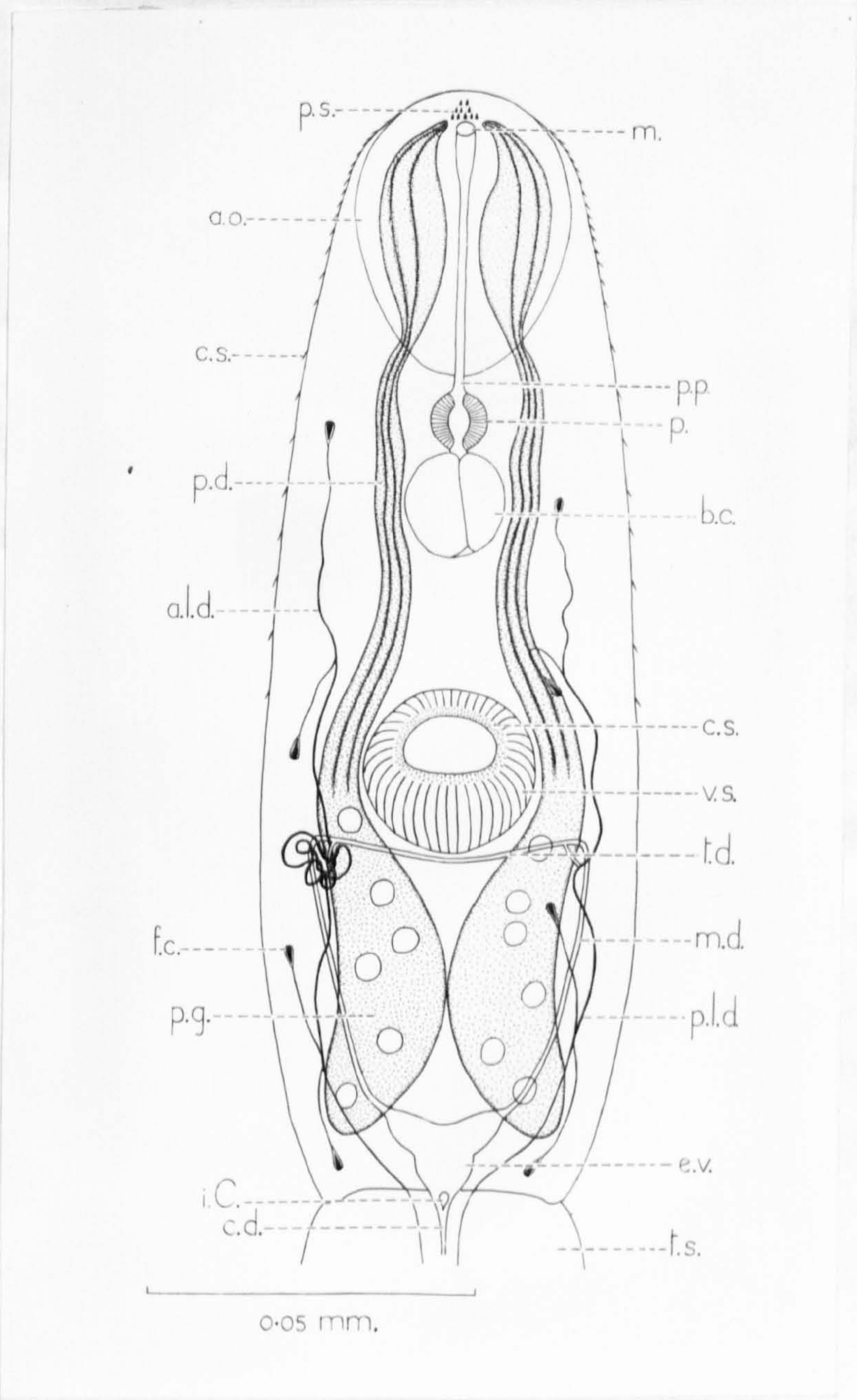


Fig. 27, Body of Cercaria hirsuta.

into the ducts which diverge round the ventral sucker and lead forward more or less parallel until they enter the anterior organ. Here they dilate before opening to the exterior lateral to the mouth.

The excretory vesicle (Fig.27,e.v.) is bilobed and situated at the posterior end of the body - its lateral lobes give rise to the main collecting ducts (Fig.27,m.d.) which extend to the level of the posterior border of the ventral sucker where they turn inwards and fuse, thus forming a transverse duct (Fig.27,t.d.) As they turn inwards each gives off a branch that coils profusely before it divides into antero- and postero-lateral ducts. (Fig. 27,a.l.d.,p.l.d.)

Each antero-lateral duct terminates in a flame cell, usually situated lateral to the pharynx and receives a capillary from one other flame cell. Each postero-lateral duct terminates in a flame cell situated in the tail stem, and receives the capillaries of two flame cells in the body. In the tail stem the flame cell on the left is usually anterior to the one on the right, but the distance between the two is variable. There are thus five pairs of flame cells in the cercaria and the flame cell formula is $2\left[2 \times 2\ (+1)\right] = 10$. There are no ciliated areas in the main ducts, but an island of Cort (Fig.27,i.C.) is present.

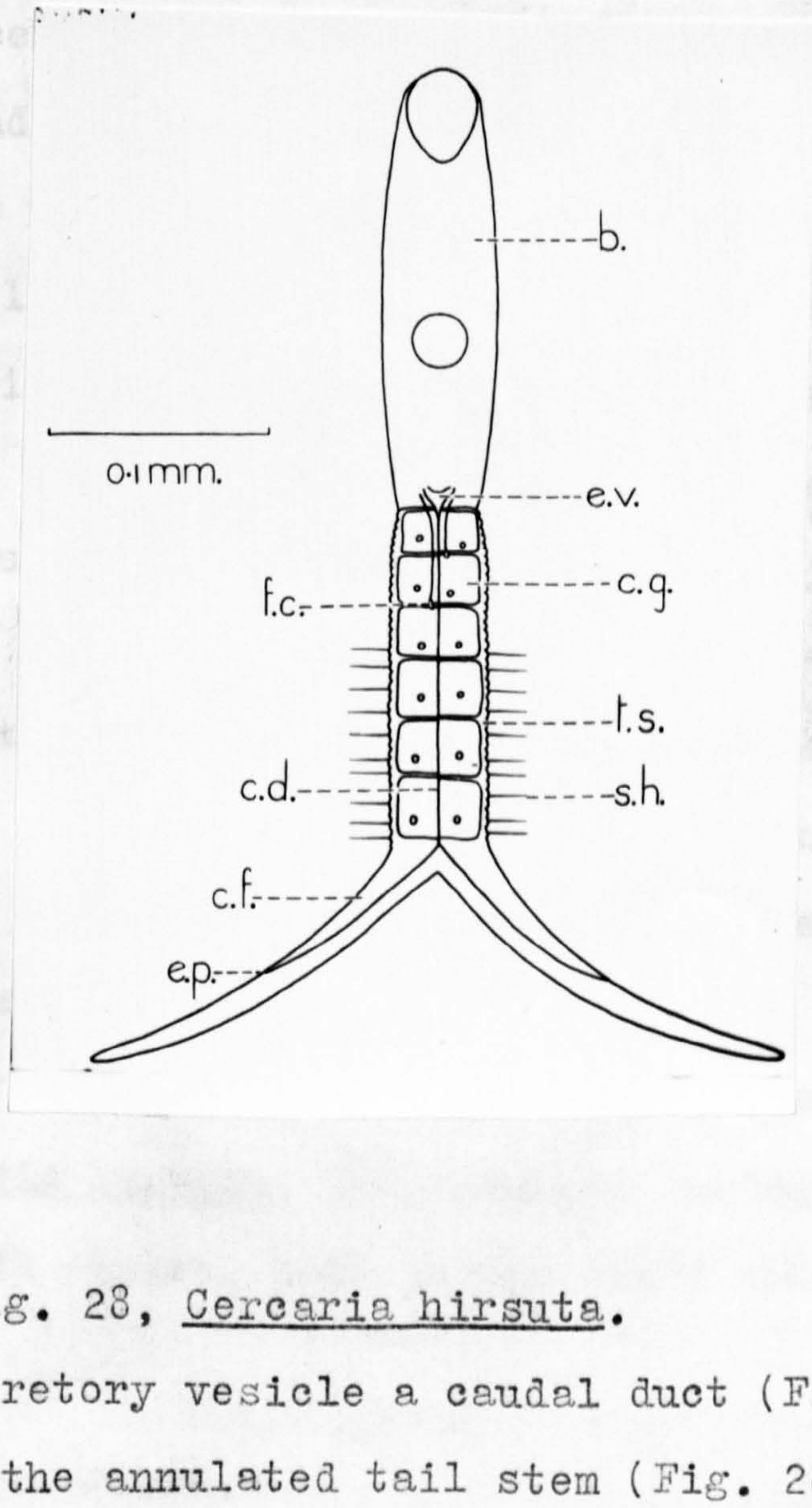


Fig. 28, <u>Cercaria hirsuta</u>.

From the excretory vesicle a caudal duct (Fig.28,c.d.) passes down the annulated tail stem (Fig. 28,t.s.) dividing into two branches at the junction of the tail stem and caudal furcae. Each branch opens to the exterior by an inconspicuous excretory pore (Fig. 28,e.p.) situated about half way along the anterior edge of the furca.

On one occasion two flame cells were observed in the position of the posterior flame cell in the body of the cercaria. The arrangement on the other side of the body was normal, similar variations have been reported for Cercaria granula Miller, 1927 by H.M. Miller (1927.)

No spines were observed on either the body or the caudal furcae, but the ten pairs of long sensory hairs (Fig.28,s.h.) were observed on the posterior half of the tail stem. Miller (1927) states that the tail stem and furcae are spinated and shows twelve pairs of "fine setae" on the tail stem.

The tail stem contains six pairs of caudal gland cells (Fig.28,c.g.) and a feature of many of the cercariae examined, was the remarkable uniformity of these cells as is shown in Fig. 28.

The cercaria although positively phototactic is not strongly so, and has a very characteristic swimming reaction, which contrasts with the behaviour of many other Furcocariae. Observation of large numbers of cercariae gives the impression of incessant swimming activity and of great vitality. When individuals are observed it is seen that the cercaria swims tail foremost, until it contacts some solid object when it ceases swimming movements, alternately elongates and contracts its body, with the anterior organ maintaining contact with the substratum. The cercaria then immediately begins swimming again. This appears to be a deliberate attempt to penetrate any object which it may contact.

Miller (1927) makes no mention of the post cercarial stages of Cercaria hirsuta, and attempts during the present work to find such stages, both in the field and experimentally have failed.

(c) Occurrence.

The cercaria was obtained from three localities namely, Ibbotson and Leather Mill Dams, and Peakshole Water, Castleton, but it was spasmodic in its appearance, as can be seen from Tables 1,3,5, and 16. It was regularly present in the early collections from Ibbotson Dam, but was not found after June 1949.

At Leather Mill Dam a single snail in each of the collections of June 1949, May and July 1950 were infected, whilst at Castleton a further single infected snail was taken in June 1950. Thus the distribution of the cercaria was localised even in the valley of the Porter, which together with the fact that the cercariae were present in the first half of the year suggests that the definitive host may be present for only part of the year - as would occur if the host were a migrant bird.

(d) Discussion.

The description of the cercaria given here agrees with Miller's description of Cercaria hirsuta regarding size, alimentary system, penetration gland cells, excretory system and swimming behaviour. Disagreement exists over the spination of the cercaria, number of sensory hairs, the normal state of the cercaria, and the presence of a birth pore in the sporocyst. Such disagreement however, is concerned with the " finer " structure of the cercaria, - the literature abounds with similar slight differences, as is seen on page 54 in the case of Opisthioglyphe ranae.In view of this, together with the absence of knowledge regarding post cercarial stages, the cercaria described here is considered synonymous with Cercaria hirsuta. The cercaria as was shown by Miller (1926) belongs to the pharyngeal longifurcate distome group of cercariae.

(iii) Cercaria helvetica XXXI Dubois, 1929.

The cercaria described below corresponds closely regarding measurement, structure, and behaviour with Cercaria helvetica XXXI and Cercaria pygocytophora, Brown 1931. These two descriptions differ with regard to the excretory system, although both cercariae have the same excretory formula. Special attention has therefore, been given to the excretory system of the cercaria studied here, and it has been found to vary slightly in different specimens. This variation although not as great as that given in the descriptions of Dubois (1929) and Brown (1931), links them together, and it seems probable that Cercaria pygocytophora is synonymous with Cercaria helvetica XXXI. The descriptions of all three cercariae show that there is close relationship with Cercaria burti Miller, 1923, described by H.M.Miller (1923) and by Cort & Brooks (1928).

(a) Sporocyst.

Measurements (mm.)

Length up to 8.9

The sporocysts are elongate, irregular in shape, and form a tangled mass in the liver of Limnaea pereger. The sporocyst is white in colour and mobile when young, development of the cercariae occurring in pockets., no birth por~~s~~e was observed, although one was reported by Dubois (1929)

(b) Cercaria.

Measurements (mm.)

	Extended	Contracted
Body Length	0.22	0.071
Breadth	0.04	0.077
Tail Stem Length	0.143	0.132
Breadth	0.04	0.033
Caudal Furca	0. 15	
Ventral Sucker	0.023 - 0.27	
Oral Sucker	0.022 x 0.033	
Pharynx	0.012 x 0.012	

The cercaria is almost transparent and has an extremely contractile body as is demonstrated by the fact that when it is contracted it is broader than long. In its normal condition the body is less in length than the tail stem, which in turn is less than the caudal furcae.

The anterior organ (Fig.29,a.o.) is pear shaped when the body is extended, and rounded when contracted. The ventral sucker (Fig. 29,v.s.) is situated just inside the posterior half of the body and bears four alternating rows of cuticular spines, (Fig. 29,c.s.) along its inner edge, compared with three rows as given by Dubois (1929). There are no anteriorly projecting spines in association with the mouth, but the body is covered with transverse rows of backwardly directed cuticular spines

(Fig.29,c.s.) except for a circumoral spineless area. The cuticular spines are most dense in the region of the anterior organ, their density decreasing towards the posterior end of the body. The tail stem (Fig.30,t.s.) bears eight pairs of long sensory hairs (Fig.30,s.h.) two pairs situated near the junction of body and tail stem, the remaining six pairs being found on the posterior half of the tail stem. Brown (1931) gives the number as four pairs for Cercaria pygocytophora. The caudal furcae (Fig.30,c.f) are finely spinated along their entire length.

The mouth (Fig.29,m.) is sub-terminal and leads through the anterior organ into a very small prepharynx (Fig.29,p.p.) which is only evident when the cercaria is extended. The conspicuous globular pharynx (Fig. 29,p.) is followed by the oesophagus (Fig.29,oes.) which dilates slightly before it bifurcates into two short intestinal caeca (Fig.29,c.) The precise location of the bifurcation and extent of the intestinal caeca is variable, depending on the state of contraction of the cercaria, but the septate intestinal caeca never extend below the posterior border of the ventral sucker.

There are four pairs of finely granular penetration gland cells (Fig.29,p.g.) situated postero-lateral to the ventral sucker, both Dubois (1929) and Brown (1931) describe the gland cells as being behind the ventral sucker. From each gland cell a duct (Fig.29,p.d.) runs forwards lateral to the oesophagus, bending round the pharynx and entering the posterior margin of the anterior organ. Each duct dilates as it passes through the anterior organ and opens to the exterior lateral to the mouth. When the cercaria is fully extended the most anterior penetration gland cell occasionally occupies a position anterior to the ventral sucker, between it and the intestinal caecum.

The excretory system consists of a small bilobed excretory vesicle (Fig.29,e.v.) situated along the posterior edge of the body. On each side of the body a main excretory duct (Fig.29,m.d.) leads antero-laterally from the excretory vesicle to the level of the ventral sucker, where it turns inwards and divides into three ducts. The anterior duct (Fig.29,b.d.) leads forwards round the ventral sucker, almost reaching its partner from the other side before terminating blindly. The middle duct which may be regarded as a continuation of the main excretory duct passes behind the ventral sucker and except on rare occasions joined the corresponding duct from the other side, thereby, forming a transverse duct (Fig.29,t.d.) Rarely the two ducts did not meet and ended blindly like the two ducts anterior to the ventral sucker. The posterior duct becomes intensely coiled after branching from the main excretory duct before dividing into antero- and postero-lateral ducts. (Fig.29, a.l.d.,p.l.d.) Each antero-lateral duct terminates in a

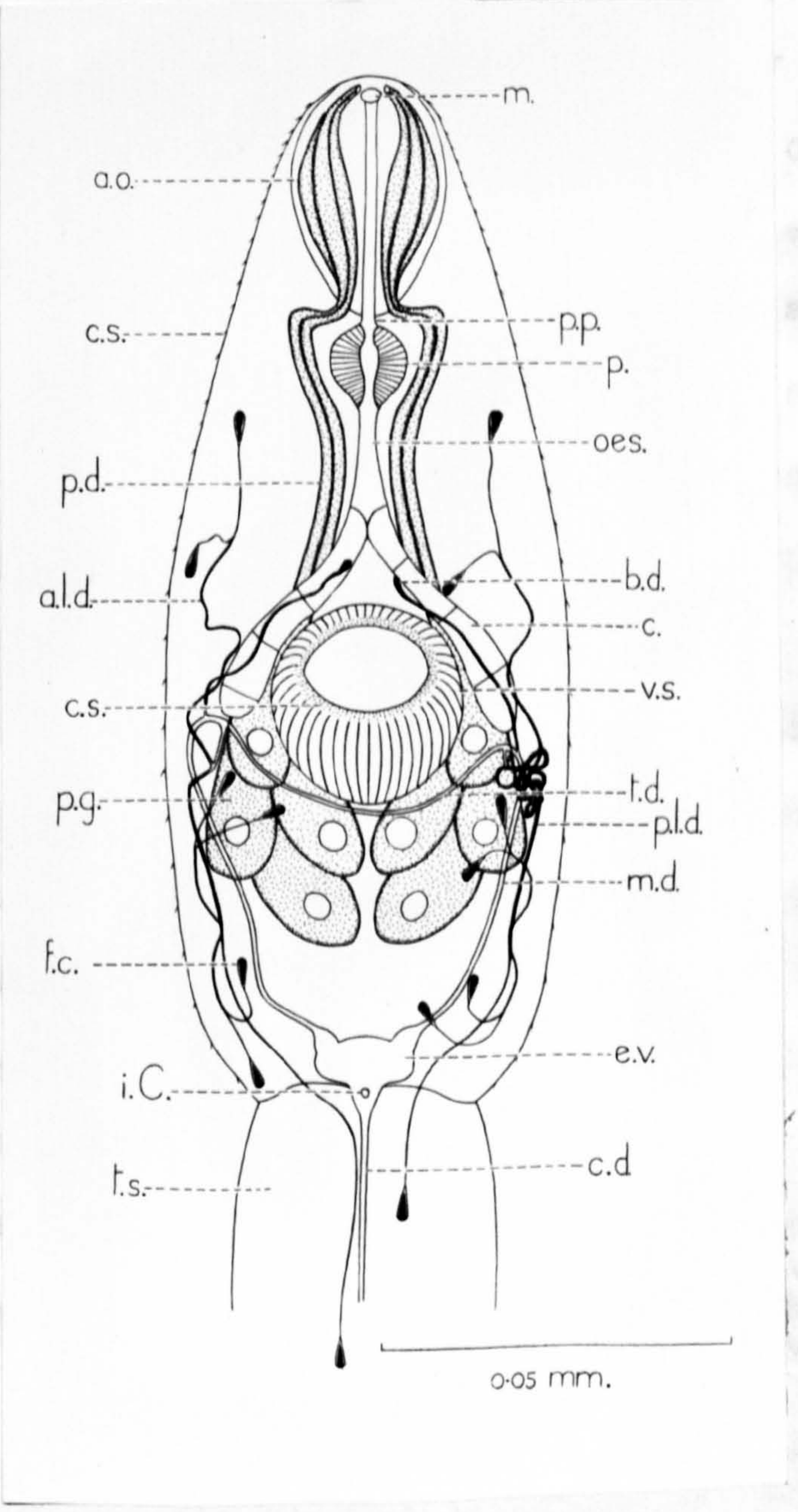


Fig. 29, Body of *Cercaria helvetica XXXI* single flame cell postero-lateral to the pharynx and receives a capillary from a flame cell anterior to the ventral sucker. Each postero-lateral duct terminates in a single flame cell situated in the tail stem. Usually the left flame cell is anterior to its right counterpart, but this is variable as is the distance between them. In addition each postero-lateral duct receives capillaries from four flame cells in the body. There are thus twelve flame cells in the body and two in the tail with a flame cell formula of $2\left[(2) + (2 + 2) + (1)\right] = 14$.

On four occasions cercariae with more than fourteen flame cells were observed. Three specimens had the normal arrangement for the body, but had one of the flame cells in the tail paired. The fourth specimen had a pair of flame cells in the position of the last flame cell in the body.

An island of Cort (Fig.29,i.C.) is situated at the junction of the caudal duct and the excretory vesicle. The caudal duct (Fig.30,c.d.) passes down the centre of the tail stem, and divides at its base, a branch entering each caudal furca. Each branch opens to the exterior through an excretory pore (Fig.30,e.p.) halfway down the furca on its anterior edge. Brown (1931) indicates the opening on the posterior edge.

Ten pairs of caudal gland cells (Fig. 30,c.g.) are found in the tail stem, but the number is not constant. Frequently only eight pairs are seen. The last pair of gland cells occasionally are found at the entrance of the caudal furcae where they join the tail stem. A similar occurrence was noted by Brown (1931) who gives the number of caudal gland cells as eight, and by Cort & Brooks (1928) for Cercaria burti. During the free life of the cercaria the caudal gland cells decrease in prominence and become increasingly irregular. It was only in newly emerged cercaria that ten pairs of caudal gland cells were observed.

The cercaria is negatively phototactic as was also noted by Brown (1931) and swims tail foremost. It spends

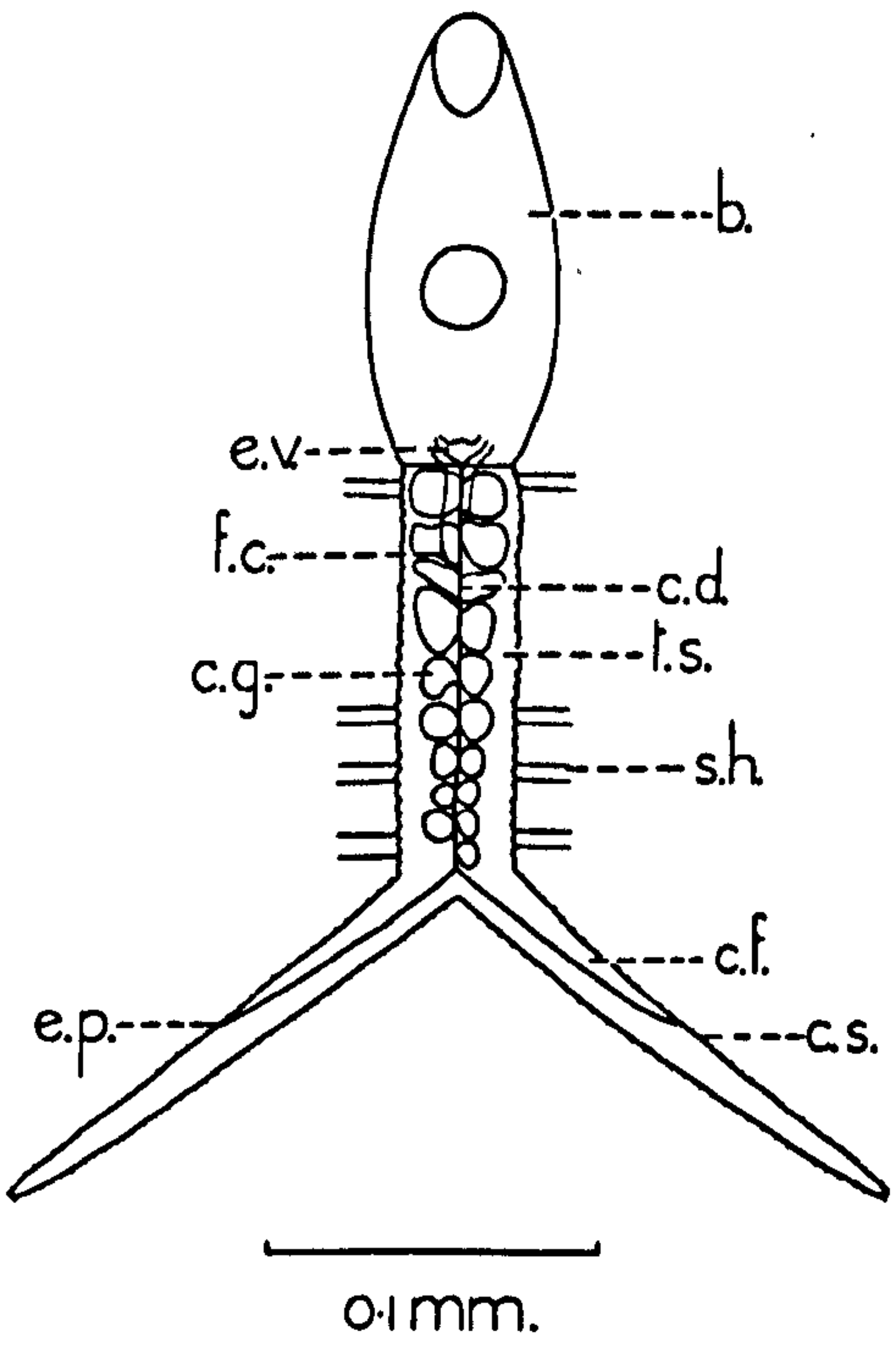


Fig. 30, Cercaria helvetica XXXI

most of its time however, floating with the furcae outstretched at right angles to the body. Active swimming and floating ceases after about ten hours, after which the cercaria sinks to the bottom of the containing vessel, where decaudation and finally death occurs.

(c) Metacercaria.

The next stage in the life history has been obtained, but was not studied. The cercaria was observed to penetrate into the leeches Helobdella stagnalis and Herpobdella atomaria developing into the "Tetracotyle" stage. Leeches infected with a few specimens of "Tetracotyle" were fed to one duck without result. Penetration into the leech is remarkably rapid. The cercaria appears to make chance contact, it then clings on to the leech with its ventral sucker. The forebody is arched, the anterior organ applied to the integument of the leech and there follows a period of active pulsation by the extreme anterior region of the body. Throughout this period the tail is lashed vigorously from side to side. After about three minutes the anterior organ has usually succeeded in affecting an entry and the ventral sucker releases its grip. The whole body then pulsates in an effort to enter the host, the tail is shed, and entry is usually completed in about seven minutes.

(d) Occurrence.

The cercaria was the most widespread of the Furcocercariae found occurring in seven habitats namely, Monsaldale, Lathkill Dale, Leather Mill, Shepherd, Ibbotson, and Holme Dams on the River Porter, and Ford Dam as is shown in Tables 1,3,4,5,6,7,14, and 17. Its occurrence was not confined to any season of the year. During 1949 it was obtained each month from at least one habitat, except for the early collections of 1949 from Ibbotson Dam where the cercaria was regularly present in considerable numbers. Its occurrence over the area under survey was occasional.

Leeches were collected on several occasions, only one sample taken from Ford in the December of 1949 contained "Tetracotyle" stages. It was not ascertained whether they were post cercarial stages of the species just described.

(e) Discussion.

The excretory system of Cercaria pygocytophora described by Brown (1931) differs from that of the cercaria described above in the absence of the transverse duct posterior to the ventral sucker. The position and nature of the blind ducts anterior to the ventral sucker are identical.

The excretory system of Cercaria helvetica XXXI as described by Dubois (1929) possesses complete transverse ducts anterior and posterior to the ventral sucker. The flame cell pattern according to all three descriptions is the same, thus the differences are solely concerned with the nature of the two ducts, anterior and posterior to the ventral sucker. This part of the system has been shown in the present work to be variable, and is evidently undergoing development during the cercarial stage. In view of this, and since the three descriptions form a graded series of development stages, it is concluded that all three descriptions refer to the same species.

Both Brown (1931) and Dubois (1929) found the snail host to be _Planorbis carinatus_ Müller, 1774, although Dubois also found it developing in _Limnaea limosa_ (Linnaeus, 1758).

The cercaria belongs to the pharyngeal longifurcate distome group of Furcocercariae, its position being discussed on page 115

(iv) _Cercaria A_ n.sp.

This cercaria belongs to a well known group of cercariae which develop into diplostomum larvae. They are all similar in structure, certain organs being uniform throughout the group, thus they all have four penetration glands situated behind the ventral sucker, long wide intestinal caeca, and a group of forwardly directed spines in close association with the mouth. Similarities exist regarding the excretory system, size and behaviour of the cercaria and structure of the sporocyst. The group includes _Cercaria furcata_ Nitzsch, _Cercaria chrysenterica_ Miller, 1923 _Cercaria flexicauda_ Cort & Brooks, 1928, _Cercaria laruei_ Cort & Brooks, 1928 _Cercaria modicella_ Cort & Brooks 1928, _Cercaria C_ Szidat, 1924, _Cercaria helvetica XIII_ Dubois 1929, _Cercaria helvetica XV_ Dubois 1929, _Cercaria X_ Baylis, 1930 and _Cercaria chromatophora_ Brown, 1931.

(a) Sporocyst.

Measurements (mm.)
Length up to 5.9

The sporocysts were found in the liver of _Limnaea pereger_. They are faintly yellow in colour, of uniform diameter and possess a sub-terminal birth pore.

(b) Cercaria.

Measurements (mm.)

Organ.	Contracted		Extended
Body Length	0.16		0.29
Breadth	0.085		0.055
Tail Stem Length		0.23	
Tail Stem Breadth		0.037 - 0.038	
Caudal Furca		0.232 - 0.26	
Ventral Sucker		0.027 - 0.033	
Anterior Organ		0.040 x 0.061	

The body of the cercaria is capable of considerable changes in size, but under normal conditions the body is about the same length as the caudal furcae which are somewhat longer than the tail stem.

Associated with the mouth is a group of about seven forwardly directed spines (Fig.31,p.s.) behind which is a circumoral spineless region. The remainder of the body is spinated in the form of transverse rows of backwardly directed cuticular spines (Fig.31,c.s.) These spines are most dense in the region of the anterior organ immediately behind the circumoral spineless region. The ventral sucker (Fig.31,v.s.) bearing three or four rows of spines on its inner free edge is situated just behind the mid-line.

The sub-terminal mouth (Fig.31,m.) leads into a tube which passes through the partly protrusible anterior organ (Fig.31,a.o.) to a small prepharynx (Fig.31,p.p.). The pharynx (Fig.31,p.) is oval in shape, and leads into a long wide oesophagus (Fig.31,oes.) which divides into two intestinal caeca (Fig.31,c.) immediately in front of the ventral sucker. The intestinal caeca are highly conspicuous and extend almost to the posterior end of the body.

The four penetration gland cells (Fig.31,p.g.) are arranged in the characteristic pattern of the group, an anterior pair situated in the mid-line, one behind the other, immediately posterior to the ventral sucker; and a posterior pair situated side by side. Their ducts (Fig.31,p.d.) (one from each cell) lead forward in a slightly sinuous path, curving round the pharynx before entering the anterior organ. In the anterior organ they dilate slightly before opening lateral to the mouth. The protoplasm of the penetration gland cells is finely granular and the nuclei are prominent.

From the excretory vesicle (Fig.31,e.v.) situated at the posterior end of the body, a pair of main excretory ducts (Fif.31,m.d.) lead forward to the level of the ventral sucker where each exhibits a double loop. Each double loop contains a single ciliated area (Fig.31,c.a.) The division of the main excretory duct into antero- and postero-lateral branches was not observed. Each antero-lateral duct (Fig.31, a.l.d.) terminates in a single flame cell lateral to the anterior organ. It receives a capillary from a second flame cell situated at the level of the pharynx, and most probably receives the capillary of a third flame cell which is situated behind the ventral sucker although its actual connection with the antero-lateral duct was not observed.

Each postero-lateral duct (Fig.31,p.l.d.) extends into the tail stem, where it receives the capillaries from a pair of flame cells. In the body each receives capillaries from three flame cells. The flame cell formula is probably

$$2\left[(3)+(2+1)+(2)\right] = 16.$$

From the excretory vesicle there extends posteriorly a caudal duct (Fig.32,c.d.) which divides at the base of the tail stem, a branch passing down each furca and opening to the exterior through an excretory pore (Fig.32,e.p.) situated about half way down the furca on its anterior edge.

The annulated tail stem (Fig.32,t.s.) bears eight pairs of long sensory hairs (Fig. 32,s.h.) and contains six pairs of caudal gland cells (Fig. 32,c.g.)

The cercaria shows the characteristic behaviour which has been noted for other cercariae in the group and studied in detail by Szidat,(1924) and by Wesenburg-Lund (1934) for <u>Cercaria C</u> Szidat,1924. The cercariaeremain hanging in the water for long periods, exhibiting periodic bursts of swimming so as to maintain their position.

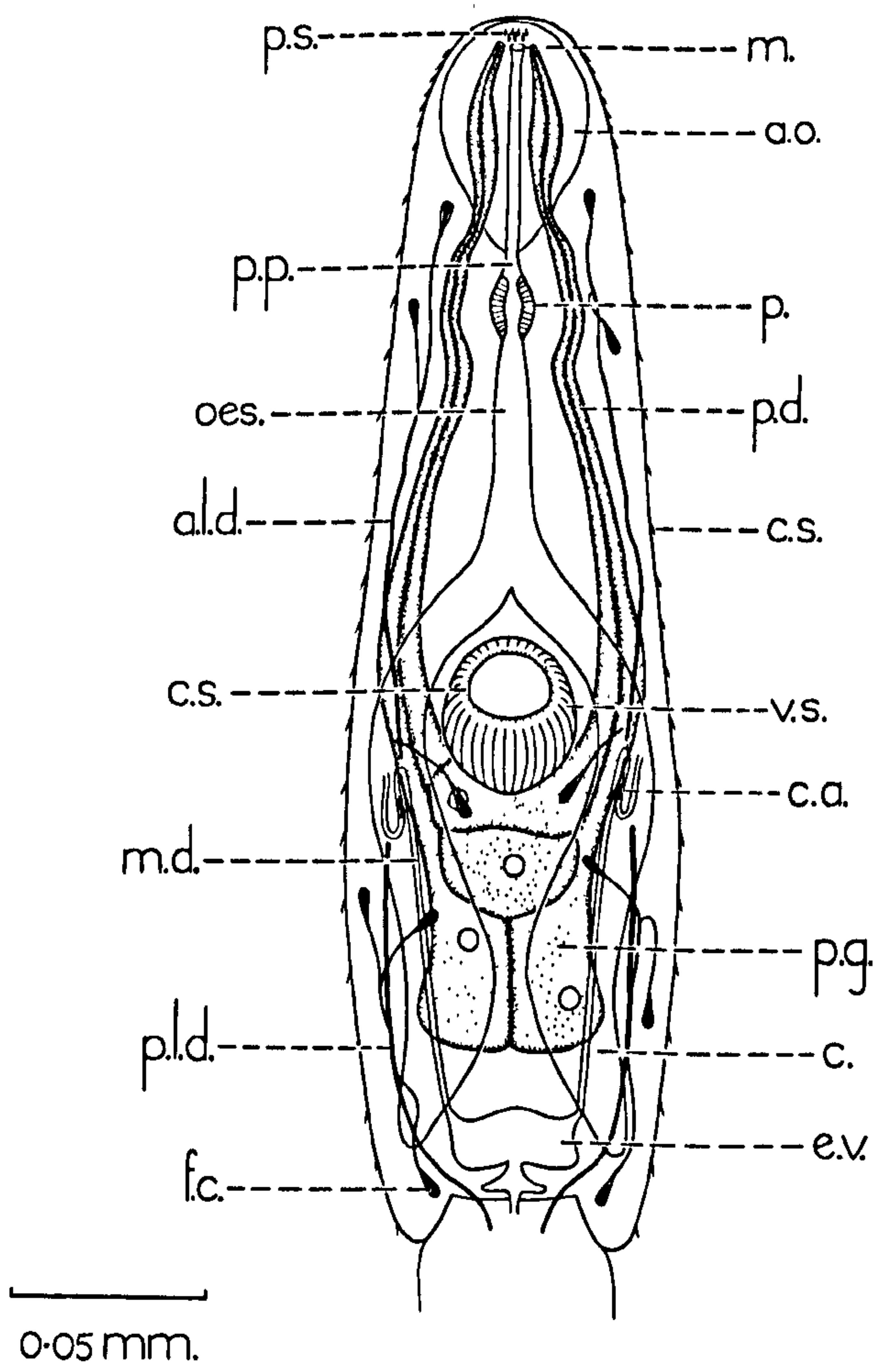


Fig. 31, Body of <u>Cercaria A</u>.

(c) Metacercaria.

During September and October of 1949 out of nineteen sticklebacks taken from the Round Dam, eighteen contained diplostomum larvae located in the eye. The diplostomula were at all stages of development, yet attempts at locating the corresponding cercarial stage from the Dam, and those upstream failed. Examination of cercariae liberated from snails collected at Monsaldale in October 1949, showed that they were obviously related to the diplostomula already observed. These cercariae were therefore, placed in contact with sticklebacks which from previous examination were known to be free of infection. The sticklebacks were readily infected and their behaviour was similar to that observed by Wesenburg-Lund (1934) when <u>Carassius carassius</u> (Linnaeus, 1758) was placed in contact with <u>Cercaria C</u> Szidat, 1924. A

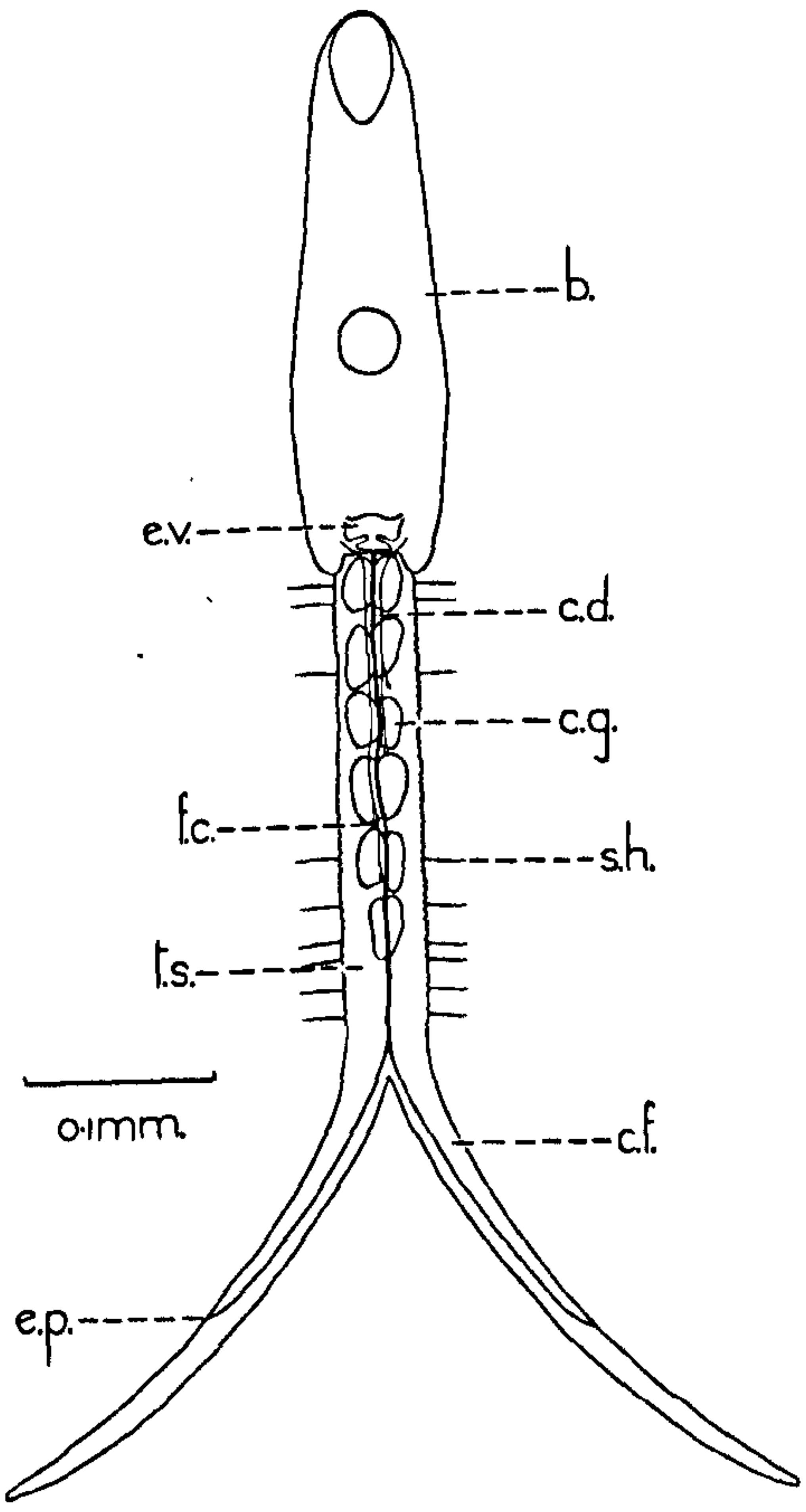


Fig. 32, Cercaria A.

short time after contacting the cercariae, whose behaviour undoubtedly facilitates chance contact with the fish, the sticklebacks became extremely restless, darting about the acquarium. After this initial period, the fish remained almost stationary for long periods, and during this time, which lasted several days, many fish died, particularly if the concentration of cercariae was heavy. Those fish remaining alive after this period were easily maintained in the laboratory. Two fish were kept thirteen months before being killed. The eyes of some of these fish became distended following infection, partial blindness was also evident in many of the fish. The passage of the cercariae to the eye was not studied, but cercariae were found in the eyes of fish six hours after contact with cercariae.

The diplostomum larva was located in the retina of the eye. It was never found in the lens. In this, it resembles Diplostomum emarginatae Cort, 1917 as reported by Oliver & Cort (1942) and Diplostomum flexicaudum as reported by Van Haitsma (1931).

Two days after penetration the structure of the cercaria is still predominant in the developing metacercaria. The penetration gland cells and ducts are easily seen, the metacercaria has commenced to feed however, black pigment being obvious in their intestinal caeca. Its concentration was heaviest in fourteen day metacercariae, but it was observed in decreasing amounts in metacercariae up to fifty nine days old.

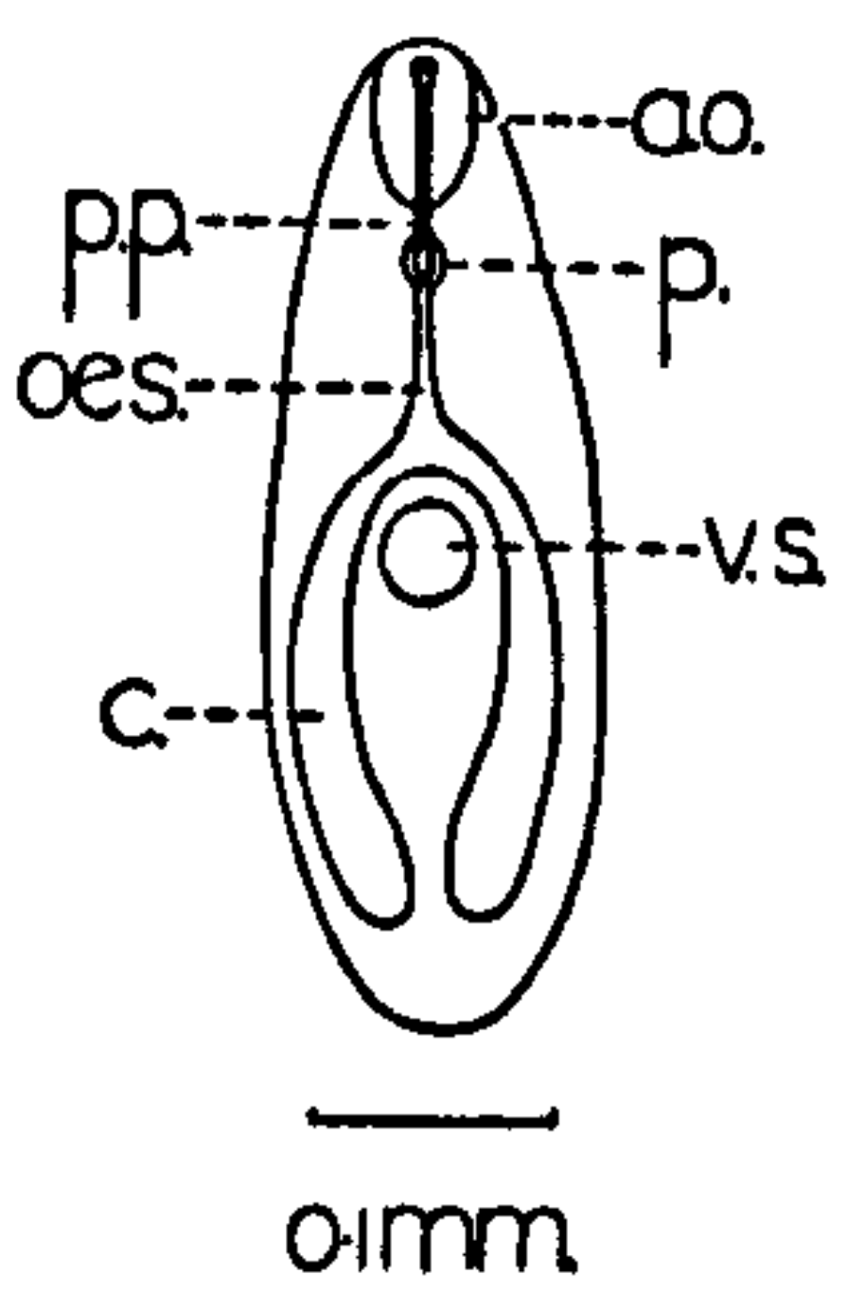


Fig. 33 Nine Day Metacercaria.

In nine day old metacercariae (Fig. 33) no new organs have formed, although those organs present are undergoing modification, and an increase in size has occurred. The living metacercaria which is of variable shape measures 0.174 - 0.406 mm. long x 0.040 - 0.116 mm. broad. The anterior organ (Fig. 33,a.o.) shows some modification towards a true oral sucker, and the oesphagus (Fig.33,oes.) is relatively shorter and more slender than in the cercaria. The last vestiges of the penetration ducts were present in some of the nine day metacercariae examined as is shown in (Fig. 34 . A twenty four day old metacercaria has almost completed its metamorphosis. The adhesive organ (Fig. 34,ad.o.) which was first observed in fifteen day old metacercariae is conspicuous behind the ventral sucker. The lateral epaulettes (Fig. 34,e.pr.) on either side of the true oral sucker (Fig.34,o.s.) are developing, and the excretory system which was not studied in detail is much elaborated. The oesophagus (Fig. 34,oes) is short and bifurcates well in front of the ventral sucker into the long intestinal caeca.

The body is dorso-ventrally flattened and is almost twice as large as the nine day metacercaria.

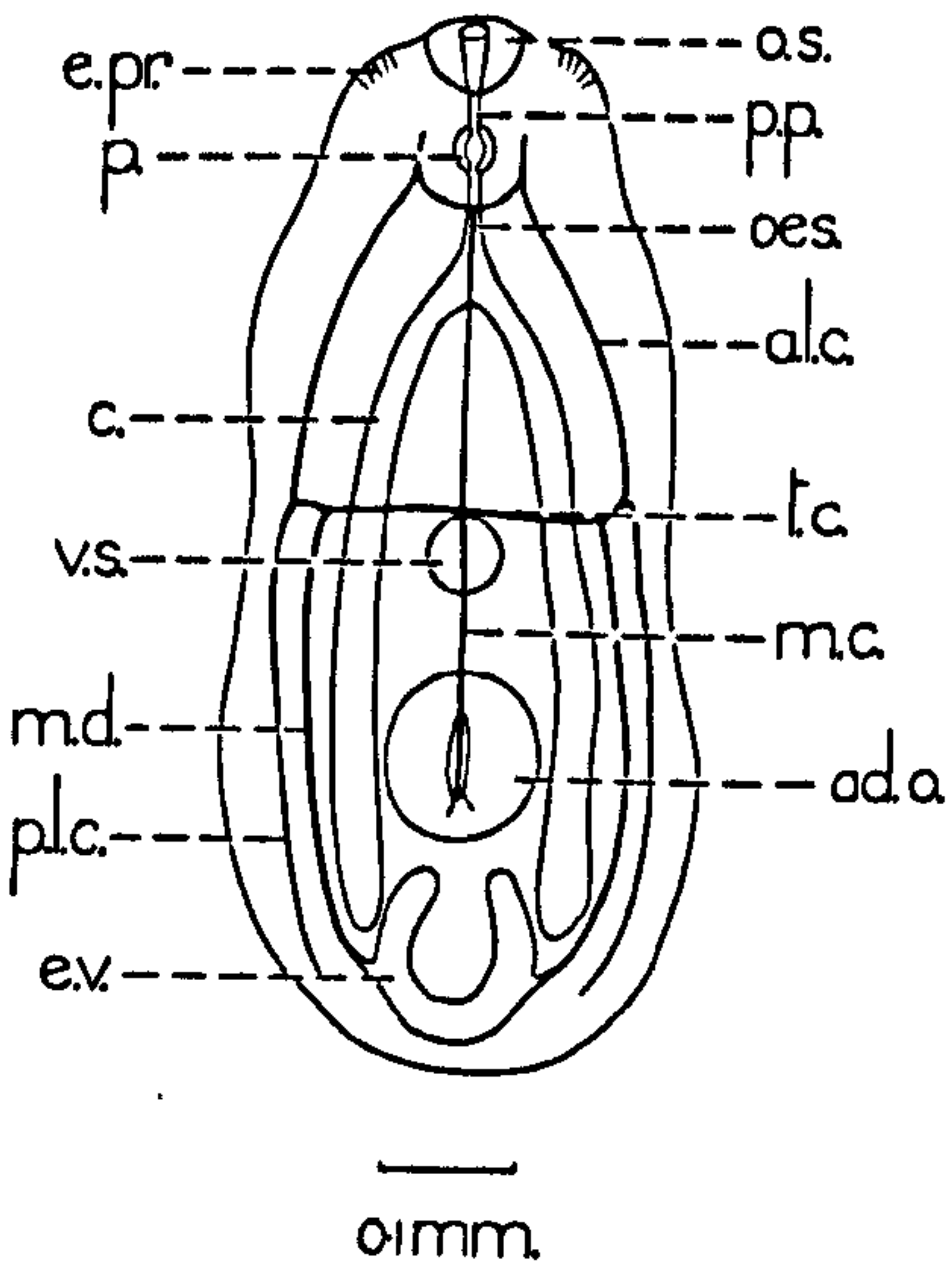


Fig. 34, Twenty Four Day Old Metacercaria.

Measurements of twenty four day old metacercaria (mm.)

Length	0.67 - 0.75
Breadth	0.30 - 0.38
Oral Sucker	0.055 - 0.057 x 0.066 - 0.071
Ventral Sucker	0.055 x 0.055 - 0.060
Adhesive Organ	0.105 - 0.116 x 0.121 - 0.145

In thirty five day old metacercariae a dorsal outgrowth from the posterior end of the body was observed (Fig.35,h.b.) This hind-body was very variable in shape, almost disappearing in fixed specimens. On the adhesive-organ a fine striation was observed, and in forty two day old specimens this same striation was found over the whole surface of the body. The solitary excretory granules found in the endings of blind ducts of the excretory system which are a feature of diplostomum larvae were first observed in forty two day old metacercariae. The measurements of thirty five day old metacercariae are very similar to those of twenty four day old specimens indicating that growth is about completed. The lateral epaulettes (Fig.35,e.pr.) are more prominent in thirty five day old metacercariae; in fixed specimens they appear as small concavities on either side of the oral sucker.

Live metacercariae were obtained from the two sticklebacks killed after being infected for thirteen months. Apart from the very numerous excretory granules present, no other differences from the thirty five day old metacercariae were observed. No development of the hind-body had occurred. It is this region which in the adult grows enormously and contains the reproductive organs.

Four sticklebacks containing mature diplostomula were fed to one duck which was killed four weeks later, but no flukes were found.

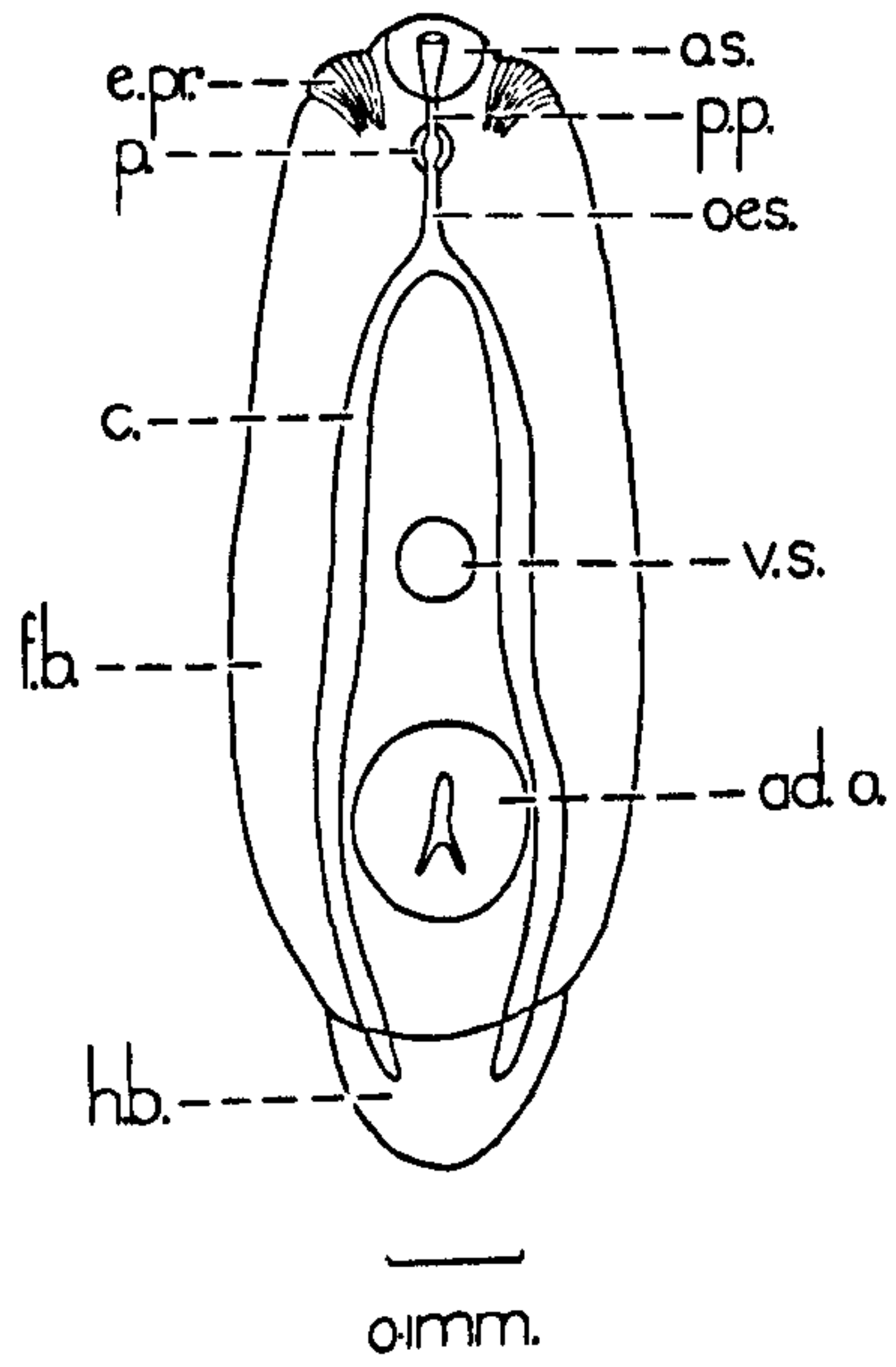


Fig. 35, Thirty Five Day Old Metacercaria.

(d) Occurrence.

Only six specimens of <u>Limnaea pereger</u> containing <u>Cercaria A</u> have been collected. Four of these snails were obtained from Monsaldale in October 1949. The remaining two from Holme Dam in September and October of 1950. The metacercarial stage in the stickleback was more common as has been noted on page 97 and they were also found in Holme Dam before the cercarial stage was obtained, also, during October 1950 metacercariae were found in two sticklebacks of a sample of seven taken from Ibbotson Dam. From these results it is evident that the cercarial stage was more prevalent in the area than the collection of infected snails show, and they also demonstrate that the cercaria was highly successful in reaching the intermediate host.

(e) Discussion.

The cercaria and its development into a diplostomum larva as has been described above is typical of the large group of cercariae (given on page 94) to which it belongs. However, it does not correspond exactly to any of these cercariae and as such, must be considered a new species. The only other British cercariae in the same group are Cercaria X Baylis, 1930, and Cercaria chromatophora. These two cercariae which are probably synonymous differ from the cercaria described above in the possession of an additional pair of flame cells in the body. It has been shown by Taylor & Baylis (1930) that Cercaria X Baylis, 1930 also penetrates into the stickleback, and develops into a diplostomum larva which is located in the lens. In this respect it is unlike the cercaria described above, but is similar to Cercaria C Szidat,1924, whose diplostomum stage was located in the lens of the stickleback and which was shown to be the larva of Diplostomum spathecum (Rudolphi, 1819) Olsson, 1876 emend. It is unfortunate that Szidat's description of Cercaria C Szidat, 1924 is incomplete, for although the life cycle is known, the cercaria cannot be identified from the description alone. It is undoubtedly most closely related, and perhaps identical with Cercaria X Baylis, 1930.

Other cercaria in the same group to which Cercaria A is related are Cercaria helvetica XV Cercaria helvetica XIII Cercaria flexicauda and Cercaria laruei; all these cercariae have identical flame cell formulae, but only Cercaria laruei possesses the same number of caudal glands. Although possessing the same flame cell formulae they all differ from Cercaria A in that they have three pairs of flame cells anterior to the ventral sucker. Thus, while the differences between Cercaria A and the related cercariae are small, they are as great as those between the species already described. In view of this Cercaria A is for the present considered to be a new species.

Cercaria A belongs to Miller's pharyngeal longifurcate distome group of Furcocercariae which are discussed on page 114

CLASSIFICATION OF CERCARIAE INCLUDED IN SURVEY.

(a) General.

Owing to the complex nature of the development of the digenatic trematodes it was at first impossible to correlate the most prominent larval stages namely, the cercariae with the phylogeny of the adults. Consequently attempts were made to arrange the cercariae in convenient groups, this in itself being most useful since it stimulates and helps other workers. Lühe (1909) was the first to attempt such a classification of cercariae. For his main divisions he followed those of the adults whilst his sub-divisions were based largely on the structure of the tail. Lebour (1911) formulated a different scheme in which like Lühe's the main divisions were based on that of the adults. The structure of the tail was also used in the sub-divisions but more emphasis was placed on the early developmental stages.

Lühe's terminology has been adopted in the present work as it is more comprehensive than Lebour's. It must be emphasised however, that the classification is to a large extent artificial and not of great taxonomic value in itself since the grouping together of cercariae with like tail structures shows convergence rather than true relationship. Because of this Lühe's scheme has been criticised, but it has served to stimulate interest and as knowledge of life histories is gained Luhe's scheme is giving way to a natural classification which incorporates all the developmental stages.

Lühe's original scheme for the classification of the cercariae has been modified by various workers, notably

Sewell (1922) and Dubois (1929) the latter worker formulating the concept that the digenea have a dual origin. These modifications are concerned with the details of Lühe's scheme and are therefore, discussed when dealing with the relevant cercariae. However, the inclusion of the Gymnocephalous, Echinostome, and Xiphidiocercariae in one grouping namely, the Leptocercariae does not appear consistent, a view also supported by Wesenburg-Lund (1934) and Sewell (1922). It would be more convenient to abandon the term Leptocercariae and raise the Gymnocephalous, Echinstome, and Xiphidiocercariae to groupings of their own, each of equal status with the other divisions of the Distome cercariae. The acceptance of this modification is frequently implied, but rarely mentioned.

Lühe's scheme as outlined by Dawes (1946) showing the important diagnostic characters is given below.

1. Lophocercariae - having a dorsal longitudinal fin-fold along the body.

2. Gasterostome cercariae - tail having two symmetrical processes, intestine simple and sac-like, mouth centrally situated on the ventral surface.

3. Monostome cercariae - ventral sucker absent.

4. Amphistome cercariae - ventral sucker at or very near the posterior extremity.

5. Distome cercariae - ventral sucker distant from the posterior extremity.

    (a) Cystocercous cercariae - base of the tail containing a cavity into which the body can be retraced.

    (b) Rhopalocercous cercariae - tail as wide as or wider than the body.

    (c) Leptocercous cercariae - tail straight, slender and narrower than body.

        (i) Gymnocephalous cercariae - anterior extremity rounded and lacking a piercing spine or stylet.

        (ii) Echinostome cercariae - anterior, extremity provided with a 'head collar' and a coronet of stout "head spines" or"collar spines".

(iii) Xiphidiocercariae - anterior extremity provided with a stylet.

(d) Trichocercous cercariae - tail provided with spines or "bristles."

(e) Furcocercous cercariae - tail forked distally.

(f) Microcercous cercariae - tail short and stumpy.

(g) Cercariaea - tail underdeveloped.

(h) Rat-King cercariae (marine) cercariae arranged in groups having the tips of the tails united to form a kind of colony.

(b) Classification of the Monostome cercariae.

Sewell (1922) using the term Monostome cercariae in its widest sense included all forms lacking a ventral sucker or acetabulum within this group, which was then divided into six viz. The Pleurolophocera; the Urbanensis; the Ephemera; the Lophocera; the Lophoides; and the Ubiquita sub-groups. Dubois (1929) and Wesenburg-Lund (1934) disagreed with Sewell and limited the Monostome cercariae to the Urbanensis and Ephemera sub-groups. It was further shown by Dubois (1929) that size could not be used in conjunction with the presence or abøsence of the median eyespot for distinguishing cercariae of the two sub-groups, since <u>Cercaria helvetica I</u> Dubois,1929 must be placed in the Urbanensis sub-group on the basis of size, whilst the possession of a median eyespot was characteristic of the Ephemera sub-group.

The possession of two or three eyespots had already been utilised by Faust (1917) for dividing Monostome cercariae into Binoculate and Trioculate groups, but these groups did not correspond exactly with the Urbanensis and Ephemera sub-groups since Faust included some additional forms in his Binoculate group.

Rothschild (1938) regarded the division of the Monostome cercariae based on the presence or absence of the median eyespot as unsatisfactory and sub-divided the cercariae of the super family Notocotyloidea (which includes the Ephemera and Urbanensis sub-groups) according to the position and structure of the anterior transverse portion of the excretory tubule. It was hoped that these characters might be employed in forming a natural classification of adult worms. Identification of adults however, is difficult, and consequently their classification is far from complete. In addition few life cycles are known with complete descriptions of all stages so that comparison of schemes of larval classification as mentioned above with schemes of adult classification as given by Price (1931) Mehra (1932) Harwood (1939) and Ruiz (1944) is almost impossible. Knowledge of the genus Notocotylus Diesing, (1839) is more complete and examination of members of this genus reveals that they do not fall into any one of Rothschild's divisions; thus the cercariae of Notocotylus seineti. Notocotylus thienemanni U. Szidat, 1933 and Notocotylus attenuatus are placed in the Monostomi group, whilst the cercariae of Notocotylus imbricatus and Notocotylus urbanensis Harrah, 1922 are in the Imbricata group. It would appear from this that Rothschild's scheme cannot be employed in classifying adults, and until more knowledge of life histories is known any scheme of classification will be incomplete. It is certain however, that when a suitable classification is found, larval as well as adult characters will have to be utilised as Notocotylus attenuatus. Notocotylus magniovatus Yamaguti, 1934 Notocotylus imbricatus and Notocotylus stagnicolae Herber, 1942 have adult forms which are almost indistinguishable, yet their larval forms have quite distinct characters.

(c) Classification of Echinostome Cercariae.

Lühe (1909) divided the Echinostome cercariae then known into four convenient groups, based on the size of the gut in the redia, and the presence or absence of a fin-fold on the tail.

Sewell (1922) divided the Echinostome cercariae into three groups, associating a further group of cercariae - the

Megalura with them. This division was based largely on the structure of the excretory system. In the Echinatoides and Coronata groups the main lateral canal divides into anterior and posterior branches opposite the ventral sucker, whilst in the Echinata group the main lateral canal passes to the posterior end of the body and "apparently divides into anterior and posterior collecting tubes" opposite the excretory vesicle. Cercariae in the Echinatoides group possess a fin fold on the tail, thereby distinguishing them from those in the Coronata group.

Two cercariae, Cercaria indica XII Sewell, 1922 and Cercaria indica XXIII, Sewell 1922 described by Sewell (1922) and placed together in the Echinata group were shown by him to belong to different genera - the former to the genus Echinostoma Rudolphi, 1809, the latter to Echinoparyphium Dietz, 1910. Sewell believing that there were insufficient grounds for separating them. The division of the main lateral canal into anterior and posterior branches was not observed in Cercaria indica XXIII, but it is obvious that Sewell postulated the division at the level of the excretory vesicle. It has been shown however, by McCoy (1928) for the cercaria of Echinoparyphium flexum, by Dubois (1929) for the cercaria of Echinoparyphium aconiatum Dietz, 1909 and by Wesenburg-Lund, (1934) and in the present work for the cercaria Echinoparyphium recurvatum that the main lateral canal divides into anterior and posterior branches opposite the posterior border of the ventral sucker. Thus, as has been pointed out by McCoy (1928) if all cercariae of the genus Echinoparyphium have similar excretory systems Cercaria indica XXIII is incorrectly classified, and must be placed in the Coronata group with the other cercariae of the genus Echinoparyphium. If these cercariae are to remain in the Echinata group then the group character must be modified. It can be seen then, that Sewell's classification is not satisfactory.

The classification has become further involved by the fact that Dubois (1929) although concluding that it is impossible to subdivide Echinostome cercariae into smaller natural groups suggested that two types of excretory formulae were evident, that of the cercaria of *Echinostoma revolutum* as given by Johnson (1920)

$$2\left[(3+3+3)+(3+3)+(3)+(3+3)\right]=48$$

and that of the cercaria of *Echinoparyphium aconiatum* as given by Dubois (1929)

$$2\left[(4+4+4)+(4+4)+(4)+(4+4)\right]=64$$

According to Dawes (1946) "these types would seem to represent the groups Echinata and Coronata" yet if these descriptions are correct and Sewell's groups remain unaltered both these cercariae must be placed in the Coronata group.

Further complications arise since according to Brown (1926) *Cercaria echinata* Siebold, 1837 which is regarded as the larva of *Echinostoma revolutum* has a completely different excretory system to that recorded by Johnson (1920) whilst Rees (1932) stated that the excretory formula of *Cercaria Z* Rees, 1932 may be expressed as

$$2\left[(4+4)+(4+4+4+4)\right]=48,$$ and that it is of the Echinata type.

It is evident that more implications have been put on Dubois's statement than he intended since he was in agreement with Cort (1915) that "it is impossible to subdivide them (Echinostome cercariae) into smaller natural groups". This statement is still correct and will remain so until more complete details of Echinostome cercariae are elucidated.

(d) Classification of the Xiphidiocercariae.

The cercaria of *Dolichosaccus rastellus* belongs to Lühe's group of Xiphidiocercariae. Lühe (1909) recognised four sub-groups namely, Cercariae microcotylae, Cercariae virgulae, Cercariae ornatae, and Cercariae armatae. The cercaria of *D. rastellus* on the basis of Lühe's characters clearly belongs to the cercariae armatae.

Cort (1915) erected a further sub-group of the Xiphidiocercariae called the Polyadenous cercariae containing the American forms *Cercaria polyadena* Cort, 1914, *Cercaria isocotylea* Cort, 1914, and the European forms *Cercaria limnaeae ovatae* = cercaria of *D. rastellus* and *Cercaria secunda* Sinitsîn, 1905, Cort (1915) gives the characters of the group as

1. Development in gastropods in elongate sac shaped sporocysts.

2. Tail slender and less than body length except when very much extended.

3. Acetabulum back of the middle of the body and smaller than the oral sucker.

4. Stylet about 0.030 mm. in length, six times as long as broad with a thickening one third distance from the point to the base.

5. Stylet glands six or more on each side between acetabulum and pharynx.

6. Excretory bladder bi-cornuate.

7. Very short prepharynx and small pharynx present. Oesphagus when developed short to medium length. Intestinal caeca when present reaching to the posterior end of the body.

Sewell (1922) recognised the fact that the Polyadenous cercariae formed part of the Cercariae armatae sub-group, and in addition placed two Indian forms belonging to the Cercariae armatae in the 'Daswan' division. He modified Cort's definition of the Polyadenous cercariae and also listed the characters of the Daswan cercariae, suggesting that *Cercaria tenuispina* and *Cercaria limnaeae ovatae* would eventually be placed in or near the Daswan grouping. E. L. Miller (1936) and Wesenburg-Lund (1934) disagreed with Sewell's division of the Cercariae armatae into Polyadenous and Daswan cercariae. Certainly as Miller (1936) points out, most of the characters given by Sewell (1922) for the Daswan cercariae apply equally well to the Polyadenous cercariae.

Further Sewell (1922) entirely agreed with Cort (1915) that the adults of the Polyadenous cercariae belong to the subfamily *Plagiorchiinae* Lühe, 1901 emend. Pratt, 1902 yet he still suggested that *Cercaria tenuispini* and *Cercaria* *limnaeae ovatae* (both larvae of species belonging to the subfamily *Plagiorchiinae* would in future be included in or near the Daswan cercariae. Consequently Sewell must have regarded both the Polyadenous and Daswan cercariae as containing larvae of members of the subfamily *Plagiorchiinae*, in such circumstances it is not surprising that most of the characters formulated should be common in both divisions.

The main difference between the two divisions lies in the excretory system. Taking *Cercaria polyadena* as the type species the excretory formula is $2\left[(3+3+3)+(3+3+3)\right]=36$ whilst that of Daswan cercariae is $2\left[(1+1+1)+(1+1+1)\right]=12$.

McMullen (1937b) reviewed all the known or partly known life cycles which included Xiphidiocercariae and suggested that there is sufficient evidence to recognise the super family *Plagiorchioidea* Dolphus, 1930 emended to include all trematodes which develop from Xiphidcercariae. He further suggested that the *Plagiorchiidae* Lühe, 1901 be redefined and restricted to those forms at present included in the *Plagiorchiinae*, and since the cercariae of all these species in which the life cycle is known belong to the Polyadenous cercariae, this characteristic should be included in the diagnosis.

The cercaria of *Dolichosaccus rastellus* = *Cercaria limnaeae ovatae* belongs to the Polyadenous cercariae since it possesses all the characters as originally formulated by Cort (1915) and as modified by Sewell (1922). The original placing of *Cercaria limnaeae ovatae* with the Polyadenous cercariae by Cort (1915) is thus shown to be correct even though at that time a detailed description of *Cercaria limnaeae ovatae* was not available. The fact that both *Cercaria limnaeae ovatae* and *Cercaria tenuispina* belong to the Polyadenous cercariae and are larvae of members of the subfamily *Plagiorchiinae* is in agreement with Cort's suggestion as to the position of the Polyadenous cercariae. It also substantiates McMullen's suggestion (1937b) that this characteristic should be utilised as a diagnostic feature.

(e) Classification of the Macrocercous Cercariae.

The cercaria of Phyllodistomum folium is a member of the Cystocercous group of Lühe (1909). It is not a natural grouping and consists of many varied forms, a fact which was recognised by Lühe, who therefore separated certain forms under the term Macrocercous cercariae which were known to be larval stages of members of the subfamily *Gorgoderinae* Looss, 1901. The cercariae of members of the subfamily *Anaporrhutinae* Looss, 1901 which with the subfamily *Gorgoderinae* constitute the family *Gorgoderidae* Looss, 1901 are unknown. Macrocercous cercariae are characterised by having a cylindrical non-forked tail, piercing stylet and development in sporocysts in *Sphaerium spp*. The subfamily Gorgoderinae is divided into three genera, *Gorgodera* Looss, 1899; *Gorgoderina* Looss, 1902; and *Phyllodistomum* Braun, 1899; the fourth genus *Cataptroides* Odhner in Looss, 1902 included by Lühe (1909) in the subfamily *Gorgoderinae* being shown by Dawes (1946) to be synonymous with *Phyllodistomum*.

Sewell (1922) divided the Macrocercous cercariae into Gorgodera and Gorgoderina groups, the former including the larval forms of members of the genus *Gorgodera* the latter of the genus *Gorgoderina*. He made no mention of the genus *Phyllodistomum* other than the fact that *Cercaria duplicata* which Lühe (1909) placed in the Rhopalocercous group of cercariae must be referred to a position in close proximity to the Cystocercous group. Sewell (1922) then lists the characters which define each group, the most important difference between the two groups being in the structure of the tail. The Gorgodera group have a clyindrical tapering tail without any marked swelling in the basal portion, the anterior end of the tail forming a cavity of length one tenth or less of the total tail length in which the cercaria may be entirely closed. In the Gorgoderina group the extreme anterior end of the tail forms a cavity which may enclose the distome body, behind which is a marked pear shaped swelling containing a number of spherical cells. The posterior portion of the tail is cylindrical and tapering, and the dilated anterior part is approximately one third of the total tail length.

Miller (1936) omitted this division of the Macrocercous cercariae when dealing with the Cystocerous group of Lühe "since experimental evidence for such a classification is lacking". Certainly only a few life cyles were known when Sewell formulated this division, the characters of the Gorgoderina group being based solely on the cercaria of Gorgoderina vitelliloba - Sewell refusing to agree with Lühe (1909) in calling it Cercaria macrocerca.

It has been shown in the present work that this cercaria is the larva of Phyllodistomum folium so that Sewell's division becomes untenable.

A survey of the larval forms of members of the subfamily Gorgoderinae shows that it is doubtful if any division of the cercariae can be made on the basis of the tail as envisaged by Sewell (1922). For instance in the genus Phyllodistomum, the cercaria of Phyllodistomum macrocotyle has an extremely short tail, and is placed in the Microcercous group of cercariae. Cercaria duplicata which may be the larva of a species in the genus Phyllodistomum (see page 75) has a more prominent tail, but it is still poorly developed as was shown by Wunder (1923). On the other hand the cercaria of Phyllodistomum solidum as described by Groves (1945) has characters which place it in a position between the Gorgodera and Gorgoderina groups. Here the anterior end of the tail which is one tenth the total length of the tail forms a cavity in which the body is enclosed. Behind this there is a slight swelling which contains a small pyramidal mass of cells which seem to be connected with the body. This swelling is greater than one would expect in the Gorgodera group, but less than that of the Gorgoderina. Finally there is the highly developed tail as is found in the cercaria of Phyllodistomum folium.

Comparisons of this kind show that cercariae which were formerly considered to belong to different groups can be shown to be related by studying the relevant intermediate forms. Even so, there does not appear at the present time any character which can be used to sub-divide the larval forms of members of the subfamily Gorgoderinae into groups which would correspond to the adult genera.

(f) Classification of the Furcocercous Cercariae.

Lühe (1909) distinguished between the Lophocercariae - monostome forked tail cercariae which are characterised by a median dorsal crest; the Furcocercariae - distome forked tail cercariae, and Gasterostome cercariae - forked tail cercariae with the mouth situated centrally on the ventral surface of the body. The Furcocercariae were then grouped according to characters which merely served to identify the few species then known.

Cort (1917) divided the six fully described Furcocercariae then known into three groups on the basis of the presence or absence of the pharynx, eyespots and the relative lengths of the furcal rami. Sewell (1922) like Lühe excluded the monostome forked tail cercariae from his classification of the Furcocercariae and placed them with the Monostome cercariae (see page 105). He discussed all the Furcocercariae then known modifying Cort's groups to include all the adequately described species.

Faust (1925) utilised the excretory system to classify digenetic trematodes and in this he included the monostome forked tail cercariae with the distome forked tail cercariae. Great emphasis was placed on the excretory system, Faust concluding that "all members of a natural adult group possess the same basic excretory pattern".

H.M.Miller (1926) discussed these classifications, giving a check list of all Furcocercariae, and presented a comprehensive scheme for their classification. The presence or absence of a pharynx was utilised to divide the Furcocercariae into two groups evidence being given showing that this is important in a natural classification. Other characters used in the classification were the relative lengths of the caudal furcae. Thus, four main sub-divisions were formed, the pharyngeal longifurcate; the pharyngeal brevifurcate; the apharyngeal longifurcate; and the apharyngeal brevifurcate cercariae. Where possible these divisions were divided into smaller natural groups on the basis of the presence or absence of the ventral sucker, together with extensive use of the excretory system. Even so, owing to the fact that few life histories were known and many descriptions were incomplete Miller concludes "it has been possible to make a satisfactory scheme of classification based largely on the excretory system, for only the apharyngeal brevifurcate distomes."

This classification proposed by Miller has proved extremely useful in so far as any Furcocercaria can be put into a schematic position and easily compared with related species. Of the four Furcocercariae described in the present work, one - Cercaria ocellata is an apharyngeal brevifurcate distome cercariae whilst the remaining three are pharyngeal longifurcate distome cercariae.

Miller (1926) after a detailed comparative study of the cercariae in this group was unable to sub-divide them. Cort & Brooks (1928) in a comparative study of holostome cercariae which constitute this same group thought that it was possible to define two sub-groups the Proalaria, and the Strigea sub-groups on the basis of the excretory system, position of the penetration glands and mode of development of the larva.

This division was augmented by Dubois (1929) and according to him Furcocercariae in the Proalaria sub-group develop through a Diplostomum stage and are pharyngeal longifurcate distomes with four penetration glands behind the ventral sucker, whilst those in the Strigea sub-group develop through a Tetracotyle stage, and are pharyngeal longifurcate distomes with four penetration glands in front of the ventral sucker.

So far as is known at the present time, all pharyngeal longifurcate distome cercariae with four penetration glands behind the ventral sucker develop through a Diplostomum stage, whilst those with four penetration glands in front of the ventral sucker develop through a Tetracotyle stage, yet the converse is not true. For instance *Cercaria emarginatae* possesses six penetration glands behind the ventral sucker and develops through a Diplostomum stage, whilst *Cercaria burti* according to Willey & Rabinowitz (1938) and *Cercaria helvetica XXXI* as described on page 92 both possess eight penetration gland cells latero-posterior to the ventral sucker and develop through a Tetracotyle stage. Clearly the group characters as envisaged by Dubois (1929) are not satisfactory, nevertheless the scheme is of great value. It would appear that the cercariae included by Dubois (1929) in his sub-groups form a division of two larger groups of cercariae which develop respectively through Diplostomum and Tetracotyle stages.

Cercaria A obviously belongs to the Proalaria sub-group of Dubois because of its life cycle and position of its four penetration glands, whilst *Cercaria hirsuta* cannot be placed in any special groupings of the pharyngeal longifurcate distome cercariae since nothing is known of its life history or of any closely related forms.

ECOLOGICAL.

(i) The Seasonal Fluctuations in Infection of Molluscs with Larval Trematodes.

This subject has received little attention, and the results obtained by various workers are not in agreement. Manson-Bahr and Fairley (1920) working in Egypt on the cercaria of *Schistosoma haematobium* (Bilharz,1852) Weinland,1858,and *Schistosoma mansoni* Sambon, 1907,found that the cercariae were present throughout the year, being highest in December, the infection in autumn being mature, that in spring immature. Soparkar (1921) using emerging cercariae as the indicator of the infection found that the percentage of infected snails was highest in autumn, lowest in late winter and early spring in the case of *Schistosoma spindale* Montgomery,1906 in India. Sewell (1922) also working in India found that maxima of infection occurred in December and July, whilst Miller &

Northrup (1926) working on the marine mollusc Nassa obsoleta (Say) also found a semi-annual rise and fall in the infection, the maxima again occurring in December and July. Rees (1932) likewise found a seasonal variation and discussed the underlying causes as had been done by Dubois (1929) and Sewell (1922).

On the other hand McCoy (1929) could not find any clear cut seasonal fluctuations although fluctuations did occur. McCormic (1923) and Wesenburg-Lund (1934) similarly were unable to find any regular seasonal fluctuations, whilst Cort, McMullen & Brackett (1937) studying the percentage infection in great detail over a short period of time, showed that the percentage of infected snails may vary very rapidly.

Diverse results like these are to be expected when workers deal with different molluscs, different parasites, either individually or in groups, found under widely different conditions. The results and discussion given below show that seasonal fluctuations can be expected and do occur within widelimits. The general seasonal fluctuations may be obliterated in some habitats which possess a restricted trematode fauna. However, knowledge of the life history of such trematodes enables the differences to be explained.

Analysis of the various factors which are involved in the seasonal incidence of infection is given first, thereby, facilitating examination of the actual results.

The presence of eggs is the first requisite for infection of the mollusc. The supply of eggs in any given locality is obviously dependent on many factors, such as the type and duration of the presence of the definitive host in any habitat eg. fish, migrant bird, or frog; the degree of infection of the definitive host; the number of flukes in infected hosts; the fecundity of the flukes; and possible addition of eggs from upstream if the habitat is not closed. All these factors influence the seasonal supply of eggs in a given habitat.

The hatching of the eggs is the next stage in the successful infection of the mollusc. This is of great importance in any seasonal variation, as conditions may be such, that, although the supply of eggs is uniform, the supply of active miracidia may be periodic. Hatching of the miracidium is largely dependent on temperature, as has been demonstrated by Dubois (1929) Mathias (1925) Willey (1941) although Bennett (1936) shows that hatching of eggs in the case of *Paramphistomum cotylophorum* Fischoeder, 1901 may be spread over a long period for a given temperature. In small bodies of water which may be subjected to freezing, hatching would be partially inhibited in winter, and there would be a tendancy for a maximum of active miracidia to occur in late spring, summer and early autumn.

Thus, the factors affecting the supply and hatching of eggs indicate that in no case is there found a constant supply of active miracidia each month of the year. A fish in a closed habitat infected with a trematode producing eggs which hatched on contact with water might be considered the nearest approach to uniformity of supply, yet even here the low temperature in winter decreases the supply of eggs as has been shown in the case of *Phyllodistomum folium* on page 135

Having established that the number of active miracidia present in a given habitat is subject to periodic fluctuations and that it is at a maximum in late spring, summer and autumn, as these are seasons of the year when hatching of miracidia in not inhibited and when the highest concentration of definitive hosts occur, it follows that the majority of molluscs become infected at this time, the main physical factor influencing the percentage of successful penetrations being the rate of flow of water through the habitat. Observations of the rate of flow of water in the localities studied, has shown that conditions in the late summer and early autumn (sometimes throughout autumn) are at an optimum for penetration of the miracidium. It is obvious however, that even during this period, climatic factors may cause the percentage of successful penetrations to vary from day to day even with a constant number of active miracidia.

The seasonal fluctuations in the infection of molluscs is also subject to other factors. Sewell (1922) rightly considered that the life history of the snail host was the most important factor. It can easily be seen that the timing and rate of reproduction, and length of life have a profound effect on the seasonal fluctuations. The degree of infection of say Limnaea truncatula which may have three generations in one year, is certain to be different from Limnaea pereger which in the area under survey has only one generation in a year. If reproduction of a mollusc occurred in late October one could visualise a very high incidence of infection throughout the summer and autumn, with little or no infection of the daughter generation the following winter and early spring. It can be seen therefore, that the life cycle of the molluscan host causes a seasonal variation in the degree of infection which must be regular owing to the regularity of the breeding season of the mollusc itself.

In the present work the seasonal fluctuations have largely been concerned with Limnaea pereger. This species was found to have one generation in a year, the breeding season extending from late March until the adults die during July and August. It can be seen then, that two generations of Limnaea pereger are present at a time when there is a maximum concentration of miracidia. This is obviously an advantageous mechanism since the chances of the successful completion of any life history are thereby much increased.

If penetration into the mollusc is independent of age of the mollusc, and if only adult molluscs are examined one would expect in the case of a mollusc with a life cycle like Limnaea pereger a high incidence of infection during mid-summer. By August this generation of snails are all dead, so that part of the August and the whole of the September collections consist of snails belonging to the daughter generation. These snails are quite small and very numerous - factors restricting the size of the population not having any great effect at this time, and consequently a low rate of infection would be expected with the September collection.

During autumn, conditions remain suitable for hatching and penetration of the miracidia, so that an increase in the percentage infection can be expected. Whether this increase would be maintained as winter approaches depends on several factors, such as the decrease in the number of active miracidia, the increase in rate of flow of the water, the rate of development of the parthenita in the snail, and the rate of recovery of snails after parasitisation. Decrease of temperature as winter approaches retards the rate of development of the parthenita in the mollusc. It seems likely therefore, that snails infected during autumn and early winter would remain infected, with little mortality of infected molluscs and no chance of any recovering from their infection. Consequently a gradual increase in the infection of snails throughout this period can be expected,most of the infections being immature.

With the approach of spring, one finds if the reasoning is correct, a high percentage of infected snails, furthermore, many of these infections are of long standing, so that they can be expected to be approaching maturity. At the first significant increase in temperature emergence of cercariae can be expected. The factors which influenced the infection rate during winter exert their effect again, but with opposite results. Increase in temperature accelerates development of the parthenita which, depending on the constitution of the snail host results either in its death or recovery from parasitisation. Either end has the same effect on the degree of infection namely, to reduce it. It has been shown however, that the increase in temperature also increases the number of miracidia present, thereby producing new infections. Thus, there are two opposing forces at work, if the two rates are equal, the percentage infection will stay the same, or there may be an increase or decrease in the percentage infection if the rates are dissimilar. It seems most likely that the overall percentage will decrease as there will be a longer time lag between the appearance of new infections, than there is in the decrease of old infections. For instance increase of temperature in the case of a frog has first to stumulate the fluke to activity. The eggs produced have to hatch, and development in the mollusc has to proceed several days before an infection can be detected on dissection. What is certain is that pronounced local fluctuations must be expected at this time.

As the height of summer approaches the rate of infection will increase rapidly, and an overall increase in the percentage of infected molluscs found can be reasonably expected, thus causing a second maximum in the seasonal infection. Whether this high rate of incidence of infection remains until all the snails of that generation are dead is doubtful, as one would expect the factors of age and parasitisation would produce a greater mortality rate than age alone, thus the last remaining snails in any habitat might be expected to be free of infection, thereby, decreasing the actual percentage infection. This lower rate of infection would initiate the large decrease in infection which is expected in the snails of the new generation.

Thus on the basis of the life cycle of the molluscan host and the environmental factors, the expected seasonal fluctuations in the percentage of snails parasitised have been deduced. It has been shown that in a mollusc which has one generation each year, with a breeding season in spring and summer, and with a winter temperature which occasionally results in the water freezing, a minimum of infection can be expected in newly hatched individuals in September, the percentage infection rising to a maximum in winter, with a further maximum about July. Minor fluctuations must be expected at all times, and that the above conclusions are general trends applied to a habitat or series of habitats which have a varied trematode fauna. Study of a habitat in which one species of trematode is dominant may result in a greatly different seasonal variation. Knowledge of its life history however, will explain the cause of the differences from the general trends. Many of the habitats studied fall into this category, their specific infection rates being discussed separately.

For this reason, together with the fact, that the number of snails collected from any one habitat, was not sufficiently large to give an accurate estimate of the true infection rate for that habitat as a whole, the total number of Limnaea pereger collected in any one month have been treated as a unit. Whilst this introduces the possibility of creating fluctuations owing to the differing numbers of individuals collected from each habitat in different months, it is sufficient to test the conclusions already derived regarding the seasonal infection of molluscs.

Table 21 shows the percentage infection of all the Limnaea pereger collected, and it can be seen that it agrees with the general trends already deduced. In the winter 1948 - 1949 the percentage of snails infected was at a maximum. During the spring the percentage decreased, but rose again in June. It was at a minimum in September when the daughter individuals were examined, and then rose again to another winter maximum. The infection for 1950 was similar to that of 1949.

The graph (page 171) however, of these results shows many minor fluctuations some of which are explained by examination of the detailed monthly table of infection (Table 22). The early infection percentages were dominated by the effect of the snails collected from Ibbotson Dam which at this period were highly infected. During the second maximum of 1949 all the habitats examined showed considerable infection and all contributed to the resulting maximum. The decrease during July may have been due in part to the preferential mortality of infected snails as discussed on page 120 but the presence of snails of the daughter generation obtained from Holme Dam contributed their effect. The decrease in the degree of infection continued during August - the collections from the Beauchief Dams consisting of snails of the daughter generation. In September the collections consisted entirely of daughter snails, and the percentage infection was at this time at a minimum. It can be seen however, that the degree of infection would have been much lower, but for the presence of the parthenita of Echinoparyphium recurvatum whose appearance in considerable numbers at this time is discussed on page 140

The expected increase was seen in October with a slight fall in the percentage infection for November. The low rate of infection of the snails taken from Monsaldale partly accounts for this slight fall. The winter maximum was reached in December, but the value is abnormally high as two out of the three collections that were taken in December were made at habitats with a consistently high infection. In January 1950 when collections from lightly infected habitats were made as well, the overall infection rate dropped and corresponds to that of October and November.

The infection decreased in February and a minimum was reached in March. It is interesting to record that although the overall infection was at a minimum in March, the presence of the parthenita of Dolichosaccus rastellus was considerable and accounted for nearly all the infected snails collected during that month. This demonstrates clearly that seasonal fluctuations at a restricted habitat (as instanced by one where Dolichosaccus rastellus is prevalent) may depart widely from the general trends which are at present being discussed.

The percentage infection then increased until July, followed by a sharp decline in August when the minimum was recorded. The August collection except for snails collected from Shepherd Dam consisted of snails of the daughter generation. The collections of September and October gave further proof that deductions should not be made regarding the general trend of the seasonal infection over a wide area from a study of restricted habitats. The September collection was unduly high owing to the large number of snails infected with Echinoparyphium recurvatum obtained from Holme Dam which formed a considerable part of the total collection; and conversely the October collection consisted largely of snails from two lightly infected habitats and therefore, was below the expected infection rate. The November and

and December collections were likewise small.

Specimens of Limnaea stagnalis were regularly obtained from two habitats, but the incidence of infection was very low and hence no seasonal variation could be studied.

At the Botanical Gardens all the infected snails except one contained parthenita of Dolichosaccus rastellus its distribution being discussed on page 151. It is interesting to record that Limnaea stagnalis certainly lives up to two years. Hence specimens one year old and over may be collected throughout the summer and into autumn. This is what was done with the 1950 collections from the Botanical Gardens. It showed that the percentage infection continued to rise until September, followed by a decrease in October and November. This then is further proof of the importance of the life cycle of the snail host. Although Limnaea stagnalis breeds at the same time as Limnaea pereger all the adult snails do not die by August. Hence, although a minimum of infection would be recorded in September as in the case of Limnaea pereger - if a random sample of adult and daughter individuals were taken, the minimum would not be as low as that of Limnaea pereger, owing to the presence of infected adults. This fact shows that the timing of the breeding season is the more important factor as compared with the length of life of the host on the seasonal fluctuations. It follows that the timing of the breeding season determines when a minimum in the percentage infection occurs, the length of life of the host influencing the degree of that minimum.

(ii) Ecology of the Molluscan Hosts.

Whilst the percentage of molluscs producing cercariae in a habitat is an important factor in the completion of a trematode life cycle, the absolute number of molluscs infected is of greater importance, thus the density of snails is a factor which must be considered in relation to a study of the trematode fauna. Whilst no experimental work on the ecology of the snail hosts was performed, a number of observations were made in the field. Almost every habitat proved of interest in this aspect hence each habitat will be dealt with separately. Most observations concern Limnaea pereger.

Dams in the Porter Valley.

Old Forge Dam - Only occasional visits were made to this Dam. Limnaea pereger were occasional and their size throughout the year conformed to that regarded as normal.

Wire Mill Dam is a large dam, and one of the two habitats where both L. pereger and L. stagnalis were found in numbers. Of the two species L. stagnalis was dominant, being frequent in occurrence and at times abundant. In spring and summer many individuals were to be seen browsing on the algae growing on the sides of the dam wall, whilst in winter they were present among the Elodea and on the leaves at the bottom of the dam. The numbers of L. pereger fluctuated considerably. It can be seen from Table 2 that they were found primarily in summer, and like L. stagnalis they too were more numerous on the side walls in spring and summer. Daughter snails were collected in August 1949, and the same generationcobtained until June. Thereafter, no snails of the following generation were obtained, Although specimens of L. pereger were more frequently infected with larval trematodes than L. stagnalis the disappearance of L. pereger cannot be correlated with this heavier infection, as L. pereger collected in other habitats were found with heavier infections and yet increased their numbers in the following generation (see page 126). Competition between the two species regarding food must have been inevitable, but this does not fully explain the disappearance of L. pereger.

Leather Mill Dam. This is rather a small dam, well silted up, decaying leaves being prominent on the muddy bottom. Both L. pereger and L. stagnalis occurred, the latter being observed in July 1949; in the months following it was collected sparingly in order to observe the change (if any) of the mollusc population, but no visible change in the population of either species occurred. L. stagnalis remained rare, whilst the population of L. pereger again fluctuated, being absent on some visits, and occasional at other times. Again it was found more easily in spring and early summer and these specimens were large as compared with the regarded normal, the infection rate being high and nearly always mature. Within a few days of being brought into the laboratory they would have produced enormous quantities of cercariae and died. Although the density of L. pereger in this dam was low, the high infection rate coupled with the large numbers of cercariae which each infected snail was capable of producing ensured a total number of cercariae far in excess of the expected, as indicated by the density of the snails.

Wesenburg-Lund (1934) was the first to suggest that gigantism in snails may be due to excessive growth induced by an infection, whilst Kendall (1949b) has shown the importance of nutrition of the snail in the rate of development, and production of cercariae. It may well be that both these phenomena occurred in Leather Mill Dam, the density of the snails being insufficient to create any competition for food between individual snails.

Shepherd Dam. This Dam is about the same size as Nether Mill Dam, but differs from it in that almost all the water is in the shade of surrounding trees, and that the depth of the water is much greater. Limnaea pereger occurred and it was mainly occasional in its distribution. Like the specimens of L. pereger collected from Leather Mill Dam, there was a high percentage infection mainly of Xiphidiocercariae for much of the year, yet neither dam became completely depopulated of L. pereger. Snails collected in spring and brought into the laboratory reacted very similar to those from Leather Mill Dam - producing large numbers of cercariae with the majority of snails quickly dying, but in marked contrast to the Leather Mill snails they were much smaller, with paler shells. The explanation of these differences which occurred in dams of close proximity both possessing a similar trematode fauna is not obvious. Possibly the shade limited the amount of algae which was further decreased by large numbers of Trichoptera larvae, thus inducing competition for food even though the density of snails was not great. Such factors were relatively absent in Leather Mill Dam.

Ibbotson Dam. The density of Limnaea pereger at this dam throughout the entire observation period was the highest found in any of the habitats studied. Three generations of snails were observed. The second showed a slight increase on the first, whilst the third showed a marked increase on the second, between one hundred and two hundred snails being collected with a single sweep of the net amongst bunches of Elodea which continually increased its area of colonisation. This increase in the population occurred in spite of two factors which would be considered as limiting the size of the population. From Table 5 it can be seen that the percentage infection of the snails collected in the first half of 1949 was very high, yet the number of L. pereger in the daughter generation increased. This generation too was subject to a moderate infection, together with what may be termed an artificial drought, during the months of April, May and June of 1950. During these three months the dam was drained, only a trickle of water passing down the centre of the dam, whilst the dam wall was completely repaired with concrete. In spite of this, the number of individuals in the following generation was greater than ever, many of them being found on the newly concreted wall. The snails of this generation remained very small during the time they were observed, much smaller than their counterparts of the two earlier generations, these latter snails being smaller than the considered normal. Among the small snails of the third generation were one or two large ones which probably belonged to the second generation.

Wesenburg-Lund (1934) in his study of snail populations and their trematode fauna in different years, came to the conclusion that at times the trematode infection killed off the entire population of snails. The observations made at Ibbotson Dam and at other localities show that in the area studied this was not the case, furthermore (see page 128) in habitats where snails disappeared the trematode infection was remarkably small.

Again non-operculate snails have always been regarded as being non-resistant to drought, but the work of Cawston (, 1929) on species of the family *Limnaeidae* and Kendall (1949a) on *Limnaea truncatula* shows that drought does not produce the mortality which was formerly presumed. In view of this evidence the occurrence of large numbers of snails after the draining of the dam is not surprising.

Nether Spur Gear Dam. This is a small dam and although it was repeatedly searched for snails none was ever found. A large resident population of aquatic birds was always present consisting of mallard and two swans (*Cygnus olor* Gmelin) Boycott (1936) indicates the importance of aquatic birds to any population of snails, a two fold effect being apparent - the snails being eaten for food, and the fouling of the water by the birds excreta - the latter probably being the more important factor in small bodies of water, thus, the absence of snails from this dam can readily be explained.

Holme Dam. This is a larger and deeper dam than Nether Spur Gear Dam with a small resident population of aquatic birds. No snails were found at this dam until the new generation of snails were observed in July 1949, from then on *L. pereger* was occasional to frequent in occurrence. The snails which were always dark in colour, of normal growth, and since no great fluctuation in their number was observed, it appeared that the mallard did not greatly effect the snail population. The volume of water in this dam is such that it precludes the possibility of fouling of the water by the birds' excreta, thereby, substantiating Boycott's statement that pollution is a more important factor than the eating of the snails as food on the snail population.

Beauchief Dams. The occurrence of *L. pereger* at these three dams has already been given (page 51). During the summer of 1949, the level of the water at the first dam decreased until the area of water was about one third normal. It was during this period that the snails disappeared. The change in conditions was less severe than at Ibbotson Dam when it was drained, the disappearance of snails cannot in consequence be correlated with the drying of the habitat.

The rapid colonisation and increase in the numbers of L. pereger at the second dam, after its dam wall had been repaired, parallels that already seen at Ibbotson Dam. The size of these snails was smaller than the accepted normal. The decrease in numbers of L. pereger at the third dam cannot be explained on the basis of their trematode infection, as the percentage infection was less than that at some habitats where the snails easily maintained their numbers. No pronounced change in the habitat was observed which could be considered likely to have an adverse effect on the snail population.

Botanical Gardens. This habitat was similar to Wire Mill Dam, in that both Limnaea stagnalis and L. pereger were found. The number of L. stagnalis remained high, varying from frequent to abundant. L. pereger however, was never found after August 1949. The infection of L. pereger with the parthenita of Dolichosaccus rastellus is discussed on page 131 , but it was not severe and cannot have been a major cause of the disappearance of the snail. The size of the habitat was very small and the occurrence of L. pereger occasional, so that there was every likelihood of the total number of snails being greatly reduced by those removed for sampling, this biotic factor was probably serious enough to effect the future generation.

On the other hand the disappearance of L. pereger in the presence of L. stagnalis closely parallels its disappearance at Wire Mill Dam under very similar conditions.

Ford. Two dams were visited, but from the first only bivalve molluscs were obtained. The second downstream, was much smaller, almost entirely silted up and with dense vegetation. The infection of L. pereger at this dam is discussed on page 130 Collections were first made in the summer of 1949 when the snail was occasional. There was a great increase in the number of snails in the following generation, the snail being abundant in autumn. In early 1950 the numbers decreased and the infection was very low, snails being collected throughout 1950 until May. No snails of the next generation were found.

At this dam the disappearance of snails can in no way be correlated with a high incidence of infection of the parent snails. The amount of water in the dam fluctuated considerably throughout the entire observation period, but no unusual fluctuation was observed in the summer of 1950. This dam was occasionally visited by mallard.

From the study of the two most important gastropod hosts at the major habitats visited, several general observations regarding the ecology of L. pereger and to a less extent L. stagnalis are evident.

1. The populations of L. pereger may vary enormously either way in successive years in habitats of close proximity.

2. The effect of a trematode infection in a population of L. pereger has no great influence on the numbers in the following generation.

3. Short periods of drought likewise have little effect on the population of L. pereger.

4. Recolonisation of a completely denuded habitat may not occur for several years, although recolonisation within a given habitat may be surprisingly rapid.

5. Disappearance of L. pereger from a habitat occurs between successive generations.

6. The presence of aquatic birds may be an important factor in determining the presence or absence of L. pereger.

7. L. stagnalis and L. pereger appear to be antagonistic to each other - this is to be expected as there must be competition for food. In two habitats where L. stagnalis was dominant, L. pereger disappeared, whilst in another habitat L. stagnalis only just maintained itself in the presence of more numerous L. pereger.

An impression was obtained from a study of all the habitats that the balance of nature relating to *L. pereger* was exceedingly fine. Below a critical density (occasional) *L. pereger* had difficulty in maintaining itself, whilst once above the critical density the numbers of *L. pereger* increased enormously tending to swamp the remainder of the fauna.

(iii) Biology of the Trematode.

(a) Distribution in areas studied.

Details of the distribution of the trematodes found in the area under survey are given in Tables 1 to 22. In addition the important features of their occurrence are included with the descriptions of each species. A study of these results shows that the distribution of a certain stage in the life cycle of the trematode gives no real indication as to the occurrence and distribution of other stages of the life cycle. In certain types of life cycles there does exist a very definite relationship between the distribution of the various stages, whilst in other cases such a relationship is not obvious. In the present work examples of both types have been found and these are discussed below.

It will be seen from Table 7 that at Ford many snails hatched in 1949 contained cysts of *Echinoparyhium recurvatum* whilst the number containing rediae was very small except in those collected in September 1949. The number of snails containing cysts in September was very high and remained so throughout that generation of snails. Although mature parthenita of *E. recurvatum* are found during autumn (this is discussed on page 140) it is difficult to relate this low infection of rediae in September with the high incidence of cysts which occurred at the same time. Out of sixty nine snails in the September collection, ten contained parthenita and sixty, cysts. Of these ten four infections were immature, one liberated cercariae on the day it was collected, three liberated cercariae after six days, one after seven and one after thirteen. In the previous June four out of eight snails liberated cercariae, it seems probable then that the majority of the sixty snails found containing cysts of E. recurvatum received their infection from the parent snails (which as the June collection indicates were highly infected) during the summer, when both generations existed together for a short while.

If this contention is correct, it is of the utmost importance since it shows that the definitive host can be absent from a locality for at least a year, and yet infective metacercariae may still occur. It also shows that the relationship of cysts to parthenita may be between daughter and parent snails.

The seasonal distribution of the parthenita of Dolichosaccus rastellus is interesting in view of the fact that frogs frequent the water only during the breeding season. Thus the effective production of eggs from the frog occurs annually and is of short duration. This fact is particularly important at a habitat such as the closed pond in the Botanical Gardens (see page 49). Under such conditions the greatest infection of snails can be expected to occur in the months following the frogs' breeding season - this is what happens as can be seen from Table 10. Furthermore it is of the utmost importance to the existence of the parasite that mature cercariae should be liberated into the water before the tadpoles metamphorphose at the end of June and the beginning of July so that the direct life cycle (page 45) can occur. Examination of the molluscan hosts showed that cercariae were liberated from June onwards, so that provided the adult frogs are infected and that suitable snail hosts are present there is every chance of the tadpoles becoming infected.

However, during 1950 infected specimens of Limnaea stagnalis of over one year old were found after July, whilst infected specimens of Limnaea pereger were found in the winter of 1949 at the Botanical Gardens. This might not have been expected to occur if the snail receives all its supply of eggs during a short period of the year. Bennett (1936) however, observed that in *Paramphistomum cotylophorum* the majority of eggs will hatch within a few days of the minimum time, but there are others which do not complete their development until months after. In one experiment developing embryos could be seen five months after hatching had begun - the cause of this slow development was not determined. Furthermore, snails have been kept under observation for several months without liberating cercariae, and yet on examination they have been found to be infected, whilst snails liberating mature cercariae have also been kept for similar periods. This delayed hatching of eggs may occur in many species of trematodes, and with the above observations on the development of parthenita in the molluscan host would satisfactorily account for the winter infections.

The same basic factors regarding the infection of the snail host with *Dolichosaccus rastellus* as have just been described for the Botanical Gardens apply to the three Dams at Beauchief, and also Wire Mill Dam, but they are more complex owing to the fact that these habitats receive their supply of water from natural sources. Nevertheless the maximum infection of the snails as would be expected occurs in April, May and June. The importance of the snail host is also evident, but one relationship which is not obvious is that it is not necesssary that a high percentage infection of snails should occur in order to give a high infection rate of tadpoles. A life cycle such as occurs here where the molluscan host is in close association with the tadpoles whose density is normally very high will inevitably result in little wastage of cercariae.

The details of the infection of the stickleback with *Phyllodistomum folium* at Wire Mill Dam have been given on page 73 . The relationship of a low incidence of infection of an intermediate host which causes a high degree of infection of a definitive host which selectively eats it can be readily understood, but the speed and extent of this infection in the case of Phyllodistomum folium at Wire Mill Dam during 1950 is surprising. Consideration of the important factors concerned in this indicates how this occurred. According to Regan (1911) Gasterosteus aculeatus breeds in spring and early summer, the time of spawning varying considerably in different years and according to locality. In Wire Mill Dam newly hatched sticklebacks were first observed during the latter part of June 1950 and of a sample of these newly hatched sticklebacks dissected on June 20 none were infected. Their lengths from snout to tip of the fin ranged from 12 to 21 mm. The contents of the stomachs of these fish were examined and with the exception of one fish of 21 mm. length all contained copepods and cladacerans. The exception contained two chironomids in addition to many crustacea. A further sample of fish collected on July 11 with sizes ranging from 18 to 30 mm. were examined and all were infected with Phyllodistomum folium, one fish of 23 mm. containing twenty five flukes. All contained chironomid larvae in their stomachs. It appears that the initial absence of flukes in these newly hatched sticklebacks can be correlated with the absence of chironomid larvae from their diet. Thereafter chironomid larvae are eaten and Hartley (1940) showed that the food of the stickleback consisted of 60% insects and 29% crustaceans. Of the insects eaten 80% were aquatic dipteran larvae. Hynes (1950) in a more detailed account lists the food items for varying fish sizes and for different periods of the year and shows that except for the chironomid each food item is of maximum importance at a certain season. The chironomids are equally important throughout the year as many as 40 larvae and 6 pupae occurring in one stomach. From this it can be seen that the commonest second intermediate host of Phyllodistomum folium is a very important food item of the sticklebacks, thus even with a low infection of the chironomidae a high infection rate in the definitive host could still be expected.

Also listed by Hynes (1950) as food items are oligochaetes and Sphaerium lacustre. It has already been shown (page 65) that Chaetogaster crystallinus and Sphaerium corneum may harbour the encysted stages of the cercaria so that they too may serve to infect the stickleback. The fact that they form only a minor part of the diet of the stickleback together with their limited infection (page 65) must reduce the part they play in infecting the definitive host to one of insignificance when compared with the chironomid larvae.

The life cycle of Sphaerium corneum also plays an important part in the degree of infection of the sticklebacks. They are most numerous during early summer decreasing in numbers during autumn, remaining at a low level thrgughout winter and increasing in numbers in the spring. The great increase in numbers of Sphaerium corneum which occurs during the spring is due to the production of young by those individuals which have survived the winter. They continue producing young into the summer, but during this period many of them die. Individuals born during spring grow rapidly and produce more young during summer, after which they themselves die during autumn. These individuals born during late summer grow slowly during autumn and those that survive the winter are responsible for the increase in numbers the following spring. There are thus two generations of Sphaerium corneum in a year as has been found by Thiel (1926) at Hambourg although Boycott (1936) reports that Sphaerium corneum appears to be annual. It might be expected that the production of two generations in a year would produce two.maxima in the number of individuals found. This has not been evident and must be due to two facts. Firstly that the production of the second generation overlaps the first, and secondly that the number of individuals produced in the second generation is smaller than the first generation which begin to die at this time. From the Table of infection of Sphaerium corneum (page 73) it can be seen that during spring and early summer of 1950 there was an increase in the percentage infection, this occurring at the same time that there was an increase in the number of individuals of <u>Sphaerium corneum</u>. The reason for this increase in the percentage infection is to be found in the development of the adults and eggs in the definitive host. The eggs of <u>Phyllodistomum folium</u> contain fully developed miracidia when they are laid and hatching occurs as soon as the eggs enter the water. Although eggs are found in the uteri of one month old flukes, the miracidia which develop inside are not mature till much later. Under natural conditions fully mature miracidia were not found in June hatched fish until late October and early November. During the winter when the temperature is low, examination of flukes revealed that development of the eggs is slow and that few miracidia are developing to maturity. Growth of the flukes and ~~productions~~ production of eggs continued even though at a slow rate during the winter so that with the increase in temperature in spring there were present a large number of partly developed eggs. Development during spring is rapid so that the maximum number of miracidia present at any time during the year occurs at this time. It is this large annual concentration of miracidia which was responsible for the increase in percentage infection obtained during spring and early summer of 1950.

It will have been noted (page 73) that during April there was a fall in the percentage of infection. This may be attributable to the fact that both the number of miracidia and <u>Sphaerium corneum</u> were increasing rapidly, as well as the fact that only molluscs of length 8 mm. or over were investigated. Thus the March collection consisted almost entirely of over-wintering individuals whilst the April collection contained both over-wintering and rapidly growing new individuals.

The high rate of infection of <u>Sphaerium corneum</u> was maintained until July, the maximum rate occurring in June and July which co-incided with the appearance of the newly hatched fish, this coupled with the fact that about

30% of the chironomids were infected at this time ensured a 100% infection of the definitive host.

Jones & Hynes (1950) have shown that sticklebacks live for a maximum of three and a half years, most becoming sexually mature in their first year. So far no mention of fish over one year old has been made, but it is clear that they must play some part in the bionomics of Phyllodistomum folium. They help to increase the concentration of miracidia in spring, and are responsible for all the miracidia produced during July, August, September and October. They are however, of minor importance since many die after breeding and their numbers are further reduced by children catching them especially during the "summer holidays." This minor importance of old fish together with the mortality of heavily parasitised Sphaerium corneum during August accounts for the rather conspicuous drop in the percentage infection which occurred during August and September.

Examination of aged fish show that although chironomids continue to be eaten and hence the fish acquire more flukes their number reaches a maximum - the highest yet recorded being one hundred and six from a stickleback 37 mm. in length, so that the flukes either die or are expelled from the bladder, the latter cause probably accounting for the removal of most of the flukes.

It can be seen then that June and July are the most important months for the maintainance of the species since it is during these months that all stages of the life cycle are prevalent, and it is at this time that newly hatched fish acquire infection. During the remaining months the important phase is the adult fluke in the definitive host, the intermediate hosts being of little importance as regards maintainance in respect of time.

(b) Infection of the Molluscan Host.

It is a well known fact that the miracidium exhibits a marked preference for a particular molluscan host, yet for many widely distributed trematodes there exists long lists of suitable hosts. The most common example is perhaps *Fasciola hepatica* which includes the following snails as its intermediate hosts, *Limnaea truncatula* in Europe, *Limnaea natalensis* in South Africa (Cawston, 1924) *Limnaea caillaudia* in East Africa (Van Someren, 1945) and *Limnaea brazieri* in Australia (Ross & McKay, 1929). *Cercaria ocellata* has similarly been found in a variety of hosts.

It appears then that although a miracidium may show a marked preference for a snail in a certain area a wider study of the trematode shows that it is not as specific as at first thought, and where a certain snail does not occur the miracidium is found to penetrate into a different species of snail. This is to be expected when studying distribution and specificity in widely separated areas, but the results obtained from the study of the snail hosts of *Dolichosaccus rastellus* indicate that specificity may similarly vary within a small area. From the infection records of *D. rastellus* at Wire Mill Dam and the Botanical Gardens, Tables 2, 10, 19 and 20) it is obvious that the miracidium shows a marked preference for *L. pereger*, yet when *L. pereger* disappeared from the Botanical Gardens, *L. stagnalis* was found to be the host. (Actually three specimens of *L. stagnalis* were found infected with sporocysts of *D. rastellus* before *L. pereger* disappeared). It seems then that even though development in *L. stagnalis* is possible so long as *L. pereger* is present, the miracidia do not penetrate *L. stagnalis*, the mechanism effecting such occurrences must be complex.

The other cercariae obtained, with the exception of the cercaria of *Phyllodistomum folium*, likewise showed a distinct preference for *L. pereger* although the two undescribed Xiphidiocercariae were occasionally found in *L. stagnalis*. The cercaria of *Phyllodistomum folium* was found only in *Sphaerium corneum*. The use of such a host is to be expected since Macrocercous cercariae have hitherto never been found in non-lamellibranchiate molluscs.

The findings of Sewell (1922) and Dubois (1929) on the rarity of multiple infections lead these authors to conclude that immunity occurred following the penetration of the first miracidium. Cort, McMullen, and Brackett (1937) critically analysed the results of these two workers when discussing their own detailed observations in a single habitat from which large numbers of snails of the species Stagnicola emarginata angulata (Sowerby) were taken. Their study of multiple infections was facilitated by the fact that at this period this snail was highly infected with a variety of trematodes.

They found that " a considerable number of multiple infections are present only when the incidence of positives is considerably over 50% and at least two species of cercariae have a rather high incidence," thus on the basis of this alone the results of Sewell (1922) Dubois (1929) and Rees (1932) who also found multiple infections to be rare can be explained. From their mathematical analysis of the results they were able to show that combinations of some species occurred as frequently as would be expected on the basis of chance whilst others did not, and concluded that "what evidence we have of an immunity or antagonism in the formation of double infections seems to be between individual species rather than between groups".

The occurrence of multiple infections in the present work as a whole has been rare, only eleven double infections having been found. The absence of double infections at most of the habitats studied is undoubtedly due to the overall low incidence of infection. At those habitats where double infections did occur their number was less than was expected when analysed by the method of Cort, McMullen and Brackett (1937). It is interesting to record that all these combinations except one were of cercariae which Cort, McMullen and Brackett similarly found either did not occur or were less than expected. Thus the cercaria of Echinoparyphiu recurvatum was never found associated with another cercaria, whilst the combinations of Xiphidocercariae with each other and with Furcocercariae were found to be less than expected.

It must be emphasised however, that these results were obtained from an analysis of very few snails as compared with the numbers used by Cort, McMullen and Brackett.

Even so it does appear that penetration of a miracidium does induce a resistance to the penetration and development of a second miracidium with regard to the species studied. Perhaps the most interesting double infection observation concerns the combination of *Cercaria ocellata* and the cercaria of *Notocotylus attenuatus* in a specimen of *Limnaea pereger*. Both these species were very rare in their occurrence (see Table 1). It indicates that even in small habitats the chances of infection of the snails are not equal. Much depends on the habits of the definitive host, as instanced by the same observation at two different habitats namely, the third Dam at Beauchief and Wire Mill Dam. During 1950 both frogs and toads spawned in these two Dams and it was noticed that at both dams the frogs mated and spawned mainly on one side, the toads on the other. In a closed habitat a difference such as this would greatly influence the trematode fauna of the molluscs in different parts of the habitat.

In view of this fact together with the difficulty of observing an immature stage of one species in a snail with a mature infection of another species and the possible occurrence of age resistance it seems that much doubt will exist with regard to induced immunity until the problem is studied experimentally.

(c) Effect of the larval Trematode on the Molluscan Host.

The seasonal fluctuations in the incidence of infection of the mollusc has already been discussed on (page 121) in addition to this there is a seasonal fluctuation in the emergence of cercariae. In the case of *Limnaea pereger* the emergence is low throughout autumn and winter, increases enormously in spring and remains relatively high throughout the summer. Observation of snails brought into the laboratory showed that in the autumn and winter most of the infections were immature and that the development of the parthenita was slow (c.f. page 119). The parthenita of *Echinoparyphium recurvatum* were an exception, for a considerable number of infected snails contained mature infections in September. As the winter progressed the number of snails collected containing mature infections of *E. recurvatum* was not maintained which suggests that either the snails recovered or that they died. Dissection of snails at all times failed to reveal any which had obviously previously been infected with any trematode stages, although occasionally snails would be dissected which had livers of abnormal appearance. It must therefore be concluded that the majority of infected snails in the area studied eventually died as a result of their infection (c.f.page 119). The same conclusion was reached by Thomas (1883) Manson Bahr & Fairley (1920) and Sewell (1922).

No difference was observed in the mortality rates of snails brought into the laboratory between those containing sporocysts and those containing rediae. This was undoubtedly due to the fact that the severity of an infection varies greatly from snail to snail. As the redia is much more active than the sporocyst, greater physical damage is caused by the redia, but to offset this there are usually many more sporocysts than rediae in a snail, with the consequence that a larger number of cercariae emerge from snails infected with sporocysts than those with rediae.

The cercaria is the second free living stage in the development of the trematode. The duration of this free living stage is short and if the life cycle is to be completed the cercaria has to reach the next host whether intermediate or deinitive host or encyst in a suitable place before death intervenes. No chemotoxis towards suitable hosts have been observed and consequently the cercaria is not as specific as to its choice of hosts as is the miracidium. Nevertheless cercariae do exhibit taxes and these have already been described for the cercariae studied in the present work. Analysis of the taxes exhibited by these cercariae where the next host was known shows that they facilitate contact with that host (see pages 91 and 96). Actual contact with the host however, is still a matter of chance.

(d) The Effect of the Metacercaria on the Intermediate Host.

The metacercarial stages seem to have little effect on the health of the host, except in certain cases where specific organs are heavily attacked, such as Dolichosaccus rastellus encysting in the buccal tissues of the tadpole and Cercaria A in the lens of the eye of the stickleback. Metacercariae observed under normal conditions have remained alive as long as the host lived (see page 101).

(e) Ecology of Larval Trematodes.

The study of the trematode fauna at the habitats visited reveals that it is unstable. This is to be expected when a large number of variable factors influence the complex trematode life cycle. Some habitats however, have maintained a fairly constant trematode fauna and in the area as a whole the larval trematodes found in 1949 were also found in 1950 sometimes at different habitats. The main factor which influences the trematode fauna is the instability of the mollusc population.

The results obtained from the Dams on the Porter River indicate that great differences in the trematode fauna may occur in habitats of close proximity, but it is interesting to note that there is an increase in the number of species of larval trematodes as one proceeds downstream.

The presence of certain stages of a trematode is not indicative that any other stages of the same species may be found in the same habitat. This is obvious from the fact that snails infected with Dolichosaccus rastellus were obtained from Dams on the Porter River downstream from the Dams where frogs spawned. A further example of a similar occurrence is mentioned on page 96. Thus both the miracidial and cercarial stages (particularly many Furcocercariae which are planktonic and have a relatively long free life) increase the distribution of the trematode. This spread of trematode stages from the origin of infection is of great importance in economic helminthology.

(f) On the life Cycles of the Trematoda.

The life cycles studied in the present work are typical of the great variation which occur in digenetic trematodes. The variation ranges from the simplest (though not necessarily the most primitive) as is seen in *Cercaria ocellata* to one of the most complex, that of *Phyllodistomum folium*. Between these two extremes there is a gradation in terms of complexity. It might be thought that the more complex life cycle is less likely to be completed than the more simple, but this is not so. Where penetration of the cercaria is direct into the definitive host, infection can only occur when the cercaria is present, and it has already been shown (page 139) that emergence of cercariae is discontinuous. Thus the use of an encysted stage increases the period of possible infection. The cyst may be found in the open as has been seen in the case of *Notocotylus attenuatus* - if this occurs entry into the definitive host is passive, depending entirely on the habits of the definitive host. Alternatively encystment may occur in a second intermediate host. This is of no advantage to the parasite unless the chances of the cercaria contacting the second intermediate host are greater than contacting the definitive host. Since the number of second intermediate hosts is usually much higher than the number of definitive hosts the chances of contact are correspondingly greater. Nevertheless contact with the second intermediate host has not completed the life cycle and contact between the second intermediate host and definitive host has still to be made. Since the parasite is in an inactive condition this change can only be made by the definitive host eating part or whole of the sedond intermediate host. Much depends on the feeding habits of the definitive host as to the likelihood of completion of a life cycle. Where the second intermediate host is the diet of a specialised feeder as is the stickleback of chironomid larvae then the chances of successful completion are high. Even where the percentage infection of the second intermediate host is low, a high rate of infection of the definitive host can still be maintained owing to the "concentrating" effect on the definitive host which probably has to eat large numbers of the intermediate host to maintain itself. This is clearly seen in the case of insectivorous definitive hosts as was shown by Brown (1927) in the case of Crepidostomum farionis (Müller, 1784). Where the definitive host is not a specialised feeder the chances of successfully completing the life cycle are much less than with specialised feeders.

It can be seen therefore that increasing complexity in life cycles may provide greater chances of successful completion, although there is no general rule regarding the complexity and completion of a life cycle. Much depends on the relative densities of the hosts concerned and their habits, two factors which have already been mentioned.

It does not follow that the more simple the life history the more primitive the trematode will be in the evolutionary scale. The study of the metacercaria of Dolichosaccus rastellus in the lungs of the tadpole (see page 45) which first undergoes encystment even though it has contacted the definitive host focussess attention on the importance of the metacercarial stage in the life cycle of the trematode. As has already been discussed by La Rue (1951) although little morphological change is noted in the majority of encysted metacercariae great physiological changes must occur. That this is so can be seen from the fact that the cercaria is a free living organism, whilst the adult stage lives in a very specialised habitat. The intervention of the metacercarial stage between the two enables the necessary physiological adjustments to be made. This therefore, explains the encystment of Dolichosaccus rastellus in the tadpole and the fact that it is not immediately infective. It also indicates why the Schistosome group of cercariae which penetrate directly into the definitive host ie. possessing the simplest type of life cycle cannot be regarded as being primitive.

GENERAL DISCUSSION.

Although only ten larval trematodes have been found in the area under survey, this has provided more than sufficient material for study (two Xiphidiocercariae not being described although details of their occurrence are included in the tables of infection). That this should be so, is a reflection on our present state of knowledge concerning the life histories and occurrence of digenetic trematodes. The fact that the present study has created as many problems as it has clarified, emphasis the great need for further research. There is however, an inherent disadvantage in studying most parasites, in that one must rely largely on chance as to the availability of suitable material. In view of this the writer has been fortunate in that two of the life cycles completed, concern trematodes whose life cycles were hitherto incompletely known. Further it is unfortunate that the most important structure in the trematode for determining phylogenetic relationships namely, the excretory system is the most difficult to study. Inevitably only slow progress will be made towards a natural classification and a true understanding of the Trematoda.

Table 1

Total infection of Limnaea pereger.

	C. of D. recurvatum	C. of D. rastellus	Xiphid b	Xiphid c	C. hirsuta	C. helvetica XXXI	Cercaria A	C. ocellata	C. of N. attenuatus	Infected, unidentified	Not infected	Double infection	Total
Wire Mill Dam	10	51	10	4						1	68		144
Leather Mill Dam	10	22	33	6	3	9					9	3	89
Shepherd Dam	6	74	87	13		3				16	58	1	256
Ibbotson Dam	87	45	185	42	19	66		2	1	27	317	5	786
Holme Dam	111	11	26	18		22	2	2	11	11	108	2	320
Old Forge Dam	10									1	15		26
Ford 2nd. Dam	21	8	1			4			8		196		238
Ford Stream	1	8	7							1	29		46
Ford River											3		3
Beauchief 1st. Dam		6									8		14
Beauchief 2nd Dam		23	18	6							252		299
Beauchief 3rd. Dam		44	7	1							76		128
Botanical Gardens		32	1								68		101
Round Dam		4	2						3	1	50		60
Monsal-Dale	10		2	1		4	4			2	17		40
Castleton					1						10		11
Lathkill Dale						1					3		4
Thornbridge Hall											15		15
Total	266	328	379	91	23	109	6	4	23	60	1302	(11)	2580

Table 2.

Infection of *Limnaea pereger* at Wire Mill Dam.

	C. of *E. recurvatum*	C. of *D. rastellus*	Xiphid b	Xiphid c	*C. hirsuta*	*C. helvetica XXXI*	*Cercaria A*	*C. ocellata*	C. of *N. attenuatus*	Infected, unidentified	Not infected	Double infection	Total
Dec. 1948		1									1		2
Jan. 1949		1									1		2
Feb.	1	5									3		9
Mar.											1		1
April		9	6	1						1	3		20
May													x
June													–
July	1										1		2
Aug.	4	1									10		15
Sept.											2		2
Oct.		1									1		2
Nov.		3									5		8
Dec.													–
Jan. 1950													x
Feb.	3	2		2							19		26
Mar.		14									7		21
Apl.	1	5	1	1							6		14
May		5	2								8		15
June		4	1								0		5
July													–
Aug.													–
Sept.													–
Oct													–
Nov													–
Total	10	51	10	4						1	68		144

Table 3.

Infection of *Limnaea pereger* at Leather Mill Dam.

	C. of *E. recurvatum*	C. of *D. rastellus*	Xiphid b	Xiphid c	*C. hirsuta*	*C. helvetica XXXI*	*Cercaria A*	*C. ocellata*	*C. of N. attenuatus*	Infected, unidentified	Not infected	Double infection	Total
Dec. 1948													–
Jan. 1949	1										0		1
Feb.													–
Mar.													–
April													–
May													–
June		3	3		1	3					2	1	11
July	1			1							0		2
Aug.	2										0		2
Sept.			1			1					0		2
Oct.													–
Nov.													–
Dec.			3								0		3
Jan. 1950.													x
Feb.		3	3								0		6
Mar.	1	3	1								2		7
April		3	6			2					1		12
May	2	3	3		1	1					0		10
June	1	3	7	2		2					2	1	16
July	2	2	5	1	1						2	1	12
Aug.			1	1							0		2
Sept.													–
Oct.													–
Nov.		2		1							0		3
Total	10	22	33	6	3	9					9	3	89

Table 4.

Infection of *Limnaea pereger* at Shepherd Dam.

	C. of *E. recurvatum*	C. of *D. rastellus*	Xiphid b.	Xiphid c.	*C. hirsuta*	*C. helvetica XXXI*	*Cercaria A*	*C. ocellata*	C. of *N. attenuatus*	Infected, unidentified	Not infected	Double infection	Total
Dec.1948													-
Jan.1949		6	6								3		15
Feb.	1	2	3							1	1		8
Mar.													-
April													-
May													-
June		1	6	2						1	3	1	12
July	1		2	1							6		10
Aug.			5							6	9		20
Sept.		1	5	4						5	7		22
Oct.	1	10	10							2	5		28
Nov.	2	7	8			1					2		20
Dec.		15	3								1		19
Jan.1950		5	4	3							2		14
Feb.	1	5	1								0		7
Mar.		7	1								4		12
April		7	8								3		18
May		1	5								0		6
June		6	10	1		2					1		20
July			9	2							1		12
Aug.			1								2		3
Sept.		1									2		3
Oct.											5		5
Nov.										1	1		2
Total	6	74	87	13		3				16	58	1	266

Table 5.

Infection of *Limnaea pereger* at Ibbotson Dam.

	C. of *E. recurvatum*	C. of *D. rastellus*	Xiphid b.	Xiphid c	*C. hirsuta*	*C. helvetica XXXI*	*Cercaria A*	*C. ocellata*	C. of *N. attenuatus*	Infected, unidentified	Not infected	Double infection	Total
Dec. 1948	14	1	11		2	6				4	10		48
Jan. 1949	6	4	14	5	5	15				2	10	1	60
Feb.	9	2	29	1	4	13				4	10	1	71
Mar.	10		21	2	5	8				2	4		58
April	14	1	16	6	2	10		2			11	1	61
May	5		1			1					1		8
June	9	1	5	1	1	3				3	17		40
July	4		3	1		3					4		15
Aug.	4		1			1				1	10		17
Sept.	5		3	2		1				1	39		51
Oct.		1	13	1		1				5	20		41
Nov.	1	4	13							1	26		45
Dec.		7	14	2		1				2	18		44
Jan. 1950		6	5	1							16		28
Feb.		3	11	9							17		40
Mar.		8	4	1							20		33
April		3	2	1		2					2		10
May													
June													
July		4	17	9		1			1		4	2	34
Aug.											11		11
Sept.											21		21
Oct.			2							2	40		44
Nov.											6		6
Total.	87	45	185	42	19	66		2	1	24	317	5	786

Table 6.

Infection of Limnaea pereger at Holme Dam.

	C. of E. recurvatum	C. of D. rastellus	Xiphid b	Xiphid c	C. hirsuta	C. helvetica XXXI	Cercaria A	C. ocellata	C. of N. attenuatus	Infected, unidentified	Not infected	Double infection	Total
Dec. 1948													-
Jan. 1949													-
Feb.													-
Mar.													-
April													-
May													-
June													-
July										3	8		11
Aug.	9					8				3	17		37
Sept.	10	4		1		2				1	3		21
Oct.	11		1	1						1	9		23
Nov.	6		6	2		1		1	2		4	1	21
Dec.	13	2	2								1		18
Jan. 1950	7	1	7	3					2		9		29
Feb.	10	1	3								4		18
Mar.	3	1	1					1	1		6		13
April	1	2	1	6		2			2		28		42
May			2	3		1					8		14
June	3			1		3					2	1	8
July	1		2								1		4
Aug.													-
Sept.	27		1			3	1		2	1	2		37
Oct.	7					1	1			2	2		13
Nov.	3			1		1			2		4		11
Total	111	11	26	18		22	2	2	11	11	108	2	320

Table 7

Infection of *Limnaea pereger* at Ford 2nd Dam.

	C. of E. recurvatum	C. of D. rastellus	Xiphid b	Xiphid c	C. hirsuta	C. helvetica XXXI	Cercaria A	C. ocellata	C. of N. attenuatus	Infected, unidentified	Not infected	No. with cysts of E. recurvatum	Total
Dec. 1948													x
Jan. 1949													x
Feb.													x
Mar.													x
April													x
May	3								6		10		19
June	4		1						1		2		8
July											0		1
Aug.													-
Sept.	10					4			1		54	60	69
Oct.	1	1									17	14	19
Nov.													x
Dec.													x
Jan. 1950	1	1									17	10	19
Feb.		1									15	8	16
Mar.		3									33	22	36
April	1	1									26	15	28
May	1										22	15	23
June													-
July													-
Aug.													-
Sept.													-
Oct.													-
Nov.													-
Total	21	8	1			4			8		196	144	238

Table 8

Infection of Limnaea pereger at Beauchief Second Dam.

	C. of *E. recurvatum*	C. of *D. rastellus*	Xiphid b	Xiphid c	*C. hirsuta*	*C. helvetica XXXI*	*Cercaria A*	*C. ocellata*	C. of *N. attenuatus*	Infected, unidentified	Not infected	Double infection	Total
Dec. 1948													x
Jan. 1949													x
Feb.													x
Mar.													x
April													x
May													x
June													–
July													–
Aug.											10		10
Sept.				1							7		8
Oct.			3								6		9
Nov.		2									6		8
Dec.													x
Jan. 1950		3		2							10		15
Feb.		1		1							22		24
Mar.		4	1								16		21
April		3	1								20		24
May		5	2								9		16
June		3	3								9		15
July			5	2							11		18
Aug.											12		12
Sept.			1								16		17
Oct.			2								38		40
Nov.		1									27		28
Dec.		1									33		34
Total		23	18	6							252		299

Table 9

Infection of *Limnaea pereger* at Beauchief Third Dam.

	C. of *E. recurvatum*	C. of *D. rastellus*	Xiphid b.	Xiphid c	*C. hirsuta*	*C. helvetica XXXI*	*Cercaria A*	*C. ocellata*	C. of *N. attenuatus*	Infected, unidentified	Not infected	Double infection	Total
Dec. 1948													x
Jan. 1949													x
Feb.													x
Mar.													x
April		13									22		35
May		5		1							3		9
June		3									1		4
July		2	2								2		6
Aug.											6		6
Sept.		1									6		7
Oct.		2	1								1		4
Nov.		1									6		7
Dec.													x
Jan. 1950													–
Feb.		2									1		3
Mar.											1		1
April		2									8		10
May		5									5		10
June		3	3								10		16
July													–
Aug.		4									0		4
Sept.		1									3		5
Oct.											1		1
Nov.													–
Total.		44	7	1							76		128

Table 10

Infection of *Limnaea pereger* at Botanical Gardens.

	C. of *E. recurvatum*	C. of *D. rastellus*	Xiphid b.	Xiphid c.	*C. hirsuta*	*C. helvetica XXXI*	*Cercaria A*	*C. ocellata*	*C. of N. attenuatus*	Infected, unidentified	Not infected	Double infection	Total
Dec. 1948													x
Jan. 1949		3	1								6		10
Feb.		6									18		24
Mar.		2									16		18
April		9									7		16
May		8									14		22
June		4									3		7
July											2		2
Aug.											2		2
Sept.													-
Oct.													-
Nov.													-
Dec.													-
Jan. 1950													-
Feb.													-
Mar.													-
April													-
May													-
June													-
July													-
Aug.													-
Sept.													-
Oct.													-
Nov.													-
Total		32	1								68		101

Table 11

Infection of Limnaea pereger at Round Dam.

	C. of E. recurvatum	C. of D. rastellus	Xiphid b.	Xiphid c.	C. hirsuta	C. helvetica XXXI	Cercaria A	C. ocellata	C. of N. attenuatus	Infected, unidentified	Not infected	Double infection	Total
Sept.1949		2							3	1	35		41
Oct. 1949		2	2								12		16
Nov. 1949											3		3
Total		4	2						3	1	50		60

Table 12

Infection of Limnaea pereger at Old Forge Dam

	C. of E. recurvatum	C. of D. rastellus	Xiphid b.	Xiphid c.	C. hirsuta	C. helvetica XXXI	Cercaria A	C. ocellata	C. of N. attenuatus	Infected, unidentified	Not infected	Double infection	Total
July 1949	2									1	2		5
Sept.1949	7										6		13
Mar. 1950	1										7		8
Total	10									1	15		26

Table 13

Infection of Limnaea pereger at Ford Stream.

	C. of E. recurvatum	C. of D. rastellus	Xiphid b.	Xiphid c.	C. hirsuta	C. helvetica XXXI	Cercaria A	C. ocellata	C. of N. attenuatus	Infected, unidentified	Not infected	Double infection	Total
May 1949		1	4								11		16
June 1949		5	2								5		12
July 1949		2									3		5
Sept.1949	1		1							1	10		13
Total	1	8	7							1	29		46

Table 14.

Infection of Limnaea pereger at Monsaldale.

	C. of E. recurvatum	C. of D. rastellus	Xiphid b.	Xiphid c.	C. hirsuta	C. helvetica XXXI	Cercaria A	C. ocellata	C. of N. attenuatus	Infected, unidentified	Not infected	Double infection	Total
July 1949	2		2	1							3		8
Oct. 1949	8					4	4				2		18
Nov. 1949										2	12		14
Total	10		2	1		4	4			2	17		40

Table 15

Infection of *Limnaea pereger* at Beauchief 1st. Dam.

	C. of *E. recurvatum*	[illegible] of *D. rastellus*	Xiphid b.	Xiphid c	C. *hirsuta*	C. *helvetica XXXI*	*Cercaria A*	C. *ocellata*	C. of *N. attenuatus*	Infected, unidentified	Not infected	Double infection	Total
June 1949		5									8		13
July 1949		1									0		1
Total		6									8		14

No snails ever found on subsequent visits.

Table 16

Infection of *Limnaea pereger* at Castleton

	C. of *E. recurvatum*	[illegible] of *D. rastellus*	Xiphid b.	Xiphid c	C. *hirsuta*	C. *helvetica XXXI*	*Cercaria A*	C. *ocellata*	C. of *N. attenuatus*	Infected, unidentified	Not infected	Double infection	Total
June 1950					1						10		11
Total					1						10		11

Table 17

Infection of *Limnaea pereger* at Lathkill Dale

	C. of *E. recurvatum*	[illegible] of *D. rastellus*	Xiphid b.	Xiphid c	C. *hirsuta*	C. *helvetica XXXI*	*Cercaria A*	C. *ocellata*	C. of *N. attenuatus*	Infected, unidentified	Not infected	Double infection	Total
Sept. 1950						1					3		4
Total						1					3		4

Table 18

Total infection of *Limnaea stagnalis*

	C. of *E. recurvatum*	[illegible] of *D. rastellus*	Xiphid b.	Xiphid c	C. *hirsuta*	C. *helvetica XXXI*	*Cercaria A*	C. *ocellata*	C. of *N. attenuatus*	Infected, unidentified	Not infected	Double infection	Total
Wire Mill Dam			6	5							267		278
Botanical Gardens		28		1							348		377
Leather Mill Dam											18		18
Round Dam											1		1
Total		28	6	6							634		674

Table 19

Infection of *Limnaea stagnalis* at Wire Mill Dam.

	C. of *E. recurvatum*	C. of *D. rastellus*	Xiphid b.	Xiphid c	*C. hirsuta*	*C. helvetica XXXI*	*Cercaria A*	*C. ocellata*	C. of *N. attenuatus*	Infected, unidentified	Not infected	Double infection	Total
Dec. 1948			3								30		33
Jan. 1949			2								24		26
Feb.			1								7		8
Mar.											7		7
April				1							12		13
May													x
June											11		11
July				1							9		10
Aug.											5		5
Sept.											13		13
Oct.											8		8
Nov.											16		16
Dec.											3		3
Jan. 1950													x
Feb.											12		12
Mar.													x
April											21		21
May											10		10
June											10		10
July											12		12
Aug.											15		15
Sept.											13		13
Oct.				2							10		12
Nov.				1							7	7	8
Dec.													X
Total			6	5							267		278

Table 20

Infection of *Limnaea stagnalis* at Botanical Gardens

	C. of *E. recurvatum*	C. of *D. rastellus*	Xiphid b.	Xiphid c	*C. hirsuta*	*C. helvetica XXXI*	*Cercaria A*	*C. ocellata*	C. of *N. attenuatus*	Infected, unidentified	Not infected	Double infection	Total
Dec.1948													x
Jan.1949		1									10		11
Feb.											23		23
Mar.		1									20		21
April											30		30
May		1									20		21
June											14		14
July											14		14
Aug.				1							20		21
Sept.											9		9
Oct.											20		20
Nov.											23		23
Dec.											8		8
Jan.1950											6		6
Feb.											11		11
Mar.											14		14
April											21		21
May		1									11		12
June		4									13		17
July		3									12		15
Aug.		7									15		22
Sept.		9									14		23
Oct.		1									14		15
Nov.											6		6
Total		28		1							348		377

Table 21

Aggregate of Monthly Infections of *Limnaea pereger*.

	C. of *E. recurvatum*	C. of *D. pantellus*	Xiphid b	Xiphid c	C. *hirsuta*	C. *helvetica XXXI*	*Cercaria A*	C. *ocellata*	C. of *N. attenuatus*	Infected, unidentified	Not infected	Double infections	Total	% not infected	% infected
Dec.1948	14	2	11		2	6				4	11		55	22.0	78.0
Jan.1949	7	14	21	5	5	15				2	20	1	88	22.6	77.4
Feb.	11	15	32	1	4	30				5	32	1	112	28.6	71.4
Mar.	16	2	21	2	5	8				2	21		77	27.3	72.7
April	14	32	22	7	2	10		2		1	43	1	132	32.6	67.4
May	8	14	5	1		1			6		39		74	52.7	47.3
June	13	22	17	3	2	6			1	4	44	2	110	40.0	60.0
July	11	6	9	4		3				4	31		68	45.6	54.4
Aug.	19	1	6			9				10	64		109	58.7	41.3
Sept.	33	8	10	8		8			4	9	169		249	67.8	32.2
Oct.	21	17	30	2		5	4			8	73		160	45.6	54.4
Nov.	9	17	27	2		2		1		3	64	1	126	50.8	49.2
Dec.	13	24	22	2		1				2	20		84	23.8	76.2
Jan.1950	8	16	16	9							54		105	51.4	48.6
Feb.	14	18	18	12							78		140	55.7	44.3
Mar.	5	40	8	1				1	1		111		167	66.4	33.6
April	3	26	19	8		6			2		94		158	59.4	40.6
May	3	19	14	3		2					52		94	55.3	44.7
June	4	19	24	4		7					34	2	91	37.4	62.6
July	3	6	38	14	1	1			1		19	3	80	23.7	76.3
Aug.		4	2	1							25		32	78.1	21.9
Sept.	27	2	3			4	1		2	1	47		87	54.0	46.0
Oct.	7		4			1	1			4	86		103	83.6	16.4
Nov.	3	3		2		1			2	1	38		50	76.0	24.0
Dec.		1									33		34	97.0	3.0
Total	266	328	379	91	23	109	6	4	23	60	1302	11	2580		
%	10.3	12.7	14.7	3.5	0.89	4.2	0.23	0.15	0.89		50.5				

Table 22.

Infection of *Limnaea pereger* collected in December 1948

	C. of *E. recurvatum*	C. of *D. rastellus*	Xiphid b	Xiphid c	*C. hirsuta*	*C. helvetica XXXI*	*Cercaria A*	*C. ocellata*	C. of *N. attenuatus*	Infected, unidentified	Not infected	Double infection	Total
Wire Mill Dam		1									1		2
Leather Mill Dam													–
Shepherd Dam													–
Ibbotson Dam	14	1	11		2	6				4	10		48
Holme Dam													–
Botanical Gardens													x
Ford 2nd Dam													–
Beauchief 2nd Dam													–
Beauchief 3rd Dam													x
Total	14	2	11		2	6				4	11	1	50

Infection of *Limnaea pereger* collected in January 1949.

	C. of *E. recurvatum*	C. of *D. rastellus*	Xiphid b	Xiphid c	*C. hirsuta*	*C. helvetica XXXI*	*Cercaria A*	*C. ocellata*	C. of *N. attenuatus*	Infected, unidentified	Not infected	Double infection	Total
Wire Mill Dam		1									1		2
Leather Mill Dam	1										0		1
Shepherd Dam		6	6								3		15
Ibbotson Dam	6	4	14	5	5	15				2	10	1	60
Holme Dam													–
Botanical Gardens		3	1								6		10
Ford 2nd Dam													x
Beauchief 2nd Dam													x
Beauchief 3rd Dam													x
Total	7	14	21	5	5	15				2	20	1	88

Infection of Limnaea pereger collected in February 1949

	C. of *E.* recurvatum	C. of *D.* rastellus	Xiphid b.	Xiphid c	*C.* hirsuta	*C.* helvetica XXXI	Cercaria A	*C.* ocellata	C. of *H.* attenuatus	Infected, unidentified	Not infected	Double infection	Total
Wire Mill Dam	1	5									3	1	9
Leather Mill Dam													–
Shepherd Dam	1	2	3							1	1	[illegible]	8
Ibbotson Dam	9	2	29	1	4	13				4	10	1	71
Holme Dam													–
Botanical Gardens		6									18		24
Ford 2nd. Dam													×
Beauchief 2nd Dam													×
Beauchief 3rd Dam													×
Total	11	15	32	1	4	13				5	32		112

Infection of *Limnaea pereger* collected in March 1949

	C. of *E.* recurvatum	C. of *D.* rastellus	Xiphid b.	Xiphid c	*C.* hirsuta	*C.* helvetica XXXI	Cercaria A	*C.* ocellata	C. of *H.* attenuatus	Infected, unidentified	Not infected	Double infection	Total
Wire Mill Dam											1		1
Leather Mill Dam													–
Shepherd Dam													–
Ibbotson Dam	16		21	2	5	8				2	4		58
Holme Dam													–
Botanical Gardens		2									16		18
Ford 2nd Dam													×
Beauchief 2nd Dam													×
Beauchief 3rd. Dam													×
Total	16	2	21	2	5	8				2	21		77

Infection of Limnaea pereger collected in April 1949

	C. of E. recurvatum	C. of D. rastellus	Xiphid b	Xiphid c	C. hirsuta	C. helvetica XXXI	Cercaria A	C. ocellata	C. of N. attenuatus	Infected, unidentified	Not infected	Double infection	Total
Wire Mill Dam		9	6	1						1	3		20
Leather Mill Dam													–
Shepherd Dam													–
Ibbotson Dam	14	1	16	6	2	10		2			11	1	61
Holme Dam													–
Botanical Gardens											7		16
Ford 2nd Dam													x
Beauchief 2nd Dam													x
Beauchief 3rd Dam		13									22		35
Total	14	32	22	7	2	10		2		1	43	1	132

Infection of Limnaea pereger collected in May 1949

Wire Mill Dam													x
Leather Mill Dam													–
Shepherd Dam													–
Ibbotson Dam	5		1			1					1		8
Holme Dam													–
Botanical Gardens		8									14		22
Ford 2nd Dam	3								6		10		19
Ford Stream		1	4								11		16
Beauchief 2nd Dam													x
Beauchief 3rd Dam		5		1							3		9
Total	8	14	5	1		1			6		39		74

Infection of Limnaea pereger collected in June 1949

	C. of E. recurvatum	C. of D. rastellus	Xiphid b	Xiphid c	C. hirsuta	C. helvetica XXXI	Cercaria A	C. ocellata	C. of N. attenuatus	Infected, unidentified	Not infected	Double infection	Total
Wire Mill Dam													–
Leather Mill Dam		3	3		1	3					2	1	11
Shepherd Dam		1	6	2						1	3	1	12
Ibbotson Dam	9	1	5	1	1	3				3	17		40
Holme Dam													–
Botanical Gardens		4									3		7
Ford 2nd Dam	4		1						1		2		8
Ford Stream		5	2								5		12
Ford River											3		3
Beauchief 1st Dam		5									8		13
Beauchief 2nd Dam													–
Beauchief 3rd Dam		3									1		4
Total	13	22	17	3	2	6			1	4	44	2	110

Infection of Limnaea pereger collected in July 1949

Wire Mill Dam	1										1		2
Leather Mill Dam	1			1							0		2
Shepherd Dam	1		2	1							6		10
Ibbotson Dam	4		3	1		3					4		15
Holme Dam										3	8		11
Botanical Gardens											2		2
Ford 2nd Dam		1									0		1
Ford Stream		2									3		5
Beauchief 1st Dam		1									0		1
Beauchief 2nd Dam													–

Infection of Limnaea pereger collected in July 1949

	C. of *E.* recurvatum	C. of *D.* rastellus	Xiphid b	Xiphid c	*C.* hirsuta	C.helvetica XXXI	Cercaria A	*C.* ocellata	C. of *H.* attenuatus	Infected, unidentified	Not infected	Double infection	Total
Monsal-Dale	2		2	1							3		8
Old Forge Dam	2									1	2		5
Total	11	6	9	4		3				4	31		68

Infection of Limnaea pereger collected in August 1949.

	C. of *E.* recurvatum	C. of *D.* rastellus	Xiphid b	Xiphid c	*C.* hirsuta	C.helvetica XXXI	Cercaria A	*C.* ocellata	C. of *H.* attenuatus	Infected, unidentified	Not infected	Double infection	Total
Wire Mill Dam	4										10		15
Leather Mill Dam	2										0		2
Shepherd Dam			5							6	9		20
Ibbotson Dam	4		1			1				1	10		17
Holme Dam	9					8				3	17		37
Botanical Gardens											2		2
Ford 2nd Dam													–
Beauchief 2nd Dam											10		10
Beauchief 3rd Dam											6		6
Total	19	1	6			9				10	64		109

Infection of Limnaea pereger collected in September 1949

	C. of *E.* recurvatum	C. of *D.* rastellus	Xiphid b	Xiphid c	*C.* hirsuta	C.helvetica XXXI	Cercaria A	*C.* ocellata	C. of *H.* attenuatus	Infected, unidentified	Not infected	Double infection	Total
Wire Mill Dam											2		2
Leather Mill Dam			1			1					0		2
Shepherd Dam		1	5	4						5	7		22
Ibbotson Dam	5		3	2		1				1	39		51
Holme Dam	10	4		1		2				1	3		21
Ford 2nd Dam :	10					4					54		69
Ford Stream	1		1							1	10		13
Beauchief 2nd Dam				1									

Infection of Limnaea pereger collected in September 1949

	C. of E. recurvatum	C. of D. rastellus	Xiphid b	Xiphid c	C. hirsuta	C. helvetica XXXI	Cercaria A	Cercaria ocellata	C. of N. attenuatus	Infected, unidentified	Not infected	Double infection	Total
Beauchief 3rd Dam		1									6		7
Round Dam		2							3	1	35		41
Old Forge Dam	7										6		13
Total	33	8	10	8		8			4	9	169		249

Infection of Limnaea pereger collected in October 1949

Wire Mill Dam		1									1		2
Leather Mill Dam			.										-
Shepherd Dam	1	10	10							2	5		28
Ibbotson Dam		1	13	1		1				5	20		41
Holme Dam	11		1	1						1	9		23
Ford 2nd Dam	1	1									17		19
Beauchief 2nd Dam			3								6		9
Beauchief 3rd Dam		2	1								1		4
Round Dam		2	2								12		16
Monsal-Dale	8					4	4				2		18
Total	21	17	30	2		5	4			8	73		160

Infection of Limnaea pereger collected in November 1949.

Wire Mill Dam		3									5		8
Leather Mill Dam													-
Shepherd Dam	2	7	8			1					2		20
Ibbotson Dam	1	4	13							1	26		45
Holme Dam	6		6	2				1	2		4	1	21
Ford 2nd Dam													X

Infection of *Limnaea pereger* collected in November 1949

	C. of *E. recurvatum*	C. of *D. rastellus*	Xiphid b	Xiphid c	*C. hirsuta*	*C. helvetica XXXI*	*Cercaria A*	*C. ocellata*	C. of *N. attenuatus*	Infected, unidentified	Not infected	Double infection	Total
Beauchief 2nd Dam		2									6		8
Beauchief 3rd Dam		1									6		7
Round Dam											3		3
Monsal-dale										2	12		14
Total	9	17	27	2		2		1	2	3	64	1	126

Infection of *Limnaea pereger* collected in December 1949

	C. of *E. recurvatum*	C. of *D. rastellus*	Xiphid b	Xiphid c	*C. hirsuta*	*C. helvetica XXXI*	*Cercaria A*	*C. ocellata*	C. of *N. attenuatus*	Infected, unidentified	Not infected	Double infection	Total
Wire Mill Dam												–	–
Leather Mill Dam			3									1	3
Shepherd Dam		15	3								1		19
Ibbotson Dam		7	14	2		1				2	18		44
Holme Dam	13	2	2								1		18
Ford 2nd Dam													x
Beauchief 2nd Dam													x
Beauchief 3rd Dam													x
Total	13	24	22	2		1				2	20		84

Infection of *Limnaea pereger* collected in January 1950.

	C. of *E. recurvatum*	C. of *D. rastellus*	Xiphid b	Xiphid c	*C. hirsuta*	*C. helvetica XXXI*	*Cercaria A*	*C. ocellata*	C. of *N. attenuatus*	Infected, unidentified	Not infected	Double infection	Total
Wire Mill Dam													x
Leather Mill Dam													–
Shepherd Dam		5	4	3							2		14
Ibbotson Dam		6	5	1							16		28
Holme Dam	7	1	7	3							9		29
Ford 2nd Dam	1	1									17		19

Infection of Limnaea pereger collected in January 1950

	C. of E. recurvatum	C. of D. rastellus	Xiphid b	Xiphid c	C. hirsuta	C. helvetica XXXI	Cercaria A	C. ocellata	C. of N. attenuatus	Infected, unidentified	Not infected	Double infection	Total
Beauchief 2nd Dam		3		2							10		15
Beauchief 3rd Dam													—
Total	8	16	16	9					2		54		105

Infection of Limnaea pereger collected in February 1950

	C. of E. recurvatum	C. of D. rastellus	Xiphid b	Xiphid c	C. hirsuta	C. helvetica XXXI	Cercaria A	C. ocellata	C. of N. attenuatus	Infected, unidentified	Not infected	Double infection	Total
Wire Mill Dam	3	2		2							19		26
Leather Mill Dam		3	3								0		6
Shepherd Dam	1	5	1								0		7
Ibbotson Dam		3	11	9							17		40
Holme Dam	10	1	3								4		18
Ford 2nd Dam		1									15		16
Beauchief 2nd Dam		1		1							22		24
Beauchief 3rd Dam		2									1		3
Total	14	18	18	12							78		140

Infection of Limnaea pereger collected in March 1950

	C. of E. recurvatum	C. of D. rastellus	Xiphid b	Xiphid c	C. hirsuta	C. helvetica XXXI	Cercaria A	C. ocellata	C. of N. attenuatus	Infected, unidentified	Not infected	Double infection	Total
Wire Mill Dam		14									7		21
Leather Mill Dam	1	3	1								2		7
Shepherd Dam		7	1								4		12
Ibbotson Dam		8	4	1							20		33
Holme Dam	3	1	1						1	1	6		13
Ford 2nd Dam		3									33		36
Beauchief 2nd Dam		4	1								16		21
Beauchief 3rd Dam											1		1
Old Forge Dam	1										7		8
Thornbridge Hall											15		15
Total	5	40	8	1					1	1	111		167

Infection of *Limnaea pereger* collected in April 1950

	C. of *E. recurvatum*	C. of *D. rastellus*	Xiphid b	Xiphid c	*C. hirsuta*	*C. helvetica XXXI*	*Cercaria A*	*C. ocellata*	C. of *N. attenuatus*	Infected, unidentified	Not infected	Double infection	Total
Wire Mill Dam	1	5	1	1							6		14
Leather Mill Dam		3	6			2					1		12
Shepherd Dam		7	8								3		18
Ibbotson Dam		3	2	1		2					2		10
Holme Dam	1	2	1	6		2			2		28		42
Ford 2nd Dam	1	1									26		28
Beauchief 2nd Dam		3	1								20		24
Beauchief 3rd Dam		2									8		10
Total	3	26	19	8		6			2		94		158

Infection of Limnaea pereger collected in May 1950

Wire Mill Dam		5	2								8		15
Leather Mill Dam	2	3	3		1	1					0		10
Shepherd Dam											0		6
Holme Dam			2	3		1					8		14
Ford 2nd Dam	1										22		23
Beauchief 2nd Dam		5	2								9		16
Beauchief 3rd Dam		5									5		10
Total	3	19	14	3	1	2					52		94

Infection of *Limnaea pereger* collected in June 1950

Wire Mill Dam		4	1								0		5
Leather Mill Dam	1	3	7	2		2					2	1	16
Shepherd Dam		6	10	1		2					1		20
Holme Dam	3			1		3					2		8
Beauchief 2nd Dam		3	3								9		15
Beauchief 3rd Dam		3	3								10		16

Infection of Limnaea pereger collected in June 1950

	C. of E. recurvatum	C. of D. rastellus	Xiphid b	Xiphid c	C. hirsuta	C. helvetica XXXI	Cercaria A	C. ocellata	C. of N. attenuatus	Infected, unidentified	Not infected	Double infection	Total
Castleton					1						10		11
Total	4	19	24	4	1	7					34	1	91

Infection of Limnaea pereger collected in July 1950

Leather Mill Dam	2	2	5	1	1						2	1	12
Shepherd Dam			9	2							1		12
Ibbotson Dam		4	17	9		1			1		4	2	34
Holme Dam	1		2								1		4
Beauchief 2nd Dam			5	2							11		18
Beauchief 3rd Dam													–
Total	3	6	38	14	1	1			1		19	3	80

Infection of Limnaea pereger collected in August 1950

Leather Mill Dam			1	1							0		2
Shepherd Dam			1								2		3
Ibbotson Dam											11		11
Holme Dam													–
Beauchief 2nd Dam											12		12
Beauchief 3rd Dam		4									0		4
Total		4	2	1							25		32

Infection of Limnaea pereger collected in Sept. 1950

Leather Mill Dam													–
Shepherd Dam		1									2		3
Ibbotson Dam											21		
Holme Dam	27		1			3	1		2	1	2		37
Beauchief 2nd Dam			1										

Infection of Limnaea pereger collected in Sept. 1950

	C. of E. recurvatum	C. of D. rastellus	Xiphid b	Xiphid c	C. hirsuta	C. helvetica XXXI	Cercaria A	C. ocellata	C. of N. attenuatus	Infected, unidentified	Not infected	Double infection	Total
Beauchief 3rd Dam		1	1								3		5
Lathkill Dale						1					3		4
Total	27	2	3			4	1		2	1	47		87

Infection of Limnaea pereger collected in October 1950

	C. of E. recurvatum	C. of D. rastellus	Xiphid b	Xiphid c	C. hirsuta	C. helvetica XXXI	Cercaria A	C. ocellata	C. of N. attenuatus	Infected, unidentified	Not infected	Double infection	Total
Leather Mill Dam													–
Shepherd Dam											5		5
Ibbotson Dam			2							2	40		44
Holme Dam	7					1	1			2	2		13
Beauchief 2nd Dam			2								38		40
Beauchief 3rd Dam											1		1
Total	7		4			1	1			4	86		103

Infection of Limnaea pereger collected in November 1950

	C. of E. recurvatum	C. of D. rastellus	Xiphid b	Xiphid c	C. hirsuta	C. helvetica XXXI	Cercaria A	C. ocellata	C. of N. attenuatus	Infected, unidentified	Not infected	Double infection	Total
Leather Mill Dam		2		1							0		3
Shepherd Dam										1	1		2
Ibbotson Dam											6		6
Holme Dam	3			1		1			2		4		11
Beauchief 2nd Dam		1									27		28
Beauchief 3rd Dam		1											–
Total	3	3		2		1			2	1	38		50

Infection of Limnaea pereger collected in December 1950

	C. of E. recurvatum	C. of D. rastellus	Xiphid b	Xiphid c	C. hirsuta	C. helvetica XXXI	Cercaria A	C. ocellata	C. of N. attenuatus	Infected, unidentified	Not infected	Double infection	Total
Beauchief 3rd Dam		1									33		34
Total		1									33		34.

X signifies Habitat not visited.

- signifies Habitat visited but no snails found

Graph of the results given in Table 21
showing the monthly percentage infection of *Limnaea*
pereger with larval trematodes.

SUMMARY OF RESULTS.

1. Eight species of larval trematodes together with attempts to complete their life histories, and details of their occurrence have been described.

2. The cercaria of Notocotyls attenuatus has been found to encyst on the shells of Limnaea pereger, the adult stage being located in the caeca of experimentally infected ducks.

3. The cercaria of Echinoparyphium recurvatum encysts in the tissues of Limnaea pereger, both pigeons and ducks were experimentally infected, the location of the adult being the intestine.

4. The cercaria of Dolichosaccus rastellus has been found to encyst in insects and in tadpoles. Frogs are infected by eating the insect - this being the normal life history for a member of the Xiphidiocercariae. Cercariae encysting in tadpoles, excyst after a time, reach the intestine and grow to maturity by which time the tadpole has metamorphosed into a frog. This more direct life cycle has been shown to be more successful in the habitats where it has been studied, than the more normal type, it is however, restricted to one short period in the life of the frog whilst the normal life history is not so restricted.

5. The cercaria of Phyllodistomum folium has been found to be Cercaria macrocerca and not Cercaria duplicata as previously reported. The cercaria is eaten by chironomid larvae after which it passes from the fore-gut into the haemocoel and there encysts. The life cycle is completed by the stickleback eating infected chironomid larvae, the adult being located in the ureters and bladder.

6. The cercaria of Trichobilharzia ocellata and Cercaria hirsuta have been obtained from Limnaea pereger but attempts to complete their life cycles were unsuccessful.

7. Cercaria helvetica XXXI has been found to penetrate into the leeches Herpobdella atomaria and Helobdella stagnalis and develop into the "Tetracotyle" stage.

Cercaria A n.sp. was found to develop into a diplostomum larva in the eye of the stickleback. Efforts to obtain the adult stages of both these cercariae from ducks were unsuccessful.

8. The percentage infection of *Limnaea pereger* with larval trematodes was found to have two maxima in a year. The main factors influencing the percentage being the timing of the snails breeding season and its length of life.

9. The constitution of the larval trematode population may vary greatly from season to season and from year to year in habitats of close proximity, although in the area as a whole all the larval trematodes found in 1949 were found in 1950.

10. It has been shown that many factors influence the completion of a trematode life cycle. Of those that have been studied, the most specialised namely, *Phyllodistomum folium* was the most successful, more important however, is the density and size of the hosts concerned in the life cycle.

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ABBREVIATIONS USED IN FIGURES.

a.o.	anterior organ.
a.sp.	aboral spine.
a.l.c.	antero-lateral excretory canal.
a.l.d.	antero-lateral excretory duct.
a.l.m.	antero-lateral musculature.
ad.o.	adhesive organ.
b.	body of cercaria.
b.c.	blind caecum.
b.d.	blindly ending excretory duct.
b.p.	birth pore.
c.	intestinal caecum.
c.a.	ciliated area.
c.d.	caudal duct.
c.f.	caudal furca.
c.g.	caudal gland cells.
c.p.	cirrus pouch.
c.s.	cuticular spine.
c.w.	cyst wall.
c.ev.	cells surrounding excretory vesicle.
c.mu.	circular muscle band.
c.sp.	corner spine.
ch.	anterior part of swollen globe forming chamber.
ci.	cirrus.
co.	collar.
cer.	immature cercaria.
d.l.p.	dorsal locomotor pocket.
d.v.m.	dorso-ventral musculature.
d.m.l.c.	descending portion of main lateral excretory canal.
e.	egg.
e.g.	excretory granule.
e.p.	excretory pore.
e.s.	excretory sinus.
e.v.	excretory vesicle.
e.pr.	lateral epaulette.
e.d.s.	elongated distal stem.
f.b.	fore body.

f.c.	flame cell.
f.f.	fin fold.
g.b.	germ ball.
g.c.	group of cells.
g.p.	genital pore.
g.p.g.	anterior coarsely granular penetration gland cells.
h.b.	hind body.
h.c.	head collar.
h.g.	head gland.
h.p.g.	posterior finely granular penetration gland cells.
i.	intestine.
i.C.	island of Cort.
i.l.l.	inner laminated layer.
l.	lappet.
L.c.	Laurer's canal.
l.m.	lateral musculature.
l.es.	lateral eyespot.
l.mu.	longitudinal muscle band.
l.s.c.	large cell.
m.	mouth.
m.c.	median canal.
m.d.	main collecting duct.
m.es.	median eyespot.
m.a.o.	muscular portion of anterior organ.
m.l.c.	main lateral excretory canal.
m.p.m.	median posterior musculature.
o.p.	opening of chamber enclosing body of cercaria.
o.s.	oral sucker.
o.sp.	oral spine.
o.g.l.	outer granular layer.
o.p.a.	original point of adherence.
o.t.l.	outer transparent layer.
ov.	ovary.
oes.	oesophagus.
ovd.	oviduct.
p.	pharynx.
p.d.	penetration ducts.

p.g.	penetration gland cells.
p.p.	prepharynx.
p.s.	piercing spine.
p.l.c.	postero-lateral excretory canal.
p.l.d.	postero-lateral excretory duct.
p.s.g.	proximal swollen globe.
s.	stylet.
s.h.	sensory hair.
s.p.	sensory papilla.
s.v.	seminal vesicle.
s.l.d.	secondary lateral excretory duct.
st.	stalk connecting body to tail.
t.	testis.
t.c.	transverse excretory canal.
t.d.	transverse excretory duct.
t.s.	tail stem.
ut.	uterus.
v.	vacuole.
v.c.	vacuoles coalescing.
v.d.	vas deferens.
v.e.	vas efferens.
v.s.	ventral sucker.
vag.	vagina.
vit.	vitelline glands.

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